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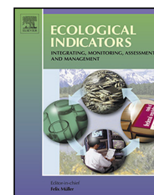

Original article

Why lichens are bad biomonitors of ozone pollution?

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ABSTRACT

The combined effects of water availability and ozone (O_3) on chlorophyll *a* fluorescence (Chl_aF) were studied in three epiphytic lichens selected for their different ecology. The samples were exposed in open top chambers (OTCs) under different watering regimes with O_3 AOT40 in the range 0–50,000 ppb. Further samples were exposed in a nearby wood, as controls. Chl_aF measurements were taken before exposure, after 3- and 6-week exposure and after a subsequent 2-day recovery period to verify the long-term effects of O_3 exposure. All species tolerated the pollutant well. However, there was a strong influence associated with the mode of exposure: the Chl_aF emission remained steady over time in the controls, whereas it varied significantly in chamber-exposed samples, with a strong decrease of F_v/F_m in non-watered and morning-watered samples, and a small decrease in evening-watered samples. Chl_aF emission characteristics were also influenced by the weather conditions of the day preceding measurements, with some species-specific differences possibly related to species ecology. The ozone-tolerance of lichens is thoroughly discussed on the basis of the cellular mechanisms that allow these organisms to overcome the oxidative burst associated with the cycles of dehydration–rehydration typical of poikilohydrous organisms.

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1. Introduction

Tropospheric ozone (O_3) is an important component of global change that is mostly determined by human activities, such as the incomplete combustion of fossil fuels (gasoline, diesel, etc.) (Logan, 1985; Wu et al., 2008). O_3 has direct, deleterious consequences on plants and animals, exerted through the fast formation of reactive oxygen species (ROS) that are particularly damaging to cell membranes, enzymes and DNA (Pell et al., 1997). In vascular plants ROS are readily formed as soon as O_3 enters the leaf through stomata by diffusion (Laisk et al., 1989), and therefore a large number of vascular plants are very sensitive to O_3. Lichens, composite organisms consisting of a symbiotic association of a fungus (the mycobiont) and one or more photosynthetic partners (the photobionts), have been studied only sporadically in relation to ozone toxicity, notwithstanding their frequent use as biomonitors of air pollution (Bargagli and Nimis, 2002). The few field studies available so far show that high concentrations of O_3 do not cause an appreciable impoverishment of the lichen flora (Lorenzini et al., 2003; Ruoss and Vonaburg, 1995), at least if peroxyacetylnitrates or other organic pollutants are not co-occurring (Egger et al., 1994; Sigal and Nash, 1983; Zambrano and Nash, 2000). Physiological studies gave more conflicting results. No significant consequence to O_3 exposure were detected by Calatayud et al. (2000), Riddell et al. (2010; 2012) and Rosentreter and Ahmadjian (1977), while limited effects were identified by Nash and Sigal (1979), Ross and Nash (1983), and Tarhanen et al. (1997). Severe damage to the integrity of photosystems, the collapse of a high percentage of photobiont cells and, occasionally, blanching of the thallus surface were instead reported by Scheidegger and Schroeter (1995). The latter study, frequently cited in the literature as a proof of lichen sensitivity to O_3 pollution, was based on lichens fumigated with O_3 in open top chambers (OTCs). Riddell et al. (2012), however, identified a clear chamber-effect associated with lichen exposure in OTCs and, therefore, it might be argued that the conclusions of Scheidegger and Schroeter (1995) were influenced as much by the exposure conditions than by the pollutant itself. A critical point is certainly the artificial rehydration process to which samples are typically subjected in this type of exposure. In contrast to vascular plants, lichens are poikilohydrous organisms, and therefore their water status varies passively according to the surrounding environmental conditions (Nash, 2008). In the OTCs lichens must be artificially watered. This process might affect lichen response to O_3 as a consequence of small differences in timing, frequency and/or intensity of sample watering. In fact, a recent field study with lichen transplants suggests that water availability is the key factor moderating O_3 resistance, because daily

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rehydrated thalli can repair O_3 damage effectively and replenish their reservoir of antioxidants (Tretiach et al., 2012b).

In this work the effects of O_3 on three epiphytic lichens with different ecologies were investigated by exposing portions of thalli subjected to different hydration regimes in OTCs with O_3 AOT40 in the range 0–50,000 ppb. To estimate the response of lichens to the different treatments, chlorophyll *a* fluorescence (Chl_aF) emission was measured. This is a non-destructive technique commonly used in plant physiology and nowadays frequently applied also to lichens (e.g. Calatayud et al., 2000; Scheidegger and Schroeter, 1995; Tretiach et al., 2007). Derived Chl_aF parameters can provide reliable information about the state of photosystems and relationships with a wide range of environmental factors, including specific pollutants (Baker and Rosenqvist, 2004; Bertuzzi and Tretiach, 2013; Nash, 2008).

2. Materials and methods

2.1. Lichen sampling and pre-treatment of samples

Three lichens, characterized by relatively well known biologies, were chosen: *Xanthoria parietina* (L.) Th. Fr., *Flavoparmelia caperata* (L.) Hale and *Parmotrema perlatum* (Huds.) M. Choisy. The species have different ecological requirements and resistance to pollutants, as shown in Table 1 and references cited therein; their photobionts are green coccoid algae of the genus T*rebouxia* de Puymaly (Ahmadjian, 1993, 2001). The samples were collected from deciduous trees in sites of NE Italy far from known air pollution sources (Table 1). Several healthy-looking thalli were detached from the bark using a sharp blade, put in open petri dishes and immediately transported to the laboratory, where the material was left to dry out at room temperature, in darkness for two days. Randomly selected thalli were then closed in petri dishes, sealed in vacuum bags and sent to the OPAL project laboratories at Imperial College's Silwood Park campus in Ascot, GB, for the following exposure experiments. Here the thalli were carefully cleaned of debris and bryophytes; 52 (*X. parietina*) and 78 (*F. caperata* and *P. perlatum*) samples were randomly cut from different thalli, numbered and photographed. Each sample (60 ± 5 mg dry weight) was tied with nylon threads to a small cork strut ($2.5 \times 4.0 \times 0.3$ cm) to facilitate moving and exposure, and kept dry under diffuse light (<10 μmol photons $m^{-2}\,s^{-1}$) until use, within a maximum of 21 days from sampling. Prior to exposure, the samples were subjected to a conditioning process for two days: they were immersed in distilled water for 3 min every 12 h and, in the remaining time, placed on rigid plastic nets within plastic boxes containing water at the bottom; the boxes were covered (but not sealed) with transparent plastic wrap (>95% RH) and placed in an incubator at 20 ± 1 °C, with a light/dark regime of 12/12 h and a light intensity corresponding to one sixth of the species-specific photosynthetic photon flux at which the quantum yield of CO_2 assimilation is the highest (see Table 1).

2.2. Sample exposure

The lichen samples were exposed for 6 weeks from July to September 2010, with a delay of one week between two successive species, starting with *P. perlatum* and finishing with *X. parietina*, in eight open top chambers (OTCs) ventilated with charcoal filtered air. In six chambers, O_3 generated using an O_3 generator (model GEN02-03, Bio-Fresh Ltd. UK), was added to filtered air to expose samples to different pollutant concentrations. Concentration measurements were continuously recorded with an O_3 monitor (model 202, 2B Technologies Inc., Boulder, Colorado, USA). The accumulated ozone exposure (AOT40) was calculated above a threshold concentration of 40 ppb as $\Sigma(c - c_0)$ for every $(c - c_0) > 40$ ppb. The maximum concentration of O_3 in the chambers were reached during the central hour of the day, but since the control of the O_3 generator did not allow, for short periods of time, to achieve concentrations below 40 ppb during the night, the AOT40 was calculated over the 24 h and not only in the interval 8:00 am–8:00 pm as indicated by the Directive 2002/3/EC (Nali et al., 2009).

The samples were positioned 2 m above ground on the north-facing portion of the internal wall of each OTC. Small black cardboard panels were placed perpendicular to the wall between three contiguous samples to avoid direct light. The samples within each OTC were divided into three groups (A–C) of three samples each, that were subjected to different hydration regimes: watered with a spray of distilled water at 9:00 am ("morning watered", group A); watered at 6:00 pm ("evening watered", group B); non-watered (group C). During exposure, therefore, group C could benefit only from the humidity of the air, whereas the two others were certainly active for some hours during the O_3 treatment (group A) or immediately after it (group B).

One further group of samples (group D; namely, 4 samples of *X. parientina* and 6 samples of *F. caperata* and *P. perlatum*) was exposed in a wood on the trunks of some oaks only 100 m away from the OTCs. These samples were exposed to normal rainfall and dew events, and served as control to check the effects of microclimatic conditions within the OTCs.

2.3. Chl_aF measurements

Chl_aF measurements were taken before and after 3 and 6 weeks of exposure, and after a further two days of recovery in the incubator under the conditions described above, to verify the long-term

Table 1
Investigated lichens, with respective photobionts, sampling sites, altitude (Alt., m above sea level), collection dates, substrata, species-specific photosynthetic photon flux at which the quantum yield of CO_2 assimilation is the highest ($PPFD_{Ik}$, μmol photons $m^{-2}\,s^{-1}$) (Piccotto and Tretiach, 2010) and categories of acidophytism (pH), hygrophytism (H), eutrophication (N), poleophobism (Pol.) according to Nimis and Martellos (2008).

Species	Photobionts	Sampling site	Alt.	Date(s)	Substratum	$PPFD_{Ik}$	pH	H	N	Pol.
Xanthoria parietina (L.) Th.Fr.	*T. irregularis* Hildreth & Ahmadjian *T. arboricola* de Puymaly	Italy, Friulan-Venetian Plain, Udine, prov., Latisana	5	29-June-2010	Northerly exposed bark of *Juglans* sp.	131	2–4	3–5	3–4	3–1
Parmotrema perlatum (Huds.) M.Choisy	*T. crenulata* Archibald *T. decolorans* Ahmadijan	Italy, Classic Karst Plateau, Trieste prov., Borgo Grotta Gigante	250	23-June-2010	Northerly exposed bark of *Quercus petraea* (Matt.) Liebl.	108	2	2–3	1–2	3–2
Flavoparmelia caperata (L.) Hale	*Trebouxia crenulata* Archibald *T. gelatinosa* Archibald	Italy, Classic Karst Plateau, Trieste prov., Borgo Grotta Gigante	260	23-June-2010	Northerly exposed bark of *Fraxinus ornus* L.	112	2–3	3	1–3	3–2

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