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Devitalization of poikilohydric lithobionts of open-air monuments by heat shock treatments: A new case study centred on bryophytes

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ABSTRACT

Heat shock treatments applied to artificially hydrated lichens have been recently proposed as a devitalization method for outdoor stone monuments. In this work their efficacy was tested against bryophytes (five mosses and one liverwort), both in the laboratory and in the field. To make a comparison, treatments with two commercial biocides commonly used by restorers were also applied at three standard temperatures. Chlorophyll *a* fluorescence emission was checked in treated and non-treated samples of all the species, and histochemical observations by confocal microscopy with a dead cell stain were carried out on one of them. Heat treatments of hydrated samples at 60 °C caused the death of all the bryophytes. Treatments at 40 °C were sufficient to significantly increase the negative effects of the biocides, even at concentrations 10 times lower than those in current use. Heat shock treatments are totally effective to kill the bryophytes, thus representing a potentially revolutionary approach in the field of stone conservation in terms of feasibility, low costs, and eco-compatibility.

1. Introduction

The identification of innovative techniques for removing the biofilms from outdoor monument surfaces is an important goal in the field of restoration. Lichens and bryophytes, are primary actors in the complex processes of biodeterioration of stone surfaces (Caneva et al., 2008), *per se* and because they favour the later invasion of vascular plants, that may cause even major concern, since they affect the stability of the buildings (Garcia-Rowe and Sainz-Jimenez, 1991). The intervention against lithobiontic biodeteriogens can be supported by indirect control methods (e.g. environmental methods) and by direct methods such as the application of biocides (Caneva et al., 1996, 2008), i.e. chemical compounds containing one or more active principles with toxic effects on living organisms (Gaylarde and Morton, 1999; Cappitelli et al., 2011). In accordance with the directive 98/8/EC, specific standard protocols indicate the correct dose-effect ratio and prescribe guidelines to protect the workers, the environment, and the substratum. Notwithstanding these best applied practices, biocides are potentially harmful, especially when they are used in massive interventions on large surfaces. Recently, a new approach against lithobiontic biodeteriogens has been proposed (Tretiach et al., 2012): it is simple, cheap and totally operator-, environment- and substratum-compatible. It consists of short heat shock treatments at 40–60 °C applied to artificially hydrated, and therefore metabolically active, organisms. According to the classification of Nugari and Salvadori (2003), this approach falls into the category of physical treatments, which generally have scarce interaction with the substratum and no toxic effects for the operator and the environment. The efficacy of the new approach was tested against lichens; these organisms are taxonomically unrelated to bryophytes but they share the same life strategy: poikilohydry, i.e. the capacity to tolerate cellular water loss to different extents. The modification in water content from the state of full turgor to that of extreme desiccation (and vice versa) causes dramatic events at cellular level, such as the progressive shrinkage of the cytoplasm, the rearrangement of the membranes, the suspension of enzymatic activities, and the partial or total deactivation of photosystems (Honegger et al., 1996). When dry, lichens and bryophytes are metabolically inactive and therefore highly thermo-tolerant; when fully hydrated they become heat-sensitive (Lange, 1955; Nörr, 1974; Meyer and Santarius, 1998; Glime, 2007), much more sensitive than the majority of homoiohydrous vascular plants. This peculiar property is well known to ecophysiologists, but it is considered as a sort of biological curiosity (e.g. Weis et al., 1988; Liu et al., 2004). No one – to the best of our knowledge – has ever exploited the potential practical implications, at least in the field of restoration, but, according to Tretiach et al. (2012), this weakness does represent an

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advantage if the goal is to kill the organisms before cleaning the stone surface in a conservative intervention (Caneva et al., 2008).

This study was specifically designed (*i*) to verify the applicability of the new approach of Tretiach et al. (2012) on selected bryophytes, and (*ii*) to find out the best protocol for treating the same species in the field.

The efficacy of the treatments was assessed by quantifying the chlorophyll *a* fluorescence (Chl_aF) emission of pre- and post-treated samples; this is a non-intrusive technique commonly used in plant physiology studies (Adams and Demmig-Adams, 2004), and recently introduced in the field of stone conservation (Tomaselli et al., 2002; Tretiach et al., 2010; Speranza et al., 2012). Histochemical observations by confocal microscopy with a dead cell stain were also carried out to verify the cell membrane integrity after the treatments.

2. Materials and methods

2.1. Laboratory exposure

Well developed gametophytes of six species of bryophytes (five mosses and one liverwort) with different ecology and substratum preference were sampled from rock outcrops in the Classic Karst plateau (NE Italy) (Table 1). The samples were put in paper bags and immediately transported to the laboratory, where the material was left to dry out on the bench of the laboratory for two days at room temperature and in darkness. In the laboratory the material was carefully cleaned from debris. For each species 15 groups of 8 samples each were randomly selected, numbered and photographed. Before measurements, the samples were subjected to a three-day-long conditioning process: they were positioned on flat limestone slabs, 1.5 cm thick, put in aluminium trays lined with absorbent paper soaked with distilled water, covered with low-density polyethylene (LDPE) food wrap, and positioned in a growing chamber at 20 °C, with a light/darkness regime of 14/10 h, and 10 μmol photons m^{-2} s^{-1}. Light was provided by neon lamps (Sylvania Grolux T8F36W/GROG13F36W), and was checked with a Micro-Quantum 2060-M Sensor (Walz, Effeltrich, D). The samples were daily watered with a spray of distilled water. Thereafter, a combination of treatments with biocides at different concentrations and/or heat shock treatments of 6 h were applied, and pre- and post-treatment Chl_aF emission was measured under standard conditions (see below). The samples were then kept in the growing chamber at the same maintenance conditions described above to observe the long term effects of the treatments. During the experiments, temperature and air humidity were continuously monitored with EL-USB-2 RH/TEMP data loggers (Lascar Electronics, Whiteparish, UK).

2.2. Field exposure

The field experiment was carried out in the Classic Karst plateau, in a pristine wood close to the village of Gropada (45°39′59″ N, 13°50′52″ E). Forty-eight well developed gametophytes of *Schistidium apocarpum* and *Tortella nitida* were sampled from rock outcrops and immediately fixed with the aid of nylon threads on 12 large stones (±20 × 40 cm). The stones were placed under the tree canopy for 3 days and during this period they were watered with a spray of distilled water in the early morning and in the late evening. At night the samples were covered with a wet black velvet blanket to keep them wet. This procedure favours the recovery of the normal activity of photosystems after a prolonged de-hydration, giving high values of Chl_aF. On the fourth day, a combination of treatments with biocides at different concentrations and heat shock treatments were applied (see below), exposing the moss-bearing stones on a sun-exposed rocky outcrop, where they remained for 16 days. The stones were then moved under a tree canopy where the samples where subjected for 3 days to the same conditioning process described above, in order to estimate the possible recovery in optimal conditions.

2.3. Biocide treatments

In the laboratory, immediately after the pre-exposure Chl_aF measurements, subsets of moistened thalli were treated with one of the commercial biocides of Table 2, at the concentrations there specified. The highest concentrations, 2% and 5% respectively for Biotin T (BT) and New Des 50 (ND50), were those suggested by the producer (C.T.S. s.r.l, Altavilla Vicentina, Italy) and normally applied by restorers, whereas the total amount of solutions per surface unit used in the laboratory treatments were those used by Tretiach et al. (2012). To measure the sample surface, the flat stones were scanned with an Epson Perfection 1260 scanner. The colour images were analyzed with the program GIMP 2.8.0 (GNU Image Manipulation Program, Copyright© 1995–2012), converted into black-and-white, and checked manually; pixels were converted into area units (cm^2).

In the field, only Biotin T was used, and the amount of solution used for unit of dry weight was 1.7 mL g^{-1}.

2.4. Heat shock treatments

In the laboratory, biocide-treated and untreated samples were exposed to heat shock treatments by introducing the material into a growth chamber at 20 ± 2, 40 ± 2, 55 ± 2 °C for 6 h in fully moistened conditions. In order to avoid dehydration, the samples were placed into aluminium trays covered with LDPE food wrap. After heat exposure, the bryophytes were returned to the original growth chamber at c. 20 °C for acclimation, and finally they were processed for the standard Chl_aF measurements.

In the field, half of the samples were watered, wrapped with black nylon foils together with the supporting stones and left under the sun from 10 am to 16 pm in a hot, windy summer day (2012, August 16). The remaining samples were not covered, and the thalli, rehydrated and eventually biocide-treated like the others, were left to dry out naturally in the sun.

2.5. Chlorophyll a fluorescence measurements

Chl_aF measurements were recorded with a portable fluorimeter handy-PEA (Plant Efficiency Analyser, Hansatech, Norfolk, UK) on

Table 1
Investigated bryophytes, with information concerning growth form, sampling sites, altitude (alt., m above sea level), collection dates and substratum. L: liverwort; M: moss.

Species		Growth form	Sampling site	Alt.	Collection date	Substratum
Calypogeia integristipula Steph.	L	Leafy	Italy, Trieste Coast, Contovello	100	13/August/2012	Sandstone
Ctenidium molluscum (Hedw.) Mitt.	M	Pleurocarpic	Italy, Classic Karst Plateau, Trieste prov., Gropada	400	11/July/2012	Limestone
Homalothecium sericeum (Hedw.) Schimp.	M	Pleurocarpic	Italy, Trieste Coast, Contovello	100	13/August/2012	Sandstone
Hypnum cupressiforme Hedw.	M	Pleurocarpic	Italy, Trieste Coast, Contovello	100	13/August/2012	Sandstone
Schistidium apocarpum (Hedw.) Bruch & Schimp.	M	Acrocarpic	Italy, Classic Karst Plateau, Trieste prov., Gropada	400	11/July/2012	Limestone
Tortella nitida (Lindb.) Broth.	M	Acrocarpic	Italy, Classic Karst Plateau, Trieste prov., Gropada	400	11/July/2012	Limestone