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A simple outdoor culturing system for the foliose macrolichens *Xanthoria parietina* (L.) Th. Fr. and *Parmelia sulcata* Taylor

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Abstract

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Thallus fragments of the foliose macrolichens *Xanthoria parietina* and *Parmelia sulcata* grew exceptionally well on especially designed ceramic supporting structures which were either glued to sheets of asbestos and incubated on the flat roof of the aquarium building of the Station Biologique de Roscoff (Brittany), or partially immersed in natural or artificial substrata contained in small terracotta pots. These were kept in large asbestos containers with peaty soil on the flat roof of our institute in the city of Zürich. The main difficulties in these outdoor culturing experiments arose not from air pollutants, but from the unpredictable chemical properties of some types of terracotta. In a search for suitable clay types, since not every commercially available brand of clay yields a ceramic with the required properties as regard to chemical stability and porosity, the best results were achieved with clinker and porcelain baked at relatively high firing temperatures.

Key words: Xanthoria parietina, *Parmelia sulcata*, ceramics, clay types, culturing technique.

Introduction

Experimental studies on lichens are often rendered difficult by the near impossibility to grow particular species in sufficient quantities in or near the laboratory. Resyntheses of symbiotic phenotypes under sterile conditions, starting with axenically cultured mycobionts and photobionts, have only seldom been successful (review: Galun 1988). A noteworth exception is the terricolous, placodioid *Endocarpon pusillum* (Verrucariales) which has been repeatedly cultured from spore to spore under sterile conditions (Stahl 1877; Bertsch and Butin 1967; Ahmadjian and Heikkilä 1970; Stocker-Wörgötter and Türk 1988). It remains unknown why most of the other compatible fungal/algal or cyanobacterial combinations of macrolichens developed only sporadically and unpredictably beyond the pre-thallus stage under sterile conditions. In these axenic cultures the fungal partner formed a crust-like, non-stratified pre-thallus but failed to

express the anatomically and morphologically distinct, placodioid, foliose or fruticose symbiotic phenotype. The foliose *Peltigera praetextata* and *P. didactyla* (Peltigerales) have been successfully cultured from ascospores or soredia (symbiotic propagules), respectively, under laboratory conditions on non-sterile, natural substrata such as clay or soil (Stocker-Wörgötter 1991). It seems that these terricolous species, like *Endocarpon pusillum*, tolerate high humidity over prolonged periods of time. Contrarily most saxicolous or corticolous macrolichens are adapted to regular wetting and drying cycles and do not express their symbiotic phenotype under continuously moist conditions. With these lichens better results were achieved in culturing experiments under natural or near-natural conditions, starting with either ascospores, soredia or thallus fragments. Such studies were designed for investigating growth and morphogenesis (e.g., Armstrong 1984, 1991 a, b; Schuster 1985; Jahns 1987; Ott 1987 a, b; Denison 1988), or for species preservation and/or recolonization of impoverished habitats (e.g., Gilbert 1988, 1991). Most of these studies were carried out in natural ecosystems, quite often far away from the laboratory.

A series of experimental studies on growth patterns, regenerative capacity, water relations and secondary metabolism in *Xanthoria parietina* and *Parmelia sulcata* necessitated a culturing system for selected thallus fragments near our laboratory in the city of Zürich. Both species are widely distributed and have been recorded in numerous saxicolous and corticolous lichen communities from the seashore to mountain areas. *Xanthoria parietina* occurs also on numerous anthropogenic substrata such as asbestos, concrete, tiles or even plastic letter boxes, as observed in coastal Brittany. It has been successfully transplanted in natural and near-natural ecosystems (Richardson 1967). The present paper aims to summarize the technical aspects of a relatively simple outdoor culturing system which can also be applied to a wide range of other lichen species. Main problems were the search for a suitable substratum and for the optimal mode of incubation. This substratum had to be chemically inert and tolerate autoclaving, at least for some types of experiments, without undergoing unpredictable chemical changes. A good water holding capacity was essential for culturing experiments in the continental climate of Zürich.

Materials and methods

Xanthoria parietina (L.) Th. Fr. (Teloschistales) was collected either on a concrete wall in the old port of Roscoff, Brittany, or on asbestos or sandstone in the botanical garden of the University of Zürich, *Parmelia sulcata* Taylor (Lecanorales) on an old pear tree (*Pyrus pyraster* (L.) Burgsdorf) in a historical garden (Abegg-Garten) near our institute in Zürich.

Manufacturing ceramic supporting structures

Ceramic supporting structures of more or less equal dimensions were modelled from different types of clay (see Table 1) and baked either in the muffler oven of our institute or in the ovens of professional ceramicists with adjustable temperature programmes (Atelier Citra or Gewerbeschule Zürich, respectively). Most of these bricks were rectangular and, with regard to future photographic documentation of growth processes at a 1:1 scale, not exceeding 35 × 22 × 10 mm in size. Visual growth monitoring was facilitated by millimetre marks scratched in the top surface of the soft clay with the aid of a serially mounted set of razor blades and appropriate aluminium spacers (Fig. 2 a). These millimetre marks were not supposed to be accurate after the firing process.

Tab. 1. Clay types tested for their suitability as a substratum for lichen cultures

Commercial name	Manufactured/ sold by	Recommended firing temperature	Selected firing temperature	Results
1) Modellierton G (modelling clay for artists)	Bodmer AG, Einsiedeln	980–1020 °C	1000 °C	Adequate porosity, large amounts of toxic, soluble salts
2) Töpfer-Klinkerton W110 (clinker)	Bodmer AG, Einsiedeln	1080–1100 °C	1100 °C	Good porosity, no soluble compounds
3) Steingutton T19 (earthenware)	Bodmer AG, Einsiedeln	980–1100 °C	1100 °C	Good porosity, soluble salts
4) Steingutton 1008 (earthenware)	Atelier Citra, Zürich	1050–1230 °C	1150 °C	Good porosity, soluble salts
5) Porzellanmasse 81298 (porcelain clay "Limoges")	Tony Güller Hägendorf	1240–1410 °C	1350 °C	Adequate porosity no soluble compounds

Transplanting selected fragments of lichens

Selected lichen fragments (e.g., marginal thallus lobes, dissected thallus stripes etc.) were fixed to the ceramic supporting structures with small quantities of Super Glue Gel, a fast-curing cyanoacrylate glue manufactured by LOCTITE (Ireland). Later the growing thallus lobes fixed themselves to the substratum with rhizinae.

The supporting structures carrying tiny lichen plantations were either glued to sheets of asbestos (Fig. 1 a) and incubated on the flat roof of the aquarium building of the Station Biologique de Roscoff, Brittany (one of the marine biology laboratories of the Université Pierre et Marie Curie, Paris), or inserted in natural (soil, peat, bark fragments etc.) or artificial substrata (e.g., Vermiculite [expanded mica]) contained either in plastic seeding trays (Fig. 1 b) or in tiny terracotta pots. These terracotta pots were partly immersed in peaty soil (Fig. 1 c, c') in large asbestos containers. Seeding trays and asbestos containers with terracotta pots were kept on the south-facing flat roof of our institute in the city of Zürich. These cultures were watered exclusively by natural precipitation (rain, snow). All transplants were regularly examined and photographed with a Zeiss Tessovar dissecting microscope.

Results and discussion

A first series of ceramic supporting structures was manufactured from a type of modelling clay (Modellierton G, Table 1) which is sold by Swiss "do-it-yourself" shops, and baked in the muffler oven of our institute. This oven should theoretically reach 1000 °C, but accurate temperature monitoring is not possible. The bricks were thoroughly washed in distilled water and dried in an oven. One series was autoclaved and subsequently inoculated with cultured *Trebouxia* photobionts under sterile conditions. Selected thallus fragments of *X. parietina* were glued to the remaining bricks (see below). After inoculation, both sterile and non-sterile supporting structures were infiltrated with

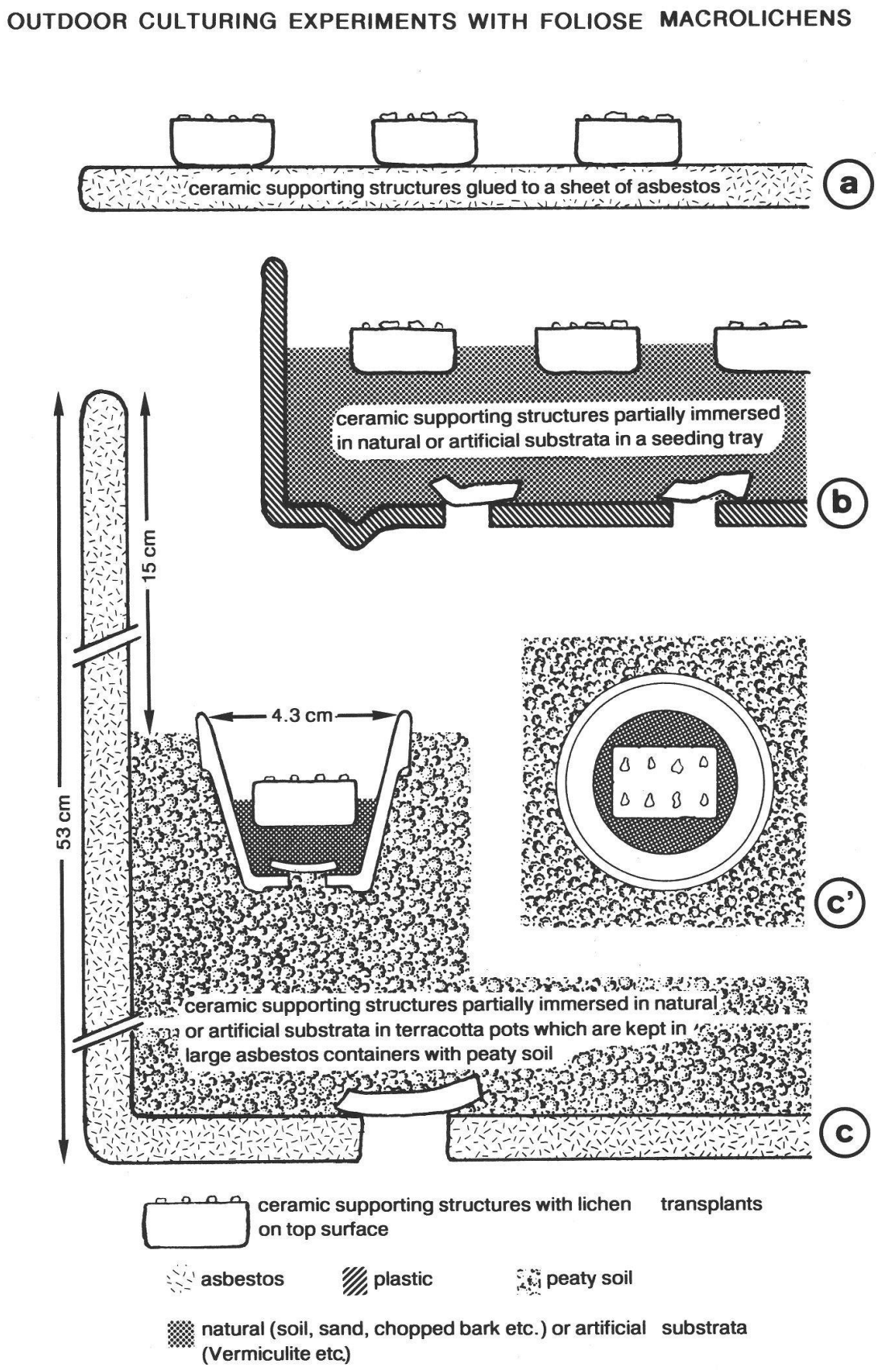

Fig. 1. Diagram illustrating the different types of outdoor incubation systems for "lichen plantations"

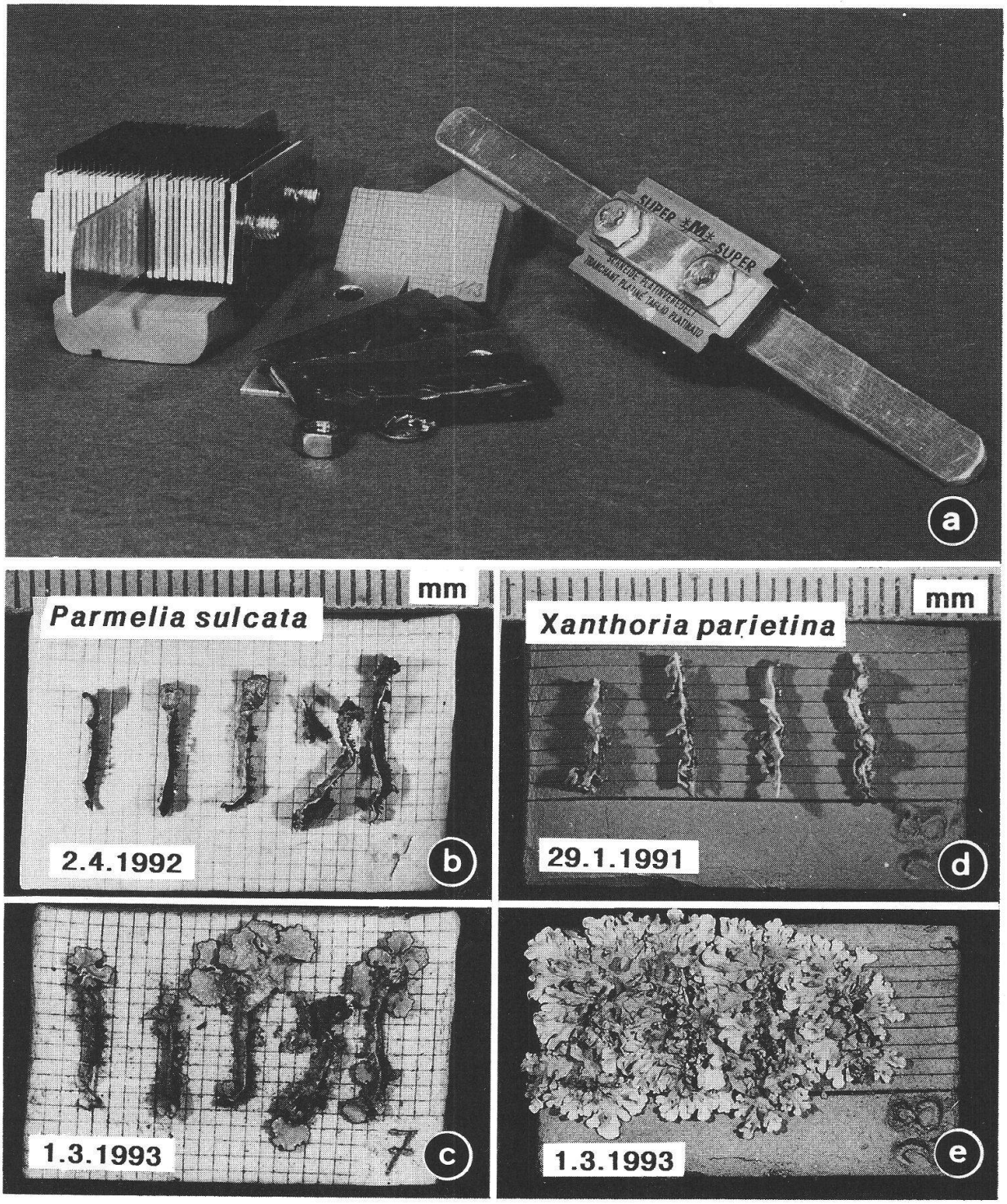

Fig. 2. (a) An instrument composed of serially arranged razor blades and aluminium spacers for scratching millimetre marks in the soft top surface of ceramic supporting structures prior to firing. Design and photograph by Jean-Jacques Pittet. (b–e) Monitoring the regenerative capacity of longitudinally dissected thallus stripes which had been glued to the surface of either a porcelain (b–c) or a clinker brick (d–e); both had been incubated in small terracotta pots on the flat roof of our Institute in the city of Zürich.

Bold's basal medium (BBM; non-nutrient mineral medium according to Deason and Bold 1960) at pH 6. All of these cultures died off within few days. The same happened to cultures on the same type of clay that had been baked in a potter's oven at a defined temperature as recommended by the manufacturer (Table 1). Evidently not every type of commercially available clay yields a suitable substratum for these culturing experiments. Four additional brands were tested, and the firing process was controlled (Table 1). Clay types 1–4 (all chemically not fully defined) contained mainly SiO_2 (approx. ⅔) and Al_2O_3 (approx. ⅓), plus low percentages of other oxides such as TiO_2, Fe_2O_3, CaO, MgO, Na_2O, K_2O. The porcelain clay (No. 5) was the only chemically defined and artificially prepared mixture. It contained 50% kaolin, 25% quartz and 25% feldspar.

Unless during firing all components of the clay mixture are chemically stabilized potentially harmful soluble salts (hydroxides) may diffuse out of the brick. These can have a devastating effect on the inoculum. The bright yellow anthraquinones of *X. parietina* transplants on bricks of modelling clay (Modellierton G) and on earthenware (Steingutton T19 and 1008) turned purple red in response to diffusible salt solutions with a high pH (around 12). Axenically cultured isolates of *Trebouxia* photobionts bleached within two days of inoculation on the same, but sterilized, supporting structures. Stabilization of all components of clay mixtures could be achieved by vitrification at very high firing temperatures, but vitrified ceramics cannot absorb and store water. This may be one of the reasons why vitrified, expanded clay particles (as used for hydroponic culturing of ornamental plants) are not a suitable substratum for culturing lichen transplants (H. M. Jahns, personal communication, and own unpublished results). The best growth rates were recorded on clinker (Töpfer-Klinkerton W110) and porcelain (Table 1). Both types of bricks were chemically stable and retained an adequate porosity. As the chemical stability and porosity of ceramics are influenced by the firing process a potter's oven with an accurately adjustable temperature programme is required for reproducible results.

The first months after transplanting were critical. Some of the lichen fragments broke off the substratum due to deformations during the regular, quite harsh wetting and drying cycles or due to heavy rain or hail. Once the transplants had formed regenerative stages along cut edges and/or fixed themselves to the substratum by means of rhizinae they started to grow continuously and reached growth rates which were comparable with those in the natural habitat (linear size increase of 4–5 mm yr^{-1}; Figs. 2 b–e). The results of these studies on the developmental biology and regenerative capacity of *Xanthoria parietina* and *Parmelia sulcata* will be presented elsewhere.

Transplants in coastal Brittany (Fig. 1 a) grew slightly faster than those which had been kept in terracotta pots in Zürich (Figs. 1 c, c'), but heavy winter storms caused large numbers of thalli to peel off the bricks. Not successful was the culturing system with plastic seeding trays (Fig. 1 b). The terracotta pots and the large amount of surrounding peaty soil in an asbestos container created a boundary layer of relatively high humidity above the cultures which was essential for adequate growth in the more continental climate of Zürich. Neither heavy frosts, snow and ice coverage for several weeks during winter nor air pollutants had an adverse effect on the cultures in Zürich. These observations are in accordance with field experiments of Schuster (1985) who demonstrated that juvenile stages of macrolichens develop even in polluted areas provided that they receive sufficient humidity.

The present culturing technique allows the maintenance of actively growing thalli in a simple outdoor incubation system even in a large city, a prerequisite for various types of experimental studies. It can be modified for growth experiments under sterile conditions.

Considering the significant differences between commercially available clay types as experienced in the present study one may conclude that qualitative differences (porosity and soluble compounds contents) between terracotta bricks are likely to be among the reasons why tiled roofs are colonized to different degrees by lichens even under favourable climatic and nutritional conditions.

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References

Ahmadjian V. and Heikkilä H. 1970. The culture and synthesis of *Endocarpon pusillum* and *Staurothele clopima*. Lichenologist 4: 259–267.

Armstrong R. A. 1984. Growth of experimentally reconstructed thalli of the lichen *Parmelia conspersa*. New Phytol. 98: 497–502.

Armstrong R. A. 1991. Competitive interactions between four foliose lichens on north and south facing rock surfaces. Environ. Exp. Bot. 31: 51–58.

Bertsch A. and Butin H. 1967. Die Kultur der Erdflechte *Endocarpon pusillum* im Labor. Planta 72: 29–42.

Deason T. R. and Bold H. C. 1960. Phycological studies I. Exploratory studies of Texas soil algae. The University of Texas Publications No. 6022, 70 pp.

Denison W. C. 1988. Culturing the lichens *Lobaria oregana* and *L. pulmonaria* on nylon monofilament. Mycologia 80: 811–814.

Galun M. 1988. Lichenization, 153–169. In: CRC Handbook of Lichenology, vol. 2. Galun M., ed., CRC Press, Boca Raton.

Gilbert, O. L. 1988. Colonization by *Parmelia saxatilis* transplanted onto a suburban wall during declining SO_2 pollution. Lichenologist 20: 197–198.

Gilbert, O. L. 1991. A successful transplant operation involving *Lobaria amplissima*. Lichenologist 23: 73–76.

Jahns, H.-M. 1987. New trends in developmental morphology of the thallus. Bibl. Lichenol. 25: 17–33.

Ott, S. 1987a. Reproductive strategies in lichens. Bibl. Lichenol. 25: 81–93.

Ott, S. 1987b. The juvenile development of lichen thalli from vegetative diaspores. Symbiosis 3: 57–74.

Richardson, D. H. S. 1967. The transplantation of lichen thalli to solve some taxonomic problems in *Xanthoria parietina* (L.) Th. Fr. Lichenologist 3: 386–391.

Schuster, G. 1985. Die Jugendentwicklung von Flechten – ein Indikator für Klimabedingungen und Umweltbelastung. Bibl. Lichenol. 20: 1–243.

Stahl E. 1877. Über die Bedeutung der Hymenialgonidien. Arthur Felix, Leipzig.

Stocker-Wörgötter E. 1991. Thallus formation of two cyanobacterial lichens: *Peltigera didactyla* and *Peltigera praetextata*, under laboratory conditions. Bull. Soc. bot. Fr. 138, Lettres, bot. (3), 179–187.

Stocker-Wörgötter E. and Türk R. 1988. Licht- und elektronenmikroskopische Untersuchungen von Entwicklungsstadien der Flechte *Endocarpon pusillum* unter Kulturbedingungen. Pl. Syst. Evol. 158: 313–328.