Water Distribution in Foliose Lichen Species: Interactions between Method of Hydration, Lichen Substances and Thallus Anatomy

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Three lichens (*Neofuscelia pokornyi*, *N. pulla* and *Xanthoria parietina*) from a semi-arid habitat were examined using low-temperature scanning electron microscopy to evaluate the effects of hydration method, lichen substances and thallus anatomy on the water distribution of hydrated thalli. In the *Neofuscelia* species, extracellular water within the thallus was observed in association with cracks in its otherwise impervious upper cortex, while *X. parietina* showed abundant extracellular water between medullary hyphae. Spraying the thalli followed by maintenance for 14–20 h in a water-saturated atmosphere led to the disappearance of the external water film in *X. parietina* but not in the *Neofuscelia* species. Surface water was abundant in specimens of all species immediately after spraying for 15 min. No extracellular water was observed inside the thallus 14–20 h after spraying, but after rinsing with acetone its presence was detected in all three species. Hydric strategy correlated with cortex hygroscopicity: *X. parietina*, an aero-hygrophytic species, had a more hygroscopic upper cortex than the *Neofuscelia* species, which are substrate-hygrophytic. The hygroscopicity of the upper cortex was linked with the amount of extracellular water in the thalline interior. Differences between *X. parietina* and *Neofuscelia* in the polarity and distribution of their lichen substances agreed with species differences in the presence and distribution of free water both as a film over the surface and inside the thallus. Lichen substances appear to play a role in the maintenance of air-filled intrathalline spaces in species whose anatomy, habitat, or both, favour water-logged conditions.   © 2000 Annals of Botany Company

Key words: Lichen, water relations, semi-arid, lichen substances, LTSEM, thallus anatomy, extracellular water, *Neofuscelia pokornyi* (Körb.) Essl., *Neofuscelia pulla* (Ach.) Essl., *Xanthoria parietina* (L.) Th. Fr.

INTRODUCTION

Water relations are of major ecophysiological interest in lichenology (Galun, 1988). Numerous studies report how lichens of different morphology respond to a frequently and rapidly changing availability of water (Kappen and Valladares, 1999). Only a limited number of thallus types have been the subject of intrathalline water localization studies (see Scheidegger *et al.*, 1997). Previous reports indicate that free water is not usually present in the internal parts of the lichen thallus and that all the stratified foliose and fruticose lichens examined to date keep their medullary space air-filled even under water-saturated conditions (Brown *et al.*, 1987; Ott and Scheidegger, 1992; Scheidegger, 1994; Scheidegger and Schroeter, 1995; Scheidegger *et al.*, 1995; Schroeter and Scheidegger, 1995). The possibility that water is able to accumulate in extracellular spaces inside the thallus has important implications for lichen physiology, in particular with regard to the increased resistance to CO_2 diffusion in water. It has been suggested that free extra-cellular water inside the thallus could be of less importance as a CO_2 diffusion barrier than superficial water films (Scheidegger, 1995). In some *Lasallia* species, certain structural features combine intercellular water storage with air-filled spaces where gas exchange is facilitated (Valladares *et al.*, 1998). In disagreement with the suggestions of other authors, it seems that not all lichen species

have a hydrophobic thalline interior (Honegger and Peter, 1994; Honegger *et al.*, 1996).

The water relations of lichens also seem to be affected by the accumulation of secondary compounds (lichen substances). In 1926, Goebel suggested that lichen substances encrusted in the walls of fungal hyphae could make the medulla water repellent. Green *et al.* (1985) suggested that certain lichen substances maintained water-free zones thus facilitating gas exchange for photosynthesis. However, Lange *et al.* (1997) recorded no change in high net photosynthesis rates maintained at high water contents in *Diploschistes muscorum* following the removal of lichen substances.

Since water relations are affected by thallus anatomy (Larson, 1979), and given the suggestion that the distribution of lichen substances may be responsible for medullary hydrophobic properties (Honegger, 1993, 1994), interactions between these two factors may make generalizations on the influence of each factor alone on water storage invalid. The present study was designed to explore these interactions by observing water storage in lichens of similar morphology that differ in their anatomy and type and distribution of lichen substances. The effects of lichen substances on water storage and distribution were assessed by extraction of the substances with acetone.

In nature, lichens undergo hydration in several ways (dew, rain, high relative humidity), and similarly in experimental studies lichens are hydrated according to different protocols. In particular, the presence of external
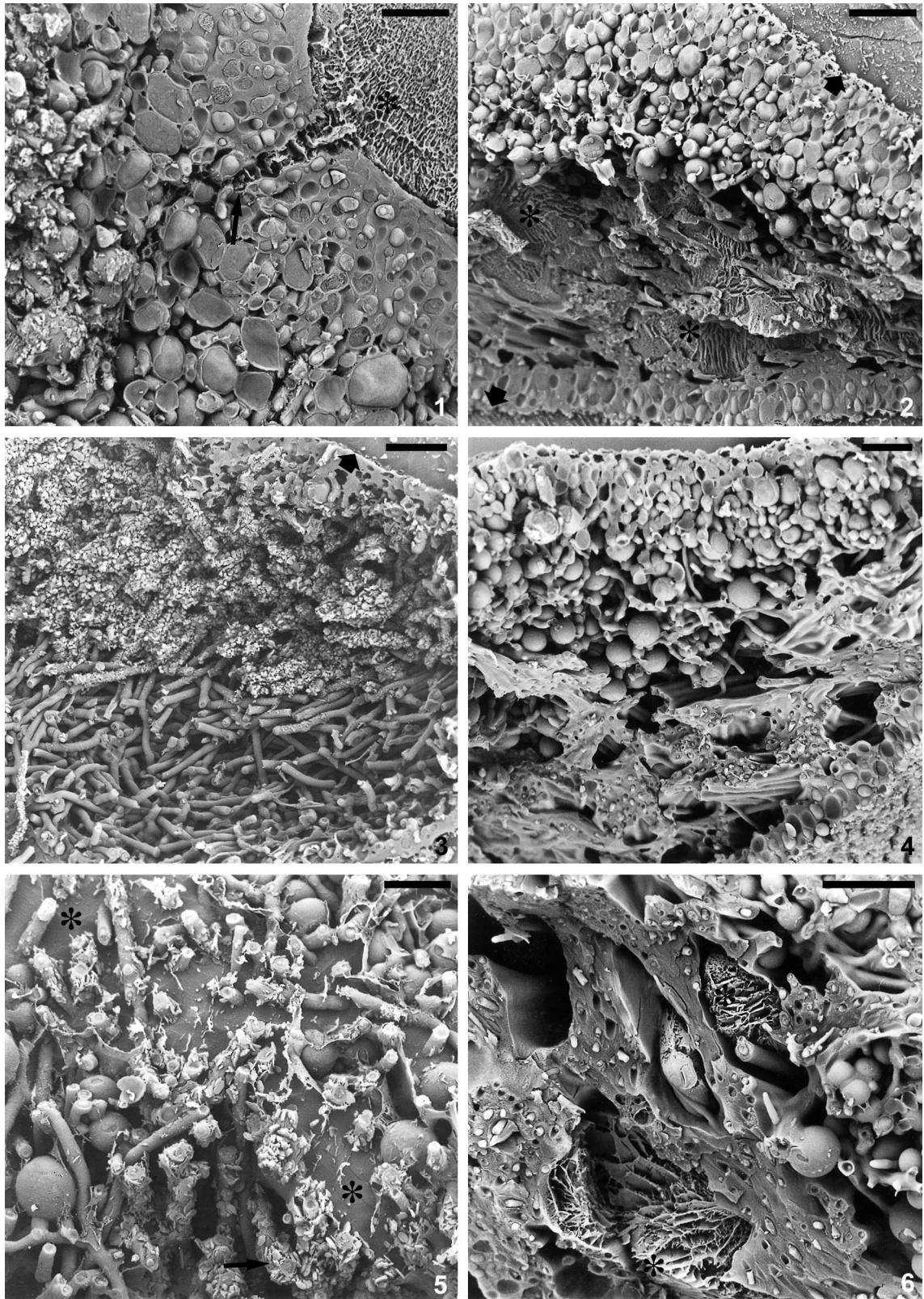
Figs 1–6. Legend on facing page.

water films and the maintenance of internal extracellular water can be affected by the time permitted for the different lichen compartments, which show different starting levels of hydration (e.g. cell walls, cell interior, extracellular spaces), to reach equilibrium. This prompted us to include the speed of hydration (rapid *vs.* slow) as an additional factor possibly affecting water distribution.

Suprasaturated conditions (high hydration levels that reduce net photosynthesis through increased resistance to diffusion) are not equally relevant for lichens of different microhabitats. Thus, it would be expected that lichens from different types of environment develop different mechanisms to avoid suprasaturation. One of the species selected for the present study (*Xanthoria parietina*) was found growing on microsites where the main source of water was the air (direct rain, water vapour) and may thus be described as an aero-hygrophytic species. The other species (*Neofuscelia* sp.) were obtained from less exposed areas where water was available mostly from the substrate (run-off) and they may therefore be considered substrate-hygrophytic. Based on these features, it was hypothesized that thallus anatomy and lichen substances could play a role in avoiding suprasaturation conditions.

MATERIALS AND METHODS

Species and habitat

Lichen specimens were collected from the semi-arid habitat of Almeria (southeastern Spain) on the Mediterranean coast. This area has a mean annual temperature of 16·5°C and mean annual rainfall in the range 250–330 mm. The coastal species *Neofuscelia pulla* (Ach.) Essl. and *Xanthoria parietina* (L.) Th. Fr. were collected from the Cerro de Enmedio, San José (UTM 30SWF80696) and the Barranco del Monsul (UTM 30SWF748657). These two species grow on volcanic rocks (andesite) on northern hill slopes; specimens of *X. parietina* were found growing on exposed rock surfaces (e.g. sharp edges of boulders), while *N. pulla* grows on relatively protected rock surfaces close to the soil. The inland, terricolous species *Neofuscelia pokornyi* (Körb.) Essl., was collected from marls of the Barranco del Cautivo, Tabernas (UTM 30SWF493962) and from gypsum crusts of the Venta de Yesos, Tabernas (UTM 30SWG629049).

Hydration method

Before observation of lichen thalli using low-temperature scanning electron microscopy (LTSEM), specimens were hydrated with distilled water via two methods. In the first, referred to as rapid hydration, dry specimens were abun-

dantly sprayed for 15 min and kept on wet filter paper at room temperature and under low light (approx. 50 μmol m^{-2} s^{-1} PPFD) until LTSEM examination. In the second, slow hydration, method, dry samples were sprayed with distilled water for 15 min as for the rapid hydration method but were subsequently incubated at 5°C in the dark in a saturated atmosphere for 14–20 h prior to LTSEM observation. The original substrate of the specimen samples was retained until the moment that a small fragment was cut for LTSEM.

Lichen substances

Lichen substances were removed according to a modification of the method of Solhaug and Gauslaa (1996) employing dry lichen fragments carefully detached from the substrate. Each fragment was rinsed in pure acetone (maximum 0·2 % water content) four times, for 5 min each time at room temperature. Fragments were then left for at least 12 h at room temperature in an open container to ensure vaporization of any residual acetone. The four acetone extracts were kept at 4°C for subsequent analysis by thin layer chromatography (TLC). The TLC protocol followed was that of Culberson (1972) with modifications by Manrique and Crespo (1983). Lichen substances were identified by TLC on the basis of their performance in three solvent systems (A, toluene–dioxane–acetic acid 180:60:8; B, hexane–diethyl ether–formic acid 130:100:20; and C, toluene–acetic acid 200:30) with respect to the performance of two standards (atranorine, Sigma Chem. Co.) and norstictic acid (extracted from *Parmelia acetabulum* (Neck.) Duby.) on 60 F$_{254}$ silica gel plates (Merck 5554). To explore the possible effects of detaching the thalli from their substrate, a second set of specimens of *N. pulla* were rinsed in acetone attached to the original substrate five times, for 5 min each time.

Low temperature scanning electron microscopy

Small fragments of hydrated and dehydrated thallus from each specimen were cut with a razor blade, mounted using O.C.T. compound (Gur BDH, UK) and mechanically fixed onto a specimen holder at room temperature. The fragments were immediately plunge-frozen in slushed nitrogen and directly transferred onto the cryo-chamber, pre-cooled to −180°C, via an air-lock transfer device. The frozen fragment was then fractured with a blade pre-cooled in the cryo-chamber to observe a transverse section of the thallus interior. The specimen was then sputter coated with gold for 2 min 15 s using a 10 mA current in the cryo-chamber, and transferred to the SEM-chamber, pre-cooled

FIGS 1–6. LTSEM micrographs of freeze-fractured saturated thalli of *Neofuscelia pulla* (Figs 1, 3 and 5) and *Xanthoria parietina* (Figs 2, 4 and 6) following rapid (Figs 1 and 2) and slow hydration (Figs 3 and 4). Figures 5 and 6 correspond to slowly hydrated thalli subsequently rinsed in acetone. Fig. 1. Asterisks indicate the surface water film; the arrow indicates extracellular water in the upper cortex; note lichen substances on algal surfaces. Figure 2 shows surface water films, indicated by arrows; internal extracellular water is indicated by asterisks. Fig. 3. The arrow indicates surface water film; note lichen substances and calcium oxalate crystals in the algal layer and upper medulla. Figure 4 illustrates the absence of a surface water film and internal extracellular water in *X. parietina* hydrated slowly. Fig. 5. Asterisks indicate internal extracellular water; the arrow points to calcium oxalate crystals on medullary hyphae. Fig. 6. Asterisks indicate internal extracellular water in the medulla. Bars = 10 μm (Figs 1 and 5) and 20 μm (Figs 2–4 and 6).

to −150–160°C, where it was observed at an accelerating voltage of 10–15 kV. Duplicate specimens were slowly heated to −90°C in the SEM-chamber after fracturing, and kept at this temperature for 2 min to sublimate the first superficial micrometers of water (etching). This step aids observation of the lichen ultrastructural elements and permits identification of the water fraction and confirmation of its location. The sublimation process was monitored on the SEM screen to confirm that the empty spaces in the etched specimens were originally water-free. After this step, the specimen was returned to the cryo-chamber, sputter coated with gold as before, and returned to the SEM-chamber. All the micrographs shown in this study were obtained from etched samples except for the dehydrated specimens. The instrumental set up consisted of a CT 1500 Cryotrans system (Oxford Instruments, UK) mounted on a Zeiss 960 scanning electron microscope.

Hygroscopic features of the thallus

The hygroscopic features of the dehydrated upper cortex were evaluated according to the method described by Valladares (1994*b*). This method simulates natural thallus hydration using liquid water. Comparison between different species shows the affinity of the upper surface for liquid water, or a lesser or greater hydrophobic/hydrophilic nature. A 5 µl drop of Quink Solv-X royal blue washable ink (Parker, UK) was deposited on the upper cortex of each lichen specimen. The sizes of the stains were compared to those produced by a drop of ink of the same volume on two reference materials: filter paper (Whatman, hardened, ashless #542) as a porous, hydrophilic surface and Parafilm© (American National Can TM) as a hydrophobic, impermeable surface. The maximum diameter attained by the stains was measured using a binocular lens with the aid of a camera lucida. The data obtained correspond to the stains produced by ten drops on five–nine thalli per species. The Student–Newman–Keuls test ($n = 10$) was used to compare data in each group.

## RESULTS

Effects off the hydration method

Surface water films. Hydrated thalli of all three species had a glossy upper surface due to a water film clearly visible by LTSEM (Figs 1, 2 and 7). In each specimen, the water film was observed on both the upper and the lower thallus surface (Fig. 2). The method of hydration had an effect on the presence of a water film in *Xanthoria parietina*, but not in *Neofuscelia pokornyi* or *N. pulla*. Spraying *X. parietina* thalli and keeping them in a water-saturated atmosphere for 14–20 h before LTSEM observation (slow hydration) led to the disappearance of the water film (Fig. 4) that was clearly visible when thalli were observed after 15 min of spraying (rapid hydration, Fig. 2). A water film, although discontinuous (Fig. 8), was always present regardless of the hydration method in the other two species (Figs 1, 2, 7 and 8).

Internal extracellular water. The hydration method had an effect on the presence or absence of free water in the extracellular spaces within the thallus. Free water was found in the three species after rapid hydration (Figs 1, 2 and 7) but not observed in slowly hydrated specimens (Figs 3, 4 and 8). The internal distribution of free water differed between species. Free water was most abundant in *X. parietina* and detected both in the medulla and the vicinity of the photobiont cells (asterisks in Fig. 2). In *N. pokornyi* and *N. pulla*, it was restricted to the upper cortex and photobiont layer (arrows in Figs 1 and 7). Free extracellular water in the upper thallus zones of *N. pokornyi* and *N. pulla* was associated with fissures or discontinuities of the upper cortex that served as conduits for the water film (Figs 1 and 7). The remaining continuous regions of the upper cortex of *N. pulla* were impervious to the water film (Figs 3 and 8).

Distribution of lichen substances and interactions with hydration method

The thalli of *X. parietina* did not lose colour after acetone rinsing, despite the intense colour of the acetone extracts. Parietin was the main substance in the thin layer chromatographs (TLCs) of *X. parietina*, while stenosporic acid was the main phenolic compound in the TLCs of both *N. pokornyi* and *N. pulla*. Parietin is more polar than stenosporic acid, as indicated by higher R_f values in two out of three solvents (7, 6, 7–8 *vs.* 5, 6, 6 for parietin and stenosporic acid in solvents A, B, and C, respectively). Parietin was mainly concentrated in the upper cortex of *X. parietina*. In the two *Neofuscelia* species, stenosporic acid and other minor crystallized lichen substances were found mainly in the medulla and the algal layer on the surface of both hyphae and algae (Figs 1 and 7). Lichen substances could be distinguished from calcium oxalates in the LTSEM micrographs by differences in shape: crystallized lichen substances formed thinner bodies than calcium oxalates, and while the former tended to be like irregular needles, the latter were polyhedral (arrow in Fig. 5). LTSEM observation of samples rinsed in acetone showed that the lichen substances had not been completely eliminated (arrow in Fig. 9). The extraction of these substances was more efficient when the specimens were detached from their substrate.

Free extracellular water was observed within the hydrated thallus of specimens of each species rinsed in acetone, irrespective of the hydration method. The main effect of the partial extraction of lichen substances with acetone was the maintenance of free extracellular water inside the thallus after slow hydration (Figs 5, 6 and 9). Qualitative differences in the amount and distribution of free water were observed between species and hydration method. *X. parietina* contained more free water than the other species. The water was observed in several spaces in the medulla (asterisks in Figs 2 and 6). In the other species, most of the water was restricted to small spaces in the upper cortex and single points within the photobiont layer (Figs 1, 5, 7 and 9). In *Neofuscelia* the quantity of water in the extracellular spaces inside the thallus depended on the distribution of the fissures in the upper cortex, while in
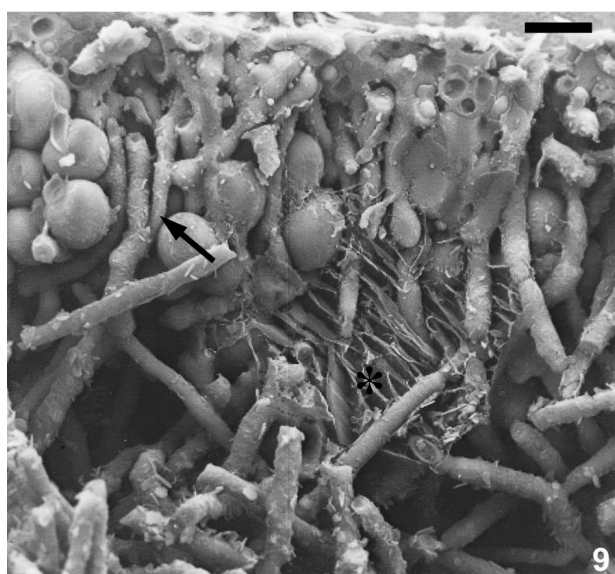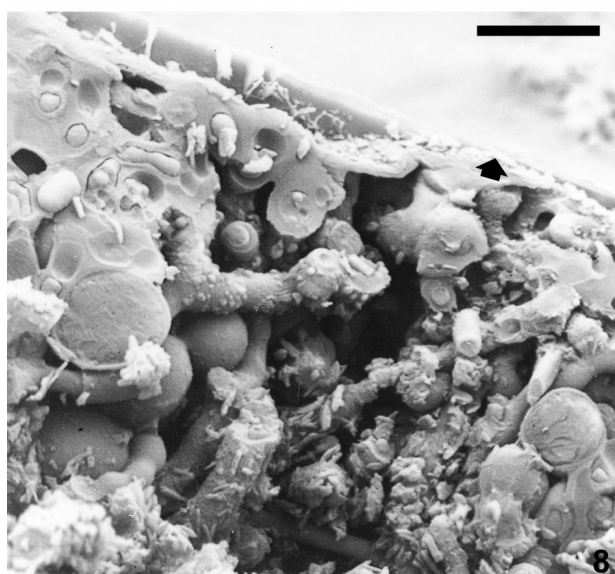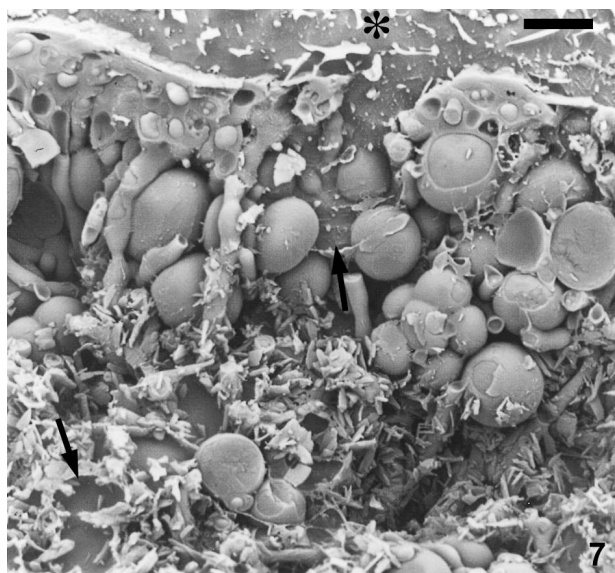
X. parietina it depended on the connection of spaces between bundles of medullary hyphae. Acetone rinsing did not modify the formation of surface films of water in any of the species. Surface water films were present whatever the hydration method in *Neofuscelia* species but only after rapid hydration in *X. parietina*, as in the samples without any previous treatment.

### Anatomy and hygroscopic features of the upper cortex

The upper cortex of all three species examined was paraplectenchymatous and made up of leptodermous fungal cells. Comparison of dry and wet images of the upper cortex revealed species differences in the gelatinous matrix surrounding the fungal hyphae. The matrix was dense and homogeneous in the *Neofuscelia* species, both in the hydrated and dry state (Figs 1 and 10), while in *X. parietina* the matrix was homogeneous when hydrated (Fig. 2) but reticulate and shrunken when dry (Fig. 11). Occasional fissures (e.g. Figs 1 and 7) interrupted the sharply defined upper cortex of *N. pulla* and *N. pokornyi*. The upper cortex of *X. parietina* was more irregular and wrinkled with areas of accumulated dead cells and necrotic material giving rise to a porous texture. The lumina of cortical hyphae were larger than those of the hyphae of other regions in *X. parietina* but were of similar size in the *Neofuscelia* species.

The hygroscopic features of the upper surfaces of the three species were different, as shown by the application of drops of water-soluble ink. The drops applied to the surface of *X. parietina* thalli produced stains that were of a significantly greater diameter than those on the *Neofuscelia* thalli surfaces (Fig. 12). The stains observed on the *X. parietina* thalli were not significantly different from those produced on filter paper, while those on *Neofuscelia* were not significantly different from those on a impermeable surface (Parafilm[©]) (see Fig. 12 all confidence limits <0·01).

DISCUSSION

The species examined in the present study share a foliose thallus morphology, but their hydric strategy differs, as shown by their water distribution pattern and microhabitat preferences. Free water was found within the extracellular spaces of the thallus interior after rapid hydration of the lichens. Species differences in the presence and amount of free extracellular water inside the thallus (*X. parietina* consistently showed more free water) can be explained by differences in the texture and hydrophilic features of the upper cortex. The reticulate upper cortex of *X. parietina* was very hygroscopic, despite the accumulation of lichen substances (mostly parietin) in this layer (Fig. 12). In contrast,

FIGS 7–9. LTSEM micrographs of freeze-fractured saturated thalli of *Neofuscelia pokornyi*. Fig. 7. Rapid hydration. Asterisk indicates the surface water film; arrows indicate internal extracellular water; note the irregular crystals of lichen substances. Fig. 8. Slow hydration. Arrow points to the discontinuous water film. Fig. 9. A slowly hydrated thallus rinsed in acetone. Asterisk indicates internal extracellular water and the arrow points to remnants of lichen substances on hyphal cell walls. Bars = 10 μm.
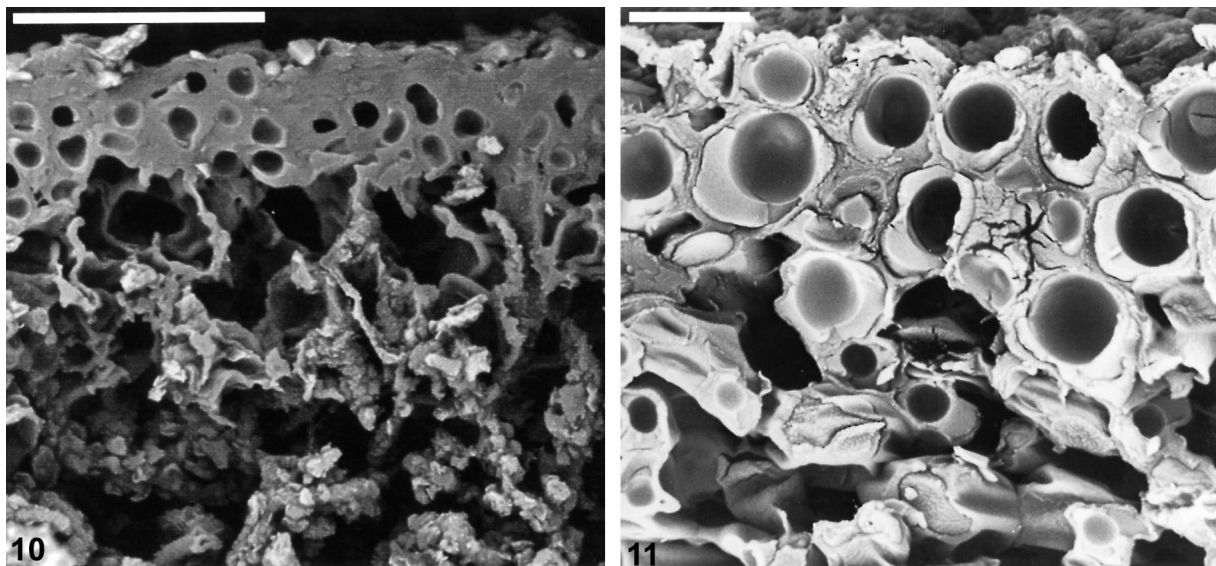
FIGS 10 and 11. LTSEM micrographs of free-fractured dehydrated thalli. Fig. 10. *Neofuscelia pulla*. Bar = 20 μm. Fig. 11. *X. parietina*. Bar = 5 μm.

the upper cortex of *N. pokornyi* and *N. pulla* was impervious and only permitted the entry of liquid water through cracks in the surface (Figs 1 and 7). Since lichen substances accumulate in the medulla and not in the upper cortex in these two species, hydrophobic lichen substances cannot be responsible for the hydrophobic nature of this surface. The waterproof cortex of *Neofuscelia* minimizes the risk of internal water-logged spaces in terricolous and saxicolour species exposed to run-off. This impermeability might be related to films of hydrophobins—hydrophobic proteins secreted by many fungi (DeVries *et al.*, 1993; Wessels, 1996).

In *X. parietina*, the external water film disappears on slow hydration since this surface shows more permeable and hydrophilic features that favour water absorption. Liquid water cannot accumulate on the upper cortex as occurs when this layer is more impervious and hydrophobic.

A porous upper cortex, like that of *X. parietina*, improves water uptake from the air but is not an efficient barrier against water loss by evaporation as discussed in Rundel (1982) and Valladares *et al.* (1998). Periods of metabolic activity (hydration under natural conditions) might be extended in *X. parietina* due to water storage in the medulla observed in the present study, which could counteract the fast dehydration that is associated with a permeable and porous upper surface and exposed microhabitat conditions.

This distinction has led to the defining of aero-hygrophytic and substrate-hygrophytic species (Sancho and Kappen, 1989). In agreement with the present findings, aero-hygrophytic species of *Umbilicaria* have porous upper cortices, while the reverse is true of substrate-hygrophytic species (Larson, 1979, 1981, 1987; Scott and Larson, 1984; Sancho and Kappen, 1989; Valladares, 1994*a*,*b*; Valladares *et al.*, 1998). Moreover, aero-hygrophytic strategy can also be linked to the structural and functional features of the thallus. For instance, the two aero-hygrophytic lichens with lichen substances in the upper cortex, *X. parietina* examined

here and *Pseudevernia furfuracea* explored by Scheidegger *et al.* (1995), show upper cortices of different hygroscopicities (*X. parietina* very hygroscopic, *P. furfuracea* impervious) possibly due to differences in the nature of the substances involved. The structural-functional differences between these two aero-hygrophytic species can be explained by water availability in their respective habitats. Water is very limited in the arid Mediterranean habitats of *X. parietina*, while it is much more abundant in the temperate and mountain forest habitats of *P. furfuracea*.
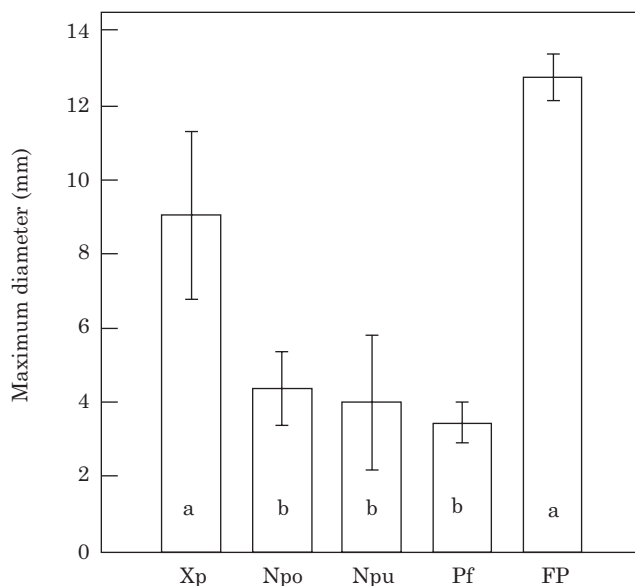

FIG. 12. Hygroscopic features of the upper cortex of *Xanthoria parietina* (Xp), *Neofuscelia pokornyi* (Npo) and *N. pulla* (Npu) in comparison with impermeable Parafilm[©] (Pf) and porous Whatman filter paper (FP) estimated as the maximum diameter (mm) of the stain produced by a 5 μl drop of water-soluble ink. Species and materials sharing the same letter showed no significant difference (ANOVA on ranks $P < 0.01$).

Thus, the risk of suprasaturation is minimized in *P. furfuracea* with an impervious upper cortex while, in the case of *X. parietina*, suprasaturation conditions are rare and brief, due to the exposed nature of its microhabitat.

Free extracellular water was observed within the thallus of slowly hydrated specimens of the three species rinsed in acetone. This finding suggests that even though lichen substances are not capable of conferring hydrophobic properties to the upper cortex and medulla of *X. parietina* or the photobiont layer of the *Neofuscelia* species, in the long run, the presence of lichen substances reduces water-logging of the thallus interior and provides for a relatively water-free photobiont layer.

In conclusion, the effects of lichen substances on water distribution within the thallus differ according to species since: (1) not all lichen substances are equally hydrophobic due to differences in their polar and chemical nature; and (2) each species has a particular type, amount and thalline distribution of lichen substances. These findings suggest that lichen substances may play a role in the maintenance of air-filled intrathalline spaces in species whose anatomy, habitat, or both, favour suprasaturation conditions. Complex interactions between the method of hydration, the type and distribution of lichen and other substances present in the cell walls, together with the anatomy of the thallus, give rise to a highly different thalline distribution of free water in each species. The complexity of the interactions between the three factors examined here may explain the discrepancies between previous findings with regard to the existence of free water inside fully-hydrated lichens and the influence of this free water on thallus gas exchange.

ACKNOWLEDGEMENTS

LITERATURE CITED

Brown DH, Rapsch S, Beckett A, Ascaso C. 1987. The effect of desiccation on cell shape in the lichen *Parmelia sulcata*. *New Phytologist* **105**: 295–299.

Culberson CF. 1972. Improved conditions and new data for the Thin Layer Chromatographic method. *Journal of Chromatography* **92**: 113–125.

DeVries O, Fekkes M, Wosten H, Wessels J. 1993. Insoluble hydrophobin complexes in the walls of *Schizophyllum commune* and other filamentous fungi. *Archives of Microbiology* **159**: 330–335.

Galun M. 1988. *Handbook of lichenology.* Boca Raton: CRC Press.

Goebel K. 1926. Morphologische and Biologiste Studien VII. Ein Beitrag zur Biologie der Flechten. *Annales Jardin Botanique Buitenzorg* **36**: 1–83.

Green TGA, Snelgar WP, Wilkins AL. 1985. Photosynthesis, water relations and thallus structure of *Stictaceae* lichens. In: Brown DH, ed. *Lichen physiology and cell biology.* New York: Plenum Press, 57–75.

Honegger R. 1993. Tansley Review No. 60. Developmental biology of lichens. *New Phytologist* **125**: 659–677.

Honegger R. 1994. Experimental studies with foliose macrolichens: fungal responses to spatial disturbance at the organism level and to spatial problems at the cellular level during drought stress events. *Canadian Journal of Botany* **73**: S569–S678.

Honegger R, Peter M. 1994. Routes of solute translocation and the location of water in heteromerous lichens visualized with cryotechniques in light and electron microscopy. *Symbiosis* **16**: 167–186.

Honegger R, Peter M, Scherrer S. 1996. Drought induced structural alterations at the mycobiont–photobiont interface in a range of foliose macrolichens. *Protoplasma* **190**: 221–232.

Kappen L, Valladares F. 1999. Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autotrophs. In: Pugnaire FI, Valladares F, eds. *Handbook of functional plant ecology.* New York: Marcel Dekker, Inc, 10–80.

Lange OL, Green TGA, Reichenberger H, Hesbacher S, Proksch P. 1997. Do secondary substances in the thallus of a lichen promote CO_2 diffusion and prevent depression of net photosynthesis at high water content? *Oecologia* **112**: 1–3.

Larson DW. 1979. Lichen water relations under drying conditions. *New Phytologist* **82**: 713–731.

Larson DW. 1981. Differential wetting in some lichens and mosses: the role of morphology. *Bryologist* **84**: 1–15.

Larson DW. 1987. The absorption and release of water by lichens. In: Peveling E, ed. *Progress and problems in lichenology in the eighties.* Berlin, Stuttgart: J. Cramer, 351–360.

Manrique E, Crespo A. 1983. Sobre *Melanelia acetabulum* (Neck.) Essl. en la Península Ibérica: caracterización química y distribución. *Lazaroa* **5**: 269–275.

Ott S, Scheidegger C. 1992. The role of parasitism in the co-development and colonization of *Peltula euploca* and *Glyphopeltis ligustica*. *Symbiosis* **12**: 159–172.

Rundel PW. 1982. The role of morphology in the water relations of desert lichens. *Journal of Hattori Botanical Laboratory* **53**: 315–320.

Sancho LG, Kappen L. 1989. Photosynthesis and water relations and the role of anatomy in Umbilicariaceae (lichens) from Central Spain. *Oecologia* **81**: 473–480.

Scheidegger C. 1994. Low temperature scanning electron microscopy: the localization of free and perturbed water and its role in the morphology of the lichen symbionts. *Cryptogamic Botany* **4**: 290–299.

Scheidegger C. 1995. Reproductive strategies in *Vezdaea* (Lecanorales, lichenized Ascomycetes): a low temperature scanning electron microscopy study of a ruderal species. *Cryptogamic Botany* **5**: 163–171.

Scheidegger C, Schroeter B. 1995. Effects of ozone fumigation on epiphytic macrolichens: ultrastructure, CO_2 gas exchange and chlorophyll fluorescence. *Environmental Pollution* **88**: 345–354.

Scheidegger C, Frey B, Schroeter B. 1997. Cellular water uptake, translocation and PSII activation during rehydration of desiccated *Lobaria pulmonaria* and *Nephroma bellum*. In: Kappen L, ed. *Bibliotheca lichenologica.* Berlin, Stuttgart: J. Cramer, 105–117.

Scheidegger C, Schroeter B, Frey B. 1995. Structural and functional processes during water vapour uptake and desiccation in selected lichens with green algal photobionts. *Planta* **197**: 399–409.

Schroeter B, Scheidegger C. 1995. Water relations in lichens at subzero temperatures: structural changes and carbon dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica. *New Phytologist* **131**: 273–285.

Scott MG, Larson DW. 1984. Comparative morphology and fine structure of a group of *Umbilicaria* lichens. *Canadian Journal of Botany* **62**: 1947–1964.

Solhaug KA, Gauslaa Y. 1996. Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. *Oecologia* **108**: 412–418.

Valladares F. 1994a. Form-functional trends in Spanish Umbilicariaceae with special reference to water relations. *Cryptogamie, Bryologie et Lichénologie* **15**: 117–127.

Valladares F. 1994b. Texture and hygroscopic features of the upper surface of the thallus in the lichen family Umbilicariaceae. *Annals of Botany* **73**: 493–500.

Valladares F, Sancho L, Ascaso C. 1998. Water storage in the lichen family Umbilicariaceae. *Botanica Acta* **111**: 99–107.

Wessels J. 1996. Fungal hydrophobins: proteins that function at an interface. *Trends in Plant Sciences* **1**: 9–14.