Water Storage in the Lichen Family Umbilicariaceae

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Abstract: Quantitative relationships between thallus structure and water storage and retention capacities in 12 species of the lichen family Umbilicariaceae were explored using three recent techniques for plant structure analysis: stereology (3D quantification of microscopic images), mercury intrusion porosimetry (determination of pore size distribution of tissues) and low-temperature scanning electron microscopy (LTSEM). Water storage capacity of the thallus was related neither to thallus density nor surface area of the thallus; it was directly related to the total porosity of the thallus and inversely related to the proportion of thallus volume occupied by cell walls and gelatinous substances. Water retention capacity increased with increasing thallus density and was decreased by slight increases in the surface area of the upper side of the thallus. Water storage and retention capacities exhibited a positive correlation only when the storage capacity was expressed on a surface area basis. LTSEM study of fully hydrated specimens revealed that many intercellular spaces of the upper cortex and upper parts of the algal layer contained liquid water. Intercellular spaces of the lower part of the algal layer and medulla were in general either air-filled or partially occupied by gelatinous substances. Species with rhizinomorphs and substrate-hygrophytic (water uptake from surface run-offs) stored more water and retained it longer than aerohygrophytic species (water uptake from the atmosphere) lacking rhizinomorphs. Thallus structure of aerohygrophytic species seems to facilitate rapid gas exchange with the environment, improving water uptake and carbon gain when atmospheric moisture is available but accelerating dehydration when the atmosphere becomes dry.

Key words: Lichen, water storage, stereology, low temperature scanning electron microscopy (LTSEM), functional structure, mercury intrusion porosimetry, Umbilicariaceae.

Introduction

Lichen-forming fungi and their photobionts, as poikilohydric terrestrial organisms, are reported to have limited ability to avoid water vapour fluxes to dry atmospheres. But, although lichens were considered unable to control the physical processes of water relations (Blum, 1973; Harris, 1976), there is increasing evidence of the importance of thallus morpholo-

gy and anatomy in controlling the rates of water uptake and loss (Larson, 1987; Rundel, 1988; Sancho and Kappen, 1989; Palmer and Friedmann, 1990). That is, lichens of any type will eventually come into equilibrium with the relative humidity of the air, but in the short-term response, thallus structure has a very strong influence on its water relations (Rundel, 1982). However, in most environments lichens cannot maximize water storage capacity and they have to reach a balance between water storage and air-filled pathways for CO₂ simply because CO₂ diffusion is 10 000 times slower in water than in air. Vascular plants have avoided this problem separating water, supplied internally through special tissues, from pathways for gas exchange. It has been postulated that an analogous situation exists in lichens, where a gas-filled thalline interior surrounded by a water-repellant coat (cuticle equivalent) would maximize gas exchange (Honegger, 1991). However, it has been known for more than a century that there is a net photosynthetic depression at high water contents (Lange et al., 1996) and the very few exceptions to this rule have been explained by unusual anatomical arrangements (Thomas et al., 1996). This photosynthetic depression at high water contents can be due to either increased resistances to CO₂ diffusion or increased thallus respiration but the former hypothesis of increased resistance has been more widely accepted (Lange and Tenhunen, 1981; Lange et al., 1993).

Low-temperature scanning microscopy (LTSEM) is a recent, very suitable technique to view plant samples without altering their water content (Echlin, 1992). LTSEM studies of lichens have shown a great scarcity or even a total absence of intercellular water in the thallus of certain species, discarding some expected sites for diffusion resistances and raising doubts about the interpretation of gas exchange studies (Honegger and Peter, 1994; Scheidegger, 1994; Honegger et al., 1996). Recently, Lange et al. (1996), combining gas exchange with fluorescence techniques, have confirmed that depression in net photosynthetic rates at high water contents is a real fall in photosynthesis due to increased CO₂ diffusion resistance. But the actual location of these diffusion resistances, or, in other words, the location of water within the lichen thallus, is a matter of debate. In some studies, free water has been assumed to fill the medulla (Bachmann, 1923; Jahns, 1984; Sancho and Balaguer, 1989). We have found correlations between intrathalline pores, medullary anatomy and water storage capacity in several species of Umbilicariaceae (Valladares et al., 1993). Nevertheless, Honegger et al. (1996) maintained that water was confined to the symplast and

apoplast of the algal and medullary layers of heteromerous lichens and that it did not accumulate intercellularly, at least in specimens wetted by spraying. Free water has been observed with LTSEM in the upper cortex of soaked thalli of *Cetraria islandica* (Scheidegger, 1994), but it seems not to accumulate extracellularly in the thalline interior of most macrolichen species studied. Lichens belonging to the family Umbilicariaceae exhibit a rather unusual medullary layer, which is sometimes conglutinate, compact and with thick walled hyphae (Valladares and Sancho, 1995). These and other peculiarities of Umbilicariaceae might be responsible for a water storage pattern different from that found in lichens belonging to other families. Consequently, the influence on water relations, and in particular on water storage, of the anatomy of lichens of different growth form and structural features requires further research.

Studies of lichens of a wide range of growth forms pointed to an important role of morphology in the control of thallus water relations (Larson, 1981; Rundel, 1982), while thallus internal anatomy arose as an important factor in comparisons among lichens of the same growth form or taxonomically close to each other (Kunkel, 1980; Snelgar and Green, 1981; Jahns, 1984; Sancho and Kappen, 1989; Palmer and Friedmann, 1990). Within the family Umbilicariaceae, the main morphological feature associated with water relations is the presence of rhizinomorphs in the lower surface of the thallus (Larson, 1981; Sancho and Kappen, 1989) while the main anatomical features affecting water relations are the structure of the medulla and the texture of the upper cortex (Larson, 1984a; Sancho and Kappen, 1989; Sancho et al., 1994; Valladares, 1994b). However, very few attempts to quantitatively link structure with water storage can be found in the lichen literature in general and in the literature dealing with Umbilicariaceae in particular (Valladares et al., 1994). In the present study we aimed to explore quantitative relationships between thallus structure and certain thallus hydric properties in 12 species of the lichen family Umbilicariaceae. More specifically, we wanted to test possible influences of thallus density, thickness, porosity, surface area and volume of gelatinous substances on water storage and retention capacities. A special effort was made to combine different, recent techniques. Quantification of thallus structural parameters was accomplished by using new, unbiased stereological tools (Ascaso and Valladares, 1994) and by mercury intrusion porosimetry (Valladares et al., 1993). LTSEM was also used to correlate quantitative measurements of thallus structure with the anatomy of fully hydrated thalli and to investigate the location of water within the thallus. The hydric resource exploited by the different species of Umbilicariaceae studied here varied from water vapour, in those growing in overhangs, to liquid water, in those growing in runoffs. Consequently, another objective of the study was to relate general structural trends of the thallus with the different hydric strategies of the lichens of this family.

Materials and Methods

Biological material

The Umbilicariaceae studied were collected in the Spanish Sistema Central (Sierra de Guadarrama, Madrid and Segovia, and Sierra de Gredos, Avila) within the period 1989–1991. The 12 species selected are *Lasallia hispanica* (Frey) Sancho &

Crespo, *L. pustulata* (L.) Mrat, *Umbilicaria carpetana* prov., *U. cinereorufescens* (Schaer.) Frey, *U. crustulosa* (Ach.) Frey, *U. cylindrica* (L.) Del. ex Duby, *U. freyi* Codogno, Poelt & Puntillo, *U. grisea* Hoffm., *U. havaasii* Llano, *U. spodochoa* (Hoffm.) DC. in Lam. & DC., *U. polyphylla* (L.) Baumg., *U. polyrrhiza* (L.) Fr. *U. carpetana* is a provisional name for *U. spodochoa* var. *carpetana* described in Sancho (1986); a detailed description of this taxon is in preparation. Several specimens of the same species but of different sizes were collected in order to include the effects of thallus size, which have been shown to be potentially important for the lichens belonging to this family (Larson, 1984b; Valladares et al., 1994).

Water relations

The following water relations parameters were investigated: 1, thallus water storage capacity, expressed by its maximum water content (MWC), and 2, thallus water retention capacity, expressed by the time required to reach 20% of MWC (T20) during the dehydration kinetics under the particular experimental conditions. Five thalli of each species were fully saturated by submerging them in distilled water for 30 min and then shaken to remove adherent water. MWC was calculated from the first weighing after complete saturation with water. MWC was expressed as percentage of oven dry weight and also as mg of water per cm² of thallus upper surface. For estimation of water retention capacities, five thalli of each species were placed in a growth chamber at 10 °C, 110 µmol m⁻² s⁻¹ PPFD and 75% relative humidity in circulating air (0.4 m s⁻¹), and independently weighed every 4 min until equilibrium with the relative humidity of the air.

Anatomical and porosimetric measurements

Lichen fragments of around 4 mm² were embedded in Spurr's resin (Spurr, 1969) and cut in 0.8 µm thick sections for light microscopy study and for the estimation of stereological parameters, as in Ascaso and Valladares (1994) and Valladares et al. (1994). The sections were stained with methylene blue and examined with a Zeiss Axiophot light microscope. Five thalli of each species and three fragments from each thallus were taken for systematic measurements. The three fragments per thallus were taken from three different thallus zones (marginal, close to the umbilicus, and intermediate, as in Valladares et al., 1994) in an attempt to obtain more real average values for thallus that have been shown to be of a great intrathalline variability (Larson, 1983; Larson and Carey, 1986; Valladares et al., 1994). The percentage of thallus volume occupied by cell walls and gelatinous substances ($V_{V_{cell\ walls, thallus}}$) was calculated by point counting on a grid system (Gundersen et al., 1988; Ascaso and Valladares, 1994) applied over microscopical images of the sections obtained from the thallus fragments embedded in Spurr's resin. The surface area of the upper and lower surfaces of the thallus was estimated from vertical sections (Baddeley et al., 1986; Ascaso and Valladares, 1994). Thallus density was calculated from thallus volume estimations (estimated surface area from cross sections of the thallus multiplied by thallus thickness) and oven dry weight. The total porosity of the thallus was measured by mercury intrusion porosimetry as in Valladares et al. (1993). Terminology referred to the anatomy of the medulla is explained in Valladares & Sancho (1995).

Fig. 1 Water retention capacity (T20 at 10°C, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 75% relative humidity in circulating air) versus thallus density and thallus upper surface area in the 12 lichen species studied. Bars indicate standard deviation ($n = 5$).

● *L. hispanica*, ■ *L. pustulata*, ▲ *U. polyphylla*, ▼ *U. grisea*, ◆ *U. freyi*, ● *U. cylindrica*, ○ *U. cinereorufescens*, □ *U. polyrhiza*, △ *U. carpetana*, ▽ *U. spodochoa*, ◇ *U. havaasii*, ○ *U. crustulosa*

Low Temperature Scanning Microscopy (LTSEM)

Thalli were fully hydrated by submerging them in distilled water for 30 min. They were kept on a wet filter paper in a Petri dish for another 30 min at room temperature before their manipulation for LTSEM. Small fragments of different thallus zones were cut with a razor blade, quickly mounted on copper stubs and frozen in slush liquid nitrogen. Afterwards, the samples were transferred to a CT 1500 Cryotrans system (Oxford instruments, UK) mounted on a Zeiss 960 scanning electron microscope. A side-mounted knife handle containing a scalpel blade was used to fracture the sample in the prechamber. The sample was warmed to -90°C inside the electron microscope chamber, and etched for 1–2 min. The sample was then sputter coated with gold in the prechamber and transferred to the cryostage of the microscope for observation at -135°C . Accelerating voltages between 10 and 15 kV were used to observe the frozen lichen samples.

Statistics

Linear regression analysis was used to explore the relationships between the different hydric and structural parameters considered. Sixty points (average of each of the 5 thalli of the 12 species) were used in the analyses, although only the average $\pm$ sd for each species is plotted in the figures together with the regression line and the correlation coefficient obtained.

Results

Water retention capacity (T20, time required to reach 20% of maximum water content in a dehydration curve) was significantly related to thallus density. Dense thalli held water longer than light thalli (Fig. 1). Water retention capacity was also related to the surface area of the upper side of the thalli. Although the notable increase in surface area caused by the presence of rhizomorphs on the lower surface of certain lichens did not appreciably decrease the water retention capacity of the thallus, slight increases in the surface area of the upper side of the thalli did clearly decrease it (Fig. 1).

Water storage capacity of the thallus, expressed by its maximum water content (MWC), was not related with either the thallus density nor the surface area of thallus upper side. The water storage capacity was related to internal features of the thalli, namely to the proportion of thallus volume occupied by cell walls and gelatinous substances (Fig. 2). MWC decreased with increasing thallus volume occupied by cell walls and gelatinous substances. Also, the total porosity of the thallus was inversely related to the proportion of

thallus volume occupied by cell walls and gelatinous substances (Fig. 2). Water retention and storage capacities exhibited a significant correlation but only when the storage capacity was expressed on a surface area basis (Fig. 3).

The potential influence of thallus thickness on the water relations studied was overridden by other structural features described above (thallus density, upper surface area, anatomy), as evidenced by the lack of any correlation between water retention or storage capacities and thallus thickness (Fig. 4).

Significant interspecific differences were observed in water relations and in the associated structural parameters. In general, species with rhizomorphs and substrate-hygrophytic (water uptake from surface run-offs) stored more water and retained it longer than aerohygrophytic species (water

Fig. 2 Maximum Water Content (MWC, % of dry weight) and thallus total porosity versus percentage of thallus volume occupied by cell walls and gelatinous substances ($V_{\text{cell walls, thallus}}$) for the 12 lichen species studied. Bars indicate standard deviation ($n = 5$). Symbols for species as in Fig. 1.

Fig. 3 Water retention capacity (T20 at 10°C, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 75% relative humidity in circulating air) versus water storage capacity (MWC) on a dry weight and on surface area basis in the 12 lichen species studied. Bars indicate standard deviation ($n=5$). Symbols for species as in Fig. 1.

uptake from the atmosphere) lacking rhizinomorphs (Table 1, Figs. 1–4). The dense thalli of *U. cinereorufescens* with verrucose rhizinomorphs and a large percentage of their volume occupied by cell walls and gelatinous substances exhibited a low water storage capacity (on a dry weight basis, although high on a surface area basis) but an extremely large water retention capacity. *U. polyrrhiza*, also with rather dense thalli and with ball-tipped rhizinomorphs, also exhibited a large

water retention capacity, but its water storage capacity was significantly larger than that of *U. cinereorufescens* thalli (when expressed on a dry weight basis) due to a very different internal structure, with less volume occupied by cell walls and gelatinous substances. The light thalli of the aerohygrophytic species (*U. havaasii*, *U. grisea* and *U. polyphylla*), with a large upper surface area to dry weight ratio of the thallus, dehydrated quickly and their water storage capacity varied according to their internal structure and total porosity when expressed on a dry weight basis, but was invariably low on a surface area basis. The pustulate species (*Lasallia hispanica* and *L. pustulata*) exhibited intermediate values between these two groups for most of the structural and hydric parameters studied, except for the water storage capacity on an upper surface area basis that was similar to that of the rhizinomorph species (Table 1, Fig. 3).

Low temperature scanning microscopy (LTSEM) revealed swollen fungal hyphae and turgid, globular photobiont cells in fully hydrated lichens (Figs. 5–11). Many intercellular spaces of the upper cortex and upper parts of the algal layer contained water (Figs. 5–6). Intercellular spaces of the lower part of the algal layer and medulla were either air-filled (Fig. 7) or partially occupied by gelatinous substances (Figs. 8–9). Water in the medulla of most of the species studied was confined to the interior of the cells (Figs. 7–9), but intercellular water was observed in the medulla of ca. 10% of the fully hydrated specimens studied. Medullary intercellular water was found, for instance, in the arachnoidal, loose layer of the medulla of *L. pustulata* (Figs. 10–11).

Discussion

Although thallus thickness has been shown to vary under different environmental conditions (Looman, 1964; Weber, 1977; Hyvärinen, 1992) controlling the water storage capacity of some lichens (Snelgar and Green, 1981; Jahns, 1984; Jahns, 1988), no significant correlation between thallus thickness and water relations (T20, MWC) was found for the Umbilicariaceae considered here. This fact points to a more important role of thallus internal structure than simply thallus thickness in the water relations of this lichen family. A similar conclusion on the importance of thallus anatomy for the understanding of the water relations of the Umbilicariaceae has been pointed out before (Sancho and Kappen, 1989; Valladares et al., 1993; Valladares, 1994a; Valladares and Sancho, 1995). Some species-specific water relations features have been associated with the medulla since the earlier work of Bachmann (Bachmann, 1923), an expected situation because the medulla constitutes, in general, a very large and structurally diverse layer of the thallus of the Umbilicariaceae (Valladares and Sancho, 1995). The relationship between

Fig. 4 Water retention capacity (T20 at 10°C, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 75% relative humidity in circulating air) and water storage capacity (MWC on a dry weight and on a surface area basis) versus thallus thickness in the 12 lichen species studied. Bars indicate standard deviation ($n=5$). Symbols for species as in Fig. 1.

Table 1 Summary of the main structural and water-related features of the 12 Umbilicariaceae taxa studied. Data are provided on an arbitrary scale, high (H), medium (M) and low (L), except for thallus and upper cortex thicknesses where taxa are separated in thick (TK) and thin (TN). Detailed, quantitative information can be found in Valladares et al. (1993, 1994, 1996) and Valladares (1994a, 1994b).

Lichen taxa	Whole thallus			Upper cortex		Medulla	Rhizino- morphs	Water storage	Water retention
	Thickness	Density	Porosity	Thickness	Wetability				
<i>U. cinereoruf.</i>	TK	H	L	TK	L	Ruebeliana type, dense, scleroplectenchyma	YES	L	H
<i>U. spodochoea</i>	TN	H	L	TN	L	Deusta type, dense, radial	YES	H	M
<i>U. polyrrhiza</i>	TN	L	H	TN	L	Havaasii type, loose, radial plectenchyma	YES	H	H
<i>U. carpetana</i>	TK	L	M	TK	M	Vellea type, arachnoidal	YES	H	M
<i>U. crustulosa</i>	TN	L	H	TK	L	Crustulosa type, as Deusta but with transverse hyphae	YES	H	H
<i>U. cylindrica</i>	TK	M	M	TK	M	Decussata type, 3-layered, central prosoplectenchyma	NO	L	L
<i>L. pustulata</i>	TK	M	H	TK	H	Pustulata type, 2-layered, arachnoidal and prosoplectenchyma	NO	H	M
<i>L. hispanica</i>	TK	M	H	TK	H	Pustulata type, 2-layered, arachnoidal and prosoplectenchyma	NO	M	H
<i>U. freyi</i>	TK	M	M	TN	M	Vellea type, arachnoidal	Scarce	M	L
<i>U. polyphylla</i>	TN	L	H	TN	L	Deusta type, dense, radial	NO	H	L
<i>U. grisea</i>	TN	L	M	TN	M	Vellea type, arachnoidal	NO	M	L
<i>U. harvaasii</i>	TN	L	H	TK	H	Havaasii type, loose, radial plectenchyma	NO	L	L

thallus porosity and volume occupied by cell walls and gelatinous substances with the MWC found here was undoubtedly due, at least in part, to the anatomy of the medulla. For instance, species with a scleroplectenchymatic medulla (e.g. *U. cinereorufescens*) were able to hold little water on a dry weight basis, while the reverse was true for species with prosoplectenchymatic medullas (e.g. *U. crustulosa*). We have

found here intercellular water in addition to intracellular water in all layers of the thallus, although it was more abundant in the upper cortex. Free water in the upper cortex have also been found in fully hydrated thalli of *Cetraria islandica* (Scheidegger, 1994). This finding fits with studies pointing to larger gas diffusion resistances in the cortex than in other thallus layers (Cowan et al., 1992).

Figs. 5–6 LTSEM micrographs of freeze-fractured fully hydrated thalli of two Umbilicariaceae species. Upper cortex and algal layer of *Lasallia pustulata* (Fig. 5) and *Umbilicaria crustulosa* (Fig. 6). Fungal hyphae are swollen and photobiont cells roundish. Many intercellular spaces contain water (arrows). M = mycobiont, p = photobiont. Bars = 10 μ m.

Fig. 7–11 LTSEM micrographs of freeze-fractured fully hydrated thalli of four Umbilicariaceae species. Bars = 10 μ m. In all cases, the upper part of the thallus is in the upper part of the micrograph. Algal layer and medulla of *Umbilicaria cylindrica* (Fig. 7), *U. crustulosa* (Fig. 8) and *U. polyphylla* (Fig. 9) and *Lasallia pustulata* (Figs. 10 and 11). Fungal hyphae are swollen and photobionts are turgid and globular. Transverse sections of some photobiont cells are indicated by solid arrows. Intercellular spaces are either air-filled (Fig. 7) or partially occupied by gelatinous substances (Figs. 8 and 9). Gelatinous substances are indicated by asterisks. Water in the medulla of these species is mostly confined to the interior of the cells, but intercellular water can be occasionally observed for instance in the arachnoidal, loose layer of the medulla of *L. pustula* (arrows in Figs. 10 and 11).

In our opinion, the study of the arrangement of hyphae and of the relative importance of cell walls and gelatinous substances, as in Valladares and Sancho (1995), together with the quantitative determination of intercellular space, e.g. by porosimetric measurements (Valladares et al., 1993) or by stereological measurements (Ascaso and Valladares, 1994), can provide information on the water storage capacity of the lichens and not only on the dimensions of the gas-filled interior. However, some studies of hydrated lichens using low temperature scanning microscopy found no evidence of intercellular water reservoirs in the gas-filled interior of the lichen thallus, suggesting that water was confined to the cells and to the apoplast in certain lichen species (Honegger, 1991, 1993; Honegger and Peter, 1994; Honegger et al., 1996). Honegger and colleagues claimed that previous studies on the structural basis of water storage in the lichen thallus were based only on correlation analysis and questioned the abundant ecophysiological studies of lichens showing a decline of CO₂ exchange at high levels of hydration due to waterlogged intercellular spaces (Green and Lange, 1994; Green et al., 1994). Lange et al. (1996) proved that the depression in net photosynthesis at high water content was actually due to increased thallus diffusion resistances in both green algal and cyanobacterial lichens. These discrepancies could be explained by differences in the hydration method as discussed by Lange et al. (1996); Honegger and co-workers superficially sprayed the thallus from above while others (Lange et al., the present study) submerged the thallus in water, which may have increased the penetration of water in the thalline interior. But different species might store water differently. Scheidegger (1994) observed free water in regularly soaked *Cetraria islandica* but not in *Teloschistes lacunosus*. Besides, diffusion of CO₂ in hydrated thalli can be impeded not only by extracellular water but also by the swelling of the fungal hyphae and the consequent blocking of the pores. The importance of 3D changes in fungal hyphae as a result of hydration for the maintenance or blocking of intrathalline CO₂ pathways is unknown in lichens and it is among the objectives of our current research. Finally, the possible existence of artifacts in LTSEM studies of lichens has to be admitted, since the manipulation of the samples, namely the speed of the freezing, can alter the location of water. A slow cooling leads to extracellular freezing of symplastic and apoplastic water as discussed by Scheidegger et al. (1995) and Schroeter and Scheidegger (1995). In order to speed up the process of freezing the sample must be very small, but since we have observed a very fast dehydration of the external zones of extremely small samples we did not use thallus fragments smaller than 3 × 2 mm.

The hypothesis that structural adaptations of lichens that increase rates of water uptake also produce high rates of evaporative loss (Rundel, 1982) was confirmed to a certain extent by those studies where species of a wide variety of growth forms were compared. In these studies, the surface area to weight ratio explained most of the variability found in the water uptake and loss of lichens (Larson, 1979; Larson, 1981). Our results, for the case of a foliose lichen, suggest that this generalization is only true if those structures are placed in the upper surface of the thallus. And considering the importance of the texture and permeability of the surface of the thallus and of any of its structures for the real water uptake or water loss performances (Larson, 1979; Larson, 1981; Valladares, 1994b), it has to be admitted that the

aforementioned hypothesis must be considered with caution, specially when species of a similar growth form are compared.

The dorsiventrality of foliose lichens is accentuated by the different water source that each surface of the thallus can be exposed to. Lichens belonging to the family Umbilicariaceae grow on a wide range of micro-habitats, from horizontal to vertical or overhanging rocks, and from xeric rock surfaces to runoffs. Consequently, some of these lichens use liquid water coming from the substrate (substrate-hygrophytic species), while for others the main source of water is the atmosphere (aerohygrophytic species), a situation that usually involves water in gaseous form (vapour) or constituted by particles of small size (fog, mist and drizzle, drop diameter from 0.003 to 0.1 mm, Larson, 1984a). While in the first case an almost impervious upper surface is very adaptive due to the consequent reduction in the evaporation of the water obtained from the lower surface, in the second case, the upper surface of the thallus is usually porous and hygroscopic in order to exploit the most frequent hydric resource (Larson, 1984a; Sancho et al., 1994; Valladares, 1994a, b).

In the case of aerohygrophytic species, all structural adaptations of the upper surface towards an increase in water uptake (increases in porosity and in surface area to weight ratio) will also produce high rates of evaporative loss. This rapid gas exchange as a result of their thallus structure will shorten the periods of metabolic activity, so aerohygrophytic species are forced to maximize the exploitation of these periods of activity either increasing the frequency of these periods by efficient water uptake or increasing the carbon gain during these periods by high net photosynthetic rates. On the other hand, the external and internal thallus structure of substrate-hygrophytic species allows them to remain wet for rather long periods of time. Lichens growing in runoffs are frequently active at very high thallus water content, which usually translates into a depression of net photosynthesis due to reduced diffusion of CO₂ (Lange and Tenhunen, 1981; Green et al., 1982, 1985, 1994). These facts point to substrate-hygrophytic lichens as being adapted to maximize growth by lengthening the periods of photosynthetic activity and not by intensive exploitation of these periods, as is the case for aerohygrophytic species. Rhizinomorphs represent a very adaptive solution for substrate-hygrophytic lichens since they increase the hysteresis of the hydration-dehydration cycles of these organisms, accelerating the uptake of liquid water from the substrate and decelerating the water loss by evaporation.

The species of the genus *Lasallia* constitute an interesting case within the Umbilicariaceae due to their structural peculiarities with clear ecophysiological implications. Their hydric features are similar to those of substrate-hygrophytic species (long water retention time, large water storage capacity on a surface area basis, Table 1) but their porous and highly hygroscopic thallus upper surface is a typical characteristic of aerohygrophytic species (Larson, 1984a; Sancho et al., 1994; Valladares, 1994b).

The medulla of the *Lasallia* species is unusual for the Umbilicariaceae, since it is made up of two distinct layers, an upper, loosely arachnoidal layer and a lower, densely prosoplectenchymatic one (Sancho and Crespo, 1989; Valladares and Sancho, 1995). Such a medullary structure combines the

large water storage capacity of dense, prosoplectenchymatic medullae with the large ventilation of loose, arachnoidal medullae precisely in the vicinity of the algal layer were the gas exchange is crucial. In analogy with the cyphellate lower cortex (e.g. Green et al., 1982), the pustules, characteristic of *Lasallia* species, could improve the gas exchange of the thallus since both the lower cortex and the lower, dense part of the medulla are interrupted in the pustules (Sancho and Balaguer, 1989). Moreover, certain species of this genus (*L. hispanica*, *L. pustulata*) exhibited a rapid change in opacity of thallus upper surface at optimum water content for photosynthesis, and this increase in the albedo had a significant cooling effect that was apparently able to cause an increase in the net photosynthetic rate (Sancho et al., 1994). All these ecophysiological-linked structural features of *Lasallia* species indicate that they have achieved an optimal compromise between the different structural solutions of the Umbilicariaceae to the opposing problems of increasing the water storage capacity and facilitating the CO₂ exchange. This conclusion is in agreement with the high photosynthetic capacity of the Spanish *Lasallia* species in comparison with several *Umbilicaria* species (Sancho and Kappen, 1989).

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