

Water relations in lichens at subzero temperatures: structural changes and carbon dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica

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SUMMARY

Photosynthetic activity and structural changes at subzero temperatures were monitored in the foliose lichen *Umbilicaria aprina* Nyl. from continental Antarctica. Carbon dioxide gas exchange measurements revealed that net photosynthesis and dark respiration occurred at subzero temperatures regardless of whether a lichen thallus saturated with liquid water was exposed to subzero temperatures, or if a dry thallus was re-hydrated only from snow at subzero temperatures. When water-saturated thalli of *U. aprina* were slowly cooled at subzero temperatures ice nucleation activity could be detected at -5.4°C , indicating extracellular freezing of water. Using low-temperature scanning electron microscopy (LTSEM) it was demonstrated that extracellular ice formation leads to cytorrhysis in the photobiont cells and to cavitation in the mycobiont cells. Both processes were reversible if the lichen thallus was re-warmed. When dry lichen thalli were covered with snow at subzero temperatures a substantial re-hydration from snow could be observed in LTSEM micrographs and measured gravimetrically. The final thallus water content was strongly dependent on the temperature regime and gave water contents between 20% d. wt at -21°C and 56% d. wt at -4.5°C after 16 h exposure. Carbon dioxide gas exchange measurements revealed that metabolic activity was initiated during re-hydration from snow at subzero temperatures. It is proposed that water uptake from snow at subzero temperatures occurs in the gaseous phase and depends only on the temperature-related differences in water potential between the cell contents and the surrounding atmosphere in equilibrium with snow. Photosynthetic activity and re-hydration from snow at subzero temperatures are of great ecological importance for primary production in extreme environments such as Antarctica where metabolic activity is severely limited by water availability and low temperatures.

Key words: Antarctica, lichenized ascomycetes, low-temperature scanning electron microscopy, photosynthesis, subzero temperatures.

INTRODUCTION

Low temperature is reputedly the single most limiting factor to natural plant distribution, and frost causes various types of injury directly or indirectly associated with the freezing of water in plant tissues (Burke *et al.*, 1976). Plants which are tolerant to

freezing predominantly undergo extracellular freezing (Burke *et al.*, 1976) and avoid the lethal process of intracellular formation of hexagonal ice crystals.

In alpine and polar tundra ecosystems where environmental conditions are dominated by low temperatures, snow–vegetation interactions play an important role in primary production. Tundra ecosystems have a low primary productivity and their environmental conditions are often near the limits for key metabolic processes (Bliss, 1985). The spatial distribution of plant species and communities is largely controlled by snow distribution which varies greatly with topography and wind patterns,

Abbreviations: DR, dark respiration; EF, exoplasmic fracture face of freeze-fractured membrane; INA, ice nucleation activity; LN₂, liquid nitrogen; LTSEM, low-temperature scanning electron microscopy; NP, net photosynthesis; PF, plasmic fracture face of freeze-fractured membrane; PPFD, photosynthetic photon flux density.

the duration of the snow-free period, and the position of meltwater drainages (Schroeter, 1926; Walker *et al.*, 1993). At the organism level the snow regime affects water availability, plant temperatures and surface microclimate. The duration and thickness of snow cover control the photon flux density incident at the plant level (Richardson & Salisbury, 1977) and might alter the ambient CO₂ concentration (Kelley, Weaver & Smith, 1968; Zimov *et al.*, 1993), both key factors for photosynthetic production.

In polar and alpine ecosystems, and especially in the ice-free areas of Antarctica, lichens form a major part of the vegetation. Lichens are known to resist temperatures down to -196 °C, both under dry and water-saturated conditions (Kappen & Lange, 1972). Lichens are species specifically affected by the snow regime (Larson & Kershaw, 1975; MacFarlane & Kershaw, 1980; Scott & Larson, 1985, 1986; Sonesson, 1989). Several species are often strongly injured by prolonged snow cover (Benedict, 1990, 1991), whereas chionophilous species such as the crustose lichen *Lecidea sciatrapha* (maritime Antarctic) are physiologically adapted to snow cover (Kappen, Schroeter & Sancho, 1990a) and prefer snow-influenced habitats. Snow cover shelters the cryptogamic vegetation from the harsh environmental conditions during winter. Although *Usnea antarctica* is generally found in windblown and snow-free sites in the comparatively mild maritime Antarctic (Schroeter, 1991; Schroeter, unpublished), it occurs in sheltered and snow-covered habitats where the environmental conditions become more extreme with increasing latitude (continental Antarctica; Hancock & Seppelt, 1988), thus giving another example of relative habitat constancy (Poelt, 1987). In continental Antarctica snow is the major water source for the cryptogamic vegetation (Kappen, Meyer & Böltner, 1990b; Hovenden, Jackson & Seppelt, 1994) apart from short-running melt water streams of summer. Because of their poikilohydrous nature lichens depend completely on the ambient water availability. Under changing ambient conditions they are subject to repeated drying/wetting cycles. In habitats with a subzero temperature regime such as continental Antarctica where precipitation occurs only as snow, water uptake from snow at subzero temperatures plays an important role in primary productivity in lichens.

Photosynthetic productivity at subzero temperatures and under the influence of snow and ice has rarely been investigated in lichens. Recently, it has been shown that lichens of the continental Antarctic are able to gain a significant CO₂ uptake at subzero temperatures in the field down to -10 °C (*Usnea sphacelata*, Kappen, 1989) and -17 °C (*Umbilicaria aprina*, Schroeter *et al.*, 1994). In both studies, dry lichens were found to be photosynthetically activated by snow at subzero temperatures. Lichens are known to be activated by water vapour uptake at water

potentials down to -35 MPa (Lange, 1969; Nash *et al.*, 1990; Scheidegger, Schroeter & Frey, 1995) and it seems reasonable to suppose that photosynthetic behaviour at subzero temperatures in polar lichens resembles that of a lichen thallus in equilibrium with humid air (Kappen, 1993a; Schroeter *et al.*, 1994).

In the past few years low-temperature scanning electron microscopy (LTSEM) has been found to be a valuable approach where turgor-related functional states of poikilohydrous organisms are investigated (Scheidegger *et al.*, 1991; Echlin, 1992; Scheidegger, 1994). Structural changes related to different turgor pressures at given water contents have been demonstrated in lichens using LTSEM methods (Scheidegger, Schroeter & Frey, 1995). Therefore in the present study CO₂ gas exchange measurements were combined with structural investigations using LTSEM methods to investigate the water uptake of dry lichens from snow at subzero temperatures, and water translocation during slow freezing and thawing, as to be expected under natural conditions in continental Antarctica.

MATERIALS AND METHODS

Plant material

Umbilicaria aprina Nyl. is a widespread species in continental Antarctica (Filson, 1966, 1975, 1987; Lindsay, 1969, 1972; Richter, 1985; Seppelt, Green & Schroeter, 1995). It is also found in the northern hemisphere, where it shows a disjunct distribution from arctic America and Scandinavia to the high mountains of Ethiopia (Thomson, 1984; Hestmark, 1990). It grows on exposed rocks and boulders, and in favourable habitats its monophyllous thallus can reach diameters > 10 cm. The material used here was collected at Botany Bay (77° 00' S, 162° 35' E), Granite Harbour region, southern Victoria Land, continental Antarctica, where it is the most prominent macrolichen (Schroeter *et al.*, 1994). All material was transported in air-dry conditions to the laboratory in Kiel where the samples were deep-frozen and kept in the dark at -20 °C until the measurements began. Before being used for measurements the lichen samples were activated by spraying with deionized water and cultivated for 3 d at +5 °C with 12/12 h light/dark treatment (photosynthetic photon flux density (PPFD) = 150 μmol m⁻² s⁻¹).

CO₂ gas exchange measurements

All CO₂-exchange measurements were carried out in the laboratory at Kiel, Germany, using a mini-cuvette-system (Walz GmbH, FRG) with a differential IRGA (Binos 100, Rosemount, FRG) in an open-flow system combined with a cooling trap (MGK-4, Walz GmbH, FRG) to remove water in the measuring and control gas stream before entering the CO₂-unit (see Schroeter *et al.*, 1994). All relevant

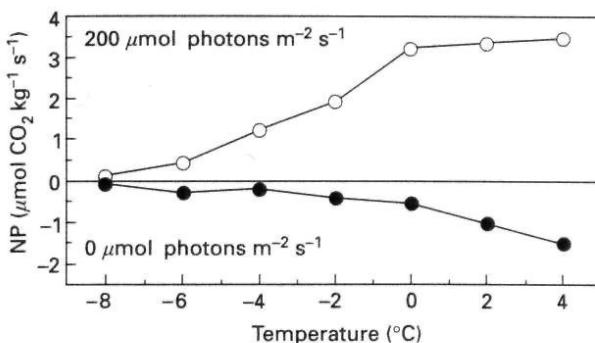


Figure 1. Temperature-dependent CO_2 gas exchange of *Umbilicaria aprina*. NP (open circles at PPFD of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$) and DR (black circles) are related to oven-dry weight. The sample was saturated with liquid water before the start of the experiment at $+4^\circ\text{C}$ and subsequently cooled down to -8°C .

parameters were recorded automatically every 120 s using an external datalogger (DES, Walz GmbH, FRG). The CO_2 zero-point of the system was checked regularly and adjusted if necessary.

The temperature-controlled measuring cuvette (GK-022, Walz GmbH, FRG) was placed in a freezing chamber (Rubarth, FRG) to maintain subzero temperatures. Teflon® tubes were used to connect the cuvette with the gas analyser and control system. A constant PPFD of c. $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ was generated by an incandescent lamp. The light intensities incident at the thallus level were measured inside the cuvette using a PAR quantum sensor (Li 190 SB, Li-Cor, USA). Temperature values were maintained by an electronic control in the mini-cuvette, and thallus temperatures were monitored with a very fine thermocouple ($< 0.1 \text{ mm}$) inserted into the lichen thallus. Carbon dioxide gas exchange data were computed on the basis of oven-dry weight (d. wt; 24 h, 105°C).

Low-temperature scanning electron microscopy studies

For LTSEM parts of the lichen thalli investigated were excised and frozen in liquid nitrogen (LN_2) within 2 s according to the different experimental designs described below. The frozen samples were then mounted and mechanically fixed on aluminium stubs in a transfer freezing device TFD (BAL-TEC, FL) at $< -80^\circ\text{C}$. The mounted specimens were cooled to -196°C and then transferred to the cold stage of the preparation chamber of a SCU 020 (BAL-TEC, FL). The specimens were fractured with a microtome at -90°C and partly freeze-dried in a high vacuum ($< 2 \times 10^{-4} \text{ Pa}$) at -90°C for 1 min. Platinum sputter coating was then undertaken after raising the pressure to 2.2 Pa in an argon atmosphere. The coating thickness was 15 nm, measured by a quartz thin film monitor. After coating, the specimens were transferred with a manipulator through the sliding vacuum valve onto the SEM cold stage in a SEM 515 (Philips, NL).

The temperature of the cold stage in the microscope was kept below -120°C . A detailed description of the method and equipment can be found in Müller *et al.* (1991) and Scheidegger *et al.* (1991).

Experiment 1 : Slow freezing of water-saturated thalli

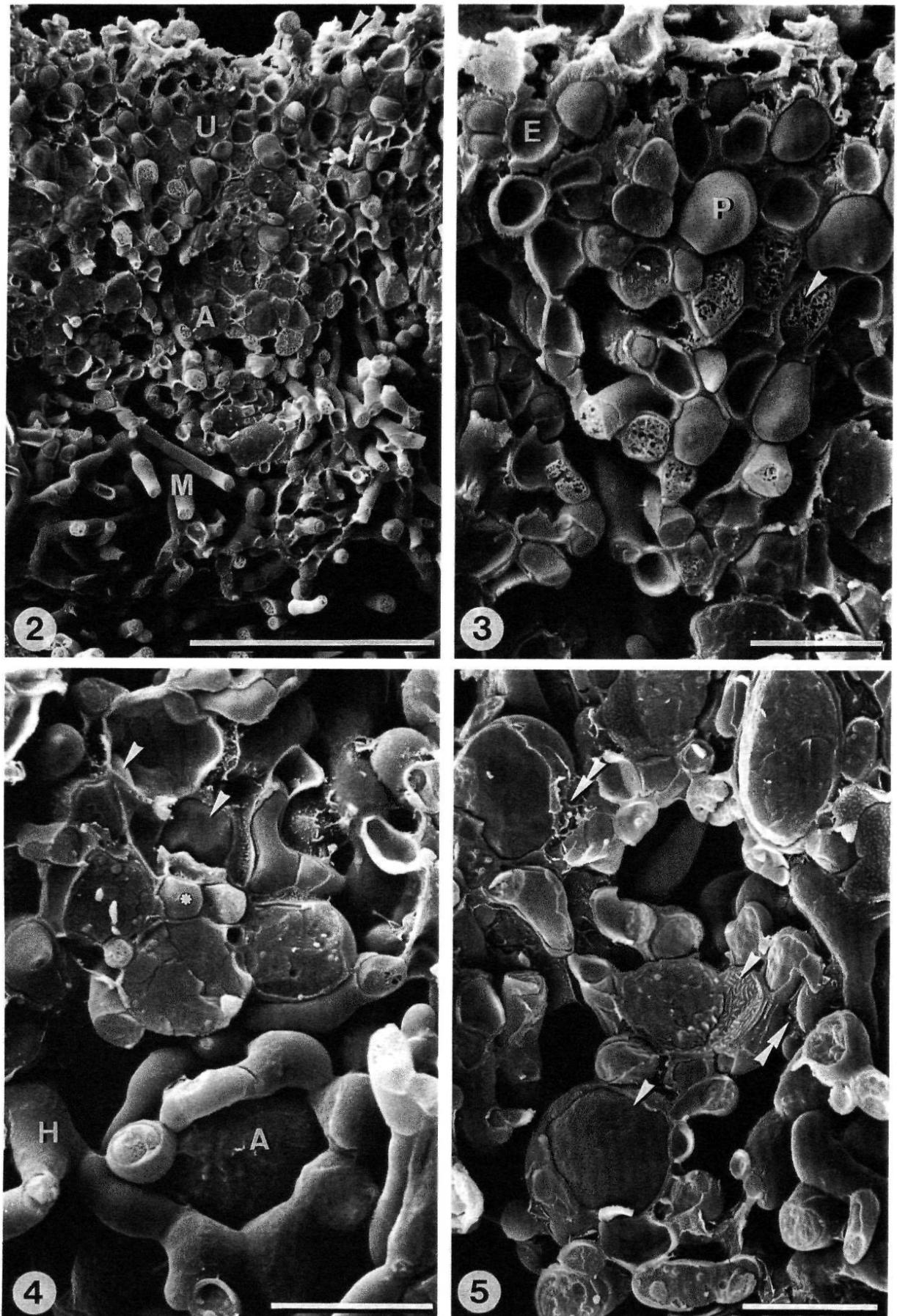
(a) CO_2 gas exchange. Dark respiration (DR) and net photosynthesis (NP) (at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$) in *U. aprina* were measured at discrete temperature steps of 2°C from $+4^\circ\text{C}$ down to -8°C . Before treatment the lichen sample was water-saturated at $+8^\circ\text{C}$ and kept at the optimum (i.e. the water content which gave maximum CO_2 gas exchange rates) during the experiments.

(b) Structural analysis. Air-dry thalli of *U. aprina* were weighed and then re-hydrated in distilled water for 1 h at $+8^\circ\text{C}$. The hydrated thalli were blotted with filter paper to remove adhering surface water and weighed again to determine their water content. For structural investigations, parts of the samples were excised with cooled tools and frozen in LN_2 within 2 s. Then the lichen samples were placed in a freezing chamber and the temperature was lowered to -20°C within 30 min. Again, parts of the samples were excised and frozen in LN_2 as described above. The lichen samples were subsequently thawed slowly to $+8^\circ\text{C}$ without removing melt water, and new samples for structural analysis were taken. The water content of the samples was determined before the subzero temperature treatment and at the end of the experiment.

(c) Thermal analysis of lichen freezing. The temperature at which water froze in specimens of *U. aprina* was determined by thermal analysis. Lichen thalli were hydrated by spraying with liquid water at $+8^\circ\text{C}$ and subsequently blotted with filter paper to remove adhering surface water. The hydrated lichen samples ($n = 6$) were placed in a computer-controlled freezing chamber (Colora, FRG). The initial temperature was $+5^\circ\text{C}$ and was subsequently lowered at 6°C h^{-1} down to -20°C . A very fine thermocouple was inserted in the lichen thalli, and thallus temperature measurements were recorded individually at 30 s intervals using a 12-bit datalogger (SQ1208, Grant, UK). During the course of the lowering of temperature an exotherm indicated freezing of water. The temperature of ice nucleation activity (INA) was noted as the sample temperature immediately before the sharp increase in the thallus temperature.

Experiment 2 : Hydration of air-dry thalli from snow at subzero temperatures

Air-dry thalli ($n = 5$) of *U. aprina* were placed in a temperature-controlled freezing chamber (Rubarth,



Figures 2–5. For legend see opposite.

FRG) at different subzero temperatures ranging between -4.5 and -21.1 °C. The temperature of the dry thalli adjusted to the ambient subzero temperature within a period of 20 min. Then, the thalli were covered with snow (which was also adjusted to the given subzero temperature before the start of the experiment). A period of 16–20 h was given for water uptake of the dry thalli from snow. Thallus temperatures were monitored using very fine thermocouples (0.15 mm) and a datalogger (SQ1208, Grant, UK).

For structural investigations, parts of the samples were excised and frozen in LN₂ within 2 s before and at the end of the hydration treatment and the water content was gravimetrically determined in parallel.

The hydration experiment was combined with measurements of the CO₂ gas exchange. Only a single thallus was used in the gas exchange cuvette. The air-dry thallus was weighed at the beginning and at the end of the gas exchange measurements. To monitor the time-dependent water uptake from snow at -4 °C the sample was regularly weighed under ambient subzero temperatures during the gas exchange measurement. Adhering snow crystals were carefully removed from the thallus surface before all weighings, to minimize water uptake from melting snow crystals.

In total more than 330 LTSEM pictures were taken in both experiments. For each temperature pictures were taken from 3–4 samples.

RESULTS

Slow freezing of water-saturated thalli

Figure 1 shows the temperature dependency of CO₂ gas exchange of *Umbilicaria aprina* hydrated with liquid water. At 200 μmol photon $\text{m}^{-2} \text{s}^{-1}$ net photosynthetic rates reached +3.4 $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ at 4 °C and dark respiration was $-1.5 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$. With decreasing temperature from +4 °C down to -8 °C gas exchange rates decreased steadily. The lower temperature compensation point was not reached in this experiment

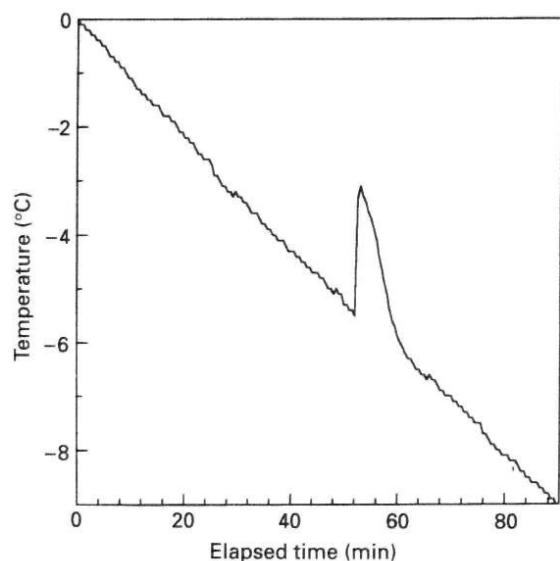


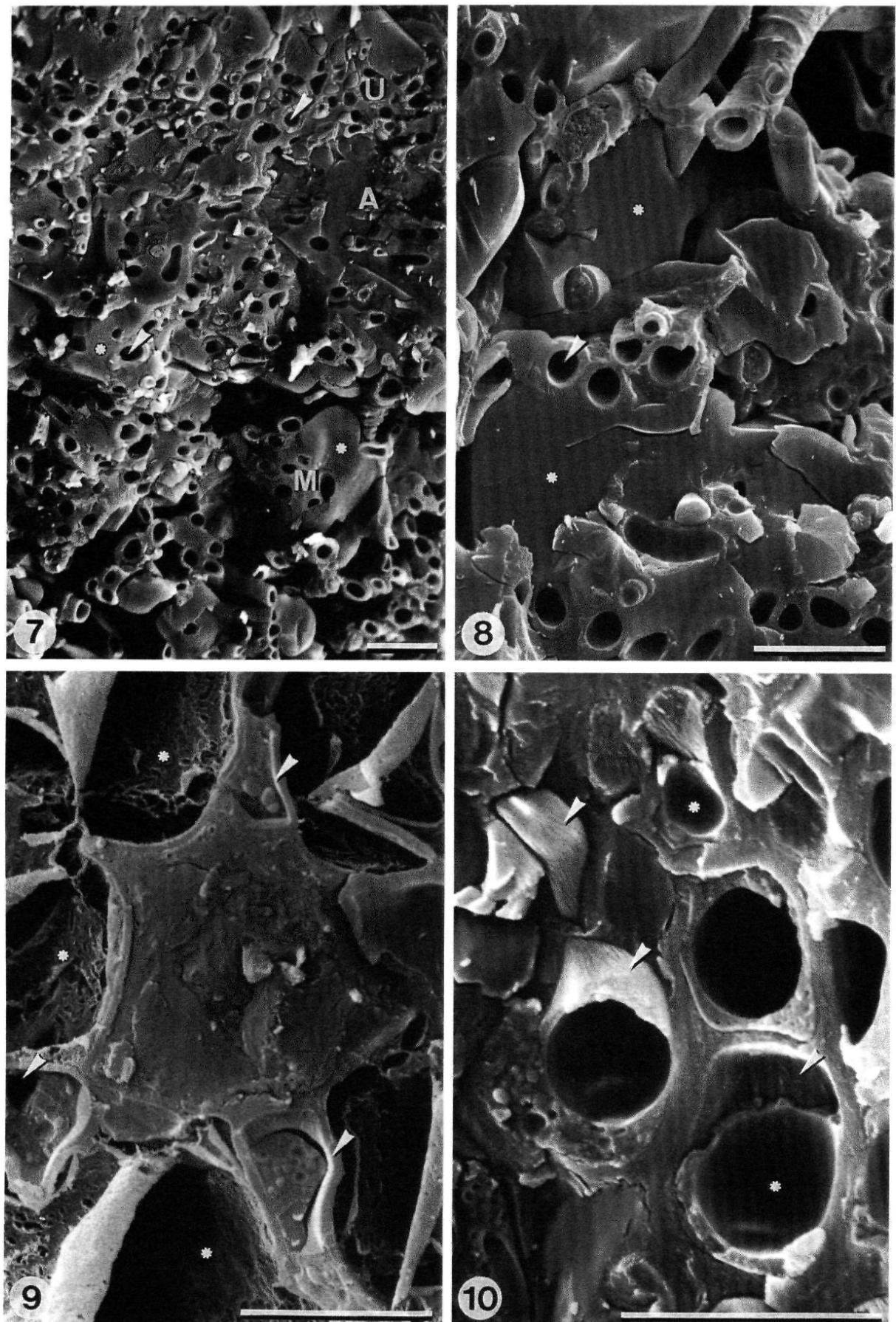
Figure 6. Thermal analysis of *U. aprina*. Water-saturated specimens were cooled down at $6 \text{ }^{\circ}\text{C h}^{-1}$ and thallus temperature was monitored individually at 30 s intervals. The freezing of water was detected as an abrupt rise in thallus temperature (exotherm) and the temperature of INA was noted as the thallus temperature immediately before that increase.

and both NP and DR gave significant rates at -8 °C, the experimental limit.

The LTSEM-micrographs in Figures 2–5 illustrate the structural relations in the thallus of *U. aprina* when saturated with liquid water above the freezing point. The upper cortex is paraplectenchymatous, and cross-fractured cortical hyphae are filled with protoplasts. In the water-saturated state paraplectenchymatous cortical cells were turgid (Fig. 2). Their cell walls were relatively thin-walled. The plasmic (PF) or exoplasmic (EF) fracture faces of the plasmalemma were smooth (Fig. 3). Only the epinecral layer consisted of dead, collapsed cortical cells (Figs 2, 3). Photobiont cells were roundish but not globular because of compressive stress from the numerous appressoria per photobiont (Fig. 4). The photobiont layer consisted of clustered *Trebouxia* cells, and strands of medullary hyphae separated the

Figures 2–5. LTSEM-micrographs of freeze-fractured water-saturated thalli of *Umbilicaria aprina*. Water content in the different thalli varied from 197–261 % d. wt.

Figure 2. Epinecral layer of dead cortical cells (arrowhead) and turgid paraplectenchymatous cells of the upper cortex (U) of *Umbilicaria aprina*. The photobiont layer consists of clustered *Trebouxia* sp. cells (A) and strands of medullary hyphae separate the photobiont clusters. The medulla (M) consists of long-celled hyphae. No extracellular free water was detectable in the photobiont or medullary layers. Bar = 50 μm . **Figure 3.** Upper cortex (U) and epinecral layer of *Umbilicaria aprina*. Transversely fractured cortical cells are filled with the protoplast. Intracellular ice crystals of hexagonal ice are freezing artefacts related to cryofixation. The intracellular ice crystals (arrowhead) were etched after freeze-fracturing PF (P) and EF (E) of the plasmalemma are smooth. Bar = 10 μm . **Figure 4.** Photobiont layer with globular cells of the *Trebouxia* photobiont (A) and moniliform hyphae (H) and appressoria (asterisk) of the mycobiont. Fractured photobiont cells are dented by the partially ensheathing appressoria (arrowheads). Bar = 10 μm . **Figure 5.** Water-saturated thallus of *Umbilicaria aprina* frozen at -20 °C at low freezing rates and re-warmed to $+15$ °C. Fungal hyphae are refilled and photobiont cells are globular. Extracellular water disappeared almost completely and is now restricted to small intercellular spaces (arrowheads). Note plasmalemma invaginations (arrowheads) on PF and EF of the photobiont-plasmalemma. Bar = 10 μm .



Figures 7–10. For legend see opposite.

photobiont clusters (Fig. 2). In *U. aprina* numerous appressoria partly ensheathed the photobiont cells (Fig. 4). In Figure 3 intracellular ice crystals could be seen in cross-fractured cortical cells. In contrast to the extracellular ice formation described below which occurs in the slow freezing experiments (see Fig. 7), the intracellular ice crystals in Figures 2 and 3 are interpreted as artefacts which were introduced during the cryofixation of the samples. However, no extracellular water was found either in the small intercellular spaces of the cortex or in the larger air space of the medulla (Figs 3, 4). This revealed that preparational freezing rates were rapid enough to induce intracellular freezing and that the cryofixation method was therefore adequate for studying natural water distribution at the cellular level. However, if the samples selected were extremely thick (> 2 mm), the cooling rate obtained by plunging the specimens in LN₂ was too slow and freezing was predominantly extracellular (data not shown).

Figure 6 shows the thermal analysis of *U. aprina*. If water-saturated specimens were cooled from +5 °C at a rate of 6 °C h⁻¹, freezing of water in the thallus could be detected as an abrupt rise in thallus temperature (exotherm). Ice nucleation temperature was -5.4 °C (mean, $n = 6$, SE = 0.1528). No second exotherm was detected down to -34 °C.

Figures 7–10 show the structural analysis of water-saturated thalli frozen with a cooling rate of 1 °C min⁻¹. In contrast to the samples which were fast-frozen in LN₂ (Figs 2–4), slow freezing resulted in a strong cellular dehydration through extracellular ice formation (Fig. 7). Ice crystals occupied a considerable amount of the intercellular air space, and often completely surrounded medullary hyphae and photobiont cells (Figs 7–10). In most fractured cortical and medullary hyphae one central cavity was found (Figs 7, 8). This cavity occupied a considerable volume of the protoplast. The cavities of the cortical cells were delimited by a smooth boundary and could be distinguished from the wrinkled plasmalemma (Fig. 10). The photobiont cells were greatly collapsed, the infolds being between the appressoria (Fig. 9). The plasmalemma was wrinkled in cavitated cells but smooth in non-cavitated cells (Figs 9, 10). This indicates a significant shrinkage of the protoplast. Because the cavitation bubble is delimited by a smooth boundary, it is probable that the shrinkage

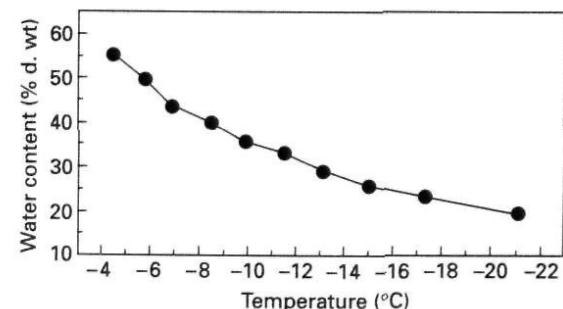


Figure 11. Temperature dependency of water uptake from snow in *U. aprina*. Air-dry thalli were adjusted to subzero temperatures and covered with snow. Water content was determined gravimetrically after 16 h exposure in the dark. Black circles represent the mean ($n = 5$). SE bars are within the circles.

of the protoplast occurred mainly before the cavitation event.

If water-saturated slow frozen thalli were thawed after extracellular ice formation had occurred and were re-warmed to +15 °C, all water which was previously frozen extracellularly on medullary hyphae and photobionts was absorbed and again no free extracellular water could be detected (Fig. 5). Cells which were cavitated in the frozen state refilled during thawing and the photobiont cells appeared turgid again. A typical compression of the photobiont cells could be observed at those sites where the photobiont cells were in contact with appressoria (Fig. 5).

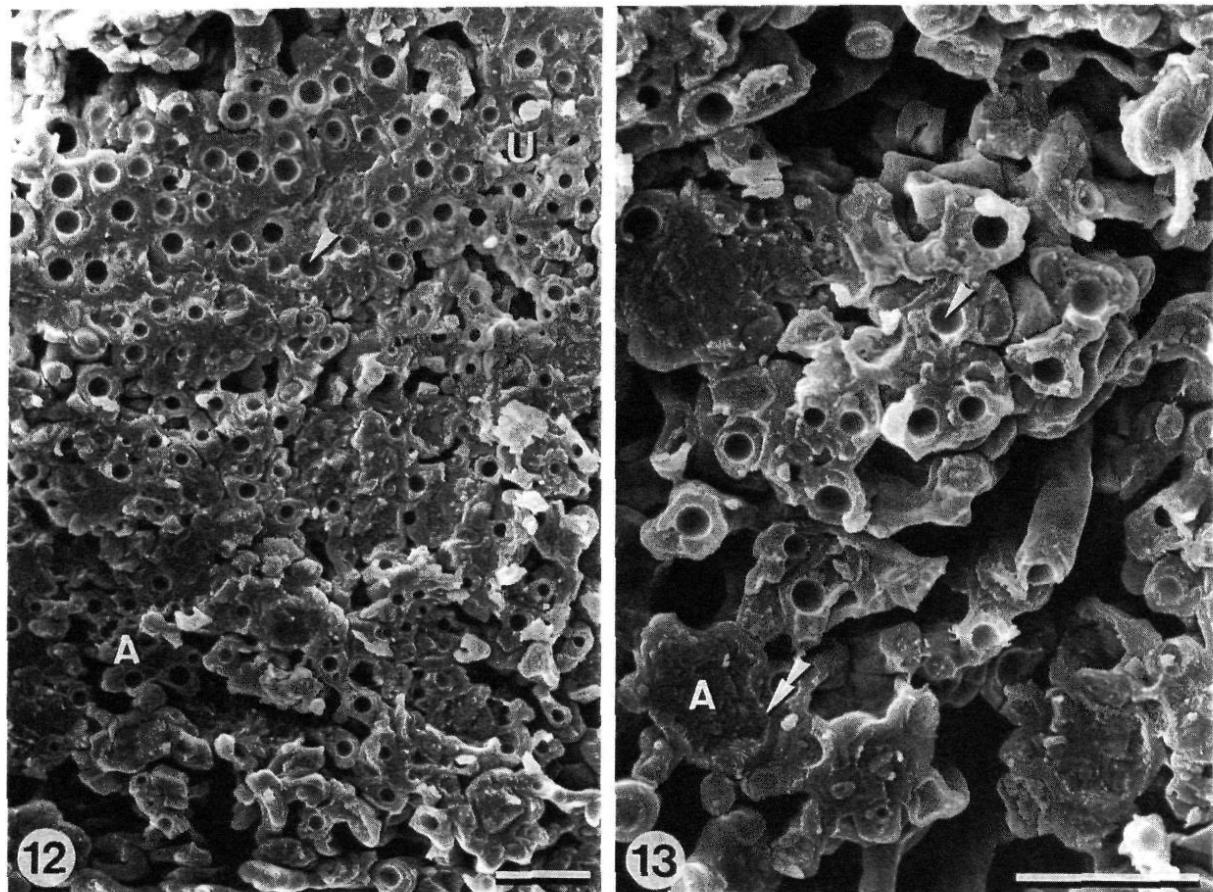
Hydration of air-dry thalli from snow at subzero temperatures

If air-dry thalli of *U. aprina* were covered with snow at subzero temperatures in the dark for 16 h or more, a considerable increase in the water content from an initial water content of c. 9% d. wt in the air-dry state up to 56% d. wt after 16 h at -4.5 °C was observed (Fig. 11). The water uptake from snow at subzero temperatures was strongly temperature dependent: whilst a water content of more than 50% d. wt was reached at temperatures above -6 °C, a water content of only 25% d. wt was obtained at temperatures below -14 °C (Fig. 11).

If air-dry thalli of *U. aprina* were equilibrated with snow at -14 °C no differences from air-dry thalli were observed in structural analysis (Figs 12,

Figures 7–10. LTSEM-micrographs of freeze-fractured water-saturated thalli of *U. aprina*, frozen at slow cooling rates to -20 °C.

Figure 7. Upper cortex (U), photobiont layer (A) and medullary layer (M) of *U. aprina*. Cortical and medullary hyphae are cavitated (arrowheads). Intercellular spaces are partly filled with extracellularly frozen hexagonal ice. Bar = 10 µm. **Figure 8.** Cavitated medullary hyphae of *U. aprina* surrounded by extracellular ice crystals (I). Bar = 10 µm. **Figure 9.** Transversely fractured collapsed photobiont cell of *U. aprina*. Infolds are between the appressoria (asterisks). Bar = 5 µm. **Figure 10.** Upper cortex of *U. aprina* with cavitated cells. One central cavity with a smooth delimitation (asterisks) is found per hyphal cell. The PF of cavitated cells is wrinkled (arrowheads). Bar = 5 µm.



Figures 12–13. LTSEM-micrographs of freeze-fractured air-dry thalli of *U. aprina* hydrated with snow at -14°C for 16 h. Final water content was 21% d. wt.

Figure 12. Upper cortex (U) and photobiont layer (A) of *U. aprina*. Cortical hyphae are cavitated. The cavity occupies the major part of the cell volumes. Bar = 10 μm . **Figure 13.** Cavitated medullary hyphae and cross-fractured cells of collapsed photobionts of *U. aprina*. EF of the algal plasmalemma is closely fitted to the cell wall (arrowhead). Bar = 10 μm .

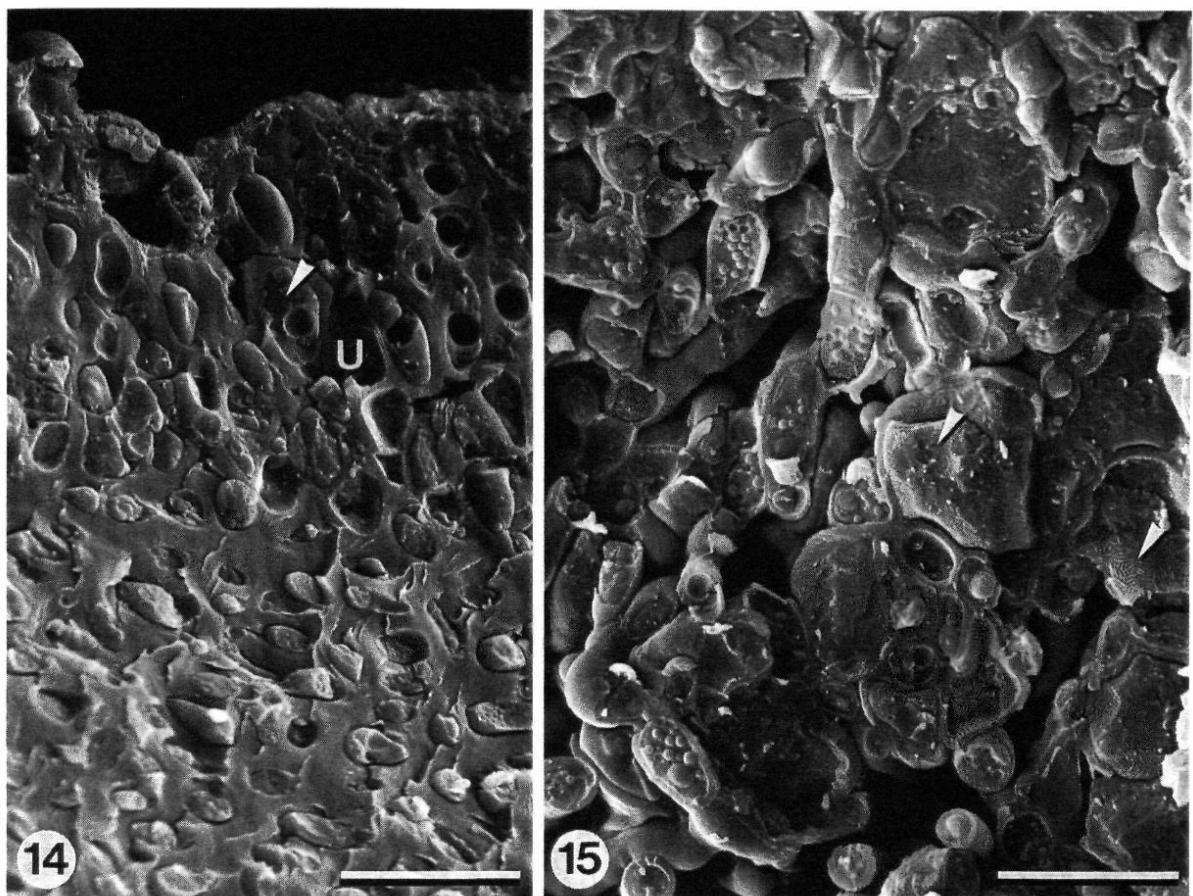
13) even though a substantial increase in water content could be observed after 16 h (see Fig. 11). At -14°C all cortical (Fig. 12) and medullary (Fig. 13) hyphae were cavitated. The hyphal cells contained one central cavity which was delimited by a smooth boundary. The volume of the protoplast was greatly reduced and was restricted to a thin film around the dominating cavitation bubble. The photobiont cells were strongly collapsed (Fig. 13) but no cavitation occurred. In the photobiont cells the plasmalemma was closely fitted to the cell wall and plasmalemma invaginations were clearly visible on the EF of the plasmalemma of the photobionts.

Structural analysis of thalli re-hydrated at -8°C as shown by the LTSEM-micrographs in Figures 14 and 15 revealed a different picture. Again, cavitated cortical cells occurred, but the surrounding protoplasts considerably increased in volume and the cavities now occupied only a minor part of the cell volume. Further, only the uppermost cortical cells were found to be cavitated. Hyphae in the lower parts of the cortex (Fig. 14) were refilled as well as the medullary hyphae (Fig. 15), and the plasma

contained numerous globular inclusions. The photobiont cells were slightly collapsed but the different shape indicates an increase of turgor in contrast to the photobiont cells at -14°C (Fig. 13).

The LTSEM-micrographs in Figures 16 and 17 show thalli re-hydrated from snow at -4°C . No cavitation in the cortical or in the medullary hyphae was observed (Fig. 16) and the shape of the photobionts indicates a further increase in turgor even though it is obvious that the photobiont cells are not fully turgid, as shown in Figure 4. In comparison with thalli of *U. aprina* hydrated from snow at temperatures below -14°C the thallus water content at -4°C was more than doubled, but still significantly lower than that of thalli fully hydrated with liquid water which reached water contents > 200% d. wt.

In a separate experiment at -4°C , re-hydration from snow in the light (PPFD = 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was paralleled by CO_2 gas exchange measurements (Fig. 18). After the air-dry thallus had adjusted to -4°C it was covered with snow in the gas exchange cuvette, and the CO_2 exchange was monitored. At



Figures 14–15. LTSEM-micrographs of freeze-fractured air-dry thalli of *U. aprina* hydrated with snow at -8°C for 16 h. Final water content was 45% d. wt.

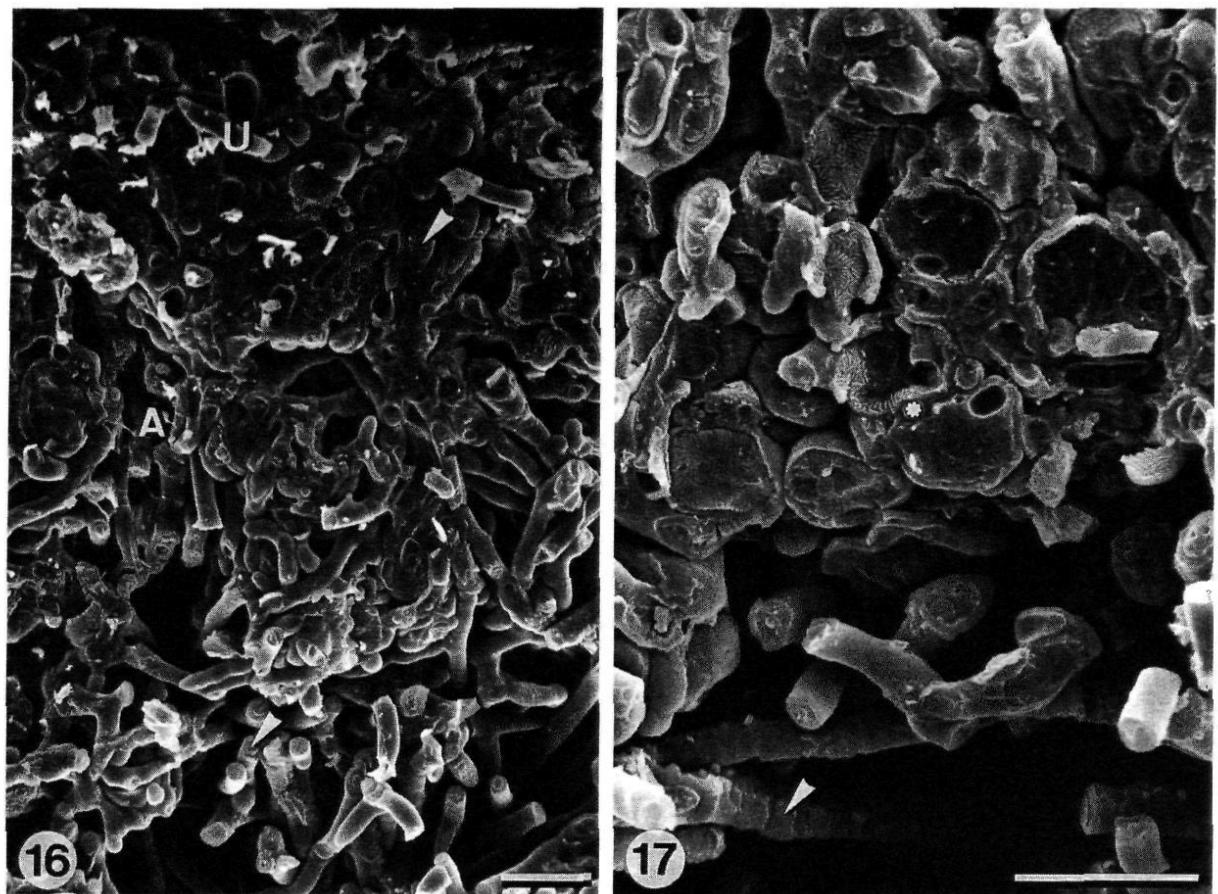
Figure 14. Upper cortex (U) of *U. aprina* with cavitated hyphal cells. Note the reduced size of the cavitated bubble and the increased volume of the protoplast (arrowhead) compared with the thallus hydrated at -14°C . Bar = 10 μm . **Figure 15.** Transversely fractured photobiont cells of *U. aprina* are greatly collapsed. The photobiont plasmalemma is closely fitted to the cell wall and plasmalemma invaginations are clearly visible (arrowheads). The medullary hyphae are refilled and turgid. Bar = 10 μm .

intervals the thallus was removed from the gas exchange cuvette, weighed at low temperatures and again covered with snow in the gas exchange cuvette. During the first hour of re-hydration a considerable increase in the water content was observed but at values below 20% d. wt no significant rates of CO_2 exchange were found. With an increase of water content up to 30% d. wt, CO_2 release indicated respiratory activity in the thallus. If the water content reached values above 40% d. wt, positive gas exchange rates were monitored. An equilibrium was almost reached after 10 h at a water content of c. 60% d. wt and the water content as well as NP increased only slightly within the next 10 h. Finally, after 20 h a photosynthetic rate of $+1.1 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ was reached.

DISCUSSION

In Antarctica, photosynthetic production in lichens is limited by water availability and low temperature. The ability of lichens to achieve considerable net

photosynthetic rates at subzero temperatures is well known from laboratory and field measurements in several species from Antarctica (Lange & Kappen, 1972; Kappen, 1989; Schroeter, 1991; Schroeter *et al.*, 1994). Schroeter *et al.* (1994) found that *Umbilicaria aprina* is able to reach significant photosynthetic rates down to -17°C in the field whilst dark respiration ceased at temperatures below -10°C . In the laboratory measurements presented here, we did not reach the absolute lower limits of NP and DR, but our gas exchange data agree well with the field measurements of Schroeter *et al.* (1994). Because of their poikilohydrous nature water availability limits severely the metabolic activity of lichens (Kappen, 1988; Schroeter, Kappen & Moldanek, 1991). In Antarctica, water appears mainly as snow and ice at subzero temperatures, and, if only hydration from liquid water could activate lichen metabolism, lichen vegetation in continental Antarctica would be restricted to sheltered habitats influenced by short-lived melt-water streams of summer. However, in continental Antarctica lichens are able



Figures 16–17. LTSEM-micrographs of freeze-fractured air-dry thalli of *U. aprina* hydrated with snow at -4°C for 16 h. Final water content was 55 % d. wt.

Figure 16. Upper cortex (U) and photobiont layer (A) of *U. aprina*. Cortical and medullary hyphae are refilled (arrowheads). Bar = 10 μm . **Figure 17.** Photobiont cells (A) of *U. aprina* are partially unfolded and the major indentations are from appressoria (asterisk). Lumpy outermost cell wall layer of medullary hyphae. Note plasmalemma invaginations on the PF of the photobiont plasmalemma (arrowheads). Bar = 10 μm .

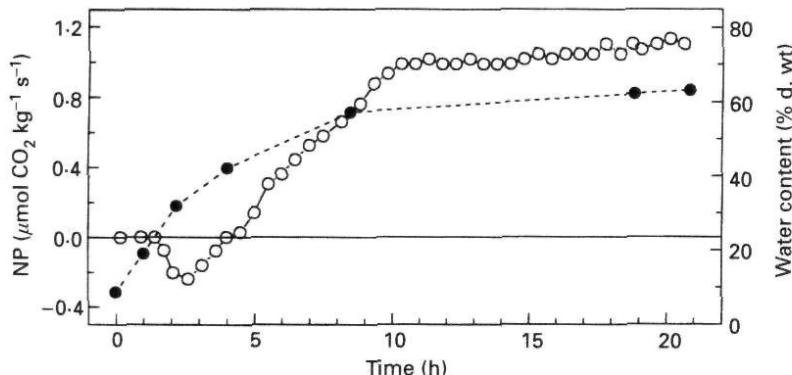


Figure 18. Net photosynthesis (open circles at $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and water content (black circles, % d. wt) during water uptake of an air-dry thallus of *U. aprina* from snow at -4°C (thallus temperature).

to colonize dry habitats such as wind-blown rock faces, and colonization seems to be more limited by availability of appropriate substrates than by a temporary availability of liquid water. In these habitats frozen water occurring as snow or ice must be considered the major water source.

In its natural habitat in continental Antarctica

Umbilicaria aprina colonizes both places close to summer melt-water streams and on the surface of northerly exposed boulders (Schroeter *et al.*, 1994). In summer, both habitats have contrasting moisture conditions: in sites influenced by melt water *U. aprina* is often wetted and metabolically active for several days or weeks. By contrast, *U. aprina* growing

on exposed rock surfaces is dry and inactive for longer periods and its metabolic activation depends entirely on snowfall. Both environmental situations are analysed in the present study: slow freezing of thalli hydrated with liquid water and re-hydration from snow at subzero temperatures.

On the sites influenced by melt water, slow freezing occurs regularly during summer. Field measurements in the Granite Harbour region, southern Victoria Land, continental Antarctica, in January and February 1992 revealed that temperatures of metabolically active *U. aprina* thalli often fall below -5°C in the diurnal course (Schroeter, Green & Seppelt, unpublished). Slow cooling of *U. aprina* below -5.4°C leads to extracellular freezing of symplastic and apoplastic water and this translocation of water leads to a rapid dehydration of the protoplast. Structural changes occurring during extracellular freezing are similar to processes observed during dehydration at ambient temperatures (Brown *et al.*, 1987; Büdel & Lange, 1991; Honegger, 1993; Scheidegger, 1994). The photobiont cells compensate the considerable reduction of the cell volume by cytorrhysis (Oertli, 1989), whereas the cortical and medullary hyphae with their rigid cell walls build up a highly negative turgor until cavitation occurs within the protoplasts (Scheidegger *et al.*, 1995). The cavitation event instantly dehydrates the protoplast and considerably reduces its water potential. It seems probable that under such highly dehydrated conditions intracellular freezing is either completely suppressed or harmless if the temperature of the lichens is further reduced, e.g. to -196°C . Therefore, as in frost-tolerant phanerogams in lichenized ascomycetes, extracellular freezing can be interpreted as a strategy to avoid frost damage.

If no liquid water occurs in the natural habitat, *U. aprina* has to utilize frozen water. The structural and physiological data presented here indicate that air-dry thalli of *U. aprina* are able to re-hydrate from snow at subzero temperatures. Even though we could not exclude the possibility that highly osmotically active substances at the lichen surface might cause thawing of snow crystals and therefore enable the uptake of liquid water from a thin water film at the lichen surface, the data on the temperature-dependent degree of re-hydration from snow in air-dry thalli of *U. aprina* indicate that water uptake from snow occurs in the gaseous phase. The physiological data presented here indicate that the behaviour of snow-covered lichens at subzero temperatures resembles that of lichens in equilibrium with humid air, as has been postulated recently (Kappen, 1993b; Schroeter *et al.*, 1994). Our data from structural analysis of thalli re-hydrated from snow at subzero temperatures are consistent with those of green algal lichen thalli equilibrated with humid air (Scheidegger *et al.*, 1995). In humid air

the cell contents equilibrate with the water potential of the atmosphere (Kaiser, 1982). Our studies show that in air-dry lichens water uptake from snow at subzero temperatures is governed by differences in the water potential of the cell contents and the surrounding atmosphere, which is in equilibrium with snow. The water potential of the system is then determined solely by the temperature: each negative degree contributes -1.2 MPa . Lichens are able to photosynthesize in equilibrium with water potentials below -35 MPa (Nash *et al.*, 1990). In our experiments on re-hydration from snow, water potentials were above -20 MPa . Therefore it does not seem surprising that *U. aprina* is able to re-hydrate from snow at subzero temperatures and that it is able to achieve considerable rates of photosynthesis under these conditions. The rates of photosynthesis at -4°C were the same, regardless of whether the thallus was water-saturated at positive temperatures and then slowly cooled down, or an air-dry thallus was re-hydrated from snow at -4°C as was shown in Figure 18. However, at -8°C , re-hydration from snow resulted only in respiratory gas exchange after 16 h (data not shown).

Ice nucleation activity (INA) close to 0°C is reported from several lichen species (Nash *et al.*, 1987; Kieft, 1988; Ashworth & Kieft, 1992). In lichens INA is related to secondary metabolites produced by the lichen mycobiont (Kieft & Ahmadjian, 1989). It is not known if INA at warm subzero temperatures is of any advantage for the lichen. At temperatures not far below zero INA might prevent damage by sudden formation of large ice crystals at lower temperatures. However, lichens are known to be extremely cold-resistant in the dry as well as in the hydrated state and this is especially true for lichens with *Trebouxia* photobionts (Kappen & Lange, 1972; Nash *et al.*, 1987). Kieft (1988) hypothesized that INA, at temperatures not far below zero as was found here in *U. aprina*, might enhance the uptake of atmospheric moisture by inducing the deposition of water vapour as ice on the lichen thallus. At temperatures around -5°C *U. aprina* is able to achieve significant net photosynthetic rates even though the formation of ice crystals might increase the CO_2 diffusion resistance of the lichen thallus. Therefore it seems reasonable to suggest that *U. aprina* profits from INA at temperatures not far below zero, especially if the snow cover produces much atmospheric water vapour. Moreover, it appears to be important to control freezing events in an environment in which temperatures of hydrated thalli frequently fluctuate around 0°C in the course of the day.

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