Postprint Version

C.A.E. Hamlett, N.J. Shirtcliffe, F.B. Pyatt, M.I. Newton, G. McHale and K. Koch, *Passive water control at the surface of a superhydrophobic lichen*, *Planta* 234 (6) (2011) 1267-1274; DOI 10.1007/s00425-011-1475-z. The following article appeared in the journal Planta and may be found at http://dx.doi.org/10.1007/s00425-011-1475-z.

Passive water control at the surface of a superhydrophobic lichen

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Abstract

Some lichens have a super-hydrophobic upper surface, which repels water drops, keeping the surface dry but probably preventing water uptake. Spore ejection requires water and is most efficient just after rainfall. This study was carried out to investigate how superhydrophobic lichen manage water uptake and repellence at their fruiting bodies, or podetia. Drops of water were placed onto separate podetia of *Cladonia chlorophaea* and observed using optical microscopy and cryo-scanning-electron microscopy (cryo-SEM) techniques to determine the structure of podetia and to visualize their interaction with water droplets. SEM and optical microscopy studies revealed that the surface of the podetia was constructed in a three-level structural hierarchy. By cryo-SEM of water-glycerol droplets placed on the upper part of the podetium, pinning of the droplet to specific, hydrophilic spots (pycnidia/apothecia) was observed. The results suggest a mechanism for water uptake which is highly sophisticated, using surface wettabilities to generate a passive response to different types of precipitation in a manner similar to the Namib desert beetle. This mechanism is likely to be found in other organisms as it offers passive but selective water control.

Key words *Apothecium, Cryo-SEM, Lichens, Soredium, Superhydrophobicity, Water uptake.*

Abbreviations:

SEM - Scanning electron microscope
Cryo - Cryoscopic, typically at liquid nitrogen temperature
Fps - frames per second

Throughout the paper to reduce confusion the term podetium/podetia is used for the wineglass-like structures found on many lichen. The term can also be used for just the leg of the structure but has not been used in this context here.

Introduction

The interaction of water with surfaces has been the subject of scientific investigation for centuries (Young 1805 and; Wenzel 1936; Cassie and Baxter 1944) and the water repellent surfaces created by nature, such as the lotus leaf reported by Barthlott and Neinhuis (Barthlott and Neinhuis 1997), has inspired a significant body of research. Water repellent surfaces have a variety of applications in nature, such as self-cleaning (Barthlott and Neinhuis 1997; Neinhuis and Barthlott 1997), controlled water flow for self-irrigation (Shirtcliffe et al. 2009) and air retention under water (Shirtcliffe et al. 2006a; Barthlott et al. 2010; McHale et al. 2010), although in many cases the advantages are unclear (Holder 2007). In addition to angiosperms, some lichens have been shown to exhibit super-hydrophobic characteristics (Shirtcliffe et al. 2006b; Hauck et al. 2008).

Lichens are generally described as symbionts with both fungal and algal components although a whole continuum of associations may be argued. The uptake of water and nutrients usually occurs over the complete surface of lichens, and no specialised structures for water gathering are observed, however the roles of cyphellae and pseudocyphellae remain poorly understood. Sievers (1908) described the wettability within a group of five *Cladonia* species as different, but emphasised that these group shows very hygroscopic species, absorbing water through pores in their podetia. In water repellent (super-hydrophobic) lichens such as *Lepraria* or *Calicium* the surface water repellency limits the uptake of water. In such species the water uptake occurs mainly over vaporized water when the air humidity is high and over the underside of the lichen in a liquid form (Henssen and Jahns 1974).

Super-hydrophobicity of the lichen surface improves gaseous exchange, because a water film on the lichen can reduce the CO_2 uptake, necessary for photosynthesis, dramatically (Lakatos et al. 2006). In addition any water, that super-hydrophobic lichens absorb, is buffered by the substrate and this is suggested to contribute to why super-hydrophobic lichens are particularly resistant to pollution (Shirtcliffe et al. 2006b; Hauck et al. 2008). Water repellence in biological and technical surfaces is caused by a hydrophobic surface chemistry in combination with one or more levels of surface sculpturing (Neinhuis and Barthlott 1997; Koch et al. 2009). Droplets on such a surface are in the Cassie-Baxter wetting regime, where air is present in the grooves of the rough surface, underneath an applied liquid droplet (Cassie and Baxter 1944). In many cases this state has a low adherence for water, allowing droplets to roll off the surface at a low angle of inclination. Consequently the contact between the water and the solid surface is restricted to the uppermost surface structures (Ensikat et al. 2009) and limited to the short time that the liquid stays on the surface.

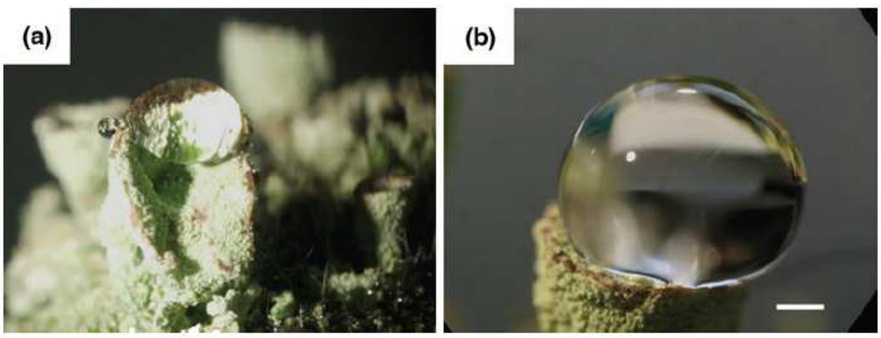

Fig. 1 Photographs of water droplets attached to *Cladonia chlorophaea* podetia. **a** A small droplet attached to the brownish upper margin of a tilted podetium (where it adhered). **b** A water droplet sitting on top of a podetium (expelled from the syringe as adhesion was not enough to cause it to detach from the needle). Scale bar 1 mm in both cases.

Cup-like bodies (Fig. 1a) on an elevated structure, as in *Cladonia spp*., can be called scyphi, although the term podetium is usually used for the entire elevated structure (e.g. Ahti 1982) and will be used here as such although it is also used for the stalk part of the structure only. Reproduction of lichens includes vegetative structures (soredia) which contain both the fungal and photosynthetic partner, and asexual conidia, or vegetative propagules and sexual fungal spores. Reproductive procedures have been reviewed in various papers, and an overview is presented by Pyatt (1974).

Lichen spores are primarily discharged into the air when humidity is high, during or following precipitation, firing the spores into the air as a response to maturity and hydrostatic pressure (Pyatt 1969). As the spores are small a thin layer of air would significantly retard their velocity; it is conceivable that super-hydrophobic cups evolved to reduce this problem. Although small drops of water may reside until they become large enough to pour over the edge (Fig. 1b), large drops are unlikely to become trapped in this way.

Cladonia chlorophaea (Floerke ex Sommerf.) Spreng has both a thallus and clearly defined upright podetia. The soredia are located within the cups or towards their upper edges and tend to be positioned externally. Soredia represent joint propagules and can effectively, following discharge by wind erosion or droplet transport, lead to the establishment of new thalli. The pycnidia were located on the cup margins whilst the sexual apparatus or apothecium, which produce the ascospores, are also generally located at the margins of the podetial cups, depending upon environmental conditions. The structure of podetia of *C. chlorophaea* and their interaction with water were studied to investigate the mechanisms used by this species to overcome the problems of sporulation in wet conditions.

Materials and Methods

Lichen Samples

Specimens of *C. chlorophaea* (Flk. ex Sommerf.) Spreng, were collected during dry periods from the Dunstable area of the UK and showed apothecia and pycnidia of varying maturities. The samples were transported to our laboratory in sterile containers to avoid fungal contamination and then dried at ambient temperature for 3 days. Samples were handled carefully to avoid damage and/or the transfer of mature soredia.

Specimen preparation

Air dried specimens were used without further drying for scanning electron microscopy (SEM). Single podetia were fixed on aluminium specimen holders using conductive carbon tape and sputter coated with gold at 65 mV for 30 s. (Balzer SCD 040, Balzers Union) resulting in a 25-30 nm thick gold layer. SEM investigations were performed at 15 kV with a Stereoscan S200 (Cambridge Instruments GmbH) equipped with a Digital Image Scanning System 5, Point Electronics, Vienna, Austria.

The contact area of droplets of a glycerol-water mixture (1:1) on the podetia of the lichen were examined by freezing the droplet on the sample with liquid nitrogen (Ensikat et al. 2009). As in the previous work this glycerol water mixture was used to reduce crystallisation artefacts. A droplet of glycerol was placed in the “cryo holder”, the podetium was placed on top of the droplet which was then frozen *in situ*, using liquid nitrogen, the cup was then removed. The imprint on the frozen glycerol droplet represents the contact area between the glycerol droplet and the fruiting body. Investigations were performed either directly with the frozen liquid on the specimen surface or after separating the sample from the droplet. Images were taken without sputter-coating of the specimens, with a reduced acceleration voltage of 10 kV.

Wetting studies

The wetting of the podetia was further investigated using i) a digital camera equipped with a macro lens and ii) goniometry (Dataphysics SCA 2.02). Small drops (5 µL) were applied to the lichen surface using a microsyringe and a micromanipulator. The drops were imaged using the SCA goniometer and its back-lighting to highlight the drop edge. The static contact angles were automatically determined using a Laplace-Young fitting algorithm to fit the edge of the liquid drop and calculate its angle with a line drawn at the lichen surface. An average value from six drops was taken. Liquids used were pure water and a glycerol-water 1:1 mixture for comparison with the SEM studies.

Water drop impact was observed using a high speed camera (NAC HotShot 512SC, NAC Ca, USA), a 20 µL drop of deionised water was allowed to fall from a syringe needle onto a single podetium from a height of 400 mm; the drop impact was filmed at 8000 fps.

Repeatability

The experiments were all repeated in triplicate on lichen from different sources and showed good repeatability. Contact angle measurements as noted showed high scatter on both plant material and on redeposited extract. The errors for this were estimated by taking the standard deviation of 6 measurements.

Results

The hierarchical sculpturing of the apothecia surface

Surface structures of podetia in different sizes from 1-3 mm height and the thallus surface were investigated by SEM. Figure 2a shows an overview of a single podetium of *C. chlorophaea*. In Fig. 2b-f enhanced magnifications illustrate that three levels of surface sculpturing in different scale sizes can be found. The largest sculptural element of the surface, the first level of sculpturing, is created by globular structures, the soredia, approximately 50 µm diameter hemispheres projecting from the surface (Fig. 2b-d). The second level of sculpturing is built by the fungal hyphae within a single soredium. The third level is built by small rod-like structures and irregularly formed structures of approximately 1-2 µm length on top of the outermost hyphae. The surface of the uppermost cortex of the thallus was also coated with small structures similar to those observed on the podetia (not shown here).

When water drops were touched onto the surface of the lichen they did not adhere to the podetia except at the rim where the apothecia were present. Drops adhering to the apothecia could be detached from the needle, drops touched to other parts of the lichen surface remained on the needle unless they were large enough to become detached under their own weight (Fig. 1a, b). This implies that the apothecia are behaving as attachment points, having low receding contact angles as compared with the surrounding surface.

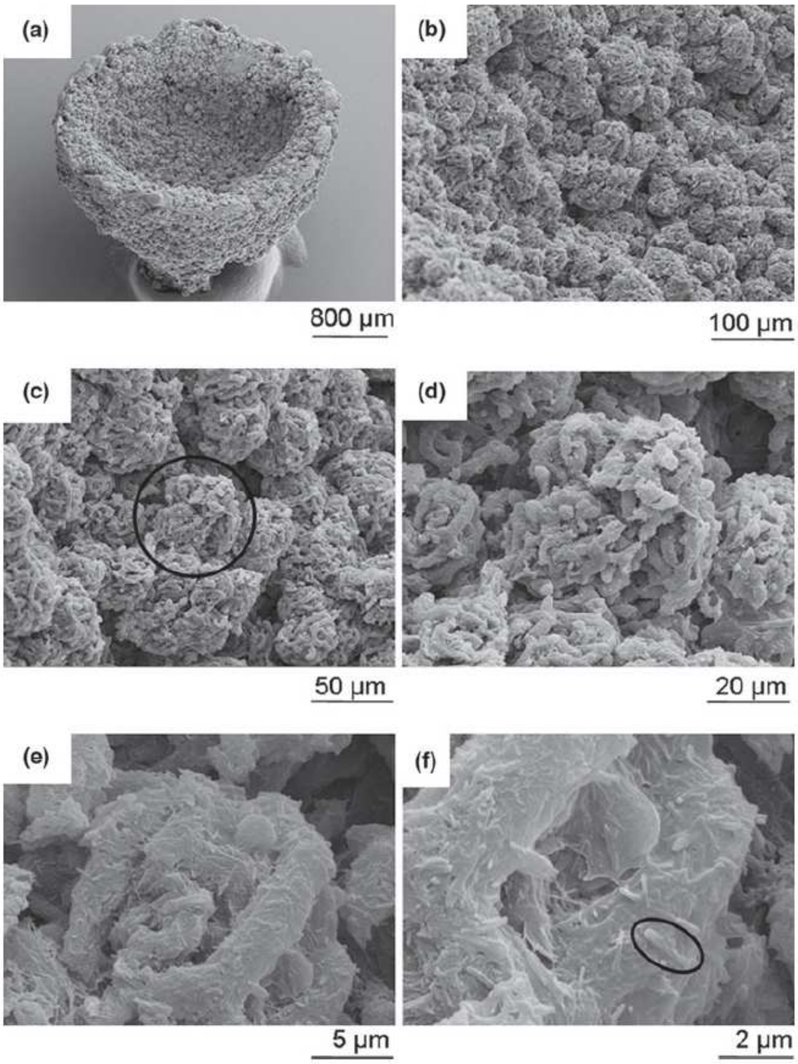

Fig. 2 SEM images of the upper part of a podetium from the lichen *Cladonia chlorophaea*. **a** The whole podetium**. b-d** the soredia inside the scyphus (one circled in c). **e** A single soredium. **f** The rod-like structures on the hyphae (one marked with an oval).

We observed that the surface structures partially dissolved by washing the podetia for 3 min. in chloroform at 70 - 75 °C. After this procedure the podetia were wettable with water. When the chloroform was allowed to evaporate on a glass slide it left a rough deposit that became smoother upon heating. The contact angle of this layer with water was around 90°, indicating that hydrophobic compounds are present (see supplementary images S1). The material was not homogenous and there is no way of ensuring that the crystalline structure is the same as on the natural surface so exact contact angle information could not be measured.

Contact angles

Contact angles for water measured on larger areas of lichen surface were 154 ± 7˚ (dry lichen) and 129 ± 11˚ (re-hydrated lichen), typical of superhydrophobic surfaces. The errors given are the standard deviation of six values; the high error compared with standard contact angle measurements is due to the heterogeneity of the samples and difficulty setting the baseline for angle measurement on the lichen surface.

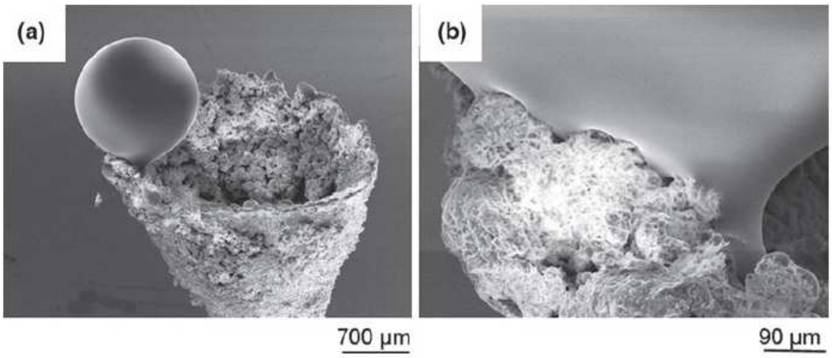

Fig. 3 SEM images of a frozen water-glycerol droplet attached to the upper portion of the inside of the podetium of *Cladonia chlorophaea*. **a** An overview of a small droplet adhering at the edge of the podetium. **b** A higher magnification image of the contact area between the droplet and the podetium.

Cryo- scanning electron microscopy (SEM)

A cryo-SEM methodology was used to freeze drops of glycerol/water to single podetia to visualize the attachment of the drop to the surface (Ensikat et al. 2009). As can be seen in Fig. 3a the droplet attached to the upper portion of the inside of the podetium, an observation that can also be seen in the photograph shown in Fig. 1a. Fig. 3b shows that the pinning of the droplet to the surface occurs at specific points, which suggests that the brown spots (pycnidia and apothecia) at the upper margin of the podetia are hydrophilic.

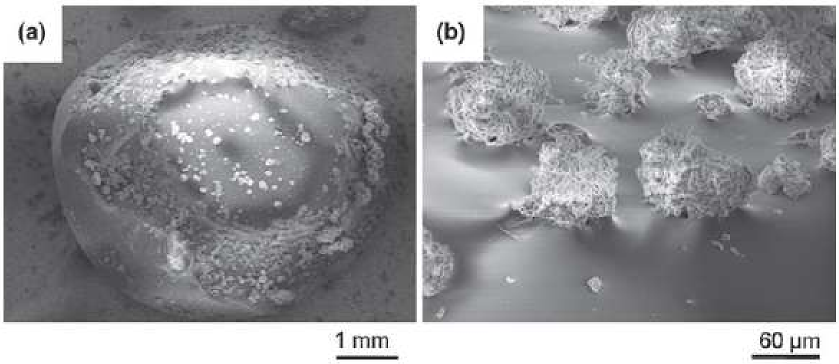

Fig. 4 SEM images showing the contact area of a glycerol-water droplet and a podetium of Cladonia chlorophaea after freezing. **a** The whole of the lower surface of the drop. **b** A higher magnification showing attached soredia.

Contact area

The SEM images in Fig. 4 show frozen glycerol droplets removed from the lichen surface. The pictures illustrate that water (or glycerol) drops on the podetia only contact the peaks of the roughness with much of the water bridging across roughness peaks (Fig. 4b) and most of the contact is made around the rim of the podetium (Fig. 4a). The non-contact area in the middle of the droplet, Fig. 4(a), is particularly contaminated with granules of loose soredia. These granules attached to the liquid and were distributed over the surface before freezing of the droplet. The attached soredia would be carried with the drop should it roll free.

The uptake of water over the podetium

The SEM investigations and contact angle measurement demonstrate that the water droplets pin at the upper rim of the podetium. Figure 1 demonstrates that the droplets adhere to the brown spots at the margin, even when they are tilted. Only small droplets, with a diameter smaller than the diameter of the podetium were found inside.

The uptake of water over the podetium of *C. chlorophaea* was studied using air dried samples. Water-saturated material, stored in water for 10 h, was used as control. A droplet of water was applied to the top of the podetium and the drop observed over time. Because of the different sizes of the podetia, the droplet volumes varied between 10 - 30 µL. The images in Fig. 5 show the water uptake within 3-20 s. after applying the droplet on the dry podetium. After 9 s. the droplet was reduced to a flat horizontal film, straddled over the edges of the podetium. Approximately 0.5 s later the liquid film retracted and formed a drop at one side. Twenty seconds after applying the water the droplet was almost gone. The lip of the cup of this podetium specimen varied between the front and the back side from 2 to 3 mm, and the diameter was between and 2.3 and 2.6 mm.

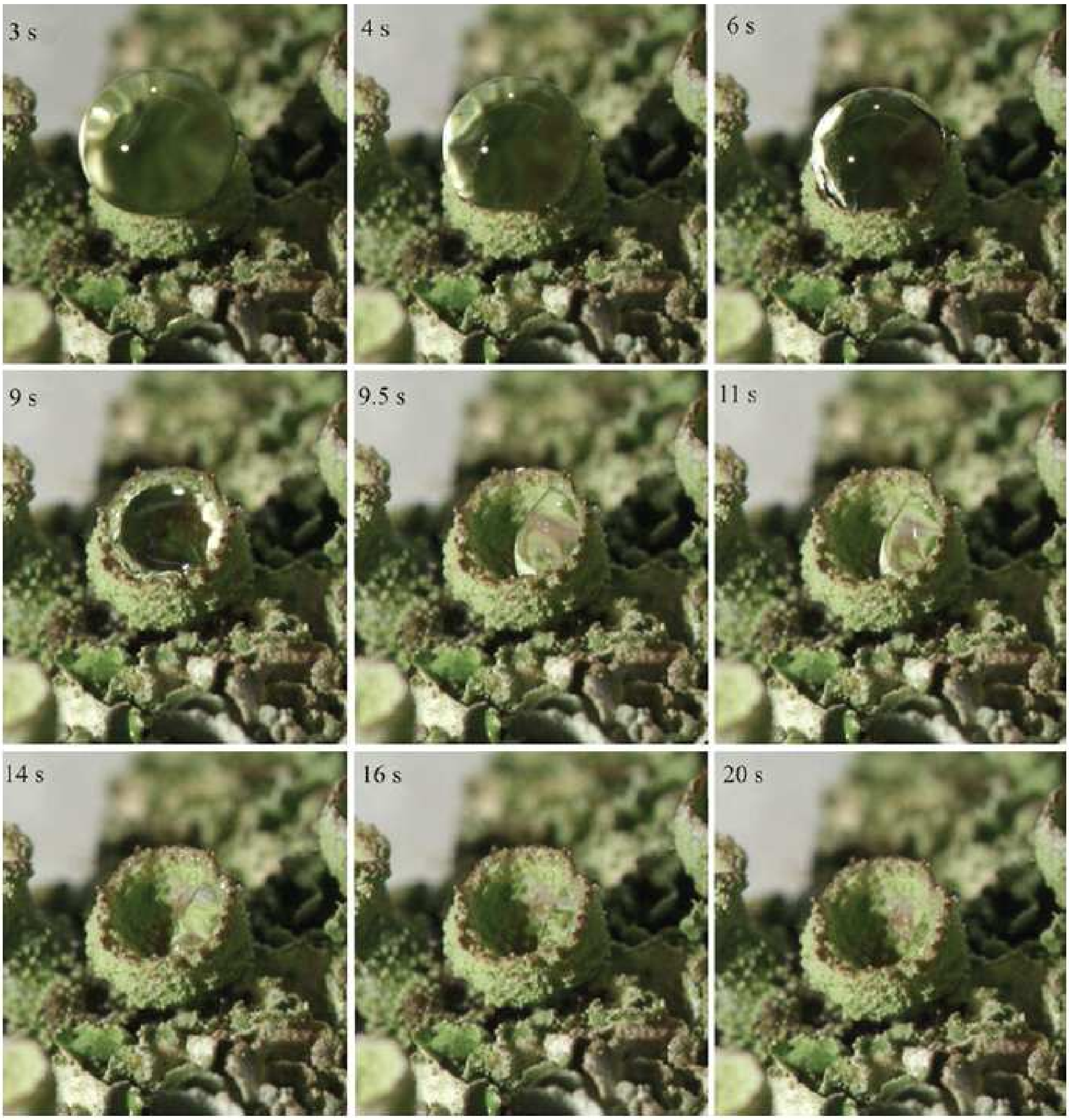

Fig. 5 Macroscopic observations of the uptake of a water droplet (20 µL) at the edge of a podetium of *Cladonia chlorophaea* at different times after application of the droplet.

Because the water was initially pinned at hydrophilic structures (shown in Fig. 6) at the edge of the podetium, the water drop formed a thinner and thinner lens as its volume decreased. After 9 s. the droplet became unstable and snapped back to one of the edges of the podetium, returning to a lower energy spherical cap. During the process the water never contacted the centre of the cups as the curvature of the drop and the super-hydrophobic surface suspended the lower edge and the pinning to the edges prevented the drop from falling in as it lost volume.

Fig. 6 Optical microscope image of the top of a podetium of species of *Cladonia chlorophaea* showing the brown apothetia or pycnidia at the upper margin of the podetium (the hydrophilic spots that absorb water) and the base of the cone being made up of granular soredia one apothecium (pycnidium) circled for clarity.

Up to five droplets were applied on the same podetium, and no difference in the speed of water absorption was found between them. After storage of the specimens in a water filled Petri dish for 10 h the experiment was repeated and no water absorption was observed. Interestingly, after storage of the specimens in a water filled Petri dish for 5 days the complete specimen surface changed from super-hydrophobic to a hydrophilic, wettable state.

To determine whether the water entered the hydrophilic brown bodies at the edge of the podetial cups different podetia were selected with high and low numbers of these structures. When water drops were placed onto these they were absorbed more rapidly on those with a greater number of the brown structures and slower on those with less. The slowest water loss rates were observed on damaged podetia, where drops could be located away from the edge. Selected videos illustrating this are available as supplementary information (S2-4). This agrees with the results of Larson (1981), who showed that *C. chlorophaea* absorbs water relatively rapidly (for its surface area).

High speed films of drops hitting podetia revealed that 20 µL drops split on impact and bounced away (Fig. 7 and supplementary video S5). This behaviour is different from when the water was supplied as a mist, when much of the water was captured.

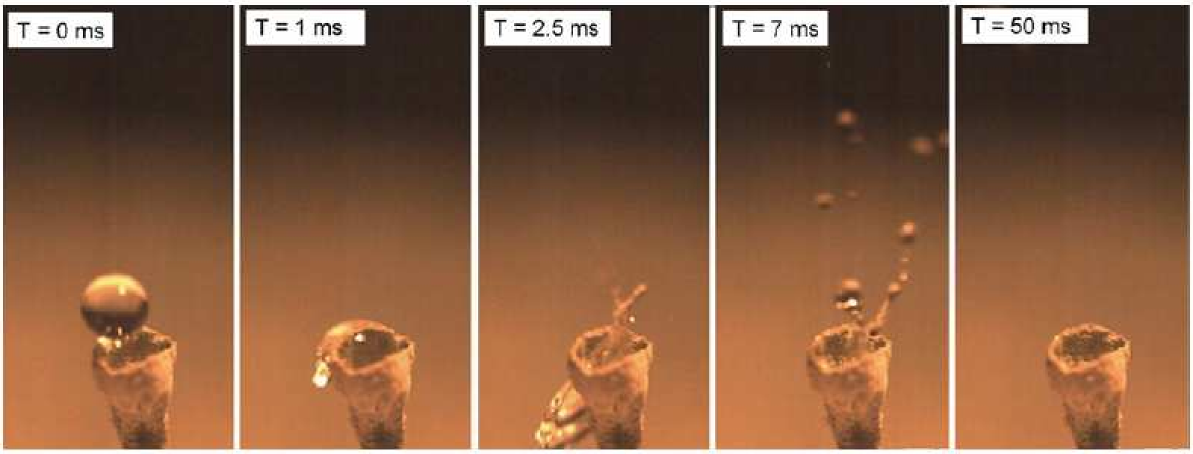

Fig. 7 Stills from a high speed video of a water drop hitting a podetium and splashing off, leaving it apparently dry.

Discussion

Surface Chemistry/Morphology

The surface of superhydrophobic plants and animals typically consists of self-organised structures on a small scale with larger structures formed by the organism more directly. The surface of the soredia is likely to be formed in the same manner. Fumarprotocetraric acid has been found in several *Cladonia* species in large amounts (Culberson 1986; Culberson et al. 1988) and is the main hydrophobic compound in this species. However lichens also produce other hydrophobic acids, proteins as hydrophobins (Wösten 2001) and phenolic compounds. Acids and phenolic compounds would be expected to dissolve in hot chloroform, hydrophobic proteins may not. Although hydrophobins are known to form rodlet structures (Wösten et al. 1993) these are considerably smaller than those observed here are.

The location of hydrophilic areas at the rim of the podetial cups makes them ideally placed to collect water from mist and drizzle. The structures will collect any drops passing and, as their surroundings are superhydrophobic, they will be able to absorb all of the water that they collect. Under heavier rain drops bounce out of the podetial cups, leaving them dry, but even if they are wetted the absorbtion of water through the apothecia at the rim of the cups rapidly causes them to soon become dry.

We propose that the distribution of water repellent soredia and water absorbent apothecia has the potential to enable the latter to subsequently increase the turgor of the asci for ascospore discharge in response to a rainfall event. Pycnidia also require water for spore discharge through a similar mechanism and could therefore also benefit.

The increased mass and impact velocity allows large drops to detach from the hydrophilic areas of the podetia. As free soredia were observed on drops gently placed and removed from a podetium Fig. 4 it is conceivable that bouncing drops carry soredia with them, dispersing them. This type of size selection is potentially of advantage to the organism because water is predominantly captured when it is scarce and deflected when larger quantities are available.

As was measured, the lichen can absorb a large quantity of water, and the final drop jumps up onto the hydrophilic podetium edge so after a rainfall event the lower surface of the podetium will be uncovered, allowing spore ejection directly into the atmosphere. The super-hydrophobicity created mainly by the soredia allows droplet based dispersal without reducing the water uptake into the apothecia, increasing the chances of success.

The podetium will increase in size as it matures, increasing its chance of catching water droplets and enhancing opportunities for the atmospheric erosion and mobilisation of propagules. The chances of catching a droplet is related to the hydrophobicity and the size of the cone compared with the capillary length of water. Immature podetia show total runoff because they are small enough that any drop hanging over the edge self-siphons all of the water away, despite the fact that the centre is lower than the edges. Additionally the density of pycnidia and apothecia can control water uptake. Furthermore, immature apothecia will hydrate with the absorbed water and become more metabolically active.

The use of hydrophilic and superhydrophobic areas is widespread in natural systems. The desert beetle (Parker and Lawrence 2001) uses hydrophilic spots to capture water droplets that then roll over a superhydrophobic base to its mouth when they are too large to remain on the hydrophilic areas, enabling it to extract water from mist. Some plants use hydrophilic areas to arrest drops of water on superhydrophobic leaves and direct them towards their roots (Shirtcliffe et al. 2009). The lichen appears to be using a similar mechanism and structure to the beetle to collect water from mist and fine rain, but to reject larger droplets of water in heavy rain.

In conclusion the structure of the podetia of the water repellent lichen, *C. chlorophaea*, and their interaction with water were investigated. Microscopy studies showed a three level structural hierarchy of the lichen body and a combination of super-hydrophobic thallus and hydrophilic spots (apothecia/pycnidia) on top of the fruiting bodies (podetium) exists. Further studies will show whether this is a unique or frequent mechanism of water uptake amongst super-hydrophobic lichens and how droplet adhesion affects the efficiency of spore dispersal.

Electronic supplementary material The online version of this article (doi:10.1007/s00425-011-1475-z) contains supplementary material, which is available to authorized users.

Acknowledgements The authors would like to thank H.J. Ensikat for technical assistance in SEM and performing of the cryo-preparation of the specimen, W. Barthlott for providing parts of the technical equipment for the studies performed at Bonn University (both Nees-Institut für Biodiversität der Pflanzen, Bonn University, D) and the EPSRC for funding NJS and CAEH under grants EP/E063489/1 and EP/H00704/1.

Conflict of Interest The authors declare that there are no conflicts of interest

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