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DOI: https://doi.org/10.1017/s002428299200015x

Lichenologist **30**(3): 193–212 (1998)
Article No. li980140

THE LICHEN SYMBIOSIS—WHAT IS SO SPECTACULAR ABOUT IT?*

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Abstract: Lichen mycobionts are typical representatives of their fungal classes but differ from non-lichenized taxa by their manifold adaptations to symbiosis with a population of minute photobiont cells. Most interesting are the morphologically complex macrolichens, the fungal partner of which competes for space above ground and contains photobiont cells optimally positioned for gas exchange and illumination. Such thalli are the product of an amazing hyphal polymorphism, with multiple switches between polar and apolar growth and hydrophilic or hydrophobic cell wall surfaces. Hydrophobic sealing of the apoplastic continuum between the partners by means of mycobiont-derived hydrophobic compounds canalizes the fluxes of solutes during the often quite dramatic de- and rehydration processes and keeps the algal layer gas-filled at any level of hydration. The impressive tolerance of drought, heat and cold stress of most lichen-forming fungi and their photobionts is due to a very interesting combination of protective and repair mechanisms at the cellular level, the molecular bases of which remain to be explored. Contemporary experimental lichenology is analysed and strategies are proposed aimed at better integration into mainstream biology.

Peculiarities of the lichen symbiosis

' Are lichens still to be considered as a cosy mixture of reasonably compatible, self supporting species of algae and fungi, or is there much more to this plant than meets the eye? ' Fox (1996)

Fungi, as heterotrophic microorganisms, depend on an external supply of fixed carbon. This they mobilize either as saprotrophs or as biotrophs in a parasitic or mutualistic symbiosis with C-auto- or C-heterotrophic hosts (Table 1). Lichens are the symbiotic phenotype of nutritionally specialized fungi that acquire, in an ecologically obligate, mutualistic symbiosis, fixed carbon from a population of minute green algal or cyanobacterial cells; these are referred to as the photobiont. Lichen-forming fungi are not a natural group, as suggested by the formerly used, now obsolete term ' Lichenes ', but a polyphyletic, taxonomically heterogenous assembly of nutritional specialists like mycorrhizal or plant pathogenic fungi (Smith 1978; Hawksworth 1988*a*; Honegger 1991, 1996*a*; Gargas *et al.* 1995). Lichenization is a common and very successful nutritional strategy, more than 20% of fungal species being lichenized (Hawksworth *et al.* 1995; Table 1). Lichen-forming fungi are normal representatives of their classes but differ from non-lichenized taxa by their manifold adaptations to symbiosis with a population of minute photobiont cells.

*Based on The 1998 Swinscow Memorial Lecture.

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0024–2829/98/030193+20 $25.00/0

TABLE 1. *Acquisition of fixed carbon by fungi and fungus-like microorganisms**

Degradation of dead organic matter	
Saprobes	*c.* 45–50%
Symbioses with C-autotrophs or C-heterotrophs	
Parasitic Symbioses (biotrophic or necrotrophic) with	
Cyanobacteria	
Algae	
Plants	
Fungi (lichenized and non-lichenized)	*c.* 20%
Animals	
Humans	
Mutualistic Symbioses	
Mycorrhizae (~8%)	*c.* 30%
Lichens (~21%)	
Mycetocytes, etc.	

*Heterotrophic *Straminipila* (oomycetes and chytridiomycetes) *sensu* Dick; Sources: Lewis (1973), Hawksworth *et al.* (1995)

The species name of lichens refers to the fungal partner, not to the symbiotic system (Greuter 1988); lichen photobionts have their own names and phylogenies. I have to admit that it took me a long time to internalize the fact that, when checking my axenic cultures of lichen-forming fungi, I am not looking at the mycobiont of *Xanthoria parietina*, *Cladonia caespiticia*, etc., but at the aposymbiotic phenotype of *X. parietina* or *C. caespiticia* proper. In nature these fungi are, as ecologically obligate biotrophs, found almost exclusively in the symbiotic state. The majority of lichen-forming fungi are physiologically facultative biotrophs: they do not depend physiologically on the symbiotic way of life and thus can be cultured under sterile conditions apart from their photoautotrophic partner.

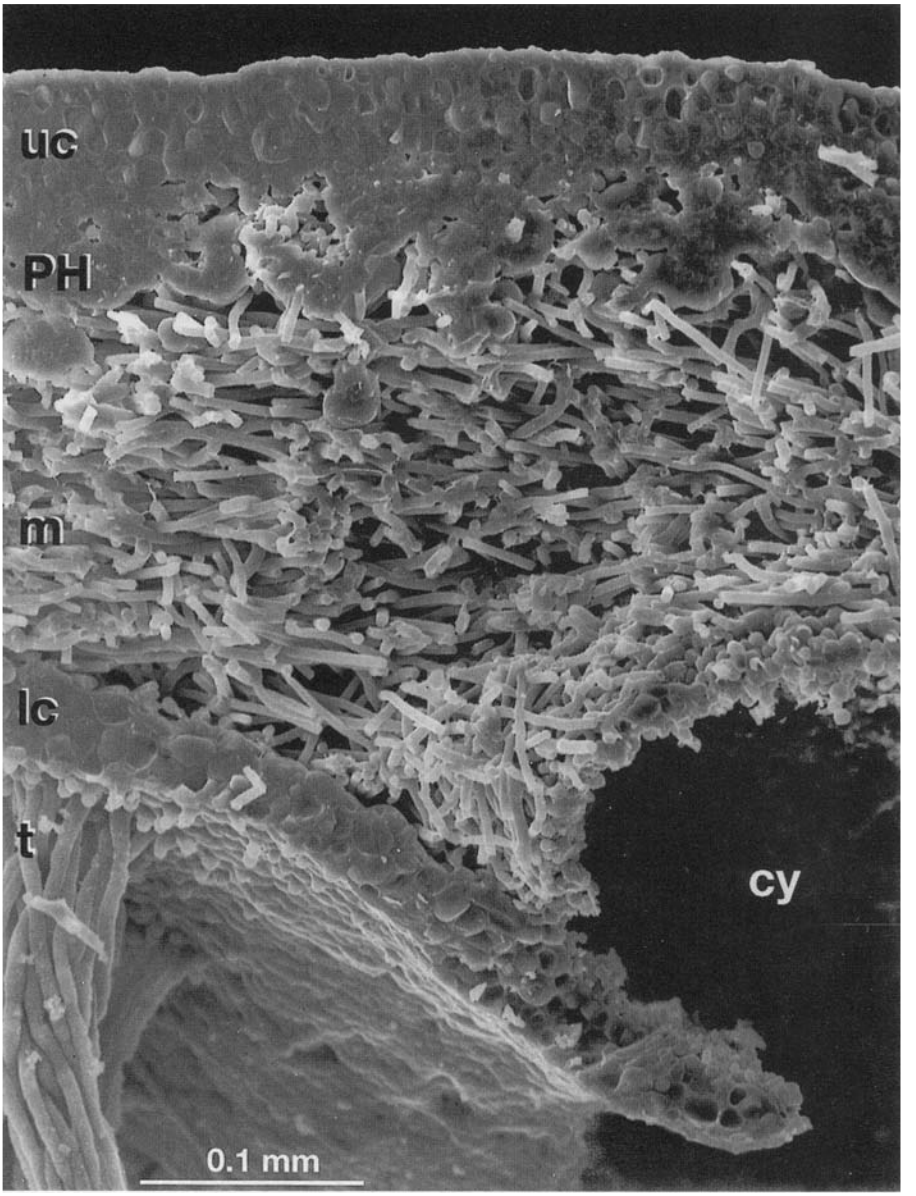
All lichen photobionts so far investigated can be axenically cultured. Some lichen photobionts are very abundant outside lichen thalli; this is the case for representatives of the Trentepohliales (filamentous green algae) many of which are considered as pests in tropical agriculture, especially when overgrowing the relatively long-lived leaves of economically important crops such as coffee, cocoa, rubber, pepper, etc. (Hawksworth 1988*b*). Others are rarely found outside lichen thalli; this is especially true of the approximately 20 species of unicellular green algal genus *Trebouxia*, which are by far the most common photobionts of temperate to arctic/alpine or antarctic lichens (Tschermak-Woess 1988). In lichen-dominated ecosystems of circumpolar areas, or in numerous deserts, *Trebouxia* species are likely to be the most common terrestrial algae. Despite contradictory reports (Ahmadjian 1988), *Trebouxia* species do occur outside lichen thalli, but they are never a common or even dominant element of aerophilic algal communities (Bubrick *et al.* 1984; Tschermak-Woess 1988; Mukhtar *et al.* 1994), the reasons for this situation being unknown. Aposymbiotic *Trebouxia* species seem to be poor competitors, like the *Chlorella* endosymbionts of fresh water protists and invertebrates. However, the latter are known to release considerable amounts of photosynthates to the environment (review: Smith & Douglas 1987), but

this does not apply for aposymbiotic *Trebouxia* species (review: Honegger 1996*b*). In nature large numbers of lichen-forming fungi are producing symbiotic propagules such as soredia, isidia, blastidia; others are capable of regenerating new thalli from fragments (see examples in Honegger 1995, 1996*c*; see Figs 2G–I). *Trebouxia* species and other lichen photobionts are thus carried along by their fungal partner and colonize ecosystems where they are hardly ever found in the free-living (aposymbiotic) state. Most biologists do not hesitate to interpret lichens as a mutualistic symbiosis in which the ecological fitness of both partners is increased in the symbiotic state, irrespective of the consumption of fixed carbon by the heterotroph (review: Smith & Douglas 1987).

Lichen-forming fungi are highly discriminative in their selection of algal or cyanobacterial species. The range of compatible algal or cyanobacterial partners per lichen-forming fungus cannot be experimentally tested since the symbiotic phenotype cannot be routinely resynthesized under laboratory conditions by combining sterile fungal and algal cultures. However, from isolation experiments it is known that most lichen-forming fungi are either moderately specific (accept several related algal species) or specific (accept only one species as compatible photobiont: Friedl 1989; Ihda *et al.* 1993; review: Honegger 1996*b*). Some lichen-forming fungi, especially those that associate with *Trebouxia* species, are highly selective with regard to photobiont acquisition. They are symbiotic with one or several photobiont species that are rarely found outside lichen thalli in natural ecosystems and, on the other hand, do not accept the most common aerophilic algal taxa (Bubrick *et al.* 1985).

The lichen thallus, especially the foliose or fruticose thalli of the so-called macrolichens, impressively illustrates the innovative force of the symbiotic way of life. In liquid or solid media aposymbiotically cultured lichen-forming fungi invariably form cartilaginous colonies; these resemble the colonies of slow-growing non-lichenized fungal taxa, but bear no similarity with the symbiotic phenotype. It has to be kept in mind that only an estimated 25% of all lichen-forming fungi are differentiating morphologically and anatomically complex, leaf- or shrub-like symbiotic phentotypes. The majority of lichen-forming fungi overgrow or ensheath algal cells on or within the substratum and thus form often quite inconspicuous crustose, microfilamentous or microglobose thalli; these are often referred to as microlichens (reviews: Honegger 1991, 1993). Macrolichens are the morphologically and anatomically most complex vegetative structures in the fungal kingdom. Their main building blocks are:

- conglutinate pseudoparenchyma, usually in the form of peripheral cortical layers and/or central strands, with often quite massive layers of hydrophilic mucilages at the wall surfaces. These conglutinate zones are involved in passive uptake of water and dissolved mineral nutrients. They provide mechanical stability to the thallus and are elastic and translucent when wet, but brittle and opaque when dry;
- loosely interwoven plectenchyma, usually as a system of aerial hyphae with hydrophobic cell wall surfaces in the thalline interior. These create a permanently gas-filled zone, a pre-requisite for gas exchange of the



photobiont cell population. Some of these aerial hyphae are in close contact with the photobiont cells.

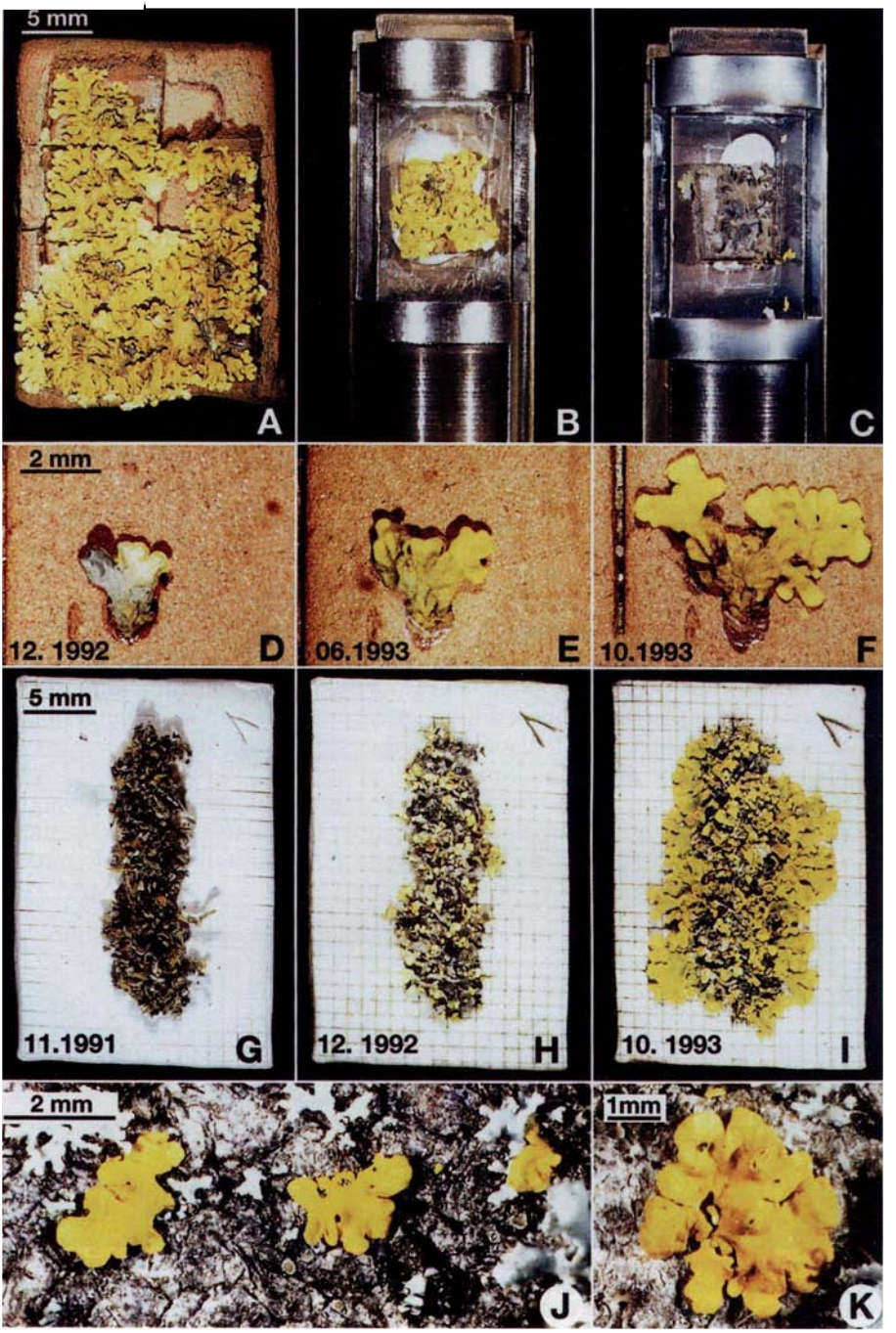
Photobiont cells are interlinked with the fungal partner via its haustorial or appressorial structures and via the mycobiont-derived hydrophobic cell wall surface layer, which spreads from the fungal surface over the algal wall surface, thus sealing the apoplastic continuum between the partners with a proteinaceous, water-repellent coat (see Figs 4A–C). This wall surface hydrophobicity may be enhanced by the deposition of mycobiont-derived phenolic secondary metabolites, which crystallize on and within the proteinaceous wall surface layer (Honegger 1986). In contrast to extracellularly secreted secondary metabolites of non-lichenized taxa the secondary products of lichen-forming fungi are almost insoluble in aqueous systems at low or neutral pH (review: Fahselt 1994). Photobiont cells receive water and dissolved mineral nutrients from the thalline surface through the fungal cell wall. Photobiont cells are actively positioned within the algal layer via growth processes of the fungal partner (reviews: Honegger 1991, 1996*b*, 1997).

The thallus of macrolichens can thus be regarded as the product of multiple switches from polar/filamentous to apolar/globose fungal growth and *vice versa* (Fig. 1). The genetic basis and external factors that trigger such transitions in fungal growth forms are currently under intensive investigation in so-called dimorphic fungi among non-lichenized taxa; these switch back and forth between the yeast form with apolar/globose growth and the hyphal form with polar/filamentous growth (Gow *et al.* 1995; Orlowski 1995). The differentiation of both growth types, polar and apolar, in close vicinity, but within functionally completely different thalline areas, as observed in lichen thalli, is a developmental process of distinctly higher complexity than the yeast to hypha transitions of dimorphic fungi.

The foliose or fruticose, internally stratified thallus of macrolichens can be regarded as a very sophisticated culture chamber for photobiont cells. In these structurally advanced lichens the mycobiont is the quantitatively predominant symbiotic partner that:

- enters the third dimension by differentiating a species-specific, morphologically complex symbiotic phenotype;
- competes for space above ground;

FIG. 1. Low Temperature Scanning Electron Microscopy (LTSEM) of a fully hydrated, cross-fractured thallus of *Sticta sylvatica* (cyanobacterial photobiont: *Nostoc* sp.) in the frozen-hydrated state. This internally stratified (heteromerous) thallus is the product of multiple switches between globose/apolar and filamentous/polar fungal growth in combination with hydrophilic or hydrophobic cell wall surfaces. Conglutinate pseudoparenchyma of the upper (uc) and lower cortex (lc) are built up by globose (apolar) fungal cells that secrete very hydrophilic extracellular material. Globose (apolar) fungal cells with hydrophobic wall surfaces line the cyphella (cy; aeration pore); numerous pores between these roundish cells facilitate gas exchange. Filamentous hyphae with hydrophobic wall surfaces create the gas-filled medullary layer (m) and contact the cyanobacterial colonies (PH); these are actively positioned by the fungal partner in the best place of the thalline interior as regards to illumination and gas exchange. A tomentum (t) is formed by filamentous outgrowths of the lower cortex; these hyphae with easily wettable wall surfaces are involved in passive water uptake and translocation towards the lower cortex.



- carries photobiont cells with it by means of growth processes and controls their cell turnover rates;
- secures adequate illumination and gas exchange of the photobiont cell population by keeping them in an optimal position within the thallus (Fig. 1). When accidentally turned upside down, the fungal partner of dorsiventrally organized lichens has been shown to correct this spatially unfavourable position by means of growth processes (Honegger 1995).
- mimics plants by forming leaf- or shrub-like structures and by keeping the photobiont cell population in a similar position to palisade parenchyma in higher plant leaves—a most fascinating situation (reviews: Honegger 1993, 1996*b*). Unicellular algal symbioses are widespread among certain groups of invertebrates such as cnidaria, flatworms and molluscs (reviews in Reisser 1992), but none of these heterotrophic exhabitants expresses a symbiotic phenotype of comparable morphological and anatomical complexity as do lichen-forming fungi in their symbiotic phenotype in order to keep the autotrophic partner photosynthetically active.

Operationally lichen thalli are an entity, but genetically they may reveal an astonishing level of complexity. A thallus may be composed of several fungal and algal or cyanobacterial genotypes, as concluded from comparative studies on isozyme patterns and on the nuclear DNA, which codes for the small subunit of ribosomal RNA (SSU rDNA) (review: Fahselt 1996). Neighbouring thalli may fuse at any age and time from the primordial stage onwards (Figs 2J–K). However, sometimes thalli of the same fungal species do not fuse when bordering upon each other. The genetic basis underlying these phenomena remains unknown.

Thalline water relations

As poikilohydric microorganisms, lichen-forming fungi and their photobionts have no means of controlling their water relations. Their symbiotic phenotype, the lichen thallus, is therefore subjected to often quite dramatic fluctuations in water content between saturation and desiccation (to <20% water content on a dry weight basis). Wetting and drying cycles may occur

FIG. 2. A–C. Preparative steps for LTSEM of *Xanthoria parietina*. A. Artificial culture on a ceramic supporting structure with detachable slabs (for methods see Honegger 1995). B. An overgrown, detached ceramic slab, mounted on the specimen holder of the LTSEM with commercially available white glue. C. The same specimen after slush freezing in subcooled LN_2, sputter-coating with an alloy of gold and palladium, followed by examination with the electron beam at 20 kV under high vacuum conditions on the cold stage of the LTSEM. D–F. Growth and development of a lobe of *X. parietina* that was glued to a new ceramic platelet after LTSEM investigation. G–I. Regenerative capacity of a mixture of finely chopped thallus fragments that had been collected at different locations and mounted on a ceramic supporting structure. J. Juvenile lobes of *X. parietina*; with high probability they would have fused, at a later developmental stage, to form a thallus rosette. K. Juvenile thallus of *X. parietina*, built up by several lobes the primordial stages of which were developing in close proximity. Same magnification in A–C, D–E, G–I. Sources: A–F from Honegger (1995), with permission of *Canadian Journal of Botany*; G–I from Honegger(1996*c*), with permission of *The New Phytologist*.

within very short periods of time. They are probably the main driving force for passive solute translocation from the thalline surface to the algal layer and *vice versa*, and especially between the partners of the symbiosis. The majority of lichen-forming fungi and their photobionts, particularly those from climatically extreme environments, survive severe drought stress unharmed in a state of dormancy but re-establish their metabolic activities very rapidly upon rehydration (review: Kappen 1988). Some drought-tolerant lichen species dominate, often in association with bryophytes, arctic/alpine, Antarctic or desert ecosystems where higher plants are at their physiological limits (review: Kappen 1988). However, there are drought-sensitive, extremely shade-tolerant lichen species in subtropical and tropical rain forests (Green *et al.* 1991).

Today, tools are available for locating free water in the thalline interior and for investigating the fate of fungal and algal cells at different levels of hydration, extreme drought stress included, at the electron microscopical level (review: Honegger & Peter 1994). Lichen thalli show no wilting symptoms under drought stress, although colour changes may occur as a consequence of increasing opacity of the peripheral cortex. However, thallus thickness may be significantly reduced due to severe shrinkage of the fungal and algal cells (examples in Honegger 1996*d*). As shown in ultrathin sections of cryofixed freeze-substituted specimens, the cellular membrane systems and organelles are dramatically deformed and the protoplast strongly condensed in desiccated algal, cyanobacterial and fungal cells (Honegger & Peter 1994; Honegger *et al.* 1996). Reversible cytoplasmic cavitation, in the form of a rapidly expanding cytoplasmic gas bubble of yet unknown origin and contents, was observed in almost every cell type of the fungal partner in response to the highly negative pressure in the course of desiccation (Figs 3A–C; Honegger 1995; Scheidegger *et al.* 1995), a cytologically most remarkable phenomenon that was first observed in drought-stressed, darkly pigmented ascospores of non-lichenized fungi (deBary 1866; Ingold 1956; Milburn 1970). Upon rehydration the cytoplasmic gas bubble disappears but it remains to be seen whether some traces are left in the cytoplasm. It has been speculated that concentric bodies, enigmatic cell organelles of unknown origin and function in lichen-forming and in a wide range of drought-tolerant non-lichenized ascomycetes, might be remnants of such cytoplasmic gas bubbles since they are round, proteinaceous shells around a gas-filled centre (Honegger 1995). They are not membrane-bound, occur usually in clusters within the cytoplasm and are approximately 300 nm in diameter.

In their desiccated state drought-tolerant lichen-forming fungi and their photobionts survive temperature extremes. Normal growth and development was recorded in the foliose *X. parietina* after slush-freezing in subcooled liquid nitrogen (LN_2; temperatures around $-200^{\circ}C$), cryofracturing, sputter-coating with an alloy of gold and palladium and examination under high vacuum conditions on the cold stage of a low temperature scanning electron microscope (LTSEM) at an acceleration voltage of 20 kV (Honegger 1995; Figs 2A–F). However, fully hydrated specimens did not survive this treatment.

Due to their impressive drought tolerance, lichens are sometimes assumed to be almost immortal, a beautiful theme for science fiction (Wyndham 1960)!

The effect of different modes of storage on the retention of viability of the symbiotic photobiont, i.e. in symbiotic propagules or thallus fragments, requires careful quantitative analysis. The isolation of viable *Trebouxia* cells from 47-year-old herbarium specimens, as reported by Keller *et al* (1995), is likely to be exceptional. No survivors were recovered among hundreds of *Trebouxia* cells isolated from specimens of *X. parietina* collected from the Brittany coast and stored for 6 years at room temperature. The fungal partner of these specimens failed to eject ascospores. However, in samples from the same location stored for 6 years at -20°C ascospores were ejected normally and their germination rate was only slightly reduced as compared with the freshly collected control (Honegger, unpublished results). The viability of desiccated thallus fragments is retained in LN_2, probably the best mode of long-term storage of lichen fragments (Honegger, unpublished results). Axenically cultured lichen-forming fungi and green algal lichen photobionts can be successfully stored for decades in cryoprotective media under LN_2 without loss of viability (Honegger 1996*a*).

Desiccation tolerance is not a peculiarity of lichen-forming fungi and their photobionts (Table 2). Drought-tolerant microorganisms and bryophytes, which all tolerate rapid desiccation and rehydration, are likely to possess constitutively induced protective mechanisms and the capacity for rapid repair of membrane damage during the rehydration process (Dhindsa 1987; Potts 1994; Scott & Oliver 1994). As in all other drought-tolerant eukaryotes the plasma membrane of lichen-forming fungi and their photobionts becomes leaky during desiccation. Approximately 10% of soluble cellular compounds have been recovered from washing fluids of dried lichens, but within minutes after rehydration the membrane integrity is re-established (MacFarlane & Kershaw 1985; Dudley & Lechowicz 1987). Drought-sensitive organisms lack the capacity for membrane repair after drought stress events (Bewley & Krochko 1982; Leopold 1986). In drought-tolerant developmental stages (pollen, spores) of pteridophytes and angiosperms, or in the drought-tolerant shoots of the so-called resurrection plants among pteridophytes and angiosperms, the protective mechanisms are established via gene activation during the comparatively slow desiccation process (Gaff 1989; Close *et al.* 1993; Schneider *et al.* 1993). The genetic and biochemical bases of desiccation tolerance are currently intensely studied in selected species of prokaryotes and vascular plants. It will be a very interesting task to explore the molecular basis of the impressive drought tolerance of lichen-forming fungi and their photobionts.

Arctic/alpine or antarctic lichens often experience very low temperatures in the fully hydrated state. How can they avoid the devastating effects of intracellular ice crystal formation? The ice nucleation potential of a range of lichen species has been measured (Kieft 1988; Kieft & Ruscetti 1990, 1992; Ashworth & Kieft 1992; Worland *et al.* 1996). In experiments with axenically cultured lichen-forming fungi and their green algal or cyanobacterial photobionts Kieft & Ahmadjian (1989) demonstrated that biological ice nuclei are formed by the fungal partner, not by the photobiont. By means of LTSEM techniques Schroeter & Scheidegger (1995) impressively visualized the extracellular freezing of water at $-5{\cdot}4$°C in Antarctic specimens of *Umbilicaria*

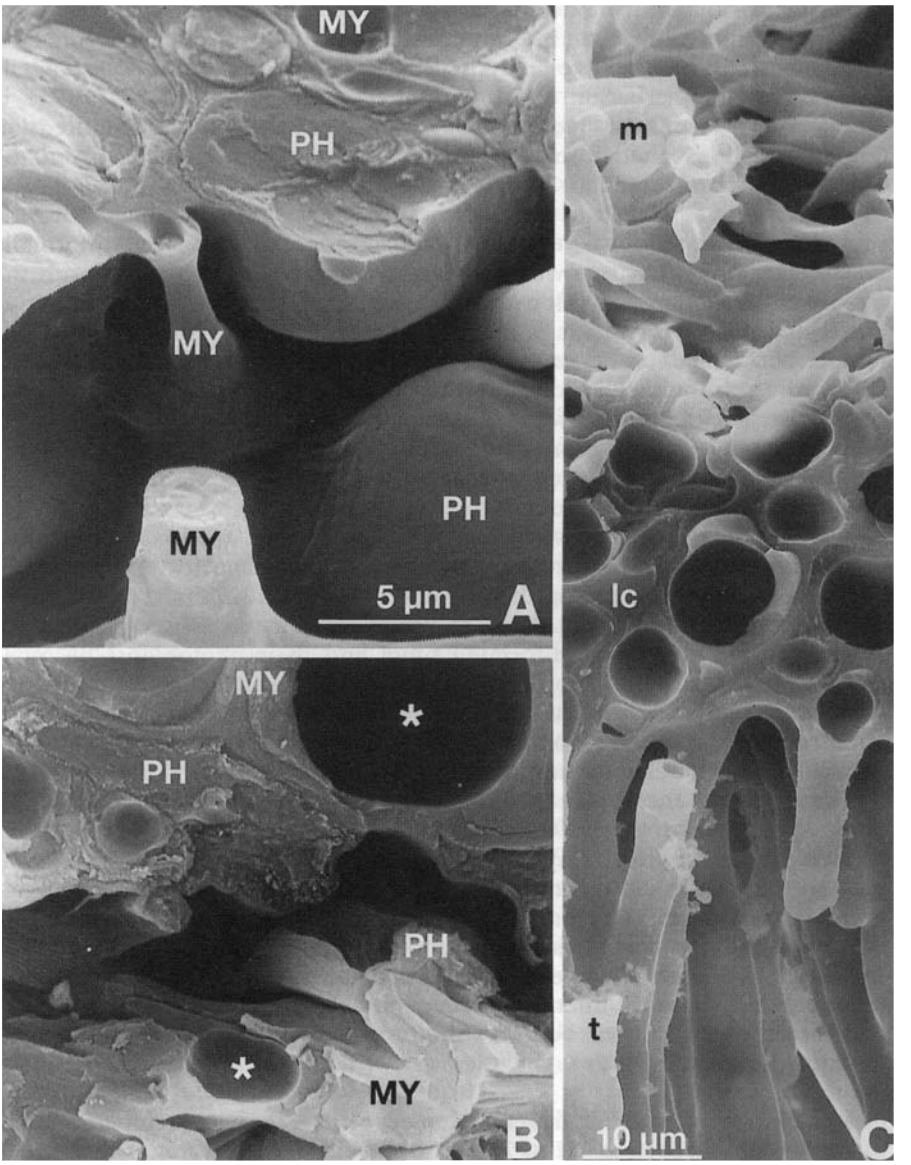


FIG. 3. Drought-stress-induced structural alterations in *Sticta sylvatica* as seen in LTSEM preparations of fully turgid (A) or desiccated specimens (B–C). Free water is confined to the fungal and cyanobacterial cells and their apoplast, but no water film is found on either the hyphal surfaces or the gelatinous sheaths of the photobiont. Both are coated with a thin, mycobiont-derived protein layer that seals the cell wall continuum between the partners with a hydrophobic coat (see Figs. 4A–C). B–C. Drought-stressed *Sticta* and *Nostoc* cells shrink dramatically; cytoplasmatic cavitation, seen as an intracellular gas bubble, occurs in all cell types of the mycobiont in response to strong negative pressures. PH: photobiont (*Nostoc* sp.); MY: mycobiont; asterisks point to cytoplasmatic gas bubbles in drought-stressed specimens. Same magnification in (B) and (C).

TABLE 2. *Drought tolerance (reviviscence after reaching air dryness) among microorganisms and plants*

Microorganisms and bryophytes: *poikilohydric* organisms (with no means of controlling their water relations)*
—numerous prokaryotes
—numerous eukaryotic microorganisms: numerous algae numerous non-lichenized fungi the majority of lichen-forming fungi and their green algal or cyanobacterial photobionts
—numerous bryophytes
Common features: Often very rapid drying and rehydration; dramatic fluctuations in cellular water contents occur within very short periods of time (seconds to minutes)
Rapid re-establishment of metabolic activities upon rehydration
Vascular plants: *Homoiohydric* organisms with controlled water relations)
—drought-tolerant developmental stages (drought tolerance lost after germination; exceptions see below)
pollen and seeds of numerous gymno- and angiosperms spores of numerous pteridophytes
—drought-tolerant foliage and/or shoots of so-called resurrection plants:
approx, 0·6% of pteridophytes approx., 0·05% of angiosperms *homoiochlorophyllous* spp. retain chloroplast structure during drought stress events *poikilochlorophyllous* spp. degrade thylakoids and chlorophyll during desiccation‡
Common features: Slow drying is essential (within one to several days). Foliage: relatively slow rehydration; full metabolic activity re-established within one (homoiochlorophyllous spp.) to several days (poikilochlorophyllous spp.)

*Reviews: Bewley & Krochko (1982); Leopold (1986); Kappen (1988); Gaff (1989); Potts (1994).
‡Examples in Tuba *et al.* (1993, 1994).

aprina that had been slowly cooled at a rate of approx. 1°C min^{-1}. This extracellular freezing due to potent ice nucleation sites on the hyphal surfaces led to desiccation and cell shrinkage in both bionts of the fully hydrated lichen and to cytoplasmic cavitation in the fungal partner.

Contemporary lichenology

'... the study of lichens is now in a phase of serious decline leading towards a trough of neglect.' Smith (1997)

Considering the biologically most interesting properties of lichen-forming fungi and their photobionts it is surprising to see that experimental lichenology is currently in a difficult situation. Experimental lichenologists have always been a minority among biologists, but today only few active teams are left worldwide. Why is this?

(1) Missing ' advocates ' of lichenology in science councils and faculties, etc.: fewer and fewer high-ranking academic positions are held worldwide by people with an active research interest in lichenology. Does this mean that this discipline attracts mainly the intellectually less gifted members of the scientific community? I don't think so, but rather see the strong trends everywhere to concentrate, in times of vigorous budget cuts, all efforts on mainstream projects, at the expense of a biodiversity in research topics. Consequently, ' advocates ' of lichenology are missing in decision-making processes in academic systems with steep hierarchies (the rule at least in central Europe). Or, as expressed by Smith (1997): ' Lichenology is out of fashion with science councils '.

(2) Missing teaching tradition: during their training most biology students are told of lichens as an example of a mutualistic symbiosis, perhaps as pioneers in harsh environments, as bioindicators, or as extremely slow-growing organisms, but basic lichenology is, to my knowledge, hardly anywhere an integral part of the curriculum of students in biology or microbiology. This is detrimental not only for the transmission of information, but also for the recruitment of good, highly motivated students; these are the rejuvenating factors *per se* and thus of central importance in any discipline!

(3) ' Identity crisis ' among lichenologists: traditionally lichens are investigated in the ill-defined sector of cryptogamic botany in botanical institutions. How are lichen-forming fungi, the quantitatively predominant element of lichen thalli, related to plants? Is it justified to refer to lichens as plants? Can lichens really be assumed to function exactly like plants, for example when gas exchange measurements are to be interpreted? Studies in molecular systematics fully support the complete separation of the fungal from the plant kingdom, as first proposed by Whittaker (1969) in his Five Kingdoms concept. No one will recommend freezing or even cutting the good relations with botanical institutions (too many of us are strongly dependent on them), but lichenologists have to define their position within life sciences in order to be able to defend their subject. Lichenology is definitely a branch of mycology and thus has primarily to be brought to the attention of mycologists and microbiologists. A better integration of lichenological research in mycology is highly desirable. Lichenologists should keep in mind that the peculiarities of the lichen symbiosis can be properly defined only in careful comparisons with non-lichenized fungal taxa or with other algal symbioses.

' The " new " techniques dominating so much of biology—such as molecular genetics and DNA sequencing—have found no novel and dramatic applications in lichens. ' Smith (1997)

Towards the end of the second millenium experimental biology is in a most fascinating period since tools are available that allow molecular geneticists and biotechnologists to:

- identify genes at the DNA sequence level;
- transfer genes horizontally, i.e. insert genes and follow their expression in unrelated organisms. By performing such transformations the species

TABLE 3. *Model organisms in molecular biology*

Organisms	Genome size Mbp/1C*	Development completed within
Multicellular		
Arabidopsis thaliana wall cress	145	5–8 Weeks
Drosophila melanogaster fruit fly	165	2–3 Weeks
Caenorhabditis elegans nematode, eel- or roundworm	100	3 Days
Unicellular		
Saccharomyces cerevisiae	13·5	Hours
Escherichia coli	4	Minutes
for comparison:		
Tricticum aestivum (wheat)	15 966	
Fritillaria assyrica (fritillary)	48 000	
Homo sapiens (man)	3 500	
Salamander	50 000	
Lichen-forming fungi	???	Years

*Mbp: millions of base pairs; 1C: DNA contents of the haploid genome equivalent. Source: Westhoff *et al.* (1996).

boundaries can be overcome that limit the possible range of conventional crossing experiments. Economically interesting compounds of slow-growing, rare or even endangered species can be produced in large quantities by transformation of suitable microorganisms.

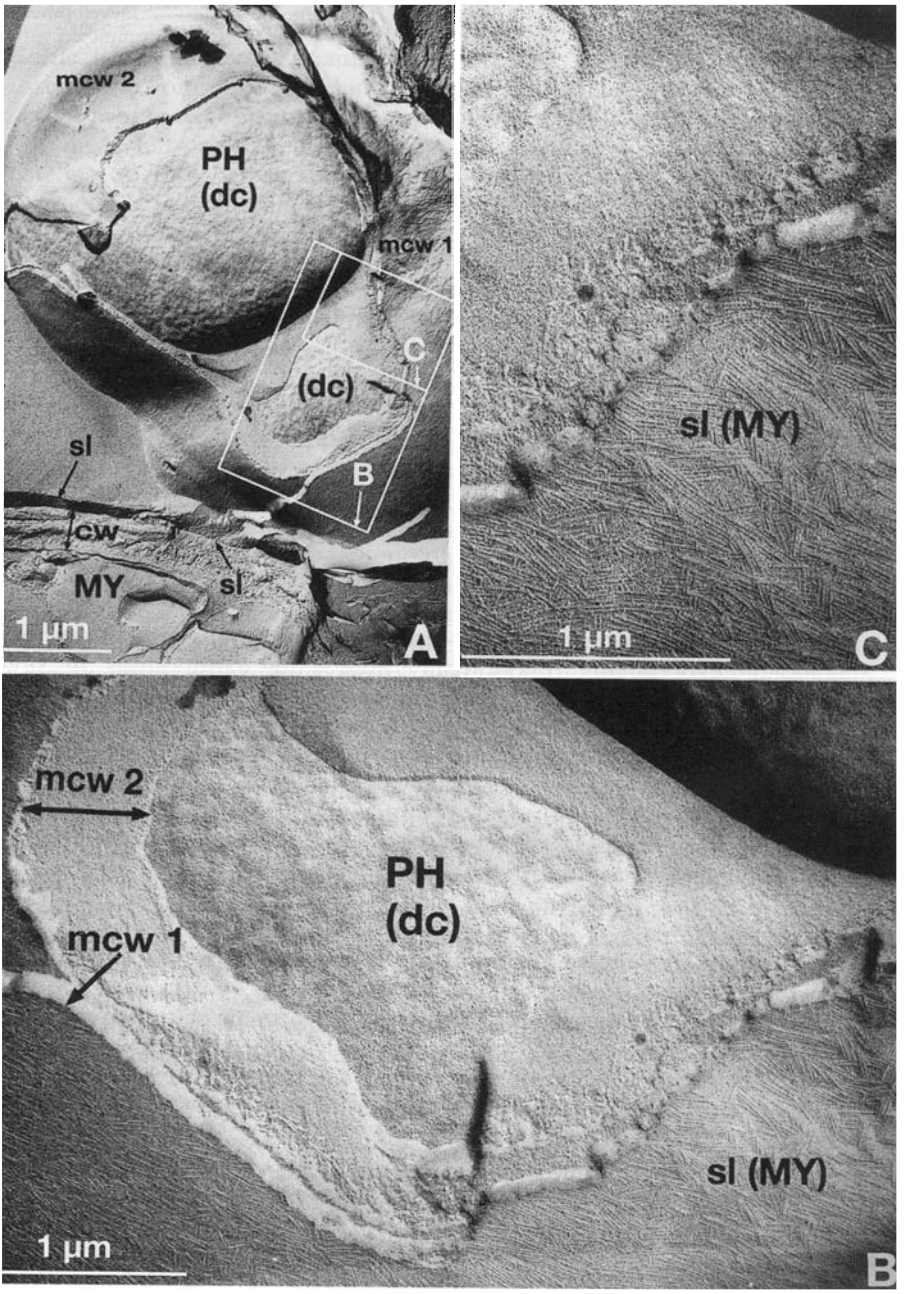
- test gene functions in mutant phenotypes; this allows a causal analysis of developmental processes.

For basic investigations in developmental biology it is essential to select model organisms (Table 3) that fulfil the following criteria:

- small genome size;
- developmental cycle completed within short periods of time (few days to few weeks);
- high regenerative capacity after mutagenic treatment or transformation.

It is quite obvious that lichen-forming fungi will hardly ever be among the organisms favoured by molecular developmental biologists for the following reasons:

- genome sizes are unknown; not even chromosome numbers have so far been identified;
- extremely slow growth, in nature (symbiotic phenotype) as well as in axenic culture;
- the sexual cycle is not completed in aposymbiotic cultures; no classical genetic studies can be performed;



- the symbiotic phenotype is only rarely and irregularly expressed in sterile cultures; this is the major handicap for experimental studies on the biology of lichens in general!

Lichen-forming fungi are far too slow-growing and too complex to serve as easy-to-handle model systems for developmental biologists.

However, modern biological techniques can be successfully applied now in lichenology. Miao & Davies (1997) used degenerate oligonucleotides (' KS ' primers) that hybridize to conserved regions near the ketosynthase domain of polyketide synthase genes as characterized in actinomycetes (Donadio *et al.* 1991) and non-lichenized fungi (Keller *et al.* 1995) to amplify homologues from lichen DNA by means of the polymerase chain reaction (PCR). Insertion of such genes of lichen-forming fungi, which code for pharmaceutically interesting secondary metabolites, into fast-growing microorganisms (e.g. yeasts) will provide a wide range of biologically and economically interesting options.

In my small research team we are currently focusing on the mycobiont-derived, hydrophobic cell wall surface proteins, which are likely to play a crucial rôle in the functioning of the symbiotic relationship between the partners (see above). Such proteins were isolated and purified and their N-terminal amino acids sequenced. Primers were designed for PCR, and the PCR product was cloned in *Escherichia coli*. Its conceptually translated amino acid sequence exhibited the characteristic hydrophobin signature as known from a range of non-lichenized fungi (PhD thesis of Sandra Scherrer; publications in preparation). Hydrophobins are small (*c.* 100 amino acids), secreted fungal proteins with little sequence homology but with 8 cysteine residues in a conserved pattern and with a typical hydropathy pattern. Class 1 hydrophobins self-assemble at the liquid-air interface into an amphipathic membrane with a characteristic rodlet pattern (reviews: Wessels 1993, 1996; Templeton *et al.* 1994; Talbot 1997). We are fully aware that the ultimate functions of this proteinaceous wall surface layer within the lichen thallus could only be tested with hydrophobin-deficient mutants. As it is not yet possible to express the symbiotic phenotype routinely in resyntheses of axenically cultured lichen-forming fungi and their compatible photobionts, we have, at the moment, no other choice than to explore functional aspects very carefully at the descriptive level.

FIG. 4. TEM micrographs of the mycobiont-photobiont interface in *Solorina saccata* (photobiont: *Coccomyxa* sp.) as seen in freeze-fractured preparations. A. Overview, showing a fungal hypha (MY) in close contact with an autosporangium of the photobiont (PH). The fracture plane follows the wall surface of two daughter cells (dc) ensheathed by two mother cell walls (mcw1 and mcw2). The fungal cell wall (cw) is cross-fractured; the very thin wall surface layer (sl) forms a strongly hydrophobic discontinuity and spreads over the older mother cell wall (mcw1). The frame indicates the position of B and C. B. Detail showing the wall surface of the daughter cell (dc), the cross-fractured mother cell wall (mcw2) with a massive amorphous layer, and the cross-fractured older mother cell wall (mcw1) that has been largely degraded except for its sporopollenin-containing, non-degradable, trilaminar sheath (for details see Honegger & Brunner 1981; Brunner & Honneger 1985; Honegger 1991). The mycobiont-derived, hydrophobic wall surface layer (sl(MY)) is spreading over the outermost, oldest mother cell wall (mcw1). C. Detail showing the characteristic rodlet pattern of the proteinaceous, mycobiont-derived wall surface layer (sl(MY)).

Lichenologists are not, by any means, the only experimental mycologists working with a difficult fungal symbiosis. Arbuscular mycorrhizal fungi, the ecologically and economically important mutualistic symbionts of more than 70% of vascular plant species, are physiologically obligate biotrophs (cannot be cultured apart from a plant host), and the same applies for the powdery mildews (Erysiphales) and rusts (Uredinales), fungal pathogens of a wide range of economically important and other host plants.

Perspectives

'. . . why is lichenology rooted in so many odd corners . . ., when will it come of age as a respected part of mainstream biology?' Farrar (1997)

This criticism, as recently expressed in an interrogative form in a book review, is likely to reflect the view of many biologists. Has lichenology ever contributed *something of relevance or interest to general mycology or symbiosis research?*

It is well known that the discovery of the dual nature of lichens in the last century had a very thought-provoking effect on open-minded contemporary scientists. Lichens were the first symbiotic system ever recognized in which no obvious signs of harm or damage were evident in either biont. Soon after the discovery of the lichen symbiosis by Schwendener (1867), numerous unicellular algal symbioses with protists and invertebrates were described by Brandt (1882), a student of Schwendener; Frank (1885) discovered ectomycorrhizae, and Hellriegel (1886) the rhizobial symbiosis in root nodules of legumes. However, it has to be admitted that the majority of lichenologists throughout Europe rejected what they called the 'algo-fungal-lichen-hypothesis' or 'Schwendenerism' (Crombie 1884). 'Schwendener's dual hypothesis excited much resistance, in part from reasons of "common sense", in part because lichenology has always been a somewhat esoteric pursuit' (Ainsworth 1976). The last attempt to disprove the dual nature of lichens was published in 1953 (Schmidt 1953) fourteen years after the first successful resynthesis of a lichen from axenically cultured fungal and algal isolates under laboratory conditions by Thomas (1939).

Lichens were the first fungal symbiosis with photoautotrophs in which the mobile photosynthates that move from the photoautotrophic to the heterotrophic partner were qualitatively determined. In a series of elegant experiments using the so-called 'inhibition technique' (or 'isotope trapping technique') Smith and co-workers identified acyclic polyols as mobile carbohydrates in green algal photobionts and glucose in cyanobacterial taxa (reviews: Richardson 1973; Smith & Douglas 1987). Their experimental design was successfully adapted by colleagues in plant pathology and mycorrhizal research and even in studies on algal symbioses of invertebrates (reviews: Smith 1978; Smith & Douglas 1987).

Lichens were the first fungal symbiosis in which the mycobiont-derived rodlet layer, the proteinaceous hydrophobic cell wall surface coat of aerial hyphae, was shown to spread from the fungal wall surface over the surface of the photoautotroph (Figs 4A–C) and to play a functionally important rôle at this particular interface (Honegger 1985). Based on published data on the

almost ubiquitous occurrence of hydrophobic rodlet layers on aerial structures of non-lichenized fungal taxa it was hypothesized that such water-repellent coats might also play an important rôle in the establishment of other fungal symbioses. In the meantime some of these hydrophobic wall surface proteins of lichen-forming fungi have been identified as hydrophobins (PhD thesis of Sandra Scherrer; publications in preparation). There are recent reports of enhanced expression of developmentally regulated genes encoding hydrophobins during the establishment of a functional interface in entomopathogenic (St. Léger *et al.* 1992), plant pathogenic (Talbot *et al.* 1993, 1996; Templeton *et al.* 1994; Spanu 1997), and ectomycorrhizal interactions (Martin *et al.* 1995; Tagu & Martin 1996). In *Magnaporthe grisea*, the most problematic fungal disease of rice, the spreading of the germ-tube-derived hydrophobin (MPG1) over the host surface was shown to be essential for the tight adhesion of the pathogen to the very hydrophobic rice cuticle prior to the very complex infection process (Talbot *et al.* 1996).

One out of five fungal species is lichenized, and approximately 8% of terrestrial ecosystems are lichen-dominated (Larson 1987). The majority of lichen-forming fungi and their photobionts display most interesting biological properties. Experimental lichenology has the potential of becoming a respected part of mainstream biology, provided that young, talented scientists are given the chance and facilities for intensive research.

My sincere thanks are due to Sandra Scherrer and Marcella Trembley for stimulating discussions, to Jean-Jacques Pittet for skilfully preparing Figs 1–4, to Verena Kutasi for doing all the darkroom work, and to the Swiss National Science Foundation for generous financial support of our research projects on water relations and on hydrophobic cell wall surface proteins (grants No. 31–32493.91 and 31–42392.94).

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Accepted for publication 4 *March* 1998