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Observing microscopic phases of lichen life cycles on transparent substrata placed *in situ*

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Abstract: The utility of plastic cover slips as a substratum for *in situ* study of lichen developmental stages is further explored in a neotropical foliicolous lichen community and in a European temperate corticolous community. Twenty-one months after placement in the tropical forest, the cover slips bore foliicolous lichen thalli with several species producing characteristic ascocarps and ascospores, indicating the suitability of the substratum for completion of the life cycle of these lichens. On cover slips placed within the temperate corticolous community, lichen propagules anchored to the substratum with relatively short attachment hyphae but did not develop further within the one year observation period. Intimately intermixed microbial communities of short-celled, mainly pigmented fungi and chlorophyte algae developed upon the transparent substratum. Among the algae, *Trebouxia* cells, often in groups showing cell division and without associated lichenizing hyphae, were commonly observed. The potential significance of the free-living populations in the life cycle of *Trebouxia* and in those of *Trebouxia*-associated lichen fungi is discussed.

Key words: foliicolous lichens, lichenization, lichen development, life cycle, *Trebouxia*.

Introduction

Although the lichen thallus may resemble an integrated plant in its structural and functional properties (Sanders 2001*b*), the individual life histories of its fungal and algal components must be considered separately as well as together. This is particularly challenging, because these components are microorganisms that by themselves are difficult to recognize and observe *in situ*. Laboratory studies confirm that ascospores of a great many lichen fungi can germinate to produce mycelia (Crittenden *et al.* 1995), and that lichen thalli can be artificially reconstituted from propagules or isolated symbionts (e.g., Ahmadjian 1993; Stocker-Wörgötter 2001*a*,*b*). However, it is difficult to study what actually happens to symbionts in nature from the time of propagule dispersal to the first appearance of a new lichen thallus. In certain cases the lichen fungi might establish an aposymbiotic mycelium until compatible algae are encountered (Frank 1876; Johnson 1940), but it is unclear how widely this occurs. Little is known, for example, about how and when sexual fusion occurs in lichen fungi, although recent works have begun to explore their mating systems indirectly by molecular analysis of meiotic progeny (Murtagh *et al.* 2000; Honegger *et al.* 2004). We are particularly ignorant of the life cycles of the most important lichen algae. In lichen symbiosis they are not known to produce sexual reproductive structures, with a few exceptional reports for *Phycopeltis* (Santesson 1952; Lücking 1994; Sanders 2002). Furthermore, it has been repeatedly asserted that *Trebouxia* species, the most important lichen algae, cannot exist in the free-living state (Ahmadjian 1989, 1993, 2001, 2002), despite several reports of aposymbiotic *Trebouxia* occurring on natural substrata (Tschermak-Woess 1978; Bubrick *et al.* 1984; Mukhtar *et al.* 1994). The question of *Trebouxia* availability is of considerable significance, since a great number of lichen fungi that require these algae disperse only aposymbiotic spores as propagules.

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A more complete understanding of the life cycles of lichen symbionts will require observations of their behaviour in the field as well as in the laboratory under controlled conditions.

In spite of the difficulties in studying microscopic phases *in situ*, a number of works have documented stages of lichen ontogeny on natural substrata using either light microscope preparations or SEM (Ward 1884; Werner 1931; Lallemant & Avnaim 1984; Jahns *et al.* 1979; Schuster 1985; Schuster *et al.* 1985; Garty & Delarea 1987; Ott 1987). One recent approach that has proven useful involves placing transparent substrata *in situ* among foliicolous lichen communities. As these lichens are adapted to grow on the leaf surface, they will readily colonize smooth artificial substrata such as plastic (Sipman 1994; Lücking 1998), including transparent materials that permit observation of developmental stages directly with the light microscope (Sanders 2001*a*, 2002; Sanders & Lücking 2002). Such observations have shown that a wide variety of reproductive strategies operate in the foliicolous lichen community, and that fungal and algal interactions differ among newly organizing thalli of different lichens (Sanders & Lücking 2002). Some further results are reported here. The ability of foliicolous lichens to complete their sexual life cycles on transparent plastic cover slips is evaluated by observing developmental stages attained 21 months after placement of the substratum *in situ*. The utility of transparent substrata for studying life cycle phases in a temperate corticolous lichen community is also explored.

Materials and Methods

Colonization of plastic cover slips was observed among foliicolous lichens within a remnant of lowland Atlantic forest in the Parque Estadual de Dois Irmãos (Recife, Pernambuco, Brazil). A synopsis of lichens occurring on leaves in the region is provided by Cáceres *et al.* (2000). To determine whether the foliicolous lichens could complete their sexual life cycles on the transparent substratum, a set of about 25 cover slips was placed in the field before leaving Recife and harvested for observation 21 months later during a return visit (August 2002). Cover slips were also placed among corticolous lichens in a temperate oak woodland of Casa de Campo, City of Madrid, Spain, during 2003–2004. About 45 cover slips were placed at that field site, with nine harvested at roughly trimonthly intervals for examination with a light microscope.

Plastic netting with a mesh of approximately 2 mm^2 was cut into strips about 4 cm wide and 20 cm long. Diagonal slits about 9 mm long were cut into these strips, such that the corners of plastic 22 mm^2 cover slips could be fitted into the slits and held fast on the surface of the mesh strips. The mesh strips were tied with polyester cord over the surfaces of the natural lichen substrata in the field (leaves of *Bactris* sp. at Dois Irmãos and branches of *Quercus ilex* at Casa de Campo).

For microscopic examination, the cover slip was placed face up over a drop of water on a glass slide, with a second drop of water and a clean glass cover slip placed over the colonized surface of the plastic cover slip.

Results

Tropical foliicolous lichen community

Cover slips examined 21 months after placement in the field showed substantial colonization by fungi, algae and lichens. There was some lichen colonization on the lower surface of the transparent plastic as well as the upper; after dissecting microscope examination, those on the lower surface were removed by wiping with a moist tissue so that light microscopic observation of a single surface would not be obstructed. Foliicolous lichens in various stages of development were observed; a number of thalli bore ascocarps. Angular black apothecia, probably belonging to *Aulaxina* sp. (*Gomphillaceae*), were developing on a thallus containing unicellular green algae (Fig. 1A). Black lirellae of *Opegrapha filicina* differentiated over broad areas containing approximately round thalli of its algal symbiont *Phycopeltis*. The lirellae usually occurred where the algal symbiont lay directly below, but their shape and orientation did not show any particular correspondence to the form of the alga (Fig. 1B). Mature asci containing ascospores were observed in the lirellae (Fig. 1C). A young thallus of *Cryptothecia* sp. produced muriform ascospores from separate asci dispersed

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within a fertile zone (Fig. 1D). A small thallus with a trebouxioid green alga bore an apothecium with muriform ascospores and epihymenial algae (Fig. 1E). Apothecia and pycnidia also occurred on several thalli recognizable as *Mazosia*, including *M. rotula* (Fig. 1F). Like *O. filicina*, *Mazosia rotula* spread over several roundish, often non-adjacent thalli of the algal symbiont *Phycopeltis*. However, *M. rotula* produced fungal structures oriented in much closer correlation with the morphology of the *Phycopeltis* symbiont below. Its apothecia and/or pycnidia were usually produced over the centre of the algal thalli, with radiating ridges containing crystalline deposits generally coinciding with the radial orientation of the underlying algal filaments (Figs 1F–G). Also present were individual lichenized *Phycopeltis* thalli showing a single fungal pycnidium or perithecium differentiating directly over their centre, while some highly lobed *Phycopeltis* had dark brown pycnidia forming along the lobe margins. The lichen *Phyllophiale* was also present, with discoid isidia produced above the margins of the *Phycopeltis* phycobiont.

Some lichens that were apparently not part of the foliicolous community were also capable of colonizing the plastic cover slips and developed to varying degrees upon the substratum. Occasionally observed were colonies of a coccoid cyanobacterium showing binary divisions within extensive gelatinous sheath material (Fig. 1H). The same alga could also be observed in a lichenized condition, although no distinctive thallus developed on the experimental substratum. In the lichenized state, the intercellular sheath material was traversed by fungal hyphae that formed haustoria penetrating into each cell, many of which appeared to continue division in apparent synchrony with haustorial growth and bifurcation (Fig. 1I). A few young foliose lichen thalli were also observed, with primordial lobes emerging obliquely upward from a loosely organized basal crust of fungal hyphae and trebouxioid cells (Figs 1J & K).

Temperate corticolous lichen community

At Casa de Campo (Madrid), a number of fungi colonized the plastic substratum within two months after placement, although typical mycelia were generally not seen until much later. The fungi observed formed moniliform chains, compact plaques or yeast-like proliferations of short, usually brown-pigmented cells (Fig. 2A & B). These appeared to be non-lichen fungi that normally colonize leaf or bark surfaces. Some of these fungi produced numerous urn-shaped sporocarps composed of spherical brown cells (Fig. 2C) These sporocarps contained hyaline spores (Fig. 2D) presumably formed conidiogenously; no asci were observed.

Lichen soredia and thallus fragments often dispersed onto the substratum and frequently produced a limited growth of germination hyphae closely adpressed to the surface (Fig. 2E). However, lichen propagule development was not observed to proceed beyond this initial stage. Of the many fungal spores, some were seen in groups of eight or fewer, apparently dispersed together from a single ascus (Fig. 2F). Occasionally, unidentified germinating spores could be seen growing towards a nearby algal cell and spreading over its surface (Fig. 2G); however, there was no clear evidence of lichen-forming interactions.

The microorganisms did not colonize the surface of the cover slips uniformly, but were more concentrated at the corners where the plastic lamina were inserted under the mesh. Diverse fungal and algal cells were usually agglutinated together rather than dispersed uniformly. Germinating spores and other fungal structures often had algal cells clustered around them (Fig. 2H). Frequently, the placoid and sporocarp-producing fungi likewise showed development of algal communities on their surfaces, with algal cells even becoming embedded within the walls of the sporocarps and tissues as the fungal cells proliferated around them. The algae included a number of aerophilic chlorophyte taxa, such as *Apatococcus lobatus* and *Desmococcus olivaceum* with parietal plastids and

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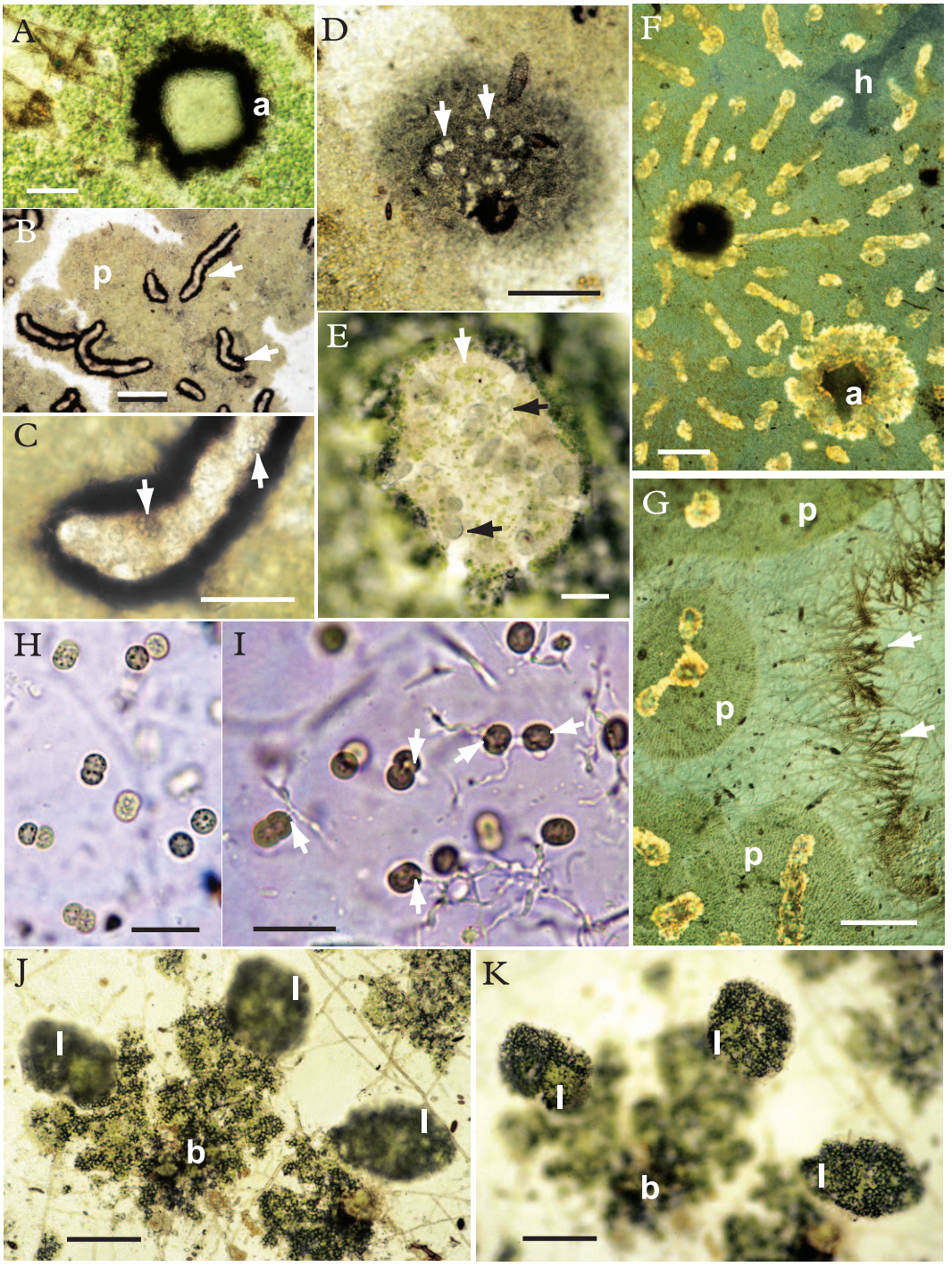

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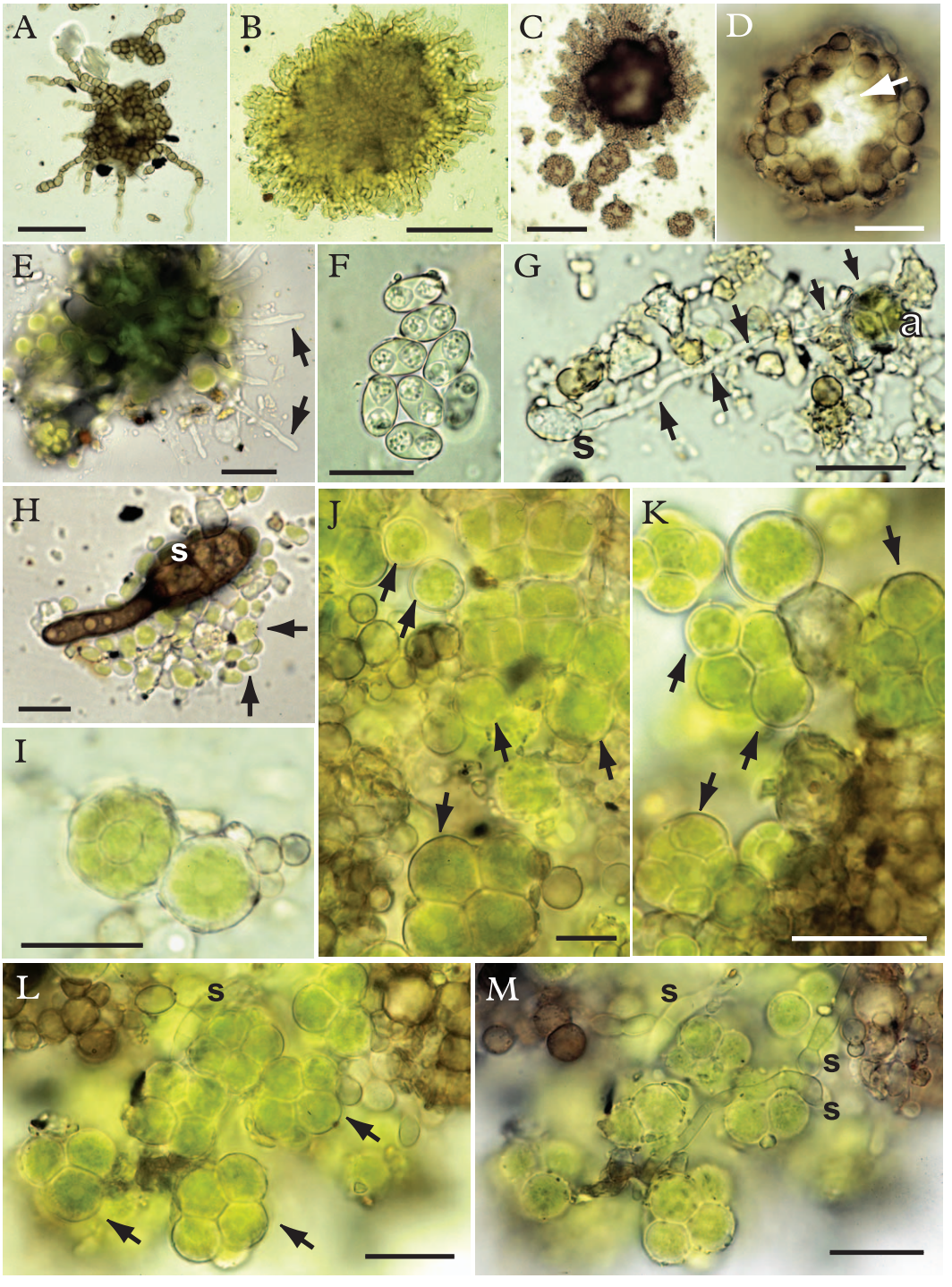
perpendicular cell divisions. Of particular relevance to lichens was the presence of *Trebouxia*, recognizable by its axial chloroplast with conspicuously lobed edges and large central pyrenoid (Fig. 2I–M). Many *Trebouxia* cells were observed in division or in adpressed clusters indicating recent division (Fig. 2I–K). They were sometimes observed in isolation but more often in contact with adjacent fungal cells and fungal thalli such as those shown in Fig. 2A–D, as well as other algae. Such contacts usually did not include any surrounding or interpenetrating fungal hyphae that might be considered as lichenizing, although such interactions were of course observable within lichen soredia and thallus fragments also present on the substratum.

Discussion

The appearance of fertile stages of foliicolous lichens on the transparent plastic cover slips indicates that this substratum is suitable for the complete development of the thalli. Production of mature ascocarps by several species within the 21-month period is consistent with results obtained in colonization experiments with opaque plastic leaves surveyed after 24 months exposure within a Costa Rican rainforest (Lücking 2001; Lücking & Bernecker-Lücking 2002). While physical sections of the fine foliicolous thalli are difficult to obtain, optical sectioning of thalli growing on the transparent substratum allows distinction of the fungal hyphae and their relationship to the algal component. The close correspondence of mycobiont structures such as ridges and fruiting bodies with phycobiont form in *Mazosia rotula* may be related to the reduced mycobiont structure relative to the phycobiont, which is typical of foliicolous lichens and especially those with *Phycopeltis*. In these compact lichens, fungal growth may simply follow the contours of the algal thalli, as also observed by Grube & Lücking (2002). On the other hand, the absence of birefringent crystals in the prothallic/hypothallic regions suggests that deposition of these substances involves a direct interaction between mycobiont and phycobiont.

Although attached lichen propagules did not develop further into basal crusts within the time period of the study, it is likely that longer exposure times may render the artificial surface more hospitable to lichen development as microorganisms and detritus accumulate. It is notable that for most of the year following substratum placement, colonizing fungi were almost exclusively cellular, moniliform or placoid; more typical hyphal growth was not commonly seen and quite limited in extent until nearly one year had passed. Although lichen development might eventually be favoured after longer exposure

FIG. 1. Development of lichens on plastic coverslips placed within a lowland tropical forest in Recife, Brazil. Lichens shown in Figs A–G and J–K occur on cover slips exposed *in situ* for 21 months. A, *Aulaxina* sp., with thick, angular, black-rimmed apothecium (a); B, *Opegrapha filicina*, showing positions of lirellae (arrows) mainly overlying the thalli of the phycobiont *Phycopeltis* (p); C, *O. filicina*, detail of lirella showing asci (arrows) containing ascospores; D, *Cryptothecia* sp., with asci (arrows) forming separately within fertile region of the thallus; E, apothecium borne on minute thallus, with muriform ascospores (horizontal arrows) and epithecial algae (vertical arrow) suggestive of *Gomphillaceae* or possibly *Sporopodium* (*Ectolechiaceae*); F & G, *Mazosia rotula* imaged in polarized light, the birefringent crystalline substances within thallus ridges appearing bright gold; F, apothecia (a) are positioned over the centre of the underlying *Phycopeltis* symbiont, with the crystal-containing ridges following the radial orientation of the underlying algal filaments and absent over hypothallic regions (h) between algal thalli; G, detail showing individual *Phycopeltis* thalli (p) and dark-pigmented prothallic hyphae at lichen margin (arrows); H & I, cyanobacteria colonizing experimental substratum 7 weeks after placement *in situ*; H, aposymbiotic condition, with no hyphae observed within gelatinous sheath; I, lichenized condition, with hyphae traversing sheath material and penetrating the dividing algal cells with haustoria (arrows), visible as pale areas; J & K, images of foliose lichen on experimental substratum in two planes of optical section, showing basal, crustose primary thallus (b) with foliose lobes (l) emerging from it. The unicellular algae are surrounded by fungal hyphae. Scales: A & C=25 μm; B, D, E, G, J & K=100 μm; C=50 μm; F=250 μm; H & I=25 μm.



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of the substratum, the build-up of microorganisms and detritus upon the cover slips renders the interactions between microorganisms increasingly difficult to study with the light microscope, thereby reducing the utility of this technique.

Nonetheless, there is reason to believe that transparent artificial substrata may prove effective for the study of non-foliicolous lichen ontogeny in certain well-chosen habitats. There have been numerous reports of (non-foliicolous) crustose and foliose lichens colonizing synthetic substrata, including rubber, plastic and painted metal, often on vertical surfaces, in temperate environments (Brightman & Seaward 1977; Gray 1999; Pedley 2000; Bennett 2002; Upreti & Dixit 2002). Why this occurs in certain environments but not in others is unclear; higher humidity might be a factor. Although in earlier studies glass proved to be a rather suboptimal substratum for foliicolous lichen development within the tropical forest (Sanders 2001*a*), non-foliicolous taxa have been reported to colonize stained and unstained glass of French churches (Mellor 1923), glass fragments in Antartica (Schroeter & Sancho 1996), and car windshields as well as supermarket window panes in New Zealand (Green & Snelgar 1977; Kappen 1994). This suggests that glass may also be useful for *in situ* studies of lichen development in communities with suitable conditions.

The ease with which populations of dividing, non-lichenized *Trebouxia* cells could be observed at the Madrid site suggests that similar findings by Tschermak-Woess (1978), Bubrick *et al.* (1984) and Mukhtar *et al.* (1994) should not be treated as reports of rare or exceptional events. Clearly, the infrequency of free-living *Trebouxia* being reported does not necessarily indicate its rarity in nature. What appears to be infrequent, despite repeated discussion, are the attempts to evaluate its presence in natural microbial communities. In an often-cited study of lichen colonization on *Fraxinus* branches, Degelius (1964) reported *Trebouxia arboricola* as rare among the free-living algae on the material examined. The author doubted its importance in lichen resynthesis and suggested that symbiotic propagules were the main means of reproduction in that community. However, Degelius's study mainly involved × 10–15 magnification observations of lichen development on hundreds of *Fraxinus* shoots measuring over 1 m long; no information was provided on how or to what extent such a vast surface area was sampled for light microscope observation of algae. Given our present state of knowledge, the reports of Tschermak-Woess (1978), Bubrick *et al.* (1984) and Mukhtar *et al.* (1994), in addition to the 'rare' sightings by Degelius (1964), are significant as positive findings and should stimulate further investigation into habitats where *Trebouxia* lichens are present. Although the smooth, exposed surface of plastic cover slips is almost certainly suboptimal for development of microbial communities compared to the microsites of the natural substratum, the simple technique applied in the present study can nonetheless provide an easy means of sampling and

FIG. 2. Microorganisms colonizing plastic cover slips at Casa de Campo, city of Madrid, Spain. A–D, development of cellular placoid fungi; C, sporocarps forming in centres of fungal plaques; D, detail of a sporocarp showing hyaline spores (arrow); E, emergence of attachment hyphae (arrows) from base of lichen soredium; F, octet of jointly dispersed ascospores, probably of lichen family *Physciaceae*; G, germination hypha (arrows) of unidentified fungal spore (s) growing toward and contacting an algal cell (a) that is dividing into a tetrad of daughter cells; H, germinating, dark-pigmented fungal spore (s) surrounded by numerous minute green algal cells, many ovate with parietal plastid (vertical arrow) and others trebouxioid with axial, lobed plastid containing a central pyrenoid (horizontal arrow); I, two large *Trebouxia* cells, one at the left dividing into spores; J–M, *Trebouxia* cells (arrows) among thalli and sporocarps of cellular, non-lichen fungi; J, with *Apatococcus* also present at upper right; K, with spore cluster at the lower left; L & M, same image at two slightly different planes of focus, with fungal spores (s) germinating over the surface of *Trebouxia* cells. Scales: A & B=50 μm; C=100 μm; D=25 μm; E, F, G, I, K, L & M=20 μm; H & J=10 μm.

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viewing at least a portion of the organisms present, including *Trebouxia*.

Even if free-living *Trebouxia* populations are ephemeral, with frequent extinction and re-establishment, they could nonetheless represent an important phase in the life cycle of *Trebouxia*, as well as a source of phycobionts for germinating fungal spores. Such populations might provide the opportunity for liberation and fusion of motile gametes. While sexually reproductive stages are generally unknown in the trebouxiophyte algae (Lewis & McCourt 2004), occasional fusion of motile gametes and formation of zygotes in *Trebouxia* cultures were reported by Ahmadjian (1967). Further observation and study of these key events are clearly needed. If the free-living *Trebouxia* populations are transient, their perpetuation may then depend on some cells being taken up within a developing lichen before the aposymbiotic population becomes locally extinguished. According to such a hypothesis, the free-living populations would be necessary for completion of the sexual cycle, while lichenization would be necessary for long-term persistence within habitats that are inhospitable to sustained aposymbiotic existence. It is also conceivable that free-living *Trebouxia* might endure certain adverse conditions by forming a thick-walled, resistant cyst-like stage, as occurs in many other algae.

Liberated *Trebouxia* need not occur in large, concentrated populations in order for encounters with germinating fungal spores to occur. *Trebouxia*, like many green algae, has flagellate motile stages known from culture (Ahmadjian 1967; Archibald 1975; Gärtner 1985; Tschermak-Woess 1989) and expressed incipiently within lichen thalli (Slocum *et al.* 1980). Thus, the transient presence of liquid water could provide the means by which algal zoospores migrate and settle near fungal spores and mycelia. The exudates of these microorganisms might be a sufficient attractant or stimulus for a zoospore to settle. The intimate clustering of a variety of green algal cells around fungal structures and germinating fungal spores was commonly observed in the present study as well as previously (Sanders 2001*a*). In such microbial communities, nutrients may be reciprocally recycled, and the clustering of the cells may reduce surface area exposed to dehydration.

If a sexual life cycle is commonly operative in *Trebouxia*, as indications of allelic recombination also suggest (Kroken & Taylor 2000), then it would also be of considerable interest to know whether vegetative cells within a lichen thallus are haploid, diploid, or a mixture of both. What remains to be resolved are not only the life cycles of the most important lichen algae, but also the question of where and how these life cycles intersect with those of lichen fungi.

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References

Ahmadjian, V. (1967) A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. *Phycologia* **6:** 127–160.

Ahmadjian, V. (1988) The lichen alga *Trebouxia*: does it occur free-living? *Plant Systematics and Evolution* **158:** 243–247.

Ahmadjian, V. (1993) *The Lichen Symbiosis.* New York: John Wiley & Sons.

Ahmadjian, V. (2001) *Trebouxia*: reflections on a perplexing and controversial lichen photobiont. In *Symbiosis* (J. Seckbach, ed): 373–389. The Netherlands: Kluwer Academic Publishers.

Ahmadjian, V. (2002) Lingering myths are hard to dispel . . . *ISS Symbiosis International* **2:** 1–2.

Archibald, P. A. (1975) *Trebouxia* de Puymaly (*Chlorophyceae*, *Chlorococcales*) and *Pseudotrebouxia* gen. nov. (*Chlorophyceae*, *Chlorosarcinales*). *Phycologia* **14:** 125–137.

Bennett, J. P. (2002) Car lichens in the United States. *British Lichen Society Bulletin* **91:** 39–42.

Brightman, F. H. & Seaward, M. R. D. (1977) Lichens of man-made substrates. In *Lichen Ecology* (M. R. D. Seaward, ed): 253–293. New York: Academic Press.

Bubrick, P., Galun, M. & Frensdorff, A. (1984) Observations on free-living *Trebouxia* de Puymaly and *Pseudotrebouxia* Archibald, and evidence that both symbionts from *Xanthoria parietina* (L) Th. Fr. can be found free-living in nature. *New Phytologist* **97:** 455–462.

Cáceres, M. E. S., Maia, L. C. & Lücking, R. (2000) Foliicolous lichens and their lichenicolous fungi in the Atlantic rainforest of Brazil: diversity, ecogeography and conservation. *Bibliotheca Lichenologica* **75:** 47–70.

Crittenden, P. D., David, J. L., Hawksworth, D. L. & Campbell, F. S. (1995) Attempted isolation and success in the culturing of a broad spectrum of lichen-forming and lichenicolous fungi. *New Phytologist* **130:** 267–297.

Degelius, G. (1964) Biological studies of the epiphyte vegetation on twigs of *Fraxinus excelsior*. *Acta Horti Gotoburgensis* **27:** 11–55.

Frank, A. B. (1876) Über die biologischen Verhältnisse des Thallus einiger Krustenflechten. *Beiträge zur Biologie der Pflanzen* **2:** 123–200.

Garty, J. & Delarea, J. (1987) Some initial stages in the formation of epilithic crustose lichens in nature: a SEM study. *Symbiosis* **3:** 49–56.

Gärtner, G. (1985) Die Gattung *Trebouxia* Puymaly (*Chlorellales*, *Chlorophyceae*). *Archiv für Hydrobiologie Supplement* **71** (*Algological Studies* **41**): 495–548.

Gray, J. M. (1999) Another good reason for buying a Lada car. *British Lichen Society Bulletin* **85:** 12–14.

Grube, M. & Lücking, R. (2002) Fine structure of foliicolous lichens and their lichenicolous fungi studied by epifluorescence. *Symbiosis* **32:** 229–246.

Honegger, R., Zippler, U., Gansner, H. & Scherrer, S. (2004) Mating systems in the genus *Xanthoria* (lichen-forming Ascomycetes). *Mycological Research* **108:** 480–488.

Jahns, H. M., Mollenhauer, D., Jenninger, M. & Schönborn, D. (1979) Die Neubesiedlung von Baumrinde durch Flechten I. *Natur und Museum* **109:** 40–51.

Johnson, G. T. (1940) Contributions to the study of the *Trypetheliaceae*. *Annals of the Missouri Botanical Gardens* **27:** 1–50.

Kappen, L. (1994) The lichen, a mutualistic system – some mainly ecophysiological aspects. *Cryptogamic Botany* **4:** 193–202.

Kroken, S. & Taylor, J. W. (2000) Phylogenetic species, reproductive mode, and specificity of the green alga *Trebouxia* forming lichens with the fungal genus *Letharia*. *Bryologist* **103:** 645–660.

Lallemant, R. & Avnaim, M. (1984) Étude de la formation du thalle de quelques lichens II. L'ontogénèse du thalle du discolichen *Pseudevernia furfuracea*. *Beiträge zur Biologie der Pflanzen* **59:** 105–111.

Lewis, L. A. & McCourt, R. M. (2004) Green algae and the origin of land plants. *American Journal of Botany* **91:** 1535–1556.

Lücking, R. (1994) A new foliicolous species of *Microtheliopsis* (lichens, *Microtheliopsidaceae*) from Costa Rica. *Mycotaxon* **51:** 69–73.

Lücking, R. (1998) "Plasticolous" lichens in a tropical rain forest at La Selva Biological Station, Costa Rica. *Lichenologist* **30:** 287–291.

Lücking, R. (2001) Lichens on leaves in tropical rainforests: life in a permanently ephemerous environment. In *Life Forms and Dynamics in Tropical Forests* (G. Gottsberger & S. Liede, eds): 41–77. Berlin & Stuttgart: Gebrüder Borntraeger Verlagsbuchhandlung.

Lücking, R. & Bernecker-Lücking, A. (2002) Distance, dynamics, and diversity in tropical rainforests: an experimental approach using foliicolous lichens on artifical leaves. I. Growth performance and succession. *Ecotropica* **8:** 1–13.

Mellor, E. (1923) Lichens and their action on the glass and leadings of church windows. *Nature* **112:** 299–301.

Mukhtar, A., Garty, J., & Galun, M. (1994) Does the lichen alga *Trebouxia* occur free-living in nature: further immunological evidence. *Symbiosis* 17: 247–253.

Murtagh, G. J., Dyer, P. S. & Crittenden, P. D. (2000) Sex and the single lichen. *Nature* **404:** 564.

Ott, S. (1987) The juvenile development of lichen thalli from vegetative diaspores. *Symbiosis* **3:** 57–74.

Pedley, I. (2000) Mobile sites, further observations on the car, and another, more curious substrate. *British Lichen Society Bulletin* **86:** 31–32.

Sanders, W. B. (2001*a*) Preliminary light microscope observations of fungal and algal colonization and lichen thallus initiation on glass slides placed near foliicolous lichen communities within a lowland tropical forest. *Symbiosis* **31:** 85–94.

Sanders, W. B. (2001*b*) Lichens: interface between mycology and plant morphology. *Bioscience* **51:** 1025–1035.

Sanders, W. B. (2002) *In situ* development of the foliicolous lichen *Phyllophiale* (*Trichotheliaceae*) from propagule germination to propagule production. *American Journal of Botany* **89:** 1741–1746.

Sanders, W. B. & Lücking, R. (2002) Reproductive strategies, relichenization and thallus development observed *in situ* in leaf-dwelling lichen communities. *New Phytologist* **155:** 425–435.

Santesson, R. (1952) Foliicolous lichens I. *Symbolae Botanicae Upsaliensis* **12:** 1–590.

Schroeter, B. & Sancho, L. G. (1996) Lichens growing on glass in Antarctica. *Lichenologist* **28:** 385–390.

Schuster, G. (1985) Die Jugendentwicklung von Flechten: ein Indikator für Klimabedingungen und Umweltbelastung. *Bibliotheca Lichenologica* **20:** 3–206.

Schuster, G., Ott, S. & Jahns, H. M. (1985) Artificial cultures of lichens in the natural environment. *Lichenologist* **17:** 247–253.

Sipman, H. J. M. (1994) Foliicolous lichens on plastic tape. *Lichenologist* **26:** 311–312.

Slocum, R. D., Ahmadjian, V. & Hildreth, K. C. (1980) Zoosporogenesis in *Trebouxia gelatinosa*: ultrastructural potential for zoospore release and implications for the lichen association. *Lichenologist* **12:** 173–187.

Stocker-Wörgötter, E. (2001*a*) Experimental lichenology and microbiology of lichens: culture experiments, secondary chemistry of cultured mycobionts, resynthesis, and thallus morphogenesis. *Bryologist* **104:** 576–581.

Stocker-Wörgötter, E. (2001*b*) Experimental studies of the lichen symbiosis: DNA-analyses, differentiation and secondary chemistry of selected mycobionts, artificial resynthesis of two- and tripartite symbioses. *Symbiosis* **30:** 207–227.

Tschermak-Woess, E. (1978) *Myrmecia reticulata* as a phycobiont and free-living – free-living *Trebouxia* – the problem of *Stenocybe septata*. *Lichenologist* **10:** 69–79.

Tschermak-Woess, E. (1989) Developmental studies in trebouxioid algae and taxonomical consequences. *Plant Systematics and Evolution* **164:** 161–195.

Upreti, D. K. & Dixit, A. (2002) Lichens on plastic net. *British Lichen Society Bulletin* **90:** 66–67.

Ward, H. M. (1884) On the structure, development and life history of a tropical epiphyllous lichen (*Strigula complanata* Fée). *Transactions of the Linnean Society of London, Botany Series* **2:** 87–119.

Werner, R. G. (1931) Histoire de la synthèse lichénique. *Memoires de la Société des Sciences Naturelles du Maroc* **27:** 7–45.

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