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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

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Abstract

Many aspects of lichen establishment and ontogeny in nature remain poorly understood. This study attempts to evaluate the potential of foliicolous lichen communities for study of lichen ontogeny *in situ*. Glass microscope slides were taped onto strips of plastic extended above lichen-colonized leaves within a coastal Atlantic forest remnant in Recife, Brazil. Within 3–4 weeks, fungi, algae, and lichen propagules were observed growing on the slides. Many developmental stages of *Phycopeltis* could be observed; this alga often showed associations with fungi. Algal cells resembling *Trebouxia* were observed free of fungal association, but usually only singly or in groups of two or three without evidence of active cell division. Apparent lichenization of coccoid green algae by germinating spores was observed; some of these associations appear to represent campylidial conidia dispersed together with the lichen's algal symbiont. Thalli of *Phyllophiale* arising from discoid isidia were by far the most developmentally accelerated of the earliest lichen colonizers, demonstrating that the isidia indeed function as propagules. The results, together with those of experiments in progress, indicate that foliicolous lichen communities offer great potential for study of lichen ontogeny *in situ*.

Keywords: Lichen development, lichenization, *Phycopeltis*, *Phyllophiale*

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1. Introduction

Many important events and processes involved in lichen formation in nature have largely eluded detailed study. Initial stages of symbiont contact and prethallus formation have been elucidated in SEM studies of resynthesis experiments under laboratory conditions (Ahmadjian and Jacobs, 1978; Ahmadjian et al., 1981), raising questions about how symbiont contacts proceed in nature. Important observations of lichen ontogeny were made by embedding and sectioning lichen-colonized *Agave* leaves (Werner, 1931) and bark (Lallemant, 1984a, b), and by SEM study of developmental stages on successive, datable twig segments (Jahns et al., 1979) and on bark substrate sown with lichen propagules (Schuster et al., 1985; Ott, 1987). Some stages of apparent lichenization *in situ* have also been documented with light microscopy (Galun, 1988). Nonetheless, our understanding of how fungi and algae manage to associate and form a lichen thallus in nature is notoriously incomplete and speculative. In particular, the direct observation of the earliest, microscopic phases of these associations under natural conditions has remained problematic. Some researchers have taken alternative and innovative approaches, such as analysis of photobiont species composition within lichen communities (Beck et al., 1998; Beck, 1999).

However, the possibility of directly observing lichen formation as it occurs *in situ* is not necessarily a lost cause. The foliicolous lichen communities which abound in tropical forests may provide a model system for such direct studies. Although they are a rather specialized group ecologically, foliicolous lichens offer several advantages for developmental study. They are simple in form and must complete their life cycle relatively quickly, as their substrate is short-lived. More importantly, while foliicolous lichens are highly adapted for growth upon leaves, there is evidence that their substrate preference may be based more on the position and orientation of the leaf rather than on its intrinsic physical, chemical or biological properties. Lücking (1998) documented 63 lichen species growing on a plastic sign within a tropical forest; the great majority of these were typical foliicolous species. Furthermore, he reported that foliicolous lichens may also colonize glass slides placed in the forest (Lücking, 1998). If foliicolous lichens will indeed readily colonize artificial and transparent substrates such as plastic and glass, their development can be monitored directly with the light microscope. This paper represents a first report of lichen colonization studies carried out within a lowland tropical forest in northeastern Brazil.

2. Materials and Methods

The study was initiated in September, 1999, in a remnant of Atlantic forest

located within the Parque Estadual de Dois Irmãos in Recife (Pernambuco state), Brazil. A detailed and well-illustrated flora of the foliicolous lichens of the region was recently compiled by Cáceres (1999). Long strips of perforated plastic of the type used for hemming curtains were tied between nearby tree trunks in the vicinity of leaves showing foliicolous lichen cover. Glass microscope slides were placed transversely on the upper surface of the plastic strips and taped down at their ends to the underside of the strips using plastic packing tape (Fig. 1). At intervals of 3–5 weeks some of the slides were removed, mounted with water under a cover slip, and examined with the light microscope. Initially, some slides were replaced in the field after observation, but young lichen thalli and algae photographed did not survive to subsequent observations. It was suspected that the intense light of the microscope lamp killed the algae.

3. Results and Discussion

Fungi

Within the first four weeks a substantial cover of fungal hyphae appeared on the slides. These hyphae varied substantially in diameter, septation, branching patterns and, in some cases, pigmentation. Germinating spores of various shapes were observed, with multicellular spores producing germ tubes from numerous cells (Fig. 2). Some mycelia developed networks of relatively short, anastomosing hyphal segments (Fig. 3).

Phycopeltis

On many slides *Phycopeltis* was the most abundant alga and among the first to appear. All stages of development described in Chapman and Good (1983) were observed. Young globose cells, presumably developing from zoospores or zygotes, developed four lobes at right angles to each other. These lobes grew radially and furcated continually, producing discoid thalli of tightly branched, appressed filaments (Fig. 4). Stalked sporangia (Fig. 5) and gametangial cells (Fig. 6) were also observed, indicating the presence of both asexual and sexual reproduction.

Fungal contacts with *Phycopeltis* were often observed, although the nature of these associations was not always clear. One type of fusiform, septate fungal spore, often containing conspicuous oil droplets and germinating from both its terminal cells, was repeatedly observed growing toward *Phycopeltis* thalli. The hyphae generally encircled part of the margin of the algal disc and penetrated between its terminal branches; the algal cells appeared to remain healthy (Fig. 7).

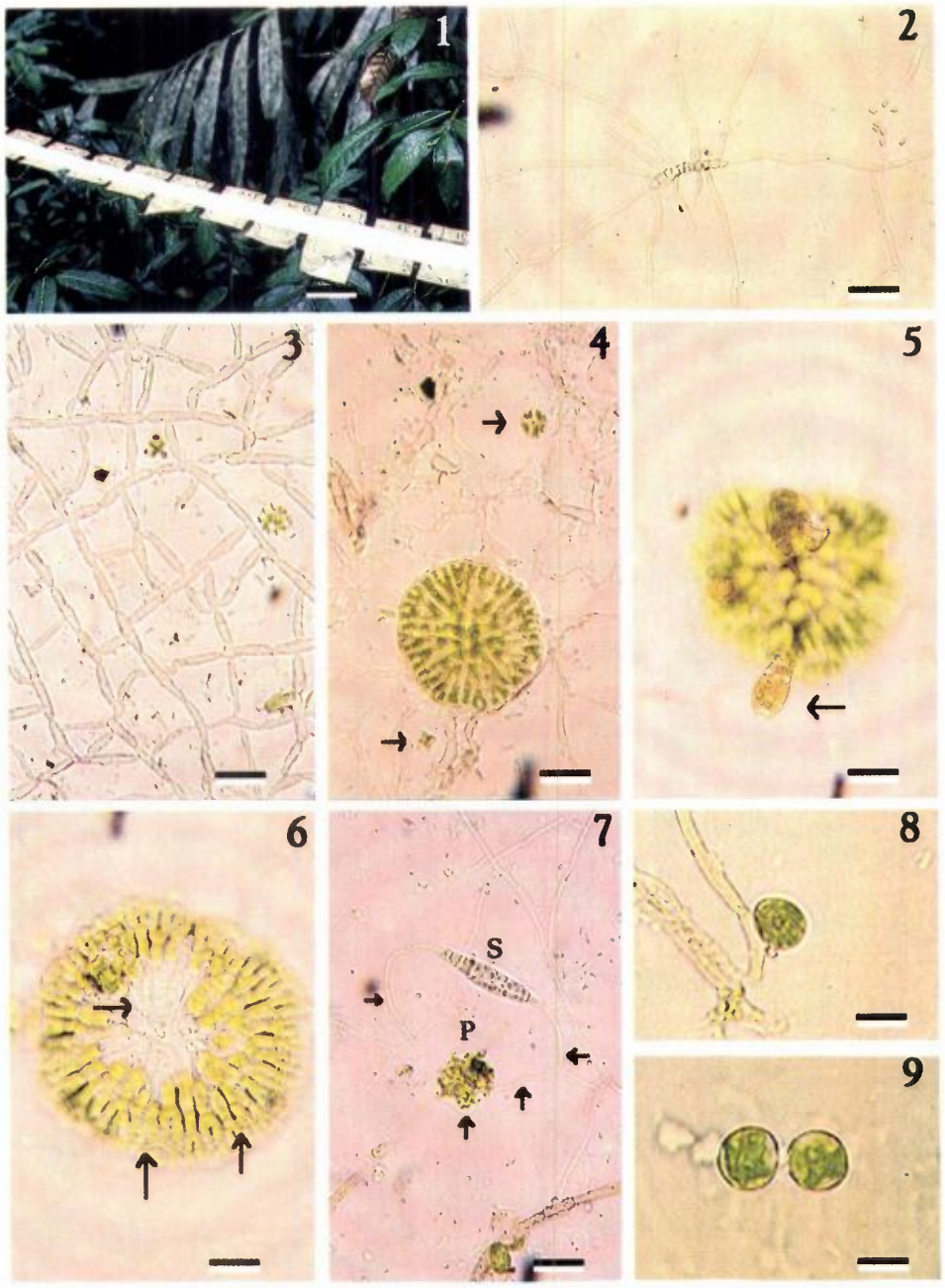


Figure 1. View of experimental set-up at field site: microscope slides taped crosswise to white plastic strip with packing tape.

Figs. 2–9. Light micrographs of organisms colonizing slides placed in field.

Coccoid green algae

A number of coccoid green algae colonized the slides. Some of these free-living alga appeared to be *Trebouxia* species: they showed a central, marginally lobed chloroplast with a conspicuous pyrenoid (Figs. 8, 9). These algae were observed singly or in very small groups, usually without evidence of active cell division or sporulation.

Not infrequently, green algae of various types were seen clustered against germinated or ungerminated fungal spores (Figs. 10, 11). The origin and significance of these contacts were unclear. The fungi and algae involved were not necessarily lichen symbionts. However, the clustering of algae around fungal spores and hyphae suggests a possible means of lichen symbiont encounter that does not seem to have received attention previously: the potential migration of motile algae to the immobile lichen fungus. The problem of relichenization in nature has generally been considered from the opposite point of view, in which the fungus must actively find the appropriate alga. Since chlorophyte lichen algae are capable of producing zoospores while lichen fungi are not, some sort of chemical attraction of the motile algal symbiont would vastly increase opportunity for symbiont contact, especially in the case of lichen algae which do not occur abundantly in the free-living state (e.g., *Trebouxia*). In such a scenario, periods of rainfall would be of great significance to lichenization, as liquid water is essential for zoospore production, release, and movement. The possibility of algal taxis toward potential mycobionts in the lichenization process is a hypothesis which deserves further attention.

Figure 2. Mycelium forming from germination of numerous cells of a septate fungal spore. Bar = 35 µm.

Figure 3. Anastomosing mycelium of segmented hyphae. Bar = 16 µm.

Figure 4. *Phycopeltis* sp. Larger discoid thallus and younger developmental stages (arrows). Bar = 16 µm.

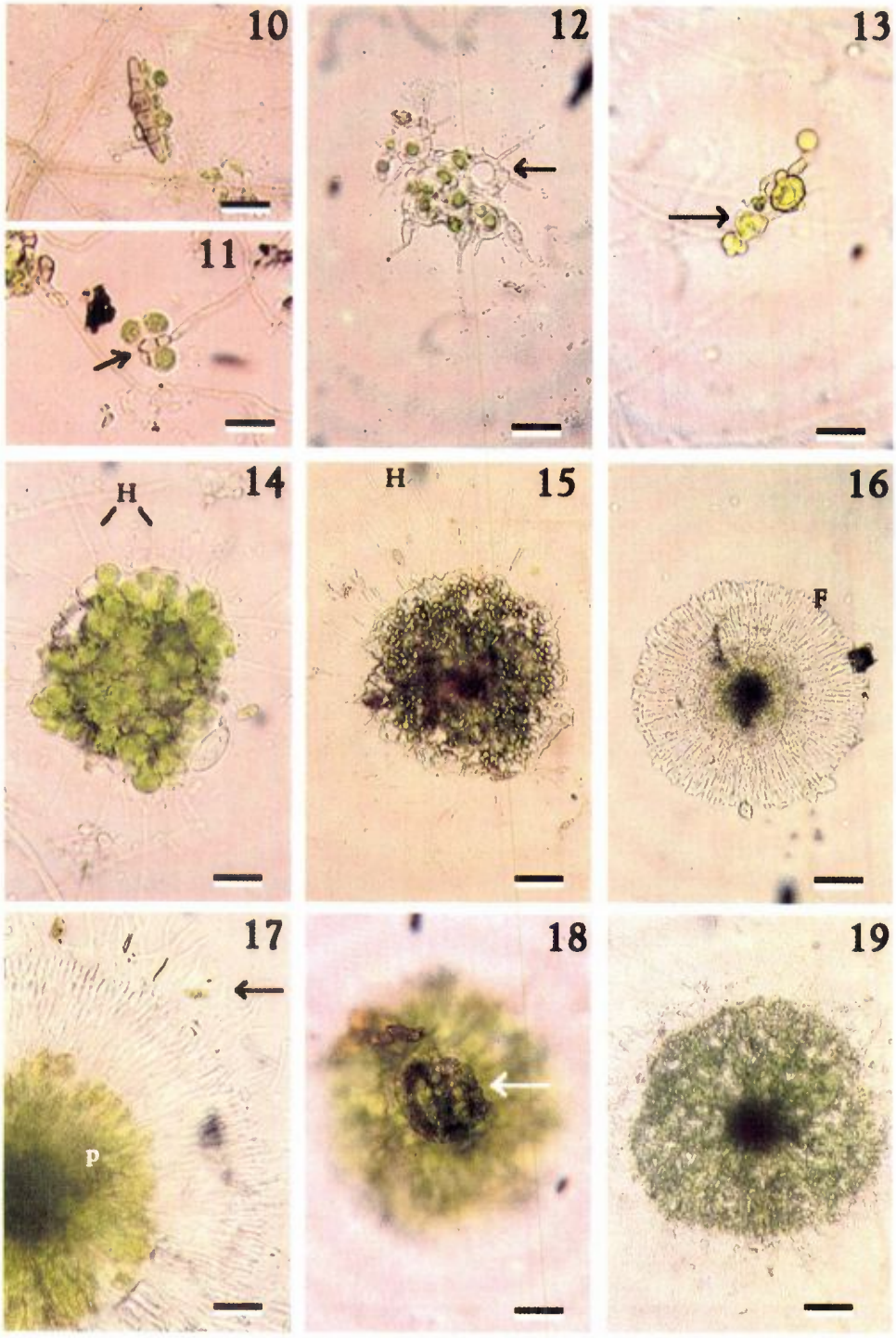
Figure 5. *Phycopeltis* sp. Stalked sporangium (arrow) in focus, with carotenoid pigmentation. Bar = 16 µm.

Figure 6. *Phycopeltis* sp. Thallus with empty gametangia located centrally. Horizontal arrow: gamete escape pore. Vertical arrows: fungal hyphae growing over thallus. Bar = 16 µm.

Figure 7. Fusiform septate spore (S), with multiple oil droplets, producing germ tubes from both ends. Arrows indicate germ tubes growing toward *Phycopeltis* thallus (P) and making intimate contact with terminal branches at thallus edge. Bar = 7 µm.

Figure 8. Alga (*Trebouxia* sp.?) in lateral contact with hypha. Bar = 9 µm.

Figure 9. Algal cells (*Trebouxia* sp.?) with deeply lobed central chloroplast, free of fungal association. Bar = 7 µm.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Lichenization of coccoid green algae

Clusters of coccoid green algae encircled by elongate, septate fungal spores were repeatedly observed (Fig. 12). The encircling spores germinated from several cells to produce radiating hyphal branches. That the algal cells and surrounding spores always occurred together in clusters suggests that the two symbionts were dispersed together. The lichen association depicted in Fig. 12 resembles germinating conidia and algal symbionts of *Tapellariopsis*, a foliicolous lichen which commonly occurs at the field site (Cáceres, 1999). *Tapellariopsis* produces filiform conidia in campylidia which also contain numerous photobiont cells within the sporogenous chamber (Lücking, 1999), suggesting that the two symbionts are dispersed together. Empty loops of

Figs. 10–18. Light micrographs of organisms colonizing slides placed in field.

Figure 10. Septate fungal spore with green algal cells appressed to its wall. The spore appears to be an ascospore of the Opegraphaceae, a family which includes a number of lichen-forming, foliicolous taxa common at the field site, such as *Mazosia* spp. and *Opegrapha filicina*. Bar = 13 µm.

Figure 11. Germinating fungal spore (arrow) with three algal cells appressed to surface. Bar = 10 µm.

Figure 12. Filiform septate fungal spores encircling coccoid green algae in probable lichen-forming association. Germination from several cells of the looped spores produces radiating hyphae (to either side of arrow). Arrow indicates looping filiform spore enclosing a space likely to have been formerly occupied by an algal cell that was subsequently dislodged. Bar = 14 µm.

Figure 13. Coccoid green algae surrounded by segmented fungal hyphae. Division of surrounded algal cells (arrow) suggests that the association may be pre-lichenic. Bar = 14 µm.

Figure 14. Cluster of coccoid green algae interpenetrated by fungus in probable lichen association. Hyphae (H) radiate from cluster over substrate. Bar = 14 µm.

Figure 15. Pre-thallus containing coccoid green photobiont. Prothallic hyphae (H) radiate over substrate. Bar = 33 µm.

Figure 16. Discoid lichenized structure identified as isidium of *Phyllophiale* sp. Note uniform radial fringe of fungal cells (F). Green radiating filaments of the photobiont *Phycopeltis* sp. are faintly visible near the center. Bar = 35 µm.

Figure 17. Germinating discoid lichenized structure attributed to *Phyllophiale* sp. Fungal hyphae developing from radial fringe, enveloping apparent germling of *Phycopeltis* sp. (arrow). Green filaments of *Phycopeltis* (P) visible near center of disk. Bar = 15 µm.

Figure 18. Germinating discoid lichenized structure attributed to *Phyllophiale* sp. End of central stalk-like structure (arrow) interpreted as original point of attachment to parent thallus. Bar = 15 µm.

Figure 19. Lichen pre-thallus containing *Phycopeltis*, with radiating hyphae, attributed to *Phyllophiale* sp. Dark central area (above plane of focus) with stalk-like structure represents original point of attachment to parent thallus. Bar = 36 µm.

fungal cells were also observed frequently in these clusters (Fig. 12, arrow); the diameter of the loops suggested that encircled algal cells had been dislodged from the fungus at this early stage. This implies that such co-dispersion of symbionts might also potentially contribute to aposymbiotic algal populations by escape from the fungal spores.

In other observations of coccoid green algal cells being encircled by fungal hyphae, division of the encircled algal cells were observed (Fig. 13), supporting the interpretation that these associations were lichenic in nature. Figs. 14 and 15 show much larger lichenized clusters of coccoid green algae, with radiating fungal hyphae.

Phyllophiale

The disc-shaped "isidia" of the sterile foliicolous lichen genus *Phyllophiale* were frequently found on the slides (Fig. 16). The discs germinated by radial growth from the hyphal fringe at the perimeter of the disc (Figs. 17, 18), thus confirming that these structures indeed serve in vegetative propagation. They were the earliest lichen colonizers on many of the slides. A remnant of the stalk by which the isidium had been attached to the mother thallus was often visible, nearly always on the surface facing upward (Figs. 18, 19). Although very efficient at early colonization of the slides, many of the *Phyllophiale* discs were also seen to degenerate after limited growth.

Methodological improvements, and recent results from ongoing studies

The slides fastened with tape showed in general a heavy colonization by non-lichen fungi. Lichenized associations observed on these slides did not usually continue developing beyond 2 or 3 months, and degeneration of prethalli such as those of *Phyllophiale* were often observed. It was suspected that organic materials in the packing tape glue leached onto the slide surface, perhaps favoring growth of non-lichen microorganisms. Modifications have been made in experimental design, and a much greater degree and variety of lichen colonization stages are currently being observed. Strips of plastic netting (mesh approximately 1 cm^2) were used as backing and the glass slides inserted directly into slits cut in the mesh, holding them fast without the use of tape. An even greater improvement in colonization is being obtained using plastic cover slips as the substrate. The cover slips are fastened to narrow strips of fine plastic netting (mesh approximately 2 mm^2) by inserting the corners into diagonal slits cut in the mesh. The strips of netting with attached cover slips are tied directly to leaf rachises of *Bactris* sp., an understory palm which is

frequently well-colonized by foliicolous lichens. The cover slips are inverted and wet-mounted on glass slides for observation.

A significant limitation of observing lichen ontogeny under uncontrolled conditions is that the identity of the pre-thalli observed can only exceptionally be determined with certainty. However, it is expected that as later developmental stages with characteristic structures are observed it will often be possible to make some reasonably reliable genus-level identifications by working backward to the younger stages observed.

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