The land flora: a phototroph–fungus partnership?
M-A. Selosse and F. Le Tacon

Numerous mutualistic associations between phototrophs and fungi exist in the extant land biota. Some are widespread, such as lichens and mycorrhizae, but some are less well known or restricted to special ecological conditions, such as endophytes in plants and algae. Recent molecular data and fossils suggest that associations arose repeatedly and that some of them are ancient, and even ancestral in the case of land plants. Mutualism, that provides various adaptations to terrestrial constraints, may have played a crucial role during terrestrialization and evolution of land phototrophs.

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since axenic cultures are possible, but the fungus seems to protect the alga against desiccation. The same fungus is also present in *Pelvetia canaliculata*, the most exposed brown alga in the tidal zone of temperate regions. Other mycophyceobioses have been reported in green (*Prasiola*, *Blidingia*) and red algae (*Apophlaea*). They are thus polyphyletic and probably recent, since they involve secondarily aquatic ascomycetes. Nevertheless, they suggest that a phototroph having the organizational complexity of a pseudoparenchymatous alga can take advantage of a mycobiont to survive terrestrial conditions. This could be an analogue of the first land plants.

**Box 1. Glossary of extant phototroph–fungi mutualisms**

Lichens are composed of a cyanobacterium or a green alga embedded in a vegetative stroma of an ascomycete or, less frequently, a basidiomycete. Geosiphon pyriforme (Fig. 1) is a non-septate fungus that harbors intracellular cyanobacteria.

Mycophyceobioses are composed of an ascomycete embedded in the thallus of a multicellular alga (Fig. 2).

Mycorrhiza are composed of septate or non-septate fungi within a root, but also exploring the surrounding soil (Box 2). In ectomycorrhiza, a septate fungus forms a sheath around the root (‘sheath-forming mycorrhiza’) and grows between cortical cells forming the so-called Hartig net. Ectomycorrhiza involves non-septate Glomales (VA mycorrhiza) or septate fungi (ericoid and orchid mycorrhiza). In both cases, the fungus penetrates the cortical cells where it forms respectively arbuscules or coils. Ectendomycorrhiza formed by basidiomycetes in some Ericales (e.g. *Arbutus*) shares features of both ecto- and endo-mycorrhiza, i.e. a sheath and intercellular coils.

Symptomless endophytes (often ascomycetes, such as Clavicipitaceae in grasses) live intercellularly in various plant organs. Some may be transmitted by seeds. The name mycophylla has been proposed for associations between leaves and endophytes.

Plants are ancestrally mutualistic with Glomales. Land colonization by multicellular phototrophs involved the radiation of the *Plantae* (a particular subgroup of green algae phylogenetically independent of terrestrial green microalgae) during the Silurian, with possible precursors during the Ordovician (Box 2). These phototrophs, probably derived from Charophyta, share an egg-protecting archegonium and are divided into two main lineages: Atrachaetae (mosses, hepatics and hornworts) and Tracheophytae (vascular plants). Vascular plants, but also hepatics and hornworts, have various mycobionts inhabiting different vegetative organs (Box 1).

Non-septate fungi form mutualistic associations with about 90% of these phototrophs. The fungus grows intercellularly and forms vesicles and intracellular arbuscules (Box 2). In ectomycorrhiza, a septate fungus forms a sheath around the root (‘sheath-forming mycorrhiza’) and grows between cortical cells forming the so-called Hartig net. Ectomycorrhiza involves non-septate (VA mycorrhiza) or septate fungi (ericoid and orchid mycorrhiza). In both cases, the fungus penetrates the cortical cells where it forms respectively arbuscules or coils. Ectendomycorrhiza formed by basidiomycetes in some Ericales (e.g. *Arbutus*) shares features of both ecto- and endo-mycorrhiza, i.e. a sheath and intercellular coils.

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**REVIEW**

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preserved arbuscules17 (Fig. 3) and established beyond doubt the existence of VA associations in the Lower Devonian. The intact host cell wall and the strictly cortical localization of the infection suggest a biotrophic association. It could explain how the rootless Aglaophyton coped with its mineral supply12, in a way reminiscent of the rootless mycorrhizal Pseudotom5, an extant endophyte of uncertain affinity, which resembles early land plants.

Older fossil plants are often compressed, making the presence of endophytes difficult to assess. However, some Silurian-Darwinian plants of unknown systematic affinities have been claimed to contain fungal structures10–12 (see above). Remembering the actual case of mycophycolobiases, it has often been speculated that land plants arose from a charophytic alga associated with a non-septate fungus; terrestrial plants showing VA associations27 and biotrophic associations within a charophyte3. In the Devonian, forms with free-living microphototrophic symbionts of uncertain affinity interpreted as lichens by some authors10–12, such as Silurian: rise of a multicellular land flora5, some fossils could be interpreted as lichens10–12. Earliest putative fossil associations3,5.

In the Devonian, the Glomales diversified during the late Paleozoic and the Mesozoic16 (two glomalean families, the Glomalaceae and the Gigasporaceae, are reported from Triassic cycad roots5), but the relation to the evolution of land flora remains poorly understood. In the course of plant and hepatic evolution, VA mycobionts were sometimes lost, leading to an apomictic habit. About 10% of plants, including whole angiosperm families (e.g. Juncaceae, Chenopodiaceae and Brassicaceae), are non-mycorrhizal and compensate by developing special root system architectures5. Disturbed habitats, where mycorrhizal inoculum is lacking, and wet or aquatic habitats, where access to mineral resources is easier and oxygen diffusion limits fungal growth, often harbour non-mycorrhizal species5. Mosses are the only high-rank taxon of Plantae where associations with fungi are lacking. Their low morphological and anatomical differentiation, together with polikohydric strategy, is reminiscent of the free-living terrestrial algae. This nutrient stress-tolerance is a successful strategy in some, but not all, terrestrial conditions.

Plants repeatedly interacted with septate fungi although the majority of advanced groups, such as grasses, retained the primitive VA mycorrhiza, other plants associated with septate fungi. Numerous trees and some shrubs (Gymnosperms, Gnetales and Angiosperms) form

Fig. 1. Geosiphon pyriforme, a non-ectomycorrhizal fungus related to the Glomalaceae, forms endosymbioses with photosynthetic and Nfixing Nostoc sp. (b) Bladders growing in the natural habitat on mineral soil (Fuessen mountains, Germany. Bar: 1 mm) – Courtesy of D. Willermetz. (c) Structure of a coenocytic bladder containing Nostoc filaments: 1, cell wall; 2, fungal plasmalemma; 3, internalized Nostoc filaments; 4, heterocyst; 5, fungal vacuoles; 6, peripheral cytoplasm; 7, section of a Nostoc filament; 8, mitochondria; 9, aquaporin membrane; 10, lipid droplets; 11, non-septate hypha linking the bladders. Courtesy of M. Kluge.

Box 2. Geological timescale and milestones in evolution of terrestrial phototrophs and fungi

Molecular clock data
A. Divergence between chytrids and terrestrial fungi
B. Divergence between Glomalaceae and other Zygomycotina5
C. Divergence between Ascocytotina and Basidiomycotina16
D. Emergence of non-mycorrhizal, among which extant ectomycorrhizal basidiomycotina15

Fossil record
i Pre cambrian: fossil palaeosols with free-living microphototrophic fossils of uncertain affinities interpreted as lichens by some authors10–12, such as Silurian: rise of a multicellular land flora
ii Carboniferous: oldest ectomycorrhiza on Pinus
iii Early Devonian: Rhynie Chert flora, with a lichen involving cyanobacteria and a zygomycete; fossil vascular plants showing VA associations27 and biotrophic associations within a charophyte3
iv Middle Triassic: evidence for diversification of VA fungi from fossil cycad roots of Antarctica
v Visean: oldest definite lichen involving extant septate fungi5 and oldest ectomycorrhiza on Picea11
Other root associations with septate fungi involve more-restricted plant taxa. Ericales associate intracellularly with fungi, mainly ascomycetes, forming endo- or ectomycorrhizae (Box 1 and Fig. 4). These associations allow utilization of dead organic matter and the successful colonization of heathland soils, where nitrogen and phosphorus are slowly or not mineralized. Intracellular coiling-forming associations with basidiomycetes occur in the Orchidaceae (Box 1); orchids are completely dependent on fungal carbohydrates at the protocorm level, and throughout life in achlorophyllous species. Both orchid and ericalean associations probably arose early in the evolution of the host taxa, in which they are highly frequent, but this remains difficult to date precisely. Mycohallus with features similar to orchid and ericalean mutualisms are also known in hepatics: basidiomycetes intracellularly colonize some Jungermanniales and Metzgeriales in peaty soils; some Jungermanniales found on organic-rich soils host ascomycetes identical to ericoïd fungi, infecting rhizoids and flagelliform subterranean axes (Fig. 4). A lateral transfer of symbiont, rather than a common ancestral association, is likely to explain such similarities. Other specialized root associations may exist; for instance, some plants of high-arctic and alpine environments are often associated with the so-called ‘dark-septate fungi’.

To summarize, as formerly hypothesized with regard to lichen evolution, septate mycobionts arose secondarily in plant evolution. This diversification of mutualism allowed colonization of new terrestrial ecosystems mainly by exploiting the organic matter of the environment. Finally, several species evolved in a heterotrophic habit with the help of mycorrhizal fungi. Plant ‘mycotrophy’ arose in monocots and dicots, but also in subterranean perennial gametophytes of some pteridophytes and in some hepatics, entailing a loss of photosynthetic capacity. The septate, or more rarely non-septate, mycobiont exploits either soil resources or other photosynthetic partners through mycorrhizal association.

However, mutualism between plants and septate fungi extends far beyond trophic relationships within root tissues. Symptomless endophytes, mainly ascomycetes, living intercellularly in plants, are increasingly being discovered. They have been extensively studied in grasses and sedges, where clavicipitaceous endophytes proved to be mutualistic. This symbiosis is interpreted as a defence mutualism, thanks to fungal alkaloids. The association was also shown to improve nitrogen utilization and drought resistance through enhanced osmotic adjustment and lower stomatal conductance. Other endophytic associations with fungi, particularly in the woody species, may await discovery: although latent pathogens and saprotrophs could be represented, some endophytes that do not colonize dead tissues may represent true biotrophs, that could protect their host against pathogens. Conversion of a pathogenic ascomycete into a symptomless and protective endophyte was shown to require a single mutation, and may thus be a rather common event.

Why 1 + 1 > 2 in terrestrialization

Adaptation to terrestrial life has often arisen through symbiosis, which brought together genetic material having different and complementary characteristics. The two partners are partly ‘preadapted’ to live on land. The mycelial habit is well adapted to three-dimensional exploration of the substrate. Some mycobionts have a huge weathering potential, that allows access to non-soluble mineral elements, or even saprotrophic ability, that compensates for the low availability of mineral resources in most soils. On the other hand, photosynthetic partners are well preadapted to gas exchange and photon absorption, as they are well adapted to three-dimensional exploration of the environment. This combination of two different and complementary adaptions results in a overflow of productive capacity, which corresponds to the increase of 1 + 1 > 2.

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The symbiotic association is thus well-suited for exploiting the interface between soil and atmosphere. The emergence of new (symbiobiotic) properties in the symbiotic association also contributes to the success of mutualism in land ecosystems. Symbiosis entails reciprocal modifications of both partners: hormone-like substances induce morphological changes in both parasitic (tumors and galls) and mutualistic symbiobioses, as described in mycorrhiza. Slow-growing and perhaps less tolerant of pathogens and ecologists of terrestrial phototrophs is rising among physiologists and these various associations. Awareness of the symbiotic status of mycorrhizae. New fossil data, mainly from early land extreme environments (e.g. mycophycobioses, lichens and eriogynes) allowed terrestrialization (e.g. VA symbiosis in plant emergence) of major consequence for vascular plants. Symbiosis-related modifications are also evident at the physiological and biochemical levels, for instance improvement of drought-resistance by systemic endophyte infection of grasses and fungi in mycophycobioses. Reciprocal induction of genes and metabolic pathways may lead to their constitutive expression, as exemplified by lichen products (aromatic compounds that are synthesized by the fungus only in the presence of the algal partner) or plant phenylpropanoids (such as flavonoids that are elicited by mycobionts such as VA fungi or ECM). Both kinds of compound improve resistance to various stresses, mainly UV radiation and parasitic or grazing organisms. Systemically induced defensive compounds could have been of significant advantage for primitive land associations, which were presumably slow-growing and perhaps less tolerant of pathogens and grazing. Stimulation of phenylpropanoid metabolism may enhance protection and increase opportunities for new entities with new abilities, was repeatedly used during evolution in many taxa and is rising among physiologists and ecologists of terrestrial phototrophs.

Acknowledgements
We thank D.G. Garbary, M. Kluge, J. Kohlmeyer and T.N. Taylor for helpful discussion, and the authors who provided illustrations. We are grateful to K.A. Priznicky for critically reading the manuscript. M.A. Selosse is on leave from École Nationale du Génie Rural, des Eaux et des Forêts.

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Can elevated CO₂ affect secondary metabolism and ecosystem function?

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It is widely known that the atmospheric concentration of CO₂ has risen about 30% from the pre-industrial concentration and it is continuously rising because of human activities. This increase is likely to affect biota indirectly, via climate change and directly, by producing changes not only in plant growth and allocation but also in plant tissue chemical composition. Among such composition changes, decreased leaf N concentration has been widely reported. The concentration of carbon-based secondary or structural compounds (CBSSC) has been shown to increase in response to elevated CO₂ concentrations. These changes may have far-reaching consequences for herbivory and plant litter decomposition. Recent experimental results provide evidence of increases in concentrations of soluble phenolics and condensed tannins but not terpenes. They also show significant effects of these plant chemical changes on herbivores and litter decomposition. However, there is no consistent evidence of any of these effects at the ecosystem level.

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