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When It Comes to *Clethra*: Roots Matter

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Roots, too often, are out of sight and out of mind, but they are critical for vigorous, healthy plant growth. All plant enthusiasts—including gardeners, farmers, foresters, and naturalists—should think about and appreciate roots if they wish to acquire a holistic understanding of plant biology. This article introduces readers to the mycorrhizal roots of the 2015 VNPS Wildflower of the Year, *Clethra alnifolia* (Sweet Pepperbush), and explores the diversity of mycorrhizae in a closely related family, Ericaceae.

Mycorrhizae, literally "fungus roots," are symbiotic associations between microscopic threadlike fungal cells (hyphae) and the superficial tissues of roots. Although structural details vary (Figure 1), mycorrhizae always involve the commingling of fungal and plant cells, allowing the symbionts to exchange vital materials. Most plants, being photosynthetic, produce sugars in leaf tissue; these sugars (and sugar-derived molecules) are transported to the roots, a process essential to keeping root cells alive. But some excess organic compounds exude from root cells and are readily taken up by the fungal partner. The fungus's hyphae permeate the soil, breaking down complex organic matter and absorbing the simpler breakdown products as a fundamental component of their nutritional process; fungal activity also liberates inorganic minerals (like nitrogen and phosphorus, which they also absorb. At the mycorrhizal interface, the fungus secretes excess minerals, which are taken up by the plant. In essence, the plant feeds the fungus and the fungus provides mineral nutrients to the plant. Both partners benefit, hence the symbiosis is mutualistic.

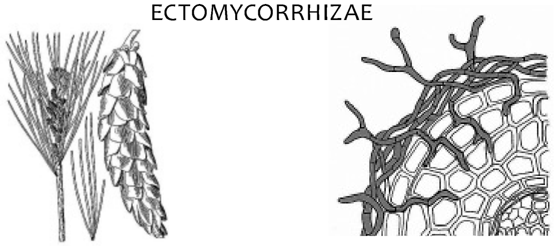
I have not been able to locate any detailed information about the roots of *Clethra alnifolia*, but there is a study of the mycorrhizal roots of a close relative, *Clethra barbinervis*, Japanese Clethra (Kubota et al., 2001). We can assume that the roots of *C. alnifolia* are similar, not only because of the close relationship between the species, but also because the type of mycorrhizae in *C. barbinervis* is, by far, the most common type. *Clethra* possesses arbuscular mycorrhizae (AM), also called endomycorrhizae. These are formed by microscopic glomeromycete fungi whose hyphae enter parenchyma cells of the root cortex and form profusely branched masses resembling small trees (hence, arbuscular). These arbuscule-containing plant cells are where water, nutrients, and organic molecules are exchanged between plant and fungus. Studies in many plants indicate that arbuscules are temporary structures, persisting for a week or two before being resorbed. Thus, arbuscule formation is a more or less continuous process in AM roots. In the roots of *Clethra* and in many other (but not all) AM roots, the fungus also forms bladderlike vesicles that come to occupy most of the cell volume.

Arbuscules have been observed in fossils of some of the oldest known vascular plants, dated at around 400 million years ago. In fact, many believe that the arbuscule type of mycorrhizal association was essential for the successful colonization of terrestrial habitats at about that point in time. It is estimated that 80 percent of vascular plants today have arbuscular mycorrhizae. Interestingly, the fungi of AM are somewhat promiscuous, capable of pairing with diverse plant hosts, and some plant roots may harbor more than one species of AM fungus. Nevertheless, the glomeromycetes are obligate symbionts, incapable of laboratory culture separate from their plant partner. The number of glomeromycete species that form arbuscular mycorrhizae is relatively small. Overall, mycorrhizal roots of *Clethra* are typical, common, and likely to involve several species of these inconspicuous glomeromycete fungi.

Another widespread fungus–root symbiosis is known as ectomycorrhizae. In contrast to AM, ectomycorrhizae involve basidiomycete fungi that mostly form a mantle on the surface of the partner's roots; some hyphae may extend between plant cells near the surface of the root, but they do not penetrate the interior of these cells. These basidiomycetes are mushroom-forming fungi, and their plant partners include conifers like pine and fir, as well as a number of temperate-zone deciduous/eudicot trees such as willow, poplar, beech, birch, and oak. Only about 3 percent of plant species have this type of mycorrhizae. In contrast to AM, ectomycorrhizal associations are species-specific, which explains why the identification of woodland mushrooms is often facilitated by taking note of tree species in their immediate vicinity. The fungal mantle covering ectomycorrhizae causes these roots to be noticeably thick and stubby, sometimes characterized as coralloid.

Clethra is closely related to Ericaceae. Although *Clethra* has common AM, its heath relatives have several different, intriguingly unique forms of mycorrhizae. Most Ericaceae have a type of mycorrhizae reminiscent of AM, at least to the extent that the fungi enter plant cells, but there are

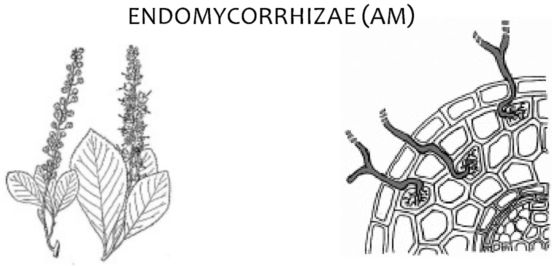
ECTOMYCORRHIZAE



Conifers, beeches, birches, oaks, poplars, willows. (Shown: pine)

Basidiomycetes. Fungal mantle on root surface and sometimes between cortex cells

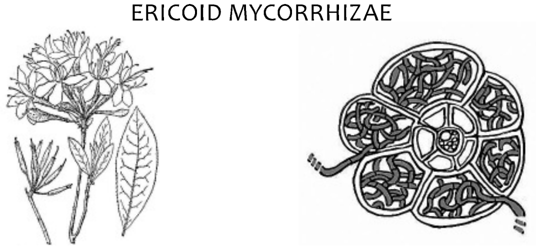
ENDOMYCORRHIZAE (AM)



Most plants. (Shown: Clethra*)*

Glomeromycetes. Hyphae enter cortex cells and form arbuscules.

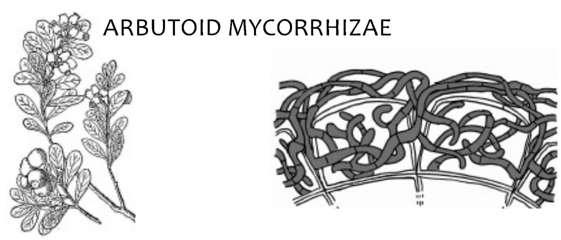
ERICOID MYCORRHIZAE



Most Ericaceae. (Shown: Rhododendron*)*

Ascomycetes. Convoluted masses of hyphae in surface cells.

ARBUTOID MYCORRHIZAE



Arbutus, Arctostaphylos, Pyrola. *(Shown:* Arctostaphylos*)*

Basidiomycetes. Fungal mantle on root surface plus convoluted masses of hyphae in surface cells.

MONOTROPOID MYCORRHIZAE

Hypopitys, Monotropa, Monotropsis. *(Shown:* Monotropa*)*

Basidiomycetes. Fungal mantle on root surface plus peglike insertions in surface cells.

Figure 1. Diversity of mycorrhizal symbionts discussed in the text. Plant illustrations from Britton and Brown's *Illustrated Flora of the Northeastern United States and Canada* (1913). Drawings of mycorrhizae by W. J. Hayden.

manifest differences. Ericaceous roots are notably slender, each consisting of a small central vascular strand, a single ring of small cortex cells, and one layer of large epidermal cells. Roots of most other plants have far more vascular and cortex cells. Also, the fungi involved are ascomycetes (sometimes called cup or sac fungi) and enter only the epidermal cells, where the hyphae form tightly curled, convoluted masses but no arbuscules or vesicles. Cortex cells remain fungus-free. Further, the ascomycete fungi of ericaceous roots are facultatively mycorrhizal—they can also exist as free-living soil microbes and can be grown alone in laboratory cultures. Plants of Ericaceae are well known for their ability to exploit soils so acidic that organic matter decomposes very slowly, resulting in the release of only meager amounts of the mineral nutrients essential for plant growth. Evidently, the special ascomycete fungi of ericaceous mycorrhizae provide these plants with critically needed amounts of nitrogen and phosphorus, allowing them to survive and even dominate peaty, acidic environments.

In addition to the widespread form of ericaceous mycorrhizae described above, this family has at least two other forms, somewhat intermediate between the typical ericaceous mycorrhizae and ectomycorrhizae. *Arbutus* (Strawberry Tree, Madroña), *Arctostaphylos* (Bearberry, Manzanita), and *Pyrola* (Shinleaf) share the so-called arbutoid form of mycorrhizae. As in ectomycorrhizae, there is an external mantle of basidiomycete fungi, but the hyphae that extend into the root grow between epidermal cells and enter some of the surface cells, where they form tightly coiled masses of hyphae. The tightly coiled hyphae of arbutoid mycorrhizae resemble the ericaceous type, despite being formed by a fungus of a different phylum.

The other form of mycorrhizae found in the heath family is restricted to a group of genera that have given up quintessential features of plant life: they have no chlorophyll, cannot photosynthesize, and, hence, are heterotrophic, not autotrophic. As distinguished in the *Flora of Virginia,* our local representatives of this odd group include *Hypopitys, Monotropa,* and *Monotropsis* (pinesaps and Indian pipes). These plants were once considered saprophytes, implying that they obtained their nutrition directly from the decomposition of organic matter in the soil. The true story became clear with the discovery that roots of these plants are sheathed with a mantle of basidiomycete fungi. As with arbutoid mycorrhizae, the fungus also enters surface cells, but penetration is restricted to a simple peglike hypha. Another distinction is that the fungi involved in these monotropoid mycorrhizae also form typical ectomycorrhizae with nearby coniferous trees. The fungus and conifer share the usual symbiotic relationship described earlier. But since Indian pipes and pinesap plants lack chlorophyll and so make no sugar of their own, it is hard to imagine what these plants provide to the fungus. The inescapable conclusion, now backed by experimental evidence, is that they obtain all their minerals and organic molecules from their fungal partners, giving back nothing in return. The plant parasitizes the fungus and, to some extent, steals from the conifer via the intermediate fungus. The term describing the unusual mode of existence exploited by Indian pipes and pinesaps is mycoheterotrophy.

If the basic idea of mycoheterotrophy is enough to make your head spin, consider that, while not exactly common, this mode of plant–fungus interaction crops up, here and there, across the breadth and depth of the plant kingdom. Examples are known from a few liverworts, some lycophytes (clubmosses), and a few ferns (all of them from haploid gametophyte generations); further, among flowering plants we also have two eudicot and eight monocot families, including, famously, *Corallorhiza* (coral root) and other orchids. But none of these other mycoheterotrophs are particularly closely related to *Clethra*.

Clearly, Ericaceae have been a hotbed of mycorrhizal diversification characterized by extremely fine-textured roots inhabited by ascomycete and some basidiomycete fungi that are otherwise not known to associate with higher plants. But one of their closest relatives, *Clethra,* conforms to the most common, prosaic form of mycorrhizal association, AM. How could an apparently ancient and eminently successful mutualistic relationship (exemplified in *Clethra*) have been abruptly replaced by different fungi in the fine-textured roots of ericaceous plants? I don't have an answer! This is a mystery. But I do have a small suggestion. Someone needs to examine the roots of *Cyrilla* (Ti-ti, Cyrillaceae), because this genus is also closely related to Ericaceae, and the mycorrhizal status of its roots, as far as I can tell, is also unknown. Will *Cyrilla* have ordinary AM, or one of the specialized types found in Ericaceae, or some intriguing intermediate? Might *Cyrilla* provide insight into what drove the shift from AM to the much less common forms of mycorrhizae found in Ericaceae?

Certainly there are lessons to be learned by looking under the surface of things. Roots matter. It is, indeed, important to remember roots! ❖

The literature on mycorrhizae and similar fungus–plant mutualisms is vast. These sources were useful in composing this article:

Kubota, M., T. P. Mcgonigle, & M. Hyakumachi. 2001. *Clethra barbinervis* in the Ericales forms arbuscular mycorrhizae. Phytopathology. 91(6 Supplement): S118, E.

Merckx, V. S. F. T., ed. 2013. Mycoheterotrophy: The Biology of Plants Living on Fungi. Springer, New York.

Moore, D., G. D. Robson, & A. P. J. Trinci. 2011. 21st Century Guidebook to Fungi. Cambridge University Press, Cambridge.

Stephenson, S. L. 2010. The Kingdom Fungi: The Biology of Mushrooms, Molds, and Lichens. Timber Press, Portland.