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THE WONDERS OF ORCHID MYCORRHIZAL ASSOCIATIONS

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Perhaps like many readers, my initial interest in orchids was in their beauty and diversity, not their reproduction and mycorrhizal associations. Then, as a mycology student, I learned that orchids have tiny seeds that depend upon infection by a fungus to germinate and that their mycorrhizae differ from those of other plants in their morphology and fungal partners. However, with so many fungi and so little time, I was happy to let others study orchid mycorrhizae—Mark Brundrett, Tom Bruns, Randy Currah, Larry Peterson, and their students, to name a few. Then along came an article by mycologist Andrus Voitk, pointing out that showy lady slipper orchids (*Cypripedium reginae*) were almost exclusively found growing around black ash trees (*Fraxinus nigra*) in his area of Newfoundland. I got involved—or, in true academic fashion, I got *other people* involved (Newfoundland botanists Henry Mann and Dmitry Sveshnikov to sample, and undergraduate students Zoe Chatzidakis, Katarina Kukolj and Rebecca Spencer to do the lab work and analyses)—and became interested in the fungal associations of orchids and other plants growing nearby. As a result, what I offer here is not based on years of my own research, but on gleanings from the work of others and some recent results of my students' studies.

First, the basics. The orchids (*Orchidaceae*) are one of the most species-rich families of plants, with about 28,000 described and accepted species worldwide (Christenhusz and Byng, 2016). Much of that species diversity is in the wet tropics, where orchids are both abundant and diverse as epiphytes growing on the trunks of trees in lowland rainforests and highland cloud forests. In Ontario, Canada, where I live, there are just over 60 species of orchids; all are terrestrial (grow on the ground, not as epiphytes) in moist forests or wetlands such as bogs and fens. Although some species were never common, many are now rare because of habitat loss, and six species are listed as threatened or endangered in Ontario. All species of orchids share a common fact of biology: none of them could reproduce in nature without the help of a fungus. Orchids produce some of the smallest seeds in the plant kingdom, most the size of a single grain of fine table salt. These seeds may be great at dispersal (especially if carried on breezes by thin, papery wings) but they bring no food reserves to power germination and growth. The energy and nutrients required for germination are provided by a fungus that penetrates the moist seed. At this stage, the plant is a nutritional parasite of the fungus: sugars,

nitrogen, phosphorus, potassium, water, and other nutrients are provided by the fungal partner. From a mycological perspective, what is very odd about these orchid associates is that they are not the typical ectomycorrhizal mushrooms (*Amanita*, *Boletus*, *Tricholoma*, etc.) that are associated with our pines, birch, and oaks, nor are they the *Glomeromycota* (*Glomus* and *Rhizophagus*, etc.) that form arbuscular mycorrhizal relationships with most other vascular plants. Instead, the fungal partners of germinating orchids range from known plant root pathogens such as *Rhizoctonia* or *Armillaria* to wood-decomposing fungi such as the polypores *Phellinus* (*sensu lato*) and *Physisporinus* (Leake, 2005). Why? What brings these fungi to penetrate an orchid seed and then stick around to provide hard-won sugars and nutrients that were stolen from other plants or obtained from decomposing wood to allow the seed to germinate?

Parents and biologists hate that three-letter word question: “Why?” It’s right up there with “Are we there yet?” Two explanations seem plausible. Sometimes, the fungus that helps the seed germinate persists as the mycorrhizal associate when the orchid grows into an adult plant, a process that can take several years. Then, if the orchid is photosynthetic, it can repay the loan of sugars used in germination and early growth with sugars it can now produce through photosynthesis. This explanation could be thought of as an “investment strategy” on the part of the mycorrhizal fungus. Of course, not all investments are successful: some orchids may never repay their loan because they may not survive to adulthood, or they may be one of the species that does not produce chlorophyll so they can never photosynthesize and will always be a parasite on one or more fungi. The other possibility is the “good things come in small packages” explanation: the tiny orchid seed may provide the invading fungus with a rare and valuable vitamin or growth factor such as PABA, or para-aminobenzoic acid. Perhaps, having tricked the fungus inside with a tasty offer, the orchid then regulates and enslaves its fungus to continue the supply of energy and nutrients.

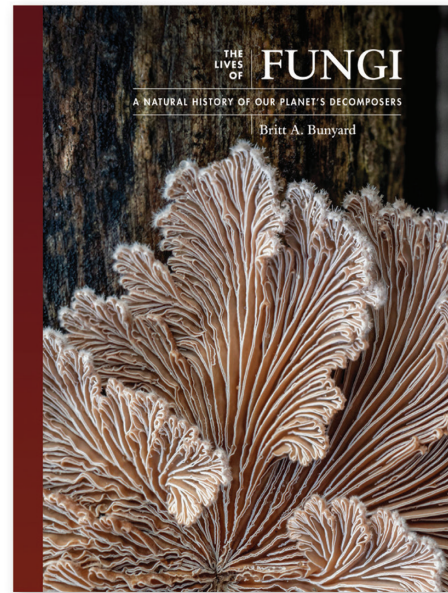
As orchids grow into adult flowering plants, some keep their fungal associates from the germination stage, but the most common associates of our temperate terrestrial orchids are fungi of the *Rhizoctonia-Tulasnella* (*Ceratobasidiaceae*) or *Sebacina-Serendipita* (*Sebacinales*) groups. Both the plant and fungal partners may be either general or very specific in their associations. The association itself takes the form of fungal hyphae penetrating orchid root cortical cells and forming hyphal coils (pelotons) inside. Bidirectional nutrient exchange (photosynthate sugars and possibly vitamins from plant to fungus, and water, nutrients and possibly sugars from fungus to plant) takes place across the plant plasma membrane surrounding the hyphal coils. Then after some time, old hyphal coils appear to be digested and the nutrients contained taken up by the plant, with new hyphal coils formed in other cortical

cells. Non-photosynthetic, achlorophyllous orchids such as the spotted or striped coral-root orchids continue to rely on their fungal partners for sugars, which their fungal partners obtain from other mycorrhizal associates or from the decomposition of wood and other plant litter. These are true parasites of a fungus, a nutritional mode described as mycoheterotrophy (myco—from a fungus; hetero—from elsewhere; trophy—feeding). Studies have shown that even chlorophyllous, photosynthetic orchids that live in shady environments may rely on their fungal partner for a continued supply of sugars into adulthood, a nutritional mode named mixotrophy (food from mixed sources).

And this is where things get interesting. My students found that the roots of showy lady slipper orchids (which should in theory have only orchid mycorrhizal fungi such as *Ceratobasidiaceae* or *Sebacinales*) and the ash tree (which should in theory have only arbuscular mycorrhizal fungi such as *Glomus* and other *Glomeromycetes*) shared root-associated fungi of many types: root pathogens, ectomycorrhizal fungi, and arbuscular mycorrhizal fungi (Weerasuriya et al., 2022). What we did *not* show was any evidence of nutrient or energy transfer among the partners, but these findings raise that good old question, “Why?” What are these fungi doing in close association with the roots of plants that they are not “supposed to be” associating with, and is there anything in their presence that could explain the initial observation that the orchids frequently grow around the ash? Please stay tuned. We now know that many plants may simultaneously or sequentially form both arbuscular and ectomycorrhizal associations (Bunyard, 2020) and that fungi that are not known to be mycorrhizal can closely associate with the roots of famously non-mycorrhizal plants in the mustard family and provide growth benefits to the plants by making mineral phosphate available to the plant roots (Almario et al., 2017). It seems likely that many fungi did not read the mycology texts that define mycorrhizae by specific plant root morphologies, and that the true nature of underground nutritional networks may be much more complicated than we have realized.

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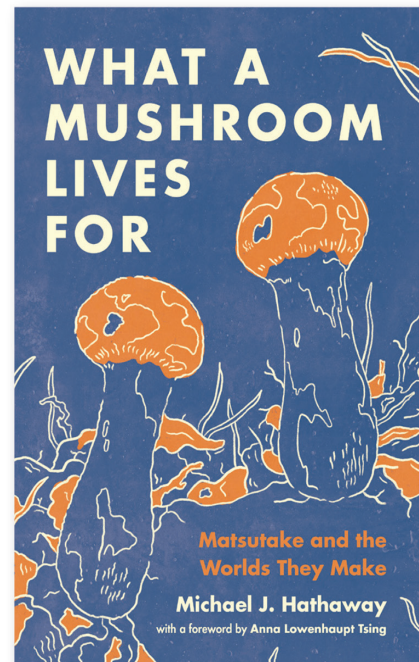
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