Reading Tree Roots for Clues: The Habits of Truffles and Other Ectomycorrhizal Cup Fungi

Rosanne Healy

Here's something to ponder: The health and regeneration of grand old oaks (*Quercus*) and majestic pines (*Pinus*) is dependent on the well-being of tiny fungi that associate with the trees' roots. Such small organisms have a big role to play not only for oaks and pines but also for many other trees that rely on their fungal partners to get them through lean and dry times. An estimated 86% of plant species benefit from (or are even dependent on) fungal root associates that transfer water and nutrients to the plant in exchange for carbohydrates (Brundrett 2009). Carbohydrates from plants are the result of atmospheric CO_2 (carbon dioxide) fixation through photosynthesis and subsequent processes, which the fungi are incapable of doing.

The fungal root associates are the mycorrhizal (myco=fungus, rhiza=root) fungi. They can be roughly sorted into two types based on how they associate with the roots. One type is mostly invisible to us because their hyphae are inside the root (endomycorrhizae), and the other can be seen as a mantle surrounding the root tip (ectomycorrhizae). The endomycorrhizal fungi are root associates of the vast majority of herbaceous plants and certain tree species. This article focuses on ectomycorrhizal fungi, which grow mostly in association with trees rather than herbaceous plants. They make their presence known to us not only because we can see them on tree roots but also because we see their fruiting bodies, particularly from midsummer into fall here in New England.

NANCY ROSE

Trees such as the red oaks (*Quercus rubra*) and eastern white pines (*Pinus strobus*) seen here benefit from ectomycorrhizal fungi.

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The color and "furry" appearance of this ectomycorrhizal red oak root tip are from the fungal symbiont, a *Scleroderma* fungus.

The ectomycorrhizal root tips (top) and fruiting bodies (bottom, at several stages of maturity) of the basidiomycete fungus *Cortinarius armillatus.*

Which fungi are they?

Thanks to ever more ingenious methods of molecular fingerprinting of fungi, and a growing database of DNA sequences for fungi of all kinds, we now know much more about what species are involved in these relationships. The ectomycorrhizal fungi include some of the largest and most colorful of the fleshy basidiomycete fungi like *Cortinarius* and *Russula,* as well as prized edibles like the king bolete and chanterelle, and deadly poisonous species such as the death cap, *Amanita phalloides.* Far less is known about the cup fungi that form ectomycorrhizae, despite their long history of study.

The term "mycorrhiza" was coined by botanist Albert Frank in 1885 while he studied the relationship of *Tuber,* a truffle cup fungus, with its host tree roots in order to determine how to cultivate this gastronomically important fungus. He and his student, Albert Schlicht, discovered that the majority of apparently healthy plants that they surveyed in Germany had fungal root associates. Frank was the first to hypothesize that the fungi observed on roots were mutually beneficial with the trees rather than parasitic (Trappe 2005), a hypothesis that has since been borne out by many studies.

Most truffles, including the economically and gastronomically important *Tuber* species that interested Frank, are ectomycorrhizal. I have been studying *Pachyphlodes,* a common but generally ignored truffle genus, for the past 15 years. During these studies I collaborated with Harvard University Herbaria cup fungus experts Don Pfister and Matthew Smith (now at the University of Florida). We noticed that the asexual form of truffles, termed sporemats here, occur most abundantly on bare or nearly bare soil. This was consistent with reports that fruiting bodies of ectomycorrhizal Pezizales (the nomenclatural order for cup fungi) tend to occur in disturbed habitats such as dirt paths or roads in the forest (Petersen 1985). I am now working with Don Pfister to test the hypothesis that ectomycorrhizal Pezizales are more prevalent in managed rather than natural environments. To do this, we are comparing the ectomycorrhizal fungi on roots of red oaks (*Quercus rubra*) in the Arnold Arboretum with those on red oaks in Harvard Forest.

A Tale of Two Sites

Why choose these two sites for this study? There are some important differences between the Arboretum and the Harvard Forest. The Arboretum habitat is more like a residential area, where much of the understory is kept clear of non-cultivated plant life and the grass is kept short. The soil organic layer is comparatively shallow, and there is not much variety in the litter layer.

In contrast, the forests here in New England are characterized by an understory of regenerat-

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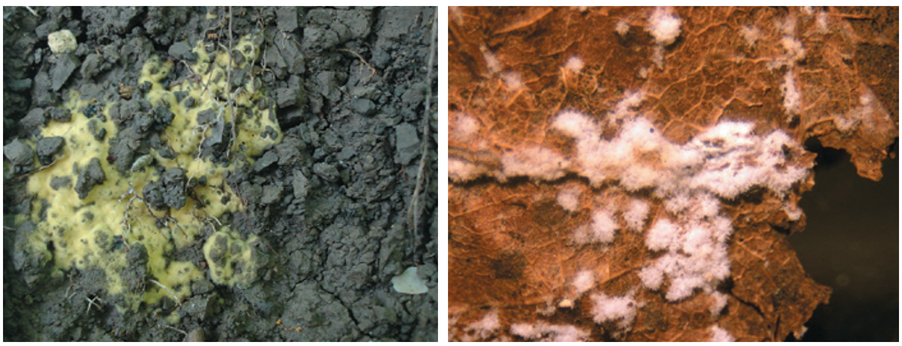
Research indicates that the ample foot paths, mowed lawns, and sparse understory in the Arnold Arboretum will favor Pezizales fungi on the root tips of the ectomycorrhizal trees.



Sporemats of truffle fungi *Pachyphlodes* sp. nov. (left) and *Tuber* sp. nov. (right).

An example of a sporemat and the truffle (*Pachyphlodes ligericus*) that its fungal barcoding sequence matches.

ing trees, native shrubs, vines, and herbs. The ground under the trees is covered by woody and leafy litter, and under that layer is a deep organic layer composed of roots, soil, and partially broken down organic matter that together form a dense mat that requires a knife to cut through it.

Compared to the forest habitat, there is not much in the Arboretum habitat to obstruct the passive transfer of fungal spores produced on the soil surface to roots and mycelia in or below the organic layer. This is possibly an important feature for the cup fungi because in order to fruit, the hyphae of outcrossing species such as *Tuber* must come in contact with a compatible mating type nucleus in another hypha. This is in contrast to most ectomycorrhizal basidiomycete species that form their mycelia with both nuclei soon after germination of their sexual spores. How do compatible mating types of truffles get together if the mycelia are underground? Perhaps the sporemats on the soil surface play a role in this event. If so, mating may be facilitated in an environment such as that found in the Arboretum over that found in a forest.

Ectomycorrhizal basidiomycete fruiting bodies (top) and their root tips (bottom) from (left to right) *Amanita rubescens, Craterellus fallax,* and *Scleroderma areolatum.*

Let's explore that idea a bit. The sporemats are produced on the soil surface, presumably from the ectomycorrhizal roots below the soil surface. They in turn produce massive numbers of spores that are small, light colored, and thin walled, and therefore probably not designed to function as survival structures. We don't know what their function is, but it makes sense that they might be involved in the mating of truffles and other cup fungi that produce them. With this in mind, as part of the study of ectomycorrhizal communities, we also collected sporemats and fruit bodies in the vicinity of the trees we sampled from.

Fungus Findings

In order to determine what species are on the roots of the trees we sampled, we utilized a technique that yields the nucleotide sequence of the fungus genome from a nuclear region that is known to mutate quickly enough to show differences in nucleotides between species, but not so quickly that they differ much within species. This region of the genome is not a coding region, and therefore, the mutations have no known impact on reproduction. It is called the internal transcribed spacer region (ITS), and is one of the most useful for studying species limits in the fungi. In fact, this region was recently adopted as the first fungal bar code marker in the recently updated International Code of Nomenclature of algae, fungi and plants (McNeill et al. 2011). There is sufficient data from this genome region available in the National Center for Biotechnology Information (NCBI) that are deposited from national and international studies to be able to place most of the sequences from our study within a genus, and in some cases feel confident about the species, or to tell if it is likely an un-named (in NCBI) species. We can also compare our sequences with others in NCBI from a geographic locality perspective, and thus analyze the likely origins of the fungi on the root tips in our study to decide whether they are native or non-native.

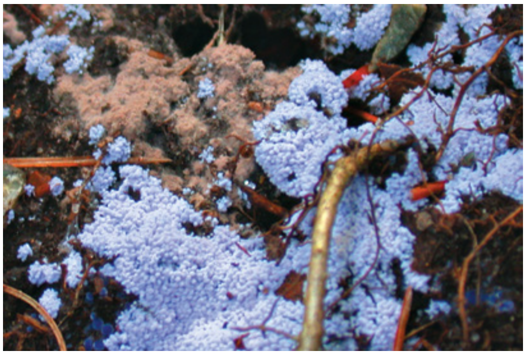
While our study is not yet complete, I would like to share several interesting vignettes that have come to light. Basidiomycetes were the most frequently sequenced from the root tips in both habitats with 59 molecular taxonomic units (MOTUs) from Harvard Forest and 56 MOTUs from the Arboretum, 17 of which overlapped in both sites. Some MOTUs could be matched to sequences in GenBank from described species or at least sequenced fruit bodies. *Russula* species were the most frequently sequenced in both habitats with 32 MOTUs. A number of our other sequences matched *Russula* sequences from a previous study by Don Pfister and Sylvia Yang, but not sequences of any described species. A distant second place for most commonly sequenced genus was *Cortinarius* (14 MOTUs) followed by *Lactarius* (9 MOTUs). Even less common (genus followed by MOTUs within parentheses): *Amanita* (4), *Boletus* (1), *Byssocorticium* (1), *Clavulina* (4), *Craterellus* (1), *Entoloma* (3), *Inocybe* (4), *Laccaria* (1), *Piloderma* (1), *Pseudotomentella* (1), *Scleroderma* (2), *Sistotrema* (1), *Strobilomyces* (1), *Tomentella* (7), *Trechispora* (1), and *Tylopilus* (1).

This *Russula* fungus (fruit body and root tip shown) has a sequence that matches root tips in this study, as well as root tips and fruit bodies from a 2006 study by Don Pfister and Sylvia Yang in which they determined that many *Russula* species are exploited by the Indian pipe plant, *Monotropa uniflora.*

Nearly equal numbers of Ascomycete MOTUs were sequenced from each site. However, there was little overlap in species. It is particularly interesting that the Pezizales had significantly greater species richness and number of root tips in the Arboretum (10 MOTUs) than in the Forest (3 MOTUs). The cup fungi detected on roots in the Arboretum included *Hydnotrya*, four species of *Pachyphlodes*, three species of *Tuber*, and two root tip sequences that have no match to a fruit body sequence. From Harvard

Ectomycorrhizal ascomycete fruiting bodies (above) and their root tips (below) from (left to right) *Elaphomyces muricatus, Pachyphlodes* sp. nov., and *Tuber separans.*

Fruiting bodies of *Leotia lubrica*, commonly known as jelly babies, were found in Harvard Forest.



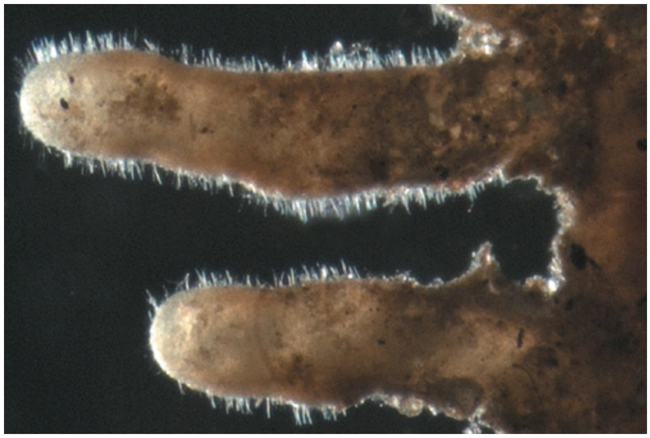
The researchers sequenced this unusual blue sporemat, which may be *Chromelosporium coerulescens* or a related species.

The distinctive black ectomycorrhiza of a *Cenococcum* fungus.

Forest we detected *Leotia lubrica* (commonly known as jelly babies) and *Elaphomyces* (hart's truffle). Cup fungi detected included *Tuber separans,* and the same species of *Tuber* (species 46) as found in the Arboretum. We also recovered a sequence that matches that of a lovely blue sporemat for which no fruiting body is known. This sporemat may be *Chromelosporium coerulescens* or a close relative. *Cenococcum,* an ascomycete not known to make a fruiting body, but with a very characteristic black ectomycorrhiza was ubiquitous on roots in both habitats.

We collected a number of truffle sporemats on the soil surface in the Arboretum, but in Harvard Forest they were found on top of the leaf litter, and even on the lower trunks of trees. Although we know from other ectomycorrhizal root studies that these species colonize roots, few of their sequences were detected on the roots sampled in this study, and none of their fruiting bodies found. The only evidence of their presence using our sampling technique was their sporemats. This may be because the Pezizales tend to be patchy in their colonization of roots, so they could easily be missed during sampling. The fact that they developed on the surface of the substantial organic layer in the forest shows that the originating mycelium is capable of navigating through the root mat and litter layer from the root tip. Where do the spores from the sporemat go and to what purpose? We don't know. We now see that they are quite capable of being formed atop heavy woodland litter, but we don't know how efficient their dispersal and ultimate journey into the soil is in either a forest or arboretum-like setting.

A second mystery came to light when one of the *Tuber* species detected on roots of a native red oak in the Arboretum was nearly identical in sequence to a species native to Europe, *Tuber borchii.* To our knowledge, this species has never been detected outside of cultivation in North America. Hannah Zurier, a Harvard undergraduate, received a Microbial Sciences Initiative fellowship to (in part) attempt to reconstruct how this truffle came to reside in the Arnold Arboretum. She found the truffle



The fruiting body and root tips of the newly named *Tuber arnoldianum*.

again on the same tree, and is in the process of looking for it on other trees in the vicinity.

A third interesting story involves another *Tuber* species. We detected a species (termed "species 46" by Tuberaceae expert Gregory Bonito, a mycologist at the Royal Botanic Gardens in Melbourne, Australia) on the roots of several trees scattered throughout the Arboretum, as well as from one of the trees sampled in Harvard Forest. Our sequences match those for an undescribed species, known previously only from orchid root tips in New York and red oak root tips from an urban area in New Jersey. We were fortunate to recover some fruiting bodies from the Arboretum so that we will now be able to describe this taxon. The Arnold Arboretum staff has chosen the name *Tuber arnoldianum* for this truffle.

While data are still being gathered, enough has been analyzed at this point (985 root tip sequences from 24 trees in each site) that I expect the pattern of Basidiomycete to Pezizales MOTUs in the two sites to hold up. This pattern continues to support the hypothesis that Pezizales are more prevalent in managed woodland sites such as the Arboretum. We can't be certain of the determining factors for this pattern, but refining the experimental parameters will help to zero in on those factors that are correlative. The well documented history of each accessioned tree, the ease of access to the rich information regarding Arboretum vegetation, and the encouragement and support of research by the staff at the Arnold Arboretum and Harvard Forest make these sites ideal for helping to resolve some of the outstanding questions regarding the ecology of ectomycorrhizal cup fungi.

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