

beneath us

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While our life is spent above ground level, myriads of other creatures spend theirs below it. There are those, too, who share their time between both worlds – like moles, ants and trees for example. Soil offers protection but it is also a great source for food. Plants and fungi are perhaps among the organisms which make the best out of this state of affairs as they forage underground for essential nutrients while, above, leaves suck in sunlight for energy and mushrooms ripen to spread spores. Seeking sustenance underground requires a system that can rummage through earth, like roots in plants or rhizomorphs in fungi. In the recent years, there has been much talk about tree roots which are able to form intricate networks underground. The same goes for the mycelia of fungi. One particular fungus, *Armillaria gallica*, created a buzz in the 1990s when scientists announced that they had found a colony whose rhizomorphs seemingly stretched over tens of acres. However, as rhizomorphs grow, they also spend a lot of time fending off microbes that also want to prosper. Consequently, fungi yield numerous antimicrobial products, among them: melleolides, whose synthesis depends on an enzyme known as protoilludane synthase, or PRO1.



Fairy Ring, by Amy Ross

Courtesy of the artist, www.amyross.com

Armillaria gallica and related *Armillaria* species are also called honey mushrooms because of its colour. Save for the patterns and hues on its body, its shape resembles the classic mushroom illustrated in fairy tales : a short stocky stem with a bulbous head. However, unlike the poisonous toadstool, honey mushrooms are edible. Found in temperate regions in Asia, North America and Europe, it is a plant parasite and attacks the roots of a broad host range of trees that have been damaged, thus gradually causing wood rot but also playing an important role in the ecosystem as it injects carbon back into the soil. It is not the mushroom *per se*, in other words the fruit body, which

attacks trees but filaments known as rhizomorphs – very similar to plant roots and from which the mushrooms bud – that zigzag their way through the soil underground to feed off roots.

In the early 1990s, scientists stumbled over a surprising colony of *Armillaria* while carrying out research on the effects that extremely low frequency radio stations, such as those used for submarine communication, could have on the environment. Further research revealed that the underground rhizomorph network, collectively known as the mycelium, from one individual *Armillaria gallica* colony seemed to stretch over an area of 37 acres – which is very roughly 15 football fields –, weigh about 95 tonnes and be about 1,500 years old. Recent molecular studies, however, have not only demonstrated that the colony is far more widespread and heavier but that it is also far more elderly; the meandering mycelium spreads underneath an astounding 75 acres, weighs 400 tonnes and has been dated back 2,500 years!

We tend to forget that beneath the ground, under mushrooms, trees or indeed any garden, there is a more or less vast and complex network continuously rooting around, literally, for nutrients. For a colony's mycelium to reach such huge proportions, it must have a system – or two – not only to make it grow but also to ward off other organisms equally eager to find nutrients in the same environment, notably microbes. Intriguingly, *A.gallica* rhizomorphs happen to be bioluminescent, and luminescence heightens when

rhizomorph growth is disturbed which may indicate that it could be a means to avert heterotrophs – although scientists suggest that it could also be used to attract insects for spore dispersal, or could simply be the by-product of another biochemical reaction.

To be able to develop to such an extent, rhizomorphs must secrete squads of cell-wall degrading enzymes to enable them to riddle their way across various layers of tree roots. They must also fire battalions of virulence factors that would begin by guiding them towards roots to sup up its nutrients while shunning the tree's own defense system. But this is not all, *Armillaria* will have needed to develop its own defense system to fight off microbes equally eager to extract nutrients from the same environment, i.e. antimicrobial products, and these are known as melleolides.

Melleolides are terpenes. Terpenes form that characteristic acrid scent released by tree resin. The name terpene originates from the Greek terbinthine meaning resin, itself extracted from terebinth, a tree species found in Greece. Interestingly, terpenes were given their name by the German organic chemist August Kekulé who was the first to lay down the revolutionary theory of chemical structure in the 1850s. Chemical formulae had been around for some time already, but no one had understood how atoms were actually located with respect to one another – until Kekulé introduced the inspired notion of chemical valence, or affinity, and was able to draft the structure of benzene. The chemical structure of terpenes themselves – of which we currently know about 50,000 – was subsequently identified by the German chemist Otto Wallach who found a bottle of essential oils lying around in Kekulé's laboratory where he was working.

How does *Armillaria* synthesize its own melleolides? It begins by the cyclization of the universal precursor farnesyl diphosphate ($C_{15}H_{28}O_7P_2$) – and this happens to be a chemical reaction which is one of the most complex that occurs in Nature. In *Armillaria*, this particular step is catalyzed by a terpene synthase,

notably delta(6)-protoilludene synthase, or PRO1, which produces an intermediate metabolite known as 6-protoilludane. 6-protoilludane does not have antimicrobial activity itself, but it constitutes the first essential step leading to the synthesis of melleolides. Subsequent steps generate yet further structural diversity ultimately giving rise to as many as 50 different melleolides – perhaps even more – and thus illustrate the major role melleolides have in *Armillaria*'s defense system.

Melleolides are very diverse and show antimicrobial activity against a wide range of microorganisms, including bacteria and viruses. As yet, their mode of action has been poorly investigated; only melleolides with antifungal activity are known to inhibit translation. Antimicrobial products are of great interest to the medical world, and fungi have been a precious reservoir for a little over a century now. Remember the revolutionary finding by the Scottish microbiologist Alexander Fleming who managed to isolate penicillin antibiotics from *Penicillium* fungi in the 1920s – the first antibiotics ever to be used against bacterial infections. The thing is, over time, bacteria are becoming more and more resistant to the antibiotics we know and we need to find other sources, or indeed engineer them ourselves – but to be able to do that you need to understand the intimacy of their molecular chemistry.

A surprising discovery: despite its good age, the genome of the *A.gallica* colony discovered in the 1990s has proved to be remarkably resistant to genetic change with an overall mutation rate that is unexpectedly low. This has made a few researchers wonder about cancer and how tumours behave. Cancer tumours also develop within a restricted environment yet their genome undergoes multiple mutations over very short periods of time. So where does the difference lie? Could it be due to a DNA repair system which is highly effective in *Armillaria*? Maybe. But in tumours, progression thrives less on a dithering DNA repair system than an overenthusiastic DNA replication system. But it is food for thought.

Cross-references to UniProt

Delta(6)-protoilludene synthase, *Armillaria gallica* (Bulbous honey fungus): P0DL13

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