Fungi and the food of the gods

Keith Clay

Plants protect themselves against attacks by microorganisms in various ways. In some circumstances at least, it turns out that they enlist the help of mutualistic fungi in defence of the home front.

Chocaholics will be pleased with the results of Arnold *et al.*[1], published last month in *Proceedings of the National Academy of Sciences*. In the long term, the findings promise a strategy for increasing production of the crop that gives rise to this delectable treat. More immediately, however, they will come as a revelation to many plant biologists. They show that a poorly understood group of microbes provide their host plants with an effective defence against pathogens. The microbes are 'endophytes', fungi that live inside leaves without causing disease, and they appear to be ubiquitous associates of all plants.

Cacao provides the raw material for chocolate, the botanical name for the source tree (Fig. 1) being *Theobroma cacao* (which literally means 'food of the gods'). Production occurs primarily in the tropics of the Old World, owing to the high disease prevalence in the cacao tree's native American tropics. Arnold *et al.* report on endophytes and disease incidence in cacao trees in Panama, and find that the trees support diverse communities of fungi that are distinct from the endophytes inhabiting other tree species. The team also experimentally manipulated fungal endophyte colonization in cacao seedlings before infecting them with a virulent pathogen. They found that endophyte inoculation significantly reduced the incidence of disease and its damaging effects.

Figure 1 **The cacao tree, *Theobroma cacao*. The pods evident in this photo contain the beans from which chocolate is made. Inset, experimental cacao plants showing the effects of disease on the leaves of plants that have been inoculated with endophytes (centre) and the much greater damage to leaves of control plants.**

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It is well known that a great variety of microorganisms associate with plant roots, so it should not be surprising that similar diversity exists in the above-ground parts of plants. Endophytic fungi have been isolated from virtually all plant samples examined[2,3]. But what, if anything, are these microbes doing? The best-understood case to date has been that of endophytes of tall fescue, *Festuca arundinacea*, and other cool-season grasses[4] (so-called because they tend to grow best in a cool, moist climate and are usually winter hardy). These fungi are vertically transmitted — that is, from generation to generation through seed. They infect the plant as a whole, and the host grass gains some protection from grazing animals as a result of alkaloid toxins that the endophytes produce.

This example is not generally representative, however. In most plants, it is individual leaves that are colonized by a diversity of endophytic fungi, each forming highly localized infections. These infections are acquired independently by horizontal transmission through wind- or water-dispersed spores. In grasses, the persistence of highly mutualistic fungi is evidently a direct consequence of their strict vertical transmission through seeds. The diversity and dynamics of horizontally transmitted endophytes, or other mutualistic microbes, is less clear[5]. Given that endophytes use their hosts' resources, they must entail some cost to the plant. If the costs outweigh the benefits, why don't plants defend themselves against infection? And if the benefits outweigh the costs, what are these fungi doing to help the plant?

Arnold and colleagues[1] examined the endophyte community of cacao by isolating the fungi from surface-sterilized leaf fragments on agar plates. They found that all samples were highly infected, with infection approaching 100% as the leaves aged (Fig. 2, overleaf). They isolated an average of 10 distinct taxonomic groups of endophyte from each leaf and a total of 344 groups from 126 leaves. The number of different endophytes per leaf also increased with leaf age. These findings agree with earlier results of Arnold *et al.*[3] showing the high diversity of endophytic fungi in tropical trees. Together, their observations indicate a staggering level of fungal biodiversity (see also ref. 6), although other studies[7,8] suggest more modest levels of diversity. If the cacao seedlings were protected from aerial deposition of spores, endophyte colonization fell to less than 1%, demonstrating that the fungal community develops over time by horizontal transmission. There was no evidence of the vertical

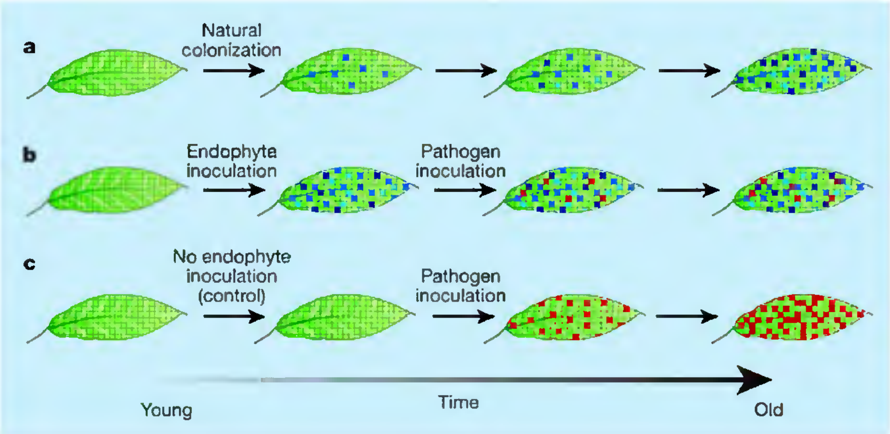

Figure 2 **The colonization of leaves by endophytes, and pathogen deterrence.** a, **The natural colonization of leaves, increasing over time, by a diverse endophyte community.** b, c, **The results of the key experiments carried out by Arnold *et al.*[1] on sterile cacao seedlings.** b, **Pathogen attack of plants that had been inoculated with endophytes resulted in comparatively little pathogen occupation of leaf cells and limited damage.** c, **By contrast, pathogens became well established in endophyte-free control leaves and caused severe damage or leaf death.**

transmission through seeds that is seen in grasses.

Arnold *et al.*[1] next looked at the distribution of endophyte diversity in cacao. The most similar communities occurred in neighbouring sites, and they became more divergent as the distance between sites increased. There was little evidence that endophyte composition reflects habitat type. The authors also sampled three tree species, including cacao, at a single site and found that most types of endophyte showed host affinity, infecting only one of the three. In fact, endophyte communities from different host species at a single site were more divergent than communities from cacao trees at separate sites. Arnold *et al.* confirmed this apparent host preference using agar plate assays in which fungal endophytes grew differentially in response to leaf extracts from the three species (although some studies[7,8] on other tropical systems found little evidence for host preference). The degree of host specificity is a critical parameter for estimating total endophyte biodiversity based on isolation of fungi from individual tree species.

The most dramatic result occurred when Arnold *et al.* inoculated sterile cacao seedlings with several groups of endophyte, and then exposed the seedlings to a species of *Phytophthora*. This is a common pathogen that causes black pod disease and is related to the agent of Irish potato blight (*Phytophthora infestans*). Endophyte-free control leaves died in greater numbers than those inoculated with fungus, and the control leaves that did survive suffered much more damage (Fig. 2). The relative advantage of endophyte inoculation was higher in older than in younger leaves. Overall, endophyte-protected leaves suffered less than half the damage seen in control leaves (Fig. 1, inset).

But how does the fungal endophyte kill or inhibit the growth of *Phytophthora*? One possibility is that the endophytes simply occupy the space that might be taken by the pathogen, or competitively consume resources; alternatively, direct antagonism might be involved. Furthermore, does inhibition result primarily from the action of a particular endophyte or do a variety of them act additively or synergistically? Would the results hold if endophytes and *Phytophthora* were inoculated simultaneously, or if the pathogen was inoculated first? In native habitats, where endophytes and pathogens occur together, do heavily diseased plants host less diverse fungal communities?

Whatever the answers to these questions, Arnold and colleagues' findings[1] point to biological control as a promising way of tackling disease in cacao trees. This could be achieved by spraying the trees with fungal spores, or perhaps more realistically by growing them alongside other trees that could serve as sources of fungal inoculum. The results also clearly demonstrate that plant–endophyte mutualistic interactions are not restricted to cool-season grasses, and they explain why host plants don't defend themselves against infection by the fungi concerned. Pathogens and endophytes are common in all plants, so carrying out similar experiments in a range of tropical and temperate species should prove highly rewarding. ■

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Nanotechnology

How does a nanofibre grow?

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Decades of research have failed to decipher the atomic-scale mechanism by which carbon nanofibres grow out of vapour. High-resolution microscopy shows that the carbon atoms have a bumpy ride.

In his historic monograph *On Growth and Form*[1], D'Arcy Thompson wrote that the form of an object is a diagram of its growth forces. Often, however, this relationship between growth and form is too complex to determine: the growth of filamentous carbon in the vapour phase is a case in point. Despite a large body of literature on the subject, replete with fascinating and varied examples, no definitive model for the growth of carbon nanofibres has evolved, owing to a lack of consistent experimental data[2,3]. Considering the substantial impact that these materials are likely to have on technology[4], it seems imperative that their growth mechanisms be understood, so that nanofibres can be manufactured with well-defined characteristics. A long-awaited solution to the mystery of nanofibre growth is presented by Helveg *et al.*[5] on page 426 of this issue.

Nanoscale carbon fibres are grown through the interaction of metal-catalyst nanoparticles with hydrocarbon vapour at high temperature. The hydrocarbon molecules dissociate at the interface between catalyst and vapour, and carbon atoms precipitate into a graphite trail in the shape of a cylindrical, multi-walled nanofibre (Fig. 1). Quite how the nanofibre forms is unknown. The catalyst particle might stay at the growing end of the nanofibre (called tip growth), or it might sit at the starting end (base growth)[6]. The state of the particle itself (such as its structure or shape) during the growth process is also unknown, but the particle size and fibre diameter are similar.

Experimentally, it has proved difficult to track the dynamics of this high-temperature catalytic reaction with spatial and temporal