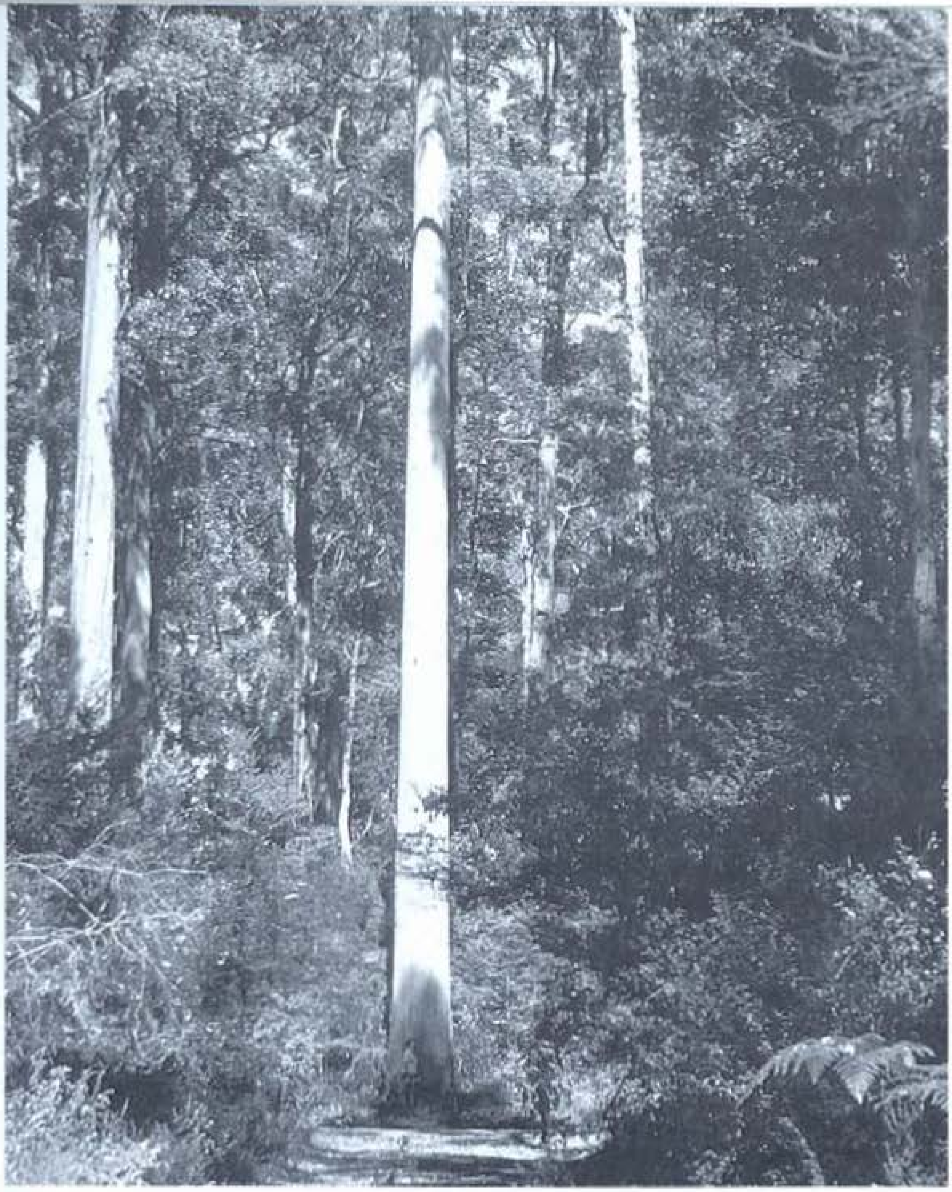


A karri forest, containing some of Australia's tallest trees, benefiting from tiny fungal threads in the soil beneath them.

Trees and fungi — a productive partnership

During the 'season of mists and mellow fruitfulness' the sight of mushrooms sprouting at the base of a tall forest tree is not uncommon. It may seem that a large tree need have little regard for those fungi that — apparently coincidentally — live at its base. But, in fact, the attentions of a mould can signal extra life or untimely death for many trees.

Plants can fall victim to fungal diseases (for example, the wilt of Tasmania's native myrtles described in *Ecos* 51). Fungi, ever-present in the soil, may enter through the roots. But often, soil fungi will actually protect a tree and aid in its nutrition, sometimes with stunning results — such as a 50% growth boost over the course of 2 years. A partnership occurs between the fungus and the tree, to the mutual benefit of the two. Such associations are called mycorrhizae, which means literally fungus-roots.

These provide good examples of symbiosis — a sort of biological 'I'll scratch your back...', or a living together for mutual benefit. Fungi cannot make their own food by the process of photosynthesis, and therefore need pre-formed organic compounds — including certain vitamins, and carbohydrates for energy. These they receive from the tree, which makes sugar, starch, and later a host of other compounds in the leaves, and transports some of them to the rest of the plant, including the roots. Being closely associated with the root cells enables the fungal mat of hyphae to sequester various needed compounds.

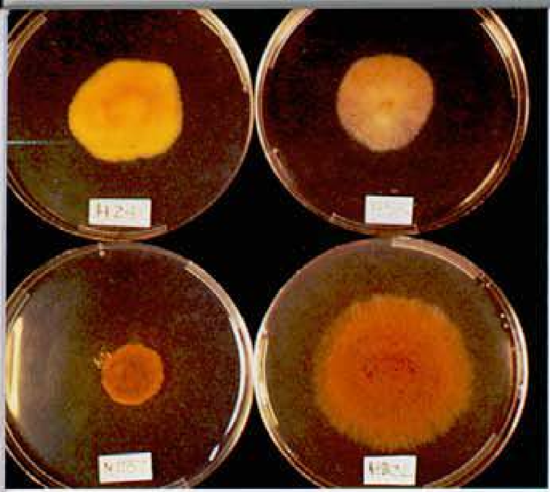
This represents a relatively small loss to a tree, in return for which it receives assistance for its roots, because the thin fungal hyphae extend in all directions through the soil and act to absorb water and nutrients. These find their way into the tree, which therefore has a vastly increased root area without itself making any more roots. At the end of the day, everybody has benefited.

Of course, this intimate association takes place under the soil, and so is little noticed. About once a year, generally when it has been wet (fungi have little protection against drying-out), the fungus will produce a fruiting body — a structure to disperse its spores. This often takes the form of the familiar mushroom or toadstool, which appears to sprout from nothing, but in fact is a collection of hyphae woven together, and represents the effort of a vast underground fungal network: the splendidly designed tip of a 'subterranean iceberg' of mould. The fruiting body grows upwards to ensure that its spores drift on the wind, and thus that they are distributed as widely as possible. After a few days the mushroom will die, its job completed, but the main body of the fungus continues to produce more hyphae in its quest for nourishment.

Not all fungi produce above-ground fruiting bodies; those that don't may instead produce an underground ball of spores (this is what the gastronomically famous truffle is), and rely on animals eating the fruiting body as a means of dispersal (see the box on page 22). Not every species of fungus in the forest floor forms mycorrhizae, but even so the conspicuous toadstools near the base of a tree are very likely to be the fruiting bodies of its underground helpers.

Compatibility

Biologists — mainly in the Northern Hemisphere — have studied mycorrhizae extensively. They now know plenty about mycorrhizae and pine trees, but *Eucalyptus* and *Acacia* species — botanically very different from the non-flowering conifers — account for the majority of trees in Australia. Do mycorrhizae have a part to play here, and if so which species of fungi are involved?



Mycorrhizal fungi cultured in the laboratory.

Helping to find answers to these questions are Dr Nick Malajczuk and a team of scientists at CSIRO's Division of Forest Research in Western Australia.

That eucalypts have mycorrhizae was recognised by botanists in the 1950s, but what Dr Malajczuk and his colleagues have done is examine and identify the fungal species and correlate them with the tree species on which they occur. He has also compared the mycorrhizal fungi from conifers with those from eucalypts to find out how specific the fungi are, and what happens when pine trees grow amid Australian soil fungi. He has now gone on to establish the effect of the mycorrhizae on the growth of some of our native vegetation.

The results are dramatic. In glasshouse experiments with karri (*Eucalyptus diversicolor*), and Tasmanian blue gum (*E. globulus*), Dr Malajczuk and Ms Treena Burgess found that 12 weeks after germination mycorrhizal seedlings had up to ten times the dry weight of seedlings without fungal partners. After 2 years, certain selected fungi had enabled trees to increase their height and diameter 50% more than

Ecological riddles

What do woylies, long-nosed bandicoots, karri, jarrah, and underground fungi have in common? All live in Western Australian forests, and they are connected by an intricate series of interrelationships that may also occur in many other temperate forests.

Dr Malajczuk, with colleagues from the United States Forestry Service, has shown that a type of fungus whose fruiting bodies develop underground, like truffles, forms mycorrhizae with karri and thereby improves the tree's growth. The fungus, called *Mesophellia*, relies on animals eating the underground mushroom it produces and so fortuitously dispersing its spores. The animals release the still-viable spores in their faeces, a nutritious place for a fungal existence to start. The network, or mycelium, of the fungus spreads into the leaf litter, and eventually into association with tree roots.

Underground fungi are not merely an unusual treat for small forest mammals, but form an important component of their diet, as Dr Per Christensen of the State Department of Conservation and Land Management has shown. He studied the diet of the woylie, *Bettongia penicillata* (also called the brush-tailed bettong or rat kangaroo), which lives in the jarrah forests. It digs out and eats underground fungi. And in the karri forest, Dr Christensen observed that species of the *Mesophellia* fungus were an important dietary component of at least two native species of forest animals — the long-nosed bandicoot and the bush rat.

Truffles are a rare and expensive delicacy, rooted out of the ground by specially trained pigs. Are there really likely to be enough underground mushrooms in Western Australia's forests for them to supply our little marsupials with sufficient sustenance? The answer seems to be yes; Dr Malajczuk and his colleagues found about 10 000 *Mesophellia* fruiting bodies per hectare under mature stands of karri forest.

So the trees rely on fungi to help them extract soil nutrients and grow. Many of the fungi, in turn, rely on the small marsupials to disperse their spores, allowing gene exchange and colonisation of new areas. The animals need the fungi for food, and need the trees to provide their forest home. The ecological precept of the interdependence of all things could not be better illustrated.

The underground fruiting bodies of a *Mesophellia* fungus — a food source for some forest-dwelling mammals.

The system closely parallels one in North America, involving pine trees, underground fungi, and squirrels and rodents. In both cases, a reduction in the animal population could affect the trees' long-term survival because of a reduction in the disperal of their symbiotic fungi.

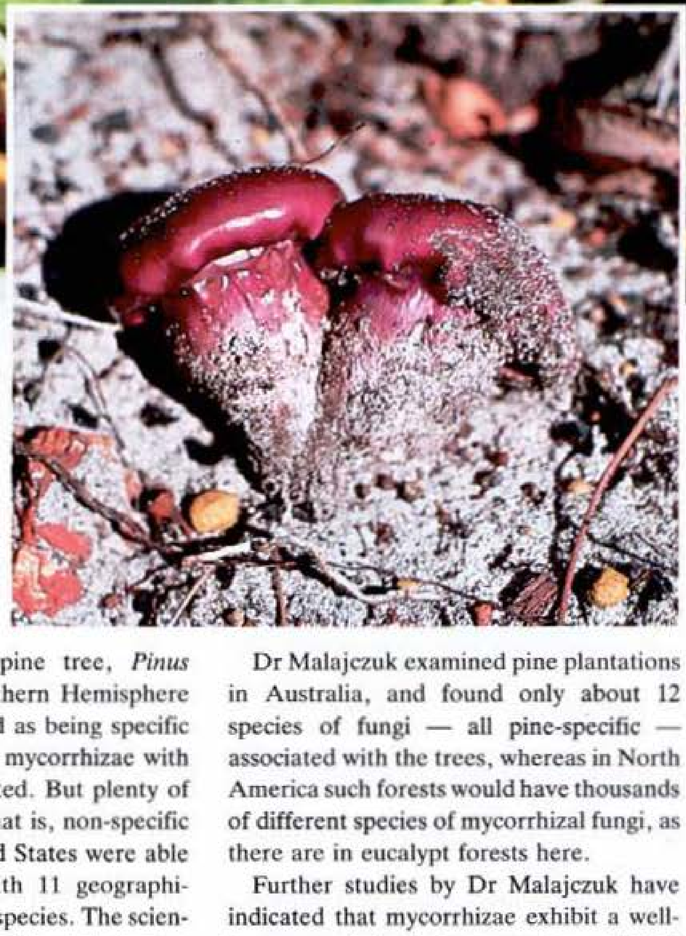
In Australia, introduced feral animals such as cats and foxes may be reducing the numbers of small native marsupials, without taking over their role of fungal feeding. For example, the numbat, Western Australia's mammal emblem, now only exists in one tiny area of the State, although it was once widespread. Although its decline is unlikely to be affecting the viability of the forests, as it is mainly a termite-eater and has not been shown to eat fungi, any decline of other less glamorous small mammals could be. And if the forests become less healthy, the mammal population will eventually fall further, resulting in a spiralling vicious circle of decline.

Another important point concerns pine plantations. Evidence from Dr Malacjzuk and his colleagues suggests that *Mesophellia* species, as well as some other Australian underground fungi, only form their fruiting bodies when they are in association with eucalypts. So the fungi-eating marsupials may perhaps be adversely affected by any reforestation programs involving introduced trees like pines.

Of course, pines have their own underground fungi, such as *Rhizopogon* species, but we are not yet sure whether marsupials would be able to exist on these as a substitute food source.

Fungi are by no means all the same nutritionally; work done by Ms Elizabeth Beckmann at the University of New England in Armidale suggests that normal cultivated field mushrooms, for example, are not an adequate food source to support rufous rat kangaroos (also called rufous bettongs — the eastern version of the brush-tailed bettong) for any length of time. In food selection experiments that included underground roots and tubers, and other foodstuffs, mushrooms were not a preferred choice of the animals. The two findings suggest that, in this case, fungi may only be a supplementary food source, perhaps only important during a particular time of year.

Obviously, we still need to know much more before we can say we have really unravelled this complex three-way association that gives life to a forest.



Some of the colourful fruiting bodies of ectomycorrhizal fungi found on pine (top and left) and eucalypts (right).

uninoculated trees that had only casually acquired natural populations of mycorrhizal fungi. Over longer time spans, the differences will presumably even out, but for a short-rotation tree crop researchers estimate that good mycorrhizae could enable harvesting to take place a year earlier, with considerable economic benefit.

Dr Malajczuk and two American collaborators — Dr Randy Molina and Dr James Trappe — found that, in general, the eucalypt forest trees in Western Australia formed mycorrhizae with fungi that were broad in their choice of plant. The researchers found many fungal species that could initiate mycorrhizae with all *Eucalyptus* species in controlled laboratory conditions.

However, three species of fungi that were known to be broad in their choice of host could not associate with *Eucalyptus* species, although they could certainly form mycorrhizae with the pine tree, *Pinus radiata*. Fungi that Northern Hemisphere researchers had reported as being specific to conifers did not form mycorrhizae with any of the eucalypts tested. But plenty of broad host-ranging — that is, non-specific — fungi from the United States were able to form associations with 11 geographically distinct *Eucalyptus* species. The scientists also isolated certain fungi that were specific to *Eucalyptus* species, but could not marry up with *Pinus radiata*.

Several interesting facts emerge from Dr Malajczuk's detailed research. Firstly, *Eucalpytus* species and *Pinus radiata* are able to form mycorrhizae with a variety of fungi, even with those found in other continents, provided those fungi are not too specific. This helps account for the relative success with which foresters have been able to establish plantations of eucalypts overseas, and of pine trees here.

Dr Malajczuk examined pine plantations in Australia, and found only about 12 species of fungi — all pine-specific — associated with the trees, whereas in North America such forests would have thousands of different species of mycorrhizal fungi, as there are in eucalypt forests here.

Further studies by Dr Malajczuk have indicated that mycorrhizae exhibit a well-known ecological phenomenon — that of succession. Over time, in almost any community of organisms, different species will in turn become the most numerous and dominant. What was suitable for one species initially may not remain so as other organisms move in. Eventually, however, a stable situation is reached and will persist — provided the environment stays constant.

Biologists can study succession on a large scale only when an event such as the creation of a new island from the ocean occurs. But among a miniature community such as symbiotic fungi living in soil, the arrival of large numbers of new plants is a dramatic event.

Just as the colonisation of bare rock proceeds first by lichens, then mosses, and then small plants, so a seedling tree when it is establishing associates with fungal species different from those it favours when it is 50 years old. Dr Malajczuk has shown that young trees form mycorrhizae with broad host-ranging fungi but that, over time, the fungal species involved change, and the more specific ones come to dominate.

This succession means that when a forest has been harvested by clear-felling, the growth of another batch of trees will not be so easy. The mycorrhizal fungi in the soil will be those that associate with mature trees, and not the species that can help a new seedling establish itself. In a native forest, if all the trees were mature it might not be easy for a young seedling to find its fungal partners, but at least some of them would be present, perhaps drifting in from outside the forest. Such a situation would rarely occur with the non-native pine forest, where the necessary 'early' fungi — if exotic — might have ceased to exist by the time a second tree crop was to be planted.

The second matter arising from the scientists' observations concerns compatibility. Why is it that some fungi can be symbiotic with eucalypts but not with pines, and *vice versa*? Curious about this, Dr Malajczuk investigated further.

Using the electron microscope he looked at the detailed structure of roots inoculated with compatible and incompatible mycorrhizal fungi and followed the progress of the symbiosis.

Some features looked the same. For example, in both cases the fungi became established in the soil around the roots (the rhizosphere), and then started forming a mantle around the outside of the roots. But the fungi compatible with the plant formed a thicker sheathing mantle of hyphae than the incompatible ones, which — after a few months, with the compatible fungi continuing to grow — suffered from break-up of their cells. Precisely why, we don't yet know. It could be that different fungal

More on mycorrhizae

Mycorrhizae occur on many tree species, especially conifers, and also on orchids — where they are essential for the plants' growth. Microscopic examination of the roots makes it possible to distinguish two types. In the first type, the fungal threads, or hyphae, form a covering around the root and make a net between some of the root cells, but do not actually go inside any cells; they are called ecto-, ectotrophic, or sheathing mycorrhizae. The mantle, or sheath, varies in thickness from about 10 to 100 μm.

In the other, less common, type of association, the hyphae ramify throughout the root cortex and penetrate some cells. Under the microscope this type is distinguishable by the production of vesicles — possibly storage structures — and 'little bushes' or arbuscules of tiny hyphae. Biologists therefore call these, rather clumsily, vesicular–arbuscular or sometimes endotrophic mycorrhizae.

Possibly a third type exists: in a survey of forests in south-western Australia from 1981 to 1983, Dr Malajczuk, together with Dr Bernie Dell of Murdoch University and Dr Neale Baugher of the University of Western Australia, found a type of ectomycorrhiza associated with eucalypts, involving fungi of the genera *Cortinarius* and *Hysterangium*, and occurring widely in both wet and dry sclerophyll forests. The structure of the association differs somewhat from that of the usual ectomycorrhizae, and Dr Malajczuk uses the term superficial ectomycorrhizae. (Almost as a bonus, the work also led to the discovery of some previously undescribed fungal species.) The fungi were so prevalent that their hyphae occupied up to 10% of the soil area.

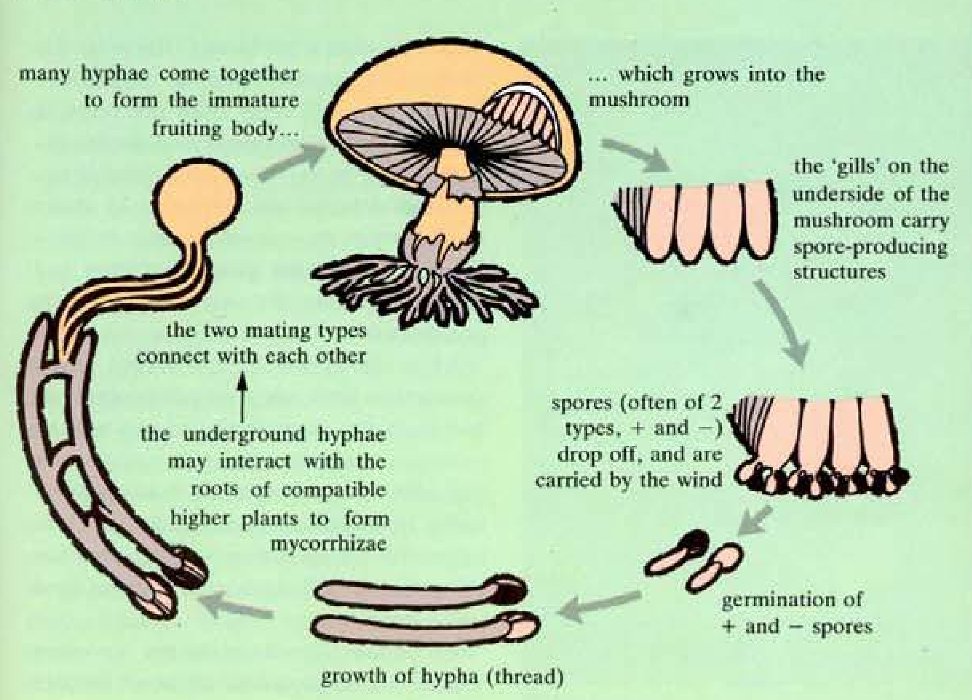
Dr Malajczuk suspects that these two fungal genera may be associated with all eucalypts, and the fact that he found them in soil from Tasmanian forests helps support this idea.

In the ectomycorrhizal symbiosis, the fungi change the structure of the host root. The tiny root hairs, responsible for providing the vast surface area for absorption of soil minerals and water, do not occur in these roots, probably being physically smothered by the fungal sheath. But the fungi may also interfere in more subtle ways. Scientists have shown that they can produce compounds very similar to the plant-growth regulator auxin. Exactly what effect, if any, this may be having remains unclear.

But the advantages that mycorrhizae can confer are clear. Back in the laboratory, Dr Malajczuk is experimenting with two of Western Australia's most important timber trees: the tall and beautiful karri (*Eucalyptus diversicolor*) and jarrah (*E. marginata*), a valuable hardwood. Comparisons between hundreds of trees, either inoculated with mycorrhizal fungi or left without, have shown that, with identical nutrient levels in the soil, the mycorrhizal plants dramatically outstrip the growth of their non-symbiotic siblings.

The response of karri (*E. diversicolor*) seedlings to inoculation with ectomycorrhizae. The controls, without mycorrhizae, are on the left in each of the two pairs. The plants are the same age, and were grown in identical soils.

Life of a fungus



Mushrooms (a term that includes toadstools), truffles, and some plant pathogens like rusts and smuts belong to the Basidiomycetes — the most highly evolved group within the fungal kingdom. The life cycles of many are far more complicated than that shown.

species have slightly different requirements for growth factors from the plant roots, and will eventually die out if these are not provided by the particular roots with which they are associating. Or, the host tree could be in some way responding and killing the unwanted fungus.

A further possibility comes from study of another microbe–plant interaction, the well-known one between legumes and the nitrogen-fixing *Rhizobium* bacteria. Here, recognition between the two partners takes place at the molecular level, by means of complementary molecules on cell membranes. If a similar system operated for mycorrhizal associations, then failure of the fungus to do more than grow on the root surface for a while could simply be due to the absence of the correct recognition molecules on either or both partners.

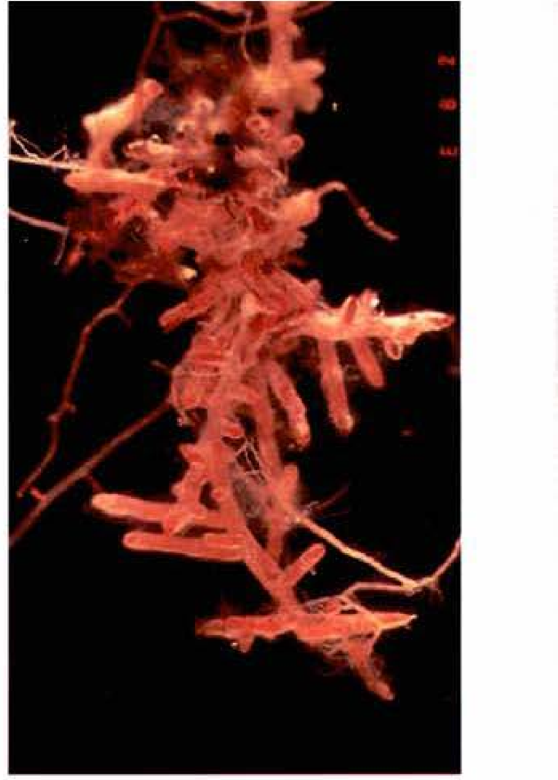
But living together is not always easy; there is a temptation for one partner to take advantage of the other. The line between being a helpful symbiont and being a disease-causing parasite is a fine one. The electron microscope showed that a tree's roots may have to act to prevent a wholesale invasion of its tissues by the fungus. The scientists saw deposits of what were probably tannins inside the root cells adjacent to the fungal hyphae. Uninfected roots did not show this. Tannin is a well-known compound in the armoury of plant defences — often occurring in leaves, where it discourages herbivores. Quite possibly an organised barrier of it would be equally discouraging to many fungi, and would prevent them from growing further into the root tissue.

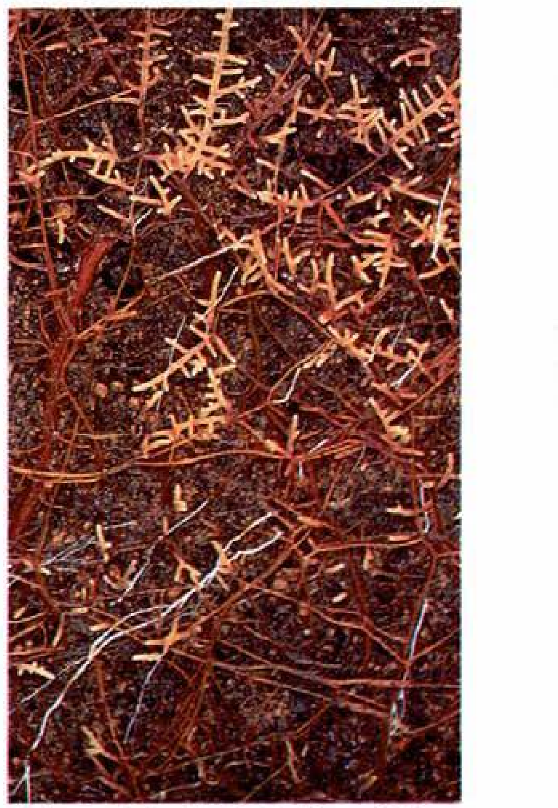
Chemistry in the soil

Dr Malajczuk and Dr Kermit Cromack of Oregon State University have made the interesting discovery that symbiotic fungi may assist in their hosts' nutrition by more than just providing an increased area for absorption. They may actively interfere in the soil chemistry to make nutrients more available. The scientists analysed the major chemicals on mycorrhizal roots of jarrah and pine, using X-ray spectroscopy. They found that calcium was the element occurring in the highest concentration in both types of mycorrhizae. Examination with the scanning electron microscope showed crystals scattered on the outside of the hyphae. To determine what the crystals were, the scientists used diffraction analysis.

In brief, crystals diffract light, or other forms of electromagnetic radiation, according to their shape and pattern of crystallisation. You can use the subsequent diffraction pattern to obtain information about the atomic structure of the crystal or, by comparison with known standards, to make a confident prediction of the chemical nature of the crystal.

The mycorrhiza's crystals turned out to be calcium oxalate, which increases the availability of soil phosphorus, an element that it's often hard to get enough of in Australian soils. Calcium oxalate works its magic by binding to compounds that themselves normally chelate — that is, make unavailable by binding to — phosphorus. Some fungi can also mobilise soil nutrients by producing enzymes called phosphatases that digest phosphorus-containing organic compounds and so release inorganic and usable phosphorus.





A tree root sheathed by the fine weft of an ectomycorrhizal fungus and, below, how such roots spread out among the leaf litter on the ground.

Disease preventers

But that's not all: further work by Dr Malajczuk and others suggests that mycorrhizal fungi are important in protecting their hosts against disease-causing fungi. A basic principle of microbiology is that if a niche, or living space, is occupied by harmless microbes — commensals that simply live on larger organisms, or symbionts that actively help in a partnership — then disease-causing microbes find it difficult to establish in that niche. Just as our own relatively harmless gut bacteria make it hard for pathogens to gain a permanent foothold inside our intestines, so a tree's mycorrhizae may well prevent the attentions of less welcome root fungi.

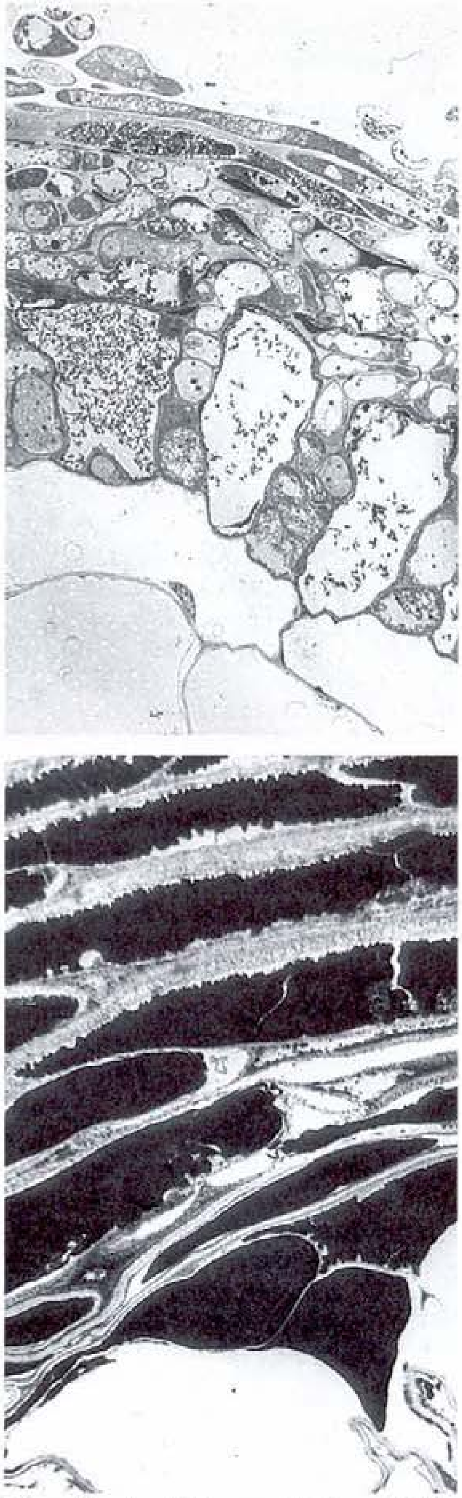
The symbiotic fungi prevent infection of the roots by various means, certainly used in combination. Firstly, many fungi produce antibiotics, as bacteria are their principal competitors in the miniature world of microbes. As well as reducing the likelihood of bacterial diseases (which are less of a problem to plants than fungal diseases), these antibiotics may also be active against pathogenic fungi.

Secondly, the compact fungal sheath around the roots provides an obvious physical barrier against an initial approach by a pathogen.

Thirdly, all roots produce organic compounds that diffuse into the soil. This so-called root exudate provides a cue to fungal spores, enabling them to home in on a root to infect. But a mycorrhizal root releases less exudate into the soil because much is absorbed by the fungal partner. As well, what little does move into the soil will be modified because of selective absorption by the fungal mantle, and will probably not be exactly the right mixture to stimulate an attack by pathogens.

Fourthly, and most subtle of all, it seems that the mycorrhizae modify the environment around the roots — the rhizosphere. The changed root exudate, as well as affecting the behaviour of potential fungal pathogens, could stimulate a bacterial population within the rhizosphere that would differ markedly from that found around non-mycorrhizal roots.

Dr Malajczuk found vast numbers of bacteria occurring between the cells of, and all around, the fungal mantle of eucalypt mycorrhizal roots. Many of these bacteria were antagonistic to certain other fungi, such as the infamous *Phytophthora* and *Pythium*, two genera that are responsible for great damage and economic loss in agriculture and forestry. It seems that the plant–fungus symbiosis is exploiting the aeons-old, and never-ending, state of war between fungi and bacteria by encouraging the growth of the natural bacterial enemies of certain pathogenic fungi.



Viewed under high magnification with the electron microscope, a normal mycorrhizal root and, below, a root a non-compatible mycorrhizal fungus has attempted to colonise. The extensive black areas represent the deposition of protective compounds secreted by the root in response to the unwelcome invasion.

Dieback

But if this is so, why do forest trees like jarrah suffer from devastating fungal diseases, such as dieback, caused by *Phytophthora cinnamomi*? Certainly the jarrah trees possess well-developed mycorrhizae, so why aren't they protected? There is no simple answer, as jarrah dieback is such a complex problem. However, Dr Malajczuk suspects that a mycorrhiza formed with soil fungi does not offer as much protection as one where the fungus component is in the forest floor litter. Controlled burns are necessary in many jarrah forests to prevent wildfire, but this practice reduces the quantity of litter and so ensures that the mycorrhizae form primarily from the soil fungi.

Also, laboratory experiments have shown that litter organisms in general are hostile to *P. cinnamomi*, and can kill the pathogen's mobile spores. Arguing teleologically, it seems obvious that creatures living in the litter would find it in their interest to act against an organism that has the power to kill the source of their food and habitat!

To be fair, fire can also have a positive side in the management of jarrah dieback — it all depends on the temperature. Hot burns, such as occur with wildfires, change the understorey vegetation over large areas, resulting in the establishment of *Phytophthora*-resistant legumes. The so-called 'cool' burns that take place during deliberate, controlled fires may cause the proliferation of *Banksia* species, which are susceptible to *Phytophthora* attack — even more so than is jarrah, because *Banksia* never forms mycorrhizae. (Its specialised root system enables it to manage its mineral and water absorption efficiently without them.)

The reason why jarrah's protection from its mycorrhizae is not adequate to prevent dieback may go beyond the effect of fire. It's worth pointing out that no biological system is ever foolproof. Nature requires the various sides in any conflict to be balanced. Although mycorrhizae are certainly protective, they can only ever reduce the likelihood of infections, not prevent them altogether. With time, a pathogenic fungus is bound to evolve that can breach a root's best defences, and the whole conflict escalates one stage further.

Manipulating mycorrhizae

With all that we now know about mycorrhizae, it is surely time to start looking for areas where we can apply our knowledge and, perhaps, deliberately encourage the initiation of mycorrhizae and influence the types that are formed.

Dr Malajczuk is attempting to do just this. He and his team have received a grant of $150 000 from the Western Australian Chip and Pulp Company to screen mycorrhizal fungi for growth promotion of eucalypts, and $500 000 from the National Biotechnology Project to screen for salt tolerance. He has already isolated some fungi that form mycorrhizae in highly saline areas.

Glasshouse experiments show that, in high-salinity soils, root tips go brown and die. In identical conditions, roots that are inoculated with the correct mycorrhizal fungi survive. Exactly how the protective effect works we don't know for sure. Dr Malajczuk suspects that it involves the fungal hyphae sequestering salt crystals into intracellular vacuoles, and so less salt actually comes into contact with the root cells.

Furthermore, scientists are starting to suspect that mycorrhizae may also offer protection against certain elements that, in some places, may exist in the soil at toxic concentrations. If the necessary fungi for these, or salt-tolerant mycorrhizae, are selected from the field and cultured in the laboratory, Dr Malajczuk hopes it will then be possible to go into commercial production of 'useful' fungal symbionts, which could be inoculated into the soil when tree seedlings are planted. In countries where eucalypts are important exotics, for example, Australian eucalypt-adapted mycorrhizal fungi would be in great demand.

He is therefore co-operating with Dr Clem Kuehn and Dr Inez Tommerup, of the University of Western Australia, to develop a means of encapsulating pieces of fungal mycelium for subsequent use as 'biological fertilisers'. A company is currently considering the possibility of full-scale production.

In this light microscope picture you can see the tiny crystals of calcium oxalate produced by some mycorrhizae that help the root extract various compounds from the soil.

Of course, the future offers other visions of rhizosphere manipulation. Many important agricultural and horticultural crops also have mycorrhizae, but unfortunately most of these are of the vesicular–arbuscular type (see the box on page 24), which at the moment scientists are unable to culture in the laboratory — but we await further developments with interest.

Roger Beckmann

More about the topic

Ectomycorrhiza formation in *Eucalyptus*. 1. Pure culture synthesis, host specificity and mycorrhizal compatibility with *Pinus radiata*. 2. The ultrastructure of compatible and incompatible mycorrhizal fungi and associated roots. N. Malajczuk, R. Molina, and J.M. Trappe. *New Phytologist*, 1982, **91**, 467–82; 1984, **96**, 43–53.

Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. N. Malajczuk and K. Cromack. *New Phytologist*, 1982, **92**, 527–31.

Procedure for inoculation of micropropagated plantlets of *Eucalyptus camaldulensis* with ectomycorrhizal fungi, and comparison with seedling inoculation using inoculum contained in a peat/vermiculite carrier. N. Malajczuk and V.J. Hartney. *Australian Forest Research*, 1986, **16**, 199–206.