10.1071_ISSN0311–4546EC103p18

Like bizarre Christmas tree baubles, the surface of many fungal spores are highly decorated. These 'ornaments', revealed here by a scanning electron microscope, are thought to help repel water and maintain spore dormancy.

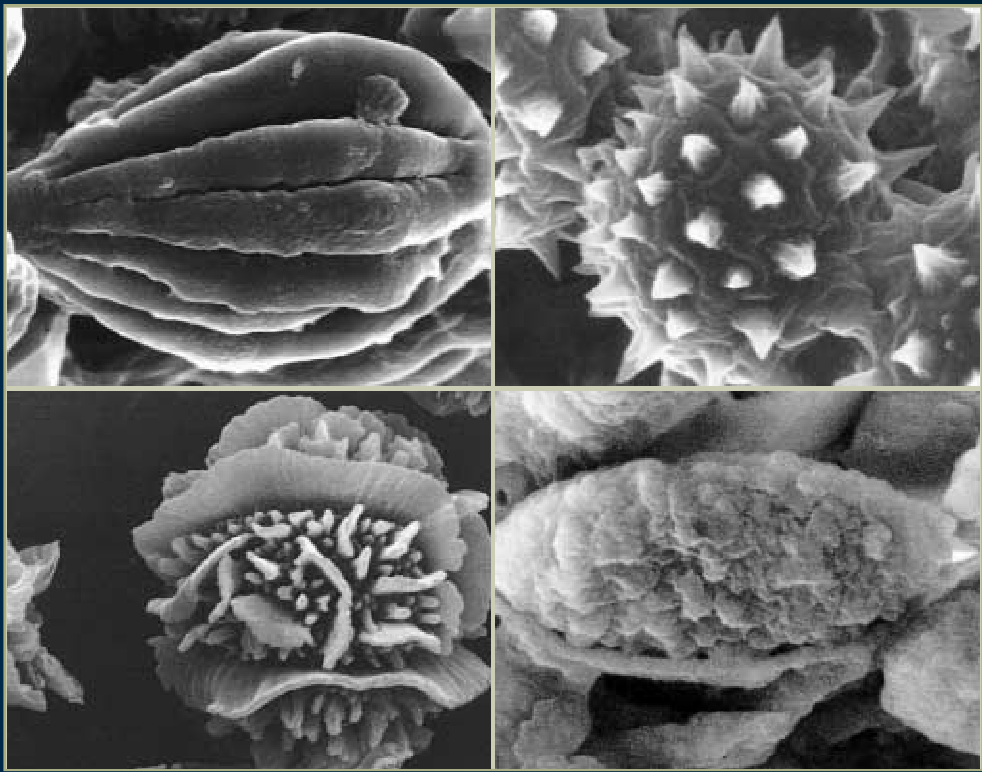
When dormant, the metabolism of the spore is substantially lowered, allowing it to survive unfavourable conditions. Until now, little has known about the processes needed to break dormancy and enable spore germination.

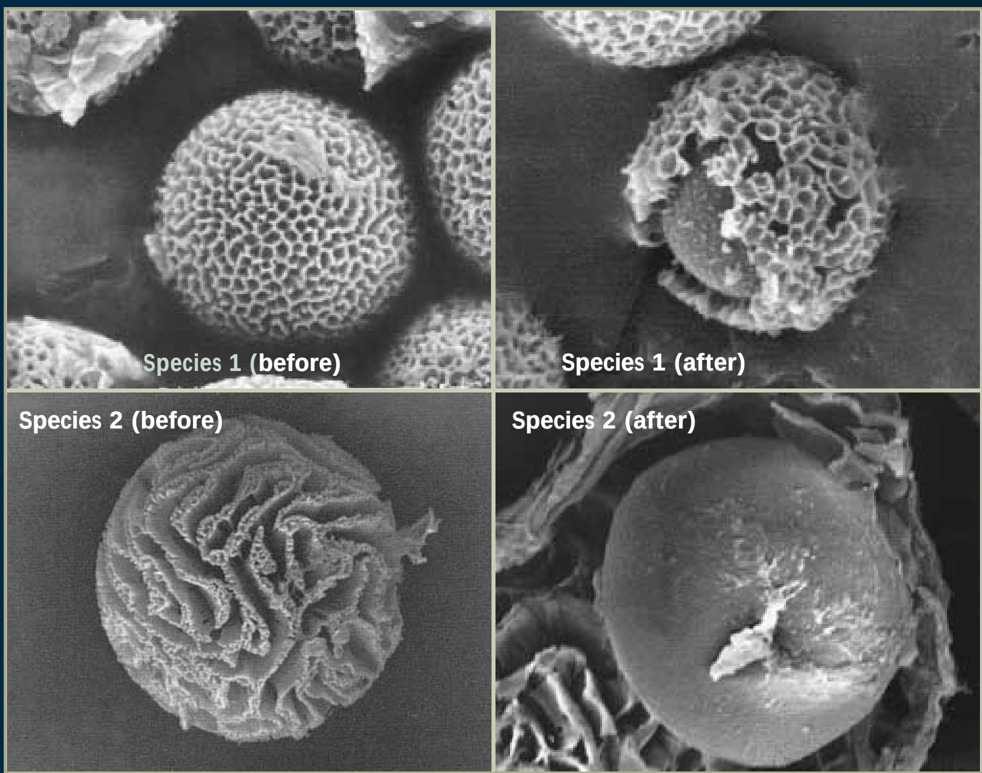
In a world first, CSIRO scientists Victoria Gordon and Paul Reddell have succeeded in breaking spore dormancy in certain ectomycorrhizal fungi. These fungi colonise the roots of native woodland plants such as eucalypts, aiding their growth and development by drawing moisture and nutrients from the soil.

Using an enzyme called 'lipase', Gordon and Reddell were able to remove the ornaments from the surface of spores. The enzyme is thought to work by breaking down a fatty layer between the spore and its ornaments, lifting the ornamental layer off and exposing the smooth spore beneath.

The discovery will lead to improved inoculation methods for woodland plants used in environmental rehabilitation and plantation forestry.

These 'before and after' images show the digestion of *Elaphomyces* spore ornaments with lipase. Removal of the ornaments increased the rate of germination from 0–12% in species 1, and from 0.8%–26% in species 2.





Fungal awakenings

Wendy Pyper describes the work of two ecologists whose tropical studies take them from sweltering rockpiles in the Top End to Queensland's squelching forest floors.

An important clue to the mysterious mechanism of fungal spore dormancy has emerged from a study of landscape rehabilitation at a Northern Territory minesite. The discovery brings scientists a step closer to developing ways of inoculating native plant seeds with their fungal partners, and improving the long-term success of efforts to re-establish woodland and forest communities.

Ecologists Victoria Gordon and Paul Reddell, of CSIRO's Tropical Forest Research Centre at Atherton in Queensland, have found that the spores of many 'ectomycorrhizal' fungi, which colonise the roots of native woodland plants, are covered with decorative 'ornaments'. Suspecting the ornaments might repel water and maintain spore dormancy, the ecologists devised a technique for their removal.

'We used an enzyme called lipase to remove ornaments from the spores of two ectomycorrhizal fungi, *Elaphomyces* species 1 and *Elaphomyces* species 2,' Gordon says. 'When we tested the germination rate of the treated spores, we found it increased from 0.8% to 26% in species 1 and from 0% to 12% in species 2.'

The breakthrough came while Gordon and Reddell were investigating the capacity of ectomycorrhizal fungi to survive and colonise the roots of eucalyptus seedlings planted in waste-rock dumps at Ranger uranium mine at Kakadu. The work is part of an Australia-wide rehabilitation process designed to return post-mine landscapes to indigenous forest and woodland.

'When environments are disturbed by mining, not only are the plants lost, but their fungal partners are too,' Gordon says. 'To successfully rehabilitate such areas, we often need to put back both the plants and their associated fungi.'

Ectomycorrhizal fungi benefit woodland plants by forming thick, protective sheaths around their roots. The sheaths are part of an extensive 'mycelial' network that draws nutrients and moisture from the surrounding soil and exchanges them at the plant root cell surface for carbohydrates. The mycelial sheaths also protect roots against pathogens and reduce the impact of salinity, pH and temperature extremes, nutrient imbalance and a limited or excessive water supply.

Despite the importance of ectomycorrhizal fungi in the growth of woodland species, surprisingly little is known about the extent of their colonisation or the range of fungi involved. Instead, research has focussed on another type of fungi, the 'vesicular arbuscular mycorrhiza', which form associations with woodland understorey plants such as grasses and acacias.

'Previously, the approach to minesite rehabilitation was to put back grasses, acacias and the vesicular arbuscular fungi

Minesites can be hostile environments. A metal spike and sledge hammer were used on the sweltering waste-rock dump at Ranger uranium mine to make holes for native seedlings.

Top: This 29-week-old seedling demonstrates the benefits of inoculation with fungal hyphae. Its growth far surpasses that of an untreated control (above).

Below: From wasteland to woodland. The ideal inoculation method for large-scale plantings would be to broadcast spores and seeds from the air, but the rate of germination success would depend on breaking fungal dormancy.

that associate with them,' Gordon says. 'This is a quick solution to the problem, but as these species are fire intolerant, they inhibit the establishment of overstorey trees such as eucalypts, resulting in a dead-end community.'

To redress this balance, Gordon and Reddell aimed to rehabilitate the Ranger site from the overstorey down, by putting back the large canopy trees and allowing the understorey to re-establish itself. But first they had to ensure that ectomycorrhizal fungi could survive and colonise the roots of eucalyptus seedlings in the hot, harsh, waste-rock environment.

The pair began by growing eucalyptus seedlings in a glasshouse and inoculating the roots with the 'hyphae' of various ectomycorrhizal fungi. The seedlings were then planted in the rock and 'protosoil' (gravel) at the minesite.

'We had to use a metal spike and sledge hammer to make holes for the seedlings, and the rocks were so hot that we couldn't touch them,' Gordon says.

At the end of the first year, treated seedlings were up to four times larger than their untreated counterparts, thanks to their tiny fungal associates. Building on this success, Gordon and Reddell began looking for a simpler inoculation method, which would cover the hundreds of hectares of waste rock more cost-effectively than the hand application of hyphae.

'The best inoculation method would be to broadcast spores stuck to seeds from an aeroplane,' Gordon says.

To simulate this process, Gordon and Reddell mixed up a spore-seed slurry in a watering can and poured it over hundreds of square meters of rock. But only about 1% of them germinated.

'Spores are difficult to work with presumably because in nature it is an advantage for them to remain dormant,' Gordon says.

'So the breaking of spore dormancy is critical to the development of a successful broadcasting inoculation technique that improves the rate of germination.'

The discovery of spore ornaments, their apparent role in maintaining dormancy, and the increase in spore germination when they are removed, are major steps towards improving inoculation techniques. Tweaking the ornament removal process further will no doubt see germination rates improve, and could see the application of spore technology on a broader scale.

'Because fungi produce spores in their billions, spores can easily be collected from the field, dried and then treated before inoculating the seeds of forest trees,' Gordon says.

'The inoculated seeds could then be used in large-scale projects such as aerial sowing of degraded landscapes, right down to small scale plantings in urban backyards. With further development, this technique could be useful to forestry managers, nurseries, tree groups and even home gardeners.'

Darwin nose best

AS DARWIN leapt from the truck, his nose was already twitching at the promise of fungal perfumes. Straining on his leash, the beagle encouraged his handler, CSIRO Tropical Forest Research Centre scientist Victoria Gordon, into the open woodland of casuarina, eucalyptus and acacia trees.

After a month of training, Darwin knew exactly what to do. Nose to ground, he scoured the carpet of casuarina needles for their elusive inhabitants: truffles. Small holes puncturing the continuity of the forest floor suggests bettongs and other small animals have already taken their share of the tasty bounty. But a sudden slackening of the leash and Darwin's motionless concentration tells Gordon truffles are at hand. Scraping back the thick mulch, she uncovers a handful of the small, round fungi.

'Truffles are the fruiting bodies of a more extensive network of fungal filaments [hyphae] that grow underground,' Gordon says. 'They belong to a group called the ectomycorrhizal fungi.'

Hyphae form 'ectomycorrhizal' associations with the roots of woody plants such as casuarinas and eucalypts, drawing moisture and nutrients from the surrounding soil for the plant's use. In return, the plant provides the fungus with carbohydrates that help it form fruiting bodies and disperse its spores.

While Darwin enjoys his reward – a piece of cheese – CSIRO ecologist Paul Reddell is still turning over the forest floor mulch with a rake. Unlike Darwin, Reddell can't smell the odour truffles release to attract animals that eat and disperse their spores.

'You can cover more ground with a dog then you can with a rake,' Gordon says. 'And I have more success with a dog'.

Gordon has trained Beagles and native bandicoots to search for truffles, which she uses in her research on the rehabilitation of mining sites (see main story). She trained Darwin by placing hand-picked truffles under leaf litter, then acting excited about the discovery and giving him a piece of cheese. Swept up in the excitement, Darwin soon learned that finding truffles led to a delicious reward.

Top: Victoria Gordon and her beagle Darwin hunt for truffles beneath a thick carpet of casuarina needles.

Above: A more laborious way of finding truffles is to rake the forest floor, as demonstrated by Paul Reddell.

Darwin inspects his find.