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# fruits of the fore

The cloying odour of insect repellent does little to deter the leeches as we squelch our way, single file, through the rainforest.

After a week of unremitting rain that left large parts of Queensland's wet tropics submerged, this sucking mud track is the last place most people would venture. But for ecologists Paul Reddell and Victoria Gordon from CSIRO's Tropical Forest Research Centre, it's just another day at the office.

Here on the Atherton Tableland, about 80 clicks south-west of Cairns, the pair is using ecology to find forest fruits and seeds containing chemical defences against predators. Their work may one day see these chemically armoured 'propagules' become a source of new bioactive compounds for medicine and agriculture.

'Evolution has made rainforests a rich source of chemical innovation,' Reddell says, stooping to pick up a fat purple 'forest walnut'.

He says medicine already benefits from more than 50 rainforest-derived drugs, including alkaloids such as cocaine, morphine, quinine and medicines used to treat heart disease and cancer.

But finding new sources of chemicals can be difficult. This is where a knowledge of ecology – the study of interactions between organisms and between organisms and their physical environment – comes in handy.

'There's a whole raft of problems you can solve by looking at ecology,' Reddell says, turning the forest walnut in his hand. Evidence of gnawing suggests a small mammal found the fruit distasteful.

'Plants can't run and hide from predators or escape harsh environmental conditions, so they must mediate their interactions with the environment and other organisms chemically,' he says.

'If you understand where these defences are likely to be well developed, and why, you can target those places.'

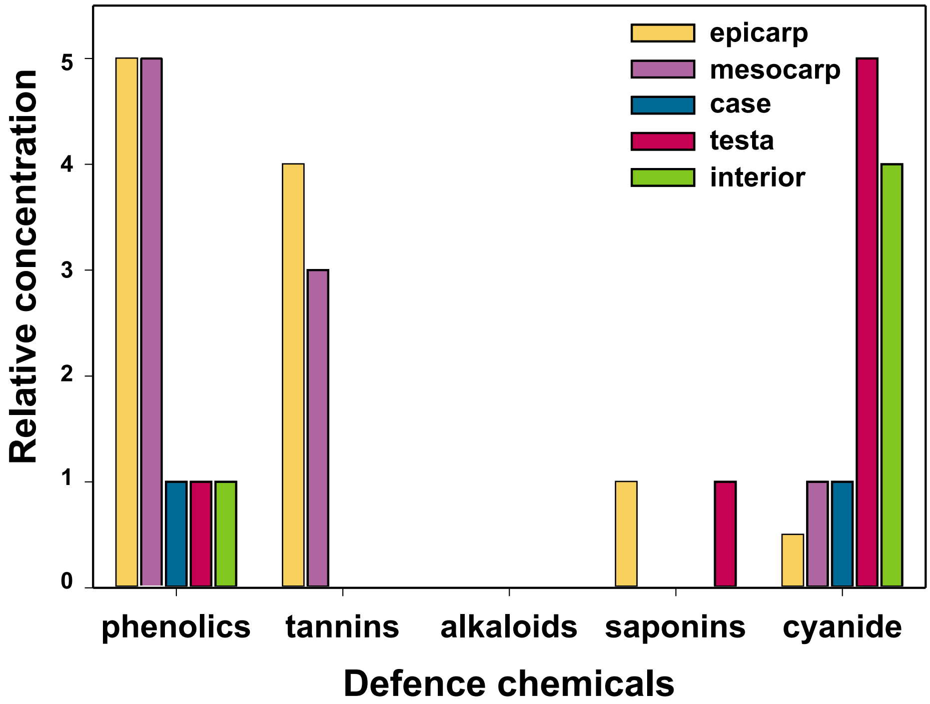
Fruits and seeds, the regenerative stage of a plant's life cycle, often concentrate chemical defences in one or many parts of their structure, making them an obvious target for Reddell and Gordon. But rather than collecting every fruit and seed in sight, the pair has focussed on two ecological groups: large seeds, which contain a significant investment of nourishing fats, oils and carbohydrates, and seeds that have been sitting untouched in the soil 'seed bank' for a long time.

Seeds that aren't being eaten or degraded must contain some deterrent compounds that repel forest-dwelling animals, fungi, insects and microbes in search of an easy and nutritious meal.

As we slither our way deeper into the rainforest, Reddell, with one eye on the ground and another on the trees, spots a cache of cassowary plums just off the track. After a quick scout around for the parent tree, he hunkers down and begins


Left: Paul Reddell and Victoria Gordon have focussed their study on two ecological groups: large fruits and seeds, and seeds from the soil 'seed bank'. Large fruits such as these cassowary plums – which linger on the forest floor, relatively untouched by insects, animals, microbes or fungi – are potential sources of bioactive compounds.

Below: Layers of bioactivity. Different parts of the fruit contain different chemical compounds, according to their particular role in plant survival (such as protection from predators, or aiding germination). An understanding of these processes has helped Gordon to extract plant compounds, some of which are more powerful than standard antibiotics and fungicides.




to peel a fruit. Each layer is a potential source of bioactive chemicals.

'Fruits and seeds are a complex system of biological engineering designed to satisfy a whole lot of requirements, some of which conflict,' he says.

For example, the outer part of a fleshy fruit, known as the epicarp, often contains compounds that are active against insects, to prevent them boring in and rotting the flesh inside. But the epicarp and the flesh it surrounds (mesocarp), may be quite tasty to an animal, ensuring a means of dispersal. In contrast, the seed coat (testa) may be active against fungi, insects or animals, while the seed itself is often well defended against most predators.

'So in terms of packaging, a propagule must deal with conflicting interests,' Reddell says. 'There might be times in its life when it needs an animal and times when it doesn't. So localising and layering its structure is how it deals with these biological tradeoffs.'

An awareness of this localisation strategy when examining fruit for bioactivity is therefore of great importance.

'Because the chemicals are localised in the fruit, it would be easy to miss them if the whole fruit were extracted and assayed,' Reddell says.

But Gordon, a chemical ecologist, knows exactly how to find them. Back at the laboratory, she freeze dries the various layers of her rainforest bounty and grinds them finely. Using a range of solvents, she extracts water and fat-soluble compounds from each fruit layer. She then analyses the extracts for five broad groups of bioactive compounds: alkaloids, saponins, tannins and phenolics, cyanide and terpenes.

Extracts are then bioassayed by placing a small paper disc, soaked in the plant extract, on cultures of *Staphylococcus aureus* and *Aspergillus niger*, both important human pathogens. Extracts that inhibit the growth of the organisms are then analysed by thin layer chromatography (TLC), which separates chemicals in the extract depending on their molecular weight and polarity.

A second bioassay is then performed over the top of the TLC plate, to identify specific groups of inhibitory chemicals. When these have been identified, the 'zone of inhibition' is cut from the TLC plate, purified and separated by high performance liquid chromatography before being sent for further analysis.

Gordon says the approach has been a great success.

'In the 18 months this project has been running, 67% of our plant extracts have shown bioactivity,' she says. And we've already identified extracts that are more powerful than standard antibiotics and fungicides currently in use.'

Reddell and Gordon have also identified extracts with herbicidal, insecticidal and cytotoxic (anti-cancer) properties. The activity of these chemicals in their crude and therefore dilute extracts indicates that they are also extremely potent.

The researchers say their work will provide a strong knowledge base for future commercial ventures to target bioactive chemicals of interest, not only in fruit, but other ecological areas such as in insects, soil microbes and fungi.

'Wood contains a lot of phenolics and tannins. So if you look at the fungi that break wood down, you could find enzymes or chemicals that could be used to clean up oil spills,' Gordon says.

Chemists could then tinker with natural bioactive molecules to mimic or enhance their activity synthetically. Compounds that proved difficult to produce synthetically could be produced in plantations. Even the power of gene technology could be harnessed.

'You could slot a gene into *E. coli* and produce it in a vat in a processing plant,' says an enthusiastic Reddell.

In the excitement of possibilities however, Reddell delivers a more sombre and important conservation message.

'Rather than seeing this work as a threat to the forest, I hope it shows what a valuable resource rainforests are. If more people are educated to understand how vital these forests are, that there's more aesthetic, spiritual and human welfare reasons to conserve them than just the furry animals that live in them, it may provide more of an impetus to conserve them.'

*This work was done in collaboration with the Rainforest CRC in Cairns.*

*Wendy Pyper*

**Above: Victoria Gordon peels the epicarp layer from the fruit of a native mangosteen (*Garcinia warrenii*). Layers must be separated before testing for bioactivity, as fruits localise their chemical defences.**

**Left: This walnut illustrates the layers found in many fruit. The epicarp consists of a thin outer layer or skin surrounding a thick, dark mesocarp. Inside the mesocarp is the seed case, a thin, light brown layer. This surrounds an even thinner testa or seed coat. The white centre is the seed itself.**

**Bottom left: Evolution has made rainforests the source of chemical innovation. Some of the bioactively promising rainforest fruits Reddell and Gordon have collected include, in front, native mangosteens and red carabeens. When cut in half, the native mangosteen reveals a fleshy mesocarp surrounding two seeds. Behind, from left to right, are cassowary plums and fruits from a native fig, walking stick palm, silver ash, Macilwraith satinash and the orange tamarind.**

**Abstract:** In separate projects, CSIRO ecologists Paul Reddell and Victoria Gordon are investigating the role of ectomycorrhizal fungi in minesite rehabilitation, and applying principles of rainforest ecology to uncover bioactive compounds. Ectomycorrhizal fungi form symbiotic associations with woodland and forest plants, and their broadscale application could improve revegetation success. But an inherent dormancy means fungal spore germination is poor. The ecologists have found the key to unlocking this dormancy lies in 'ornaments' surrounding the fungal spores and have orchestrated their removal, a vital step towards developing seed inoculation techniques. In the rainforests, the researchers are targetting likely sources of new antibiotics, anti-cancer drugs, herbicides, fungicides and insecticides. These can be found in crude extracts from different fruit layers – skin, flesh, seed coat and seed – according to the fruit's defensive and reproductive strategies.

**Keywords:** seeds; seedlings; rainforests; biochemistry mycorrhiza; fungi; ectomycorrhizal fungi; spore dormancy; landscape rehabilitation; symbiosis.