NIR reveals why koalas eat from certain *Eucalyptus* trees

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Eucalyptus trees dominate more than 90% of Australia's forests and woodlands and support a unique group of animals. There are more than 750 species of eucalypts with all but two of them found exclusively in Australia. They range from small bushy shrubs of 1–2 m to 95 m forest giants that are the tallest flowering plants on the planet. Eucalypts occur from the snows of the alpine regions, through deserts and flooded swamps and many have been planted outside Australia. One species (the river red gum) is even credited with helping to drain malarial swamps near Rome. Important though that was, a group of enterprising Benedictine monks found yet another use for these humble eucalypts and prepared a distinctive and very potent liqueur called "Eucalitino". So if you want to know what flavours a koala has to bear, come and see me at the Verona meeting—I'll try to have a bottle of "Eucalitino" on hand for a personal tasting!

Our interest in *Eucalyptus* focuses more on its value as a food source for four species of tree-dwelling marsupials. The koala is the best known and biggest of these animals, but they also include the greater glider and the ringtail and brushtail possums. Although the koala is the best known for its dependence on *Eucalyptus* leaf the other three species all rely on eucalypt leaf entirely or for a substantial part of their diet.

We are not the first to try to explain why the koala and these other marsupials have such a limited diet. There have been at least 25 studies over the past 50 years but all have been constrained by the complex chemistry of *Eucalyptus* leaves. Up to 40% of the dry mass of a *Eucalyptus* leaf is a complex mixture of terpenoids and polyphenolic compounds. These secondary plant metabolites may be toxic or distasteful and so influence animal feeding but the problem remains that ecologists do not know which components to measure—the chemistry is just too complex.

Ring-tailed possum.

Figure 1. A single tree which has been completely browsed by common ringtail possums—resistant trees of the same species in the background.

We had been using NIR spectroscopy as a rapid method of analysing the nutrient content of plants eaten by other wild herbivores for some time[1] but given the poor success of the previous studies, there seemed little point developing NIR-based models of constituents such as protein and fibre that the koalas appeared to find unimportant! However, when we carefully watched koalas and other marsupials feeding, it became clear that not all eucalypts were alike. Some trees were always favoured yet others of exactly the same species growing adjacent were always avoided. Insects treat eucalypts similarly (Figure 1). This then suggested a new approach to examine the basis of the koala's fussy diet. First, we compared the NIR spectra of plants that were always eaten with those that were apparently resistant to marsupials. These comparisons indicated that peaks at 1716 nm, 2188 nm, 2220 nm, 2268 nm and 2364 nm were particularly important in explaining the difference in utilisation of resistant and susceptible trees but it was not possible to attribute these peaks to any known group of compounds.

This approach has been used elsewhere with some success to try to identify those plant fractions that confer resistance against herbivores (see, for example, References 2 and 3) but ultimately it has to be accompanied by detailed chemical studies. In our case, this meant a laborious and time-consuming process of fractionating foliage extracts and feeding those extracts back to animals[4] but we eventually identified a new group of natural plant toxins called sideroxylonals as potent antifeedants against koalas and ringtail possums. When these were present in the foliage at concentrations of more than $15\ \mathrm{mg\ g^{-1}}$ leaf, common ringtails would refuse to eat any leaf at all. For most animals it is a combination of both the intake and the digestibility of a food that determines its nutritional quality but these toxins were so deterrent that the animals refused to eat irrespective of the levels of nutrients in the food.

Now that we knew what we should be analysing the foliage for, we developed

Figure 2. A resistant branch on an other wise susceptible *Eucalyptus sideroxylonal* tree near Cumnock NSW—in this case insects (Christmas beetles) are largely responsible for defoliation. Other resistant trees of the same species in the background.

Figure 3. Two *E. sideroxylonal* trees growing adjacent—one resistant tree and the other has been defoliated by Christmas beetles.

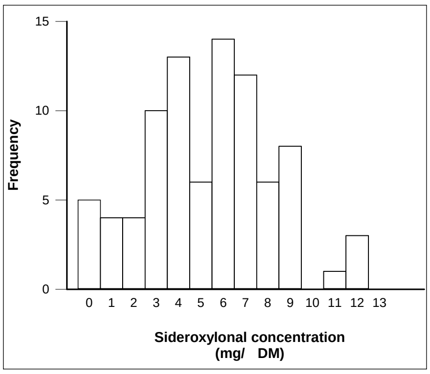

Figure 4. Distribution of foliar sideroxylonal (determined by NIR) in 84 *E. polyanthemos* trees at Captain's Flat, NSW, Australia.

an NIR spectroscopy-based model of sideroxylonal concentrations in leaves to replace the long and tedious HPLC method. There is so much variation between the food value of individual trees in a forest and even within one tree (sometimes there are resistant branches on otherwise susceptible trees!) (Figures 2 and 3) that sampling has to be intense. Without NIR spectroscopy we could not sample multiple branches on many trees over many seasons (Figure 4).

Importantly NIR spectroscopy was also able to provide an independent confirmation of our detailed chemical work. We examined the wavelengths that best correlated with the two important measures that we had made (Figure 5) First, wavelengths that were important for predicting sideroxylonal (with the direction of the effect indicated in brackets) were 1756 nm (–), 2188 nm (+), 2220 nm (–), 2260 nm (–), 2364 nm (+). The wavelengths that contributed most to calibrations of feeding by common ringtail possums were 1652 nm (+), 2188 nm (–), 2220 nm (+), 2364 nm (–) and 2404 nm (+). Note the importance of wavelengths 2188 nm, 2220 nm and 2364 nm which are used in both predictions but which have opposite signs. This result provided independent but clear evidence that foliar concentrations of sideroxylonal are a major determinant of feeding in common ringtail possums and later studies have shown this for koalas as well.

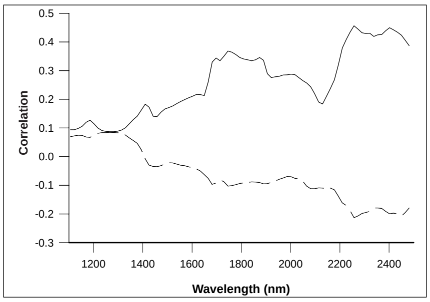

Figure 5. Importance of individual wavelengths in contributing to the prediction of foliar sideroxylonal concentration (Solid line) or prediction of standard intake of the same foliage by common ringtail possums.

So now we knew that we could use NIR spectroscopy to measure the concentration of toxins that were important to feeding but these measurements were still one step removed from telling us just how much food an animal would eat. But we then started to think—if we can measure the sideroxylonal concentration using NIR spectroscopy and since we know that foliar sideroxylonal influences how these marsupials feed, we should be able to directly predict feeding rates using NIR spectroscopy without even considering the intermediary analyses. Happily this proved to be the case and we have adequate models of food intake in koalas, ringtail possums and greater gliders. In each model the *SECV* is between 3 and 6 g of leaf which is more than satisfactory for our purposes.

Importantly, our first model of the feeding rates of koalas and common ringtail possums took two of us an afternoon to complete using a data set previously collected in conjunction with the detailed chemical work and allowed us to account for 93% of the variation in feeding. In contrast, the detailed chemical work took four people three years to complete and allowed the explanation of 75% of variation in feeding. Being able to predict how much an animal would consume in a standard experiment, independent of detailed chemical analyses, means that we can measure what really matters to an animal instead of measuring all the chemical intermediates and then drawing some conclusions about how these chemicals might affect the animals.

In agriculture several groups have developed NIR spectroscopy-based models of food intake in sheep and cattle and so this success at predicting food intake for leaf-eating marsupials is not a first. However, the types of foods eaten by wild mammals are almost always chemically complex compared to foods eaten by domestic animals. Therefore, this demonstration that NIR spectroscopy can be used to predict such a complex attribute as the intake of *Eucalyptus* leaves by koalas will be an important waypoint in convincing ecologists of the potential for NIR spectroscopy in their studies.

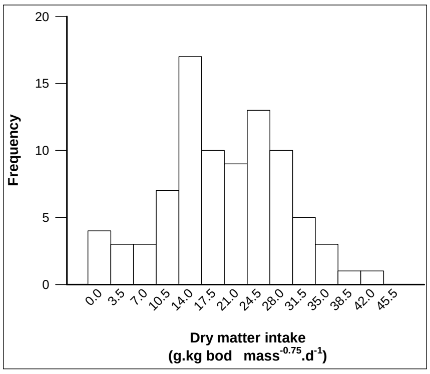

Figure 6. Distribution of potential feeding pressure by common ringtail possums as predicted by NIR in 84 *E. polyanthemos* trees at Captain's Flat NSW Australia.

Our first use of this approach was to map an area of *Eucalyptus polyanthemos* trees in a forest inhabited by ringtail possums. At first glance, this area looked as if it contained significant food resources for the animals—all the trees bore plenty of young and healthy leaves—but NIR spectroscopy allowed us to see the forest as the animals do. We collected leaf from every tree within an area equal to the size of the home range of one common ringtail possum and predicted the standard feeding rate with our NIR spectroscopy-based models. The results showed that about 60% of the trees in this forest patch were of such low palatability that the animals would not have been able to maintain weight if that was the sole food available to them (Figure 6). A further 30% were of marginal palatability that left only 6 out of the 84 trees as being palatable enough to support ringtail possums. Therefore, an area which looked favourable when viewed from a human perspective proved to be of only marginal value for animals when NIR spectroscopy was used to get the animal’s perspective.

This exciting result led us to more ambitious sampling (some in the lab would say “to bite off more than we can chew!”) and we have recently sampled 1700 trees in a forest inhabited by koalas, ringtail possums and brushtail possums on Phillip Island in Victoria. The major limitation has been in drying and grinding the samples and so we have developed good calibrations using whole fresh leaves. We are currently applying these models to produce the first palatability map of a forest for any herbivore.

Convincing ecologists of the value of NIR spectroscopy has not been straightforward. At conferences we have found that whereas most are happy to accept that NIR spectroscopy can reduce the laborious laboratory work of chemical analysis of many samples, it is harder to convince them that NIR spectroscopy is equally useful for complex behaviours such as feeding. Our argument is that although few ecologists will ever be able to do the detailed chemical work on the tree foliage eaten by endangered marsupials and primates, they need to be able to predict which habitats and patches are of superior nutritional quality urgently. NIR spectroscopy captures chemical information sufficiently well for spectral data to be more than adequate for many studies of plant-animal interactions. Hopefully we'll see more and more applications in the mainstream ecological literature as the doubters are convinced!

We are moving towards collaborations with the remote sensing community because although our lab-based NIR instrument vastly improves the numbers of samples we can analyse, we need to be able to predict habitat quality over even wider areas in order to influence conservation and land-use decisions. Barriers between those using NIR spectroscopy in laboratory settings and those trying to collect and interpret spectra either from air-borne or satellite platforms are breaking down. This is largely because laboratory-based NIR spectroscopy instruments can provide a much better definition of appropriate wavelengths to combine with the improvements in resolution of modern air and space-borne spectrometers. With the launch of the first hyperspectral imaging satellite (ARIES—Australian Resource Information and Environment Satellite) in 2000, we hope to apply some of our initial efforts in animal habitat modelling more widely.

References

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