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Diet selection in marsupial folivores of *Eucalyptus*: the role of plant secondary metabolites

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Past efforts to explain feeding by eucalypt folivores focussed on three groups of plant secondary metabolites — phenolics, tannins and terpenes. It is yet unknown if these compounds consistently limit the food available to marsupial folivores or whether they provide a better explanation of food choice or habitat quality than do nutrient concentrations alone. Recently, the formylated phloroglucinol compounds were shown to defend many eucalypts against marsupial browsers. This discovery has enhanced our understanding of the interaction between marsupial folivores and *Eucalyptus* while elaborating its complexity. Some informal subgenera of *Eucalyptus*, such as *Monocalyptus*, appear to lack formylated phloroglucinol compounds while others (e.g., *Symphyomyrtus*) contain a wide variety. Of most importance ecologically, is the huge variation in formylated phloroglucinol compound concentrations between individual trees within species, even within a small area. This makes it impossible to generalize about "food trees" across species. The concentrations of formylated phloroglucinol compounds and terpenes in foliage are positively correlated. It appears that folivores use smell to gauge terpene concentrations and hence that of formylated phloroglucinol compounds, and thus to avoid the foliage of some trees and feed from others. Thereafter, a physiological feedback mechanism, involving the emetic system, keeps the dose of formylated phloroglucinol compounds below a threshold. This understanding of formylated phloroglucinol compounds makes it pertinent to re-evaluate the roles of other plant secondary metabolites, particularly tannins, in marsupial feeding. However, the greatest challenge ahead is to extrapolate the results of feeding experiments with captive animals to predicaments of wild marsupial folivores. Near infrared spectroscopy provides a way of measuring formylated phloroglucinol compound concentrations in samples from hundreds of trees. Preliminary research suggests that it may be possible to measure formylated phloroglucinol compounds at the landscape scale with remote sensing.

INTRODUCTION

TREE foliage is generally poor food for mammals because of the high concentration of indigestible material, low concentrations of protein and other nutrients and, usually, the presence of toxic or anti-nutritional plant secondary metabolites. Those animals that depend on tree foliage face significant nutritional challenges. This is particularly so for arboreal species, which face opposing constraints on selection for small body size for mobility and access to the best foliage, and selection for large body size which should maximize returns from digestion of fibrous materials (Cork and Foley 1991). The importance of these limitations is highlighted by the paucity of mammals that feed predominantly on *Eucalyptus* foliage. Despite the ubiquity of eucalypts in Australia (Williams and Woinarski 1997), their foliage is eaten appreciably by only four marsupial species. These species — the focus of this review — are the greater glider *Petauroides volans*, the common ringtail possum *Pseudocheirus peregrinus*, the common brushtail possum *Trichosurus vulpecula* and the koala *Phascolarctos cinereus*. Some macropodids (e.g., *Wallabia bicolor* and *Thylogale billardierii*) may eat *Eucalyptus* foliage, especially seedlings in plantations grown for wood fibre (Montague 1994, 1996).

Folivores of *Eucalyptus* are known to carefully choose their food trees. Many observers have commented on the preferences shown between different *Eucalyptus* species and even between individual trees within species, especially by koalas (e.g., Fleay 1937; Pratt 1937; Eberhard 1978; Robbins and Russell 1978; Hindell and

Lee 1987, 1988; Pahl 1987; Kavanagh and Lambert 1990; Pahl and Hume 1990; Hume and Esson 1993; Melzer 1994; Moore and Foley 2000). This, and the fact that many eucalypt forests grow on poor soils, has led some authors to suggest that populations and distributions of these animals are limited by food availability, even though it may appear superabundant (Degabriele 1981; Braithwaite et al. 1983; Norton 1987; Cork and Catling 1996). Clearly, if scientists are to understand and model the effect of nutritional factors on habitat quality and population density, they must understand what chemical factors determine the food quality of foliage.

Many studies in the past two decades have tried to explain the feeding preferences of marsupial folivores of Eucalyptus. Although these preferences may be partly due to differences in foliar nutrients, particularly foliar nitrogen concentration, between species, individuals and leaf age groups (Ullrey et al. 1981; Geritz 1987; Kavanagh and Lambert 1990), some plants that appear to be nutritionally adequate are avoided. In such cases, researchers point to toxic or anti-nutritional plant secondary metabolites as an explanation (e.g., Hindell et al. 1985; Martin 1985; Pahl 1987; Hindell and Lee 1988; Comport et al. 1996). Despite studies focusing on the role of plant secondary metabolites in the food choices of marsupial folivores (McArthur and Sanson 1991; Zoidis and Markowitz 1992; Hume and Esson 1993), there is still no universal explanation.

In this chapter, we review research into the effects of plant secondary metabolites on the feeding behaviour of marsupials that browse Eucalyptus foliage. Our discussion is divided amongst the three major groups of plant secondary metabolites commonly implicated in food choice and that are commonly measured, namely total phenolics, condensed tannins and terpenes.

Sometimes, researchers have proposed other unspecified plant secondary metabolites to explain differential feeding by folivorous marsupials without the chemical studies to support such proposals (e.g., Braithwaite et al. 1983; Cork and Pahl 1984; Statham 1984; Hindell et al. 1985; Hindell and Lee 1990). Later in this review, we describe the pivotal role that a newly discovered group of plant secondary metabolites plays in determining some food choices of marsupial folivores. We describe how an approach using bioassay-guided fractionation led us to these compounds, and how by focusing on intraspecific variation in leaf chemistry we confirmed their importance in the feeding preferences of marsupial folivores. We then discuss data on the variability in concentrations of these compounds among and within species, and the implications of these for animal foraging and habitat quality. Finally, we discuss an analytical technique to minimize the logistical obstacle of conventional laboratory analyses.

REVIEW OF EARLIER STUDIES

The feeding choices made by marsupial browsers of Eucalyptus have typically been studied using a correlative process consisting of four steps:

1. Observe the feeding behaviour of animals. This may involve recording feeding by wild animals or, alternatively, the food intake of captive animals in choice or no-choice experiments, where the researcher determines the food offered to the animals.

2. Collect samples of food available to the animals. In field studies, a bulked sample representing each Eucalyptus species at a site is often used. With captive animals, samples are taken from the leaf actually offered to the animals.

3. Analyse the leaves, quantifying compounds or characteristics determined previously.

4. Relate leaf chemistry to feeding preference or food intake with a range of regression-style statistics.

Note that in step one we list recording of feeding behaviour or food intake. We have limited the discussion in this chapter to food intake as a measure of animal preference for two reasons. First, measurement of other nutritional indices, such as dry matter digestibility or metabolizable energy intake, has not been possible for free-ranging animals, whereas it is possible to measure an animal's preferences for individual trees. Secondly, despite a sound theoretical basis, there is no published evidence that metabolizability or digestibility of foliage diets varies widely. This may simply reflect the limited work done with captive animals but, nonetheless, it is clear that these animals have sufficient digestive and physiological adaptations to function across the range of digestibilities that wild animals may encounter. Therefore, in the absence of information on what constitutes adequate "performance" on a diet, we suggest that food intake is the best indicator of the suitability of a particular foliage diet. Put simply, if an animal does not eat a leaf it gains no nourishment, and digestibility is irrelevant.

There has been a focus on "total" phenolics, condensed tannins and terpenes as explanatory variables for feeding by marsupial folivores on Eucalyptus. This has probably arisen because the chemicals are abundant in Eucalyptus foliage and because studies in some other species (reviewed

in Foley *et al*. 1999) suggest that they reduce the nutritional quality of plant foods. Below we consider how each group may influence the feeding decisions of marsupial folivores.

"TOTAL" PHENOLICS

Phenolics are compounds in which the molecule includes an aromatic ring with an attached hydroxyl (-OH) group. Phenolics are often highly reactive (Morrison and Boyd 1987), which may explain why they have received attention as potential defensive compounds. The term "phenolic" covers a diverse range of compounds, including tannins. Even within eucalypt species there may be considerable qualitative variation in phenolic compounds (Hillis 1966) and a matching variance in potency as antifeedants. It is this variation that calls into question whether an inclusive measure, like "total" phenolics, can explain feeding.

We have used quotation marks when referring to "total" phenolics, because the common analyses do not measure all phenolic compounds in *Eucalyptus* foliage. In fact, perhaps the most important plant secondary metabolites in the interaction between *Eucalyptus* and marsupial folivores are phenolics that do not produce colour in Folin reagents (Lawler *et al*. 1998a). Typically, "total" phenolics refers to those phenolic compounds extracted in 50% aqueous acetone or aqueous ethanol (Cork and Krockenberger 1991) — a common measure in studies of marsupial feeding (Foley 1992; Hume and Esson 1993; Hume *et al*. 1996). These solvents primarily extract polar compounds whereas the phenols range from polar to non-polar.

Short-term studies with captive animals and field studies of koala habitat use at individual sites provide little evidence that "total" phenolics influence feeding (Cork and Pahl 1984; Hume and Esson 1993; Bednarik 1996; Lawler *et al*. 1998a). An unknown proportion of lower molecular weight phenolics may be absorbed and exert some toxic effects (Cork and Sanson 1990). However, although animals incur a cost in metabolizing these compounds, in terms of effects on acid-base balance and nitrogen excretion (Foley and Hume 1987a; Foley 1992; Foley *et al*. 1995), common ringtail possums do not eat less in response to increasing concentrations of foliar phenolics (McDowell 1999). Particular phenolics may defend trees against marsupial herbivores but this can be illustrated only by identifying the particular compound and assaying it separately from the total phenolic pool.

The only consistent trend involving total phenolics and marsupial folivores has been at the landscape scale. Cork and co-workers (Cork 1992; Cork and Catling 1996) have identified a threshold in the ratio of foliar nutrients to non-condensed tannin total phenolics below which viable populations of leaf-eating marsupials apparently cannot be sustained. However, it should be noted also that foliar nutrients alone provide a similarly strong distinction between suitable and unsuitable sites (Cork 1992; Pausas *et al*. 1995; Braithwaite 1996). Cork and Catling (1996) argue that nutrient:phenolic ratios are a better measure than nutrients alone because some phenolics bind some nutrients. This relies on the reasonable but untested assumption that the proportion of phenolics responsible for folivore deterrence varies consistently with the concentration of "total" phenolics. However, as nutrient and "total" phenolic concentrations are negatively correlated within (Moore *et al*. 2004b) and among (Cork 1992) many eucalypt species, nutrient concentrations alone will probably show a similar pattern to nutrient:phenolic ratios.

Tannins

Tannins are distinguished from other polyphenolic compounds by their ability to bind and precipitate proteins (Silanikove *et al*. 1996). Condensed tannins have been of particular interest because they resist degradation in the gut. However, the lack of a simple method to quantify hydrolysable tannins has meant that this potentially important group of tannins has received less attention than it should. Recent advances in analytical methods (Barry *et al*. 2001; Hartzfeld *et al*. 2002) should change this. In contrast to other plant secondary metabolites, the effects of tannins on the feeding of marsupial folivores of *Eucalyptus* have been intensively studied perhaps because their potential actions are well known. It was thought that the protein-precipitating capacity of tannins would reduce the availability of dietary protein, already seen as being in short supply (Cork 1986; Cork and Sanson 1990). However, few data support this idea (Foley *et al*. 1999). Tannin assays suffer the same problems afflicting those for "total" phenolics. In particular, tannins are assayed as a single group so that specific compounds of widely varying molecular structure and activity against herbivores are measured inclusively (Clausen *et al*. 1990, 1992; Ayres *et al*. 1997).

At least the three most specialized marsupial folivores of *Eucalyptus* — koalas, greater gliders and common ringtail possums, possess mechanisms to combat tannins. In common ringtail possums, tannin-protein complexes are degraded in the caecum, which may counter any reduction in protein digestibility (McArthur and Sanson 1991, 1993). McArthur *et al*. (1991) have further suggested that the similarities in protein digestive capability, gut structure and dietary habits of

koalas and greater gliders indicate a similar capacity in these species. Common brushtail possums, which do not selectively retain fluids and fine particles in the hindgut as do the other three species (Foley and Hume 1987b), show poorer protein digestion and are probably more susceptible to the deleterious effects of tannins. Indeed, W. J. Foley and S. Dalla Pozza (unpubl. data) found a large increase in apparent nitrogen digestibility in common brushtail possums when polyethylene glycol 4000 was added to a diet of *Lumnitzera racemosa* foliage. Polyethylene glycol preferentially binds many tannins and so prevents them from combining with dietary or endogenous proteins. Addition of polyethylene glycol to a diet of eucalypt foliage led to a significant increase in fibre digestion by common brushtail possums (Foley and Hume 1987c) but had no effect in common ringtail possums (McArthur and Sanson 1991). In contrast, McArthur and Sanson (1993) found that adding a "standard tannin", quebracho, to the diet of common brushtails had no impact on their ability to digest nitrogen or fibre.

The use of polyethylene glycol offers the most promising approach to studying the effects of tannins on feeding (but see Foley *et al.* 1999) and we are currently developing *in vitro* assays that mimic these effects. However, it does not overcome the need to separate the effects of tannins from those of other dietary constituents. As we will describe later in this review, the discovery of the effects of formylated phloroglucinol compounds on feeding opens the way to re-evaluating the effects of tannins on marsupial folivores.

TERPENES

Terpenes, or essential oils, are a familiar characteristic of eucalypt foliage. Although some species (e.g., *Eucalyptus* (syn. *Corymbia*) *clarksoniana*: Brophy *et al.* 1998), and individual trees within species, may have low concentrations of terpenes, their distinctive smell is a feature of most eucalypt foliage. Terpenes can intoxicicate micro-organisms and so potentially affect the gut flora of herbivores (Nagy *et al.* 1964, but see Welch *et al.* 1982) as well as being toxic to vertebrates directly by disrupting cell membranes (Foley and McArthur 1994; McLean and Foley 1997). Accordingly, many have regarded eucalypt terpenes as feeding deterrents for marsupial folivores (Betts 1978; Southwell *et al.* 1980; Zoidis and Markowitz 1992). The effects of these compounds on the gut flora of arboreal marsupial folivores are now believed to be minimal, as all are hindgut fermenters and most terpenes are absorbed before they reach the hindgut (Foley *et al.* 1987).

Until recently, no study had shown strong and consistent relationships between feeding and foliar terpene concentrations. In fact, the results of studies attempting to relate terpene concentrations to food intakes have been contradictory, including weak negative correlations, no significant relationship, and weak positive relationships (Betts 1978; Southwell 1978; Zoidis and Markowitz 1992; Hume and Esson 1993). One possible reason for this may be the reliance on comparisons between eucalypt species. There may be wide variation in the concentrations of individual terpenes and total terpene yield both among and within eucalypt species (Boland *et al.* 1991). If there is corresponding variation in the antifeedant effect of different terpenes, then the considerable qualitative differences in terpenes, between eucalypt species, may obscure trends between the species that animals favour or reject.

RECENT DEVELOPMENTS

We now describe recent work where we have put aside preconceived assumptions about what we think is important for animals and instead let them guide us in our chemical assays.

Identification of previously unrecognized feeding deterrents in *Eucalyptus* foliage

Our interest in these questions began with observations of striking intraspecific feeding preferences of common ringtail possums for *E. ovata* foliage. In Lysterfield National Park, near Melbourne, possums defoliated some individual *E. ovata* trees while leaving adjacent conspecifics untouched (Pahl 1984, 1987; Geritz 1987; W. J. Foley, pers. obs.). Elsewhere, W. J. Foley (unpubl. data) made similar observations of *E. polyanthemos*.

Recognizing the limitations of the correlative approach, Pass *et al.* (1998) adopted a technique that has proven successful in studies of other plant-herbivore systems (e.g., Bryant *et al.* 1983; Reichardt *et al.* 1984). Bioassay-guided fractionation overcomes the problems of prejudice about which foliar constituents deter animals by continually testing dietary preferences against increasingly refined extracts from resistant foliage. It is important not to rely solely on the chemical work but to ensure that the patterns discerned from feeding leaf extracts can be verified using whole leaf. In the first stage of their study, Pass *et al.* (1998) showed that common ringtail possums would not eat foliage from a particular individual of *E. ovata* even when given no alternative food. A methanolic extract of this foliage, added to a palatable artificial diet, caused the possums to drastically reduce their food intake. This initial extract was then fractionated and the response of the animals to each fraction was tested. This process

was repeated with each antifeedant fraction until macrocarpal G (Fig. 1a) was isolated as the principal chemical compound responsible for browsing resistance (Pass *et al.* 1998). Complementary experiments with *E. ovata* individuals susceptible to browsing showed no effect with the same extracts. Analysis showed that this foliage contained negligible macrocarpal G.

Macrocarpal G belongs to a well defined group of compounds known as the formylated phloroglucinol compounds, or formylated phloroglucinol compounds, which are widespread among *Eucalyptus* species (Ghisalberti 1996; Eschler *et al.* 2000) and which are potent antifeedants against marsupial browsers. Significantly, formylated phloroglucinol compound molecules consist of both terpene and phenolic moieties (Fig. 1a–d). There is experimental evidence, discussed later, that formylated phloroglucinol compounds play a predominant role in the short-term feeding decisions of marsupial browsers. We also describe a secondary role for foliar terpenes and highlight the implications of this work for future field studies of marsupial browsing on *Eucalyptus*.

Mechanistic predictions of feeding in marsupials

Since identifying the formylated phloroglucinol compounds as important plant secondary metabolites, we have adopted a correlative approach, much the same as that described above but with three important modifications:

1. The formylated phloroglucinol compounds have been identified as antifeedants without reliance on any prior notions.

2. We have concentrated our efforts on explaining variation between individual trees within species in their susceptibility or resistance to marsupial browsing.

3. Where correlative relationships exist between feeding and plant secondary metabolites, we have done bioassays with the pure compound to show cause and effect.

This second point is important because few studies have focused on how plant secondary metabolites vary between individual trees within a species. Concentrating on intraspecific variation has two advantages. First, it recognizes that animals do not necessarily feed on "Latin binomials" but are influenced by the quality of the foliage in front of them, irrespective of its taxonomic affinities. Thus, we cannot say without qualification that common ringtail possums eat *E. ovata*. Secondly, conspecifics are likely to vary less in the types of plant secondary metabolites they contain than are individuals belonging to different species. This simplifies studies of how plant secondary metabolites influence feeding.

Fig. 1. Structures of representative formylated phloroglucinol compounds.

Formylated phloroglucinol compounds: the main line of defence?

We have now found that, for many species of *Eucalyptus*, there are individuals covering the full spectrum of palatability. For common ringtail possums, intakes ranging from almost nil to their maximum daily intake, have been recorded for *E. ovata*, *E. polyanthemos* and *E. sideroxylon* (Fig. 2). The ranking of preferences is similar in common ringtails, koalas and two macropod species (Lawler and Foley 1999) but the threshold of koalas is much higher; that is they are more tolerant of the formylated phloroglucinol compounds (Lawler *et al.* 1998a). Further experiments have shown a similar range of food intakes by koalas for *E. melliodora*, *E. camaldulensis*, *E. viminalis* and *E. globulus* (B. D. Moore, unpubl. data).

In these same experiments (Lawler *et al.* 1998a, 1999a; B. D. Moore, unpubl. data), we measured various aspects of leaf chemistry, including the putative defences — terpenes, "total" phenolics and condensed tannins, as well as measures of formylated phloroglucinol compounds. We found no relationship between food intake by koalas or common ringtail possums and condensed tannins or "total" phenolics for any of the *Eucalyptus* species studied. However, we consistently found strong negative correlations between food intake and foliar terpene and formylated phloroglucinol compound concentrations.

Initially, we focused on *E. ovata* because we knew that macrocarpal G explained the resistance of individuals of this species to browsing (Pass *et al.* 1998). However, at that time

we could not quantify macrocarpal G or any other formylated phloroglucinol compounds and so we used a crude semi-quantitative measure of total formylated phloroglucinol compounds (Lawler *et al.* 1998a). This may explain why the correlations between feeding and terpenes were stronger than those between feeding and formylated phloroglucinol compounds. However, when we measured food intake in possums fed graded amounts of cineole or macrocarpal G to reflect concentrations found in leaves, only macrocarpal G reduced food intakes. We concluded that formylated phloroglucinol compounds were likely to determine the food quality of eucalypt leaf for at least common ringtail possums. We have since confirmed this finding for koalas (Lawler *et al.* 1998a; B. D. Moore, unpubl. data).

This conclusion has subsequently been reinforced by studies of other eucalypt species. Fractionation of resistant *E. polyanthemos* trees showed that another group of formylated phloroglucinol compounds, the sideroxylonals (Fig. 1b), deterred feeding by common ringtail possums (D. M. Pass and W. J. Foley, unpubl.). Sideroxylonals are among the easiest formylated phloroglucinol compounds to quantify by high performance liquid chromatography and to isolate for bioassay experiments. Consequently, we have since studied mainly tree species in which these are the principal formylated phloroglucinol compounds. We examined the relationship between food intakes by common ringtail possums fed foliage of *E. polyanthemos* and *E. sideroxylon* (Lawler *et al.* 2000) and by koalas (B. D. Moore, unpubl. data) and common brushtail possums fed *E. melliodora* (Wallis *et al.*
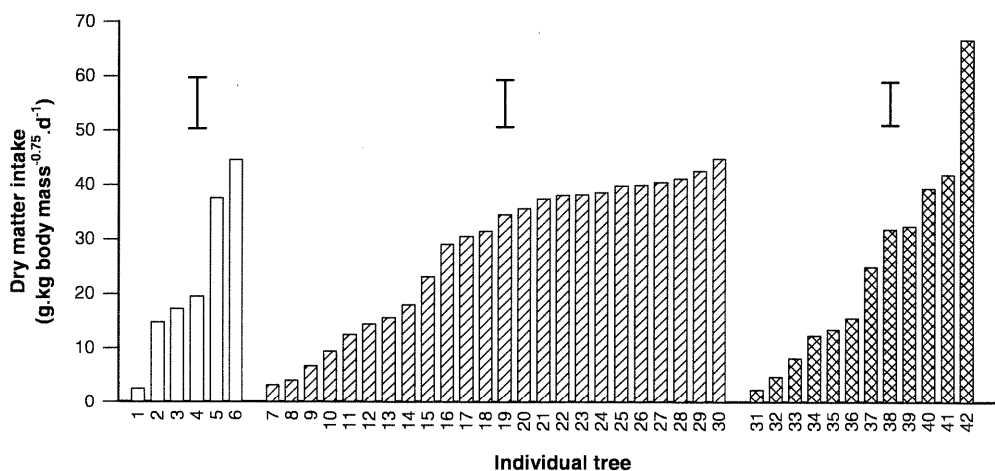

Fig. 2. Intakes of foliage of *Eucalyptus ovata* (unshaded bars), *E. polyanthemos* (bars with diagonal lines) and *E. sideroxylon* (crosshatched bars) by common ringtail possums *Pseudocheirus peregrinus*. Data are means with bars representing least significant differences. (Adapted from Lawler *et al.* 1998a, 2000).

2002) and found that the concentration of a single isomer of sideroxylonal (sideroxylonal A) could explain 65–80% of the variation in feeding. We confirmed the effect seen in foliage by adding sideroxylonal A to an artificial diet and feeding it to common ringtail possums (Fig. 3) (Lawler *et al.* 1999a).
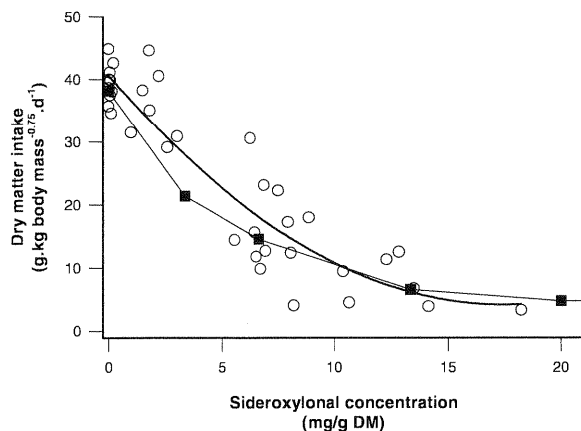

Fig. 3. Relationships between dry matter intake of common ringtail possums fed *Eucalyptus polyanthemos* foliage (open circles) or an artificial diet with sideroxylonal added (closed squares) and the sideroxylonal concentration of diets. The thick line shows the quadratic regression equation for the relationship between intake of foliage and foliar sideroxylonal concentration (r^2 = 0.86). (After Lawler *et al.* 2000).

In conclusion, we have found substantial variation between individual trees, ranging from susceptible to completely resistant to browsing, within several *Eucalyptus* species. In all cases, food intake rates have correlated strongly and negatively with foliar formylated phloroglucinol compounds and (where measured) terpene concentrations. Bioassay experiments confirm that only the formylated phloroglucinol compounds can cause resistance to browsing at typical foliar concentrations. We discuss below the variation and distribution of formylated phloroglucinol compounds among several *Eucalyptus* species, and the implications for the feeding ecology of marsupial folivores.

The action of formylated phloroglucinol compounds

We have used the simple formylated phloroglucinol compounds, jensenone (Fig. 1c), as a "model compound" to study the consequences of an animal ingesting formylated phloroglucinol compounds and their post-ingestive fate (e.g., Lawler *et al.* 1998b; Stapley *et al.* 2000). The reason for this is that jensenone was the first formylated phloroglucinol compound that we could readily isolate in sufficient quantity and purity for extensive

experiments. Jensenone has the same fully substituted aromatic ring seen in macrocarpals but with a C5 sidechain rather than a C15 terpenoid sidechain.

Adding jensenone to an artificial diet reduced food intakes by possums at similar molar concentrations to sideroxylonal A (Lawler *et al.* 1998b). Common ringtail and common brushtail possums regulate their intakes of an artificial diet containing varying concentrations of jensenone so as not to exceed a threshold dose. In order for the animals to regulate their intakes of formylated phloroglucinol compounds, across a wide range of dietary concentrations, there must be some form of feedback used as a cue to over-ingestion. We tested the hypothesis that it was due to stimulation of the emetic or nausea system, or more specifically, due to feedback from serotonin acting at the 5-HT$_3$ receptors (Lawler *et al.* 1998b). Administration of the drug ondansetron, a selective antagonist of these receptors, allowed both species of possum to eat more of a diet containing jensenone (Fig. 4). However, ondansetron does not completely ameliorate the effects of dietary jensenone (Lawler *et al.* 1998b; Stapley *et al.* 2000), indicating that some other feedback mechanism exists. This may simply be another receptor for serotonin, other neurohormones involved in the emetic response (e.g., dopamine; Grant 1987), or an unrelated pathway.
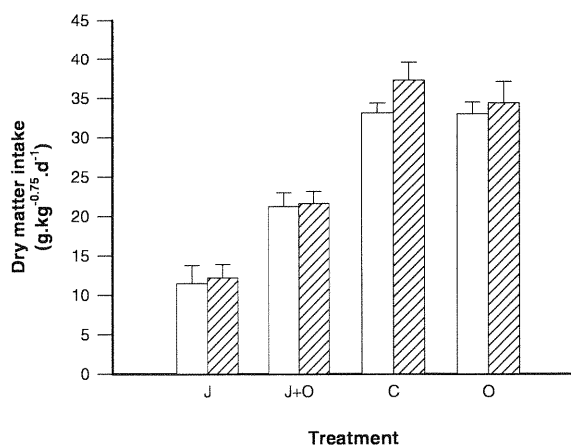

Fig. 4. The effect of administering ondansetron (O) or water (C) on the dry matter intake of common ringtail possums (open bars) and of brushtail possums (bars with diagonal lines) fed dietary jensenone (J). Data are means ± SE. (After Lawler *et al.* 1998b).

Currently, little is known of the fate of formylated phloroglucinol compounds after ingestion. They are certainly absorbed and metabolized as no trace of jensenone, macrocarpals or sideroxylonals occur in the urine or faeces of either common ringtail or common brushtail possums fed any of these compounds

(McLean *et al.* 2004). There are indications that jensenone may have a general non-specific toxic effect on cells (McLean *et al.* 2004), which causes the release of serotonin consistent with the findings described above. The toxic effects of the compounds, and the costs of their detoxification, are little known.

A different role for terpenes?

We have already described the correlation between marsupial browsing and foliar terpenes within eucalypt species. However, bioassays have shown that marsupial browsers willingly eat diets containing concentrations of terpenes that greatly exceed those measured in resistant trees. Therefore, we questioned the role of foliar terpenes in the *Eucalyptus*-marsupial interaction.

Similarities in the molecular structures of terpenes and formylated phloroglucinol compounds suggested a secondary role for terpenes. One characteristic of all of the formylated phloroglucinol compounds is an isoprene (the basic building block of terpenes) sidechain or, more commonly, a whole terpene. This suggested that formylated phloroglucinol compounds might share some biosynthetic pathways with terpenes. Moreover, if production of secondary metabolites is substrate-limited, the concentrations of the two groups of compounds should be correlated. This is the case with the relationship between the concentrations of terpenes (especially 1,8 cineole) and sideroxylonal in *E. polyanthemos*, *E. melliodora* and *E. microcorys* particularly strong (Lawler *et al.* 1999a; Wallis *et al.* 2002; Moore *et al.* 2004a).

Formylated phloroglucinol compounds have little odour compared to terpenes. Many studies have noted that marsupial folivores smell leaves before eating or rejecting them leading some researchers to conclude that a volatile cue indicates leaf palatability (e.g., Hindell *et al.* 1985; Pahl and Hume 1990). We hypothesized that because the concentrations of terpenes and formylated phloroglucinol compounds are correlated, marsupials could use the odour of the leaf terpenes as a proximal cue to assess the likely post-ingestive consequences of the formylated phloroglucinol compounds contained in the foliage (Lawler *et al.* 1998a, 1999a). Such an association between a taste and/or smell and post-ingestive consequences is termed a conditioned flavour aversion (Provenza *et al.* 1990). Animals in the wild may have existing conditioned flavour aversions to natural foods and so testing this concept is not straightforward. However, conditioned flavour aversions are dynamic and they can be reduced or eliminated by repeated exposure to the taste stimulus alone.

Lawler *et al.* (1999b) showed that both common ringtail and common brushtail possums could be conditioned to eat large quantities of cineole when it was offered in the food over a 12-day period. However, once cineole was paired with jensenone, the willingness of the conditioned animals to eat cineole was abolished. We concluded that one role of terpenes in marsupial folivore-*Eucalyptus* interactions is to act as a cue to the concentration of toxins in the leaf, rather than to act as toxins (Lawler *et al.* 1999a, 1999b).

DISTRIBUTION AND VARIATION IN FORMYLATED PHLOROGLUCINOL COMPOUNDS AMONG AND WITHIN SPECIES

Interspecific variation

The recent discovery of the importance of formylated phloroglucinol compounds in *Eucalyptus* ecology means that there are limited data on the distribution of these compounds across species (relative to the large number of *Eucalyptus* species). The initial interest in formylated phloroglucinol compounds was spurred by their potential activity in other systems, particularly as anti-bacterial agents (Murata *et al.* 1990; Yamakoshi *et al.* 1992; Singh *et al.* 1996; Osawa *et al.* 1997). This work was principally conducted by Japanese research groups, who focused on isolating and characterizing individual compounds using exhaustive studies of few species. Thus, there is substantial qualitative information on the occurrence of formylated phloroglucinol compounds in several eucalypt species but little or no information for most species.

Eschler *et al.* (2000), attempting to address more ecologically relevant questions about the distribution of formylated phloroglucinol compounds, used Fourier transform, ion cyclon resonance mass spectrometry to survey 41 eucalypt species for formylated phloroglucinol compounds. It was not possible to identify individual compounds in this manner, but different groups of formylated phloroglucinol compounds could be distinguished (macrocarpals, sideroxylonals, euglobals) and quantified crudely using the aldehyde signal in nuclear magnetic resonance. The most interesting finding was the absence of formylated phloroglucinol compounds from the 10 eucalypts selected from the informal subgenus, *Monocalyptus*. In contrast, all groups of formylated phloroglucinol compounds then known were represented among 22 species sampled from another major eucalypt group, the informal subgenus *Symphyomyrtus*. *Monocalyptus* species differ in many important ecological traits from those of the *Symphyomyrtus* (Noble 1989) and the

differences in the occurrence of formylated phloroglucinol compounds may have ecological importance. Certainly, formylated phloroglucinol compounds are likely to be important influences on herbivory in many species of eucalypts beyond the few examined.

Intraspecific variation and a technique to measure it

Standard laboratory techniques are too slow and costly to sample sufficient individual trees to assess the resources available for folivores. However, we have shown that near infrared spectroscopy can address this problem. A full description of the method is available in Foley *et al.* (1998) but, in short, near infrared spectroscopy works by analysing the interaction between near infrared light and the chemical bonds or functional groups (e.g., N-H, C-H) that comprise plant tissues. Introduction of light energy to a sample causes functional groups to vibrate at characteristic frequencies and so the light absorbed or reflected by a sample (the spectrum) must contain information about the functional groups that make up the sample. We can quantify this relationship by building a statistical model relating spectra of many samples to accurate laboratory measures of the compound(s) of interest and then use that model to predict the chemical composition of unknown samples.

The method processes large numbers of samples quickly (120 per day) with no reagents and minimal sample preparation. Also, multiple analyses can be made with little additional work using the same sample spectrum, the only requirement being a calibration equation for each component of interest.

We have found that near infrared spectroscopy is an excellent technique to estimate leaf chemistry (McIlwee *et al.* 2001) and have applied this extra sampling capacity to four surveys of intraspecific variation. The first survey was conducted on a regional scale (~300 km) and measured sideroxylonal concentrations of several populations of *E. sideroxylon* subsp. *sideroxylon* (Watson 1998). Although most of the variation observed (57% of the total) was attributable to differences between populations, a large proportion (40%) was between individual trees within populations.

In the second survey, our interest was variation in foliar sideroxylonal concentrations at a spatial scale relevant to individual animals (Lawler *et al.* 2000). We surveyed an area of forest (approx. 100 × 50 m), near Queanbeyan, New South Wales. This area is comparable to the home range of a single common ringtail possum, and an animal might encounter each tree while foraging. Sideroxylonal concentrations varied

from undetectable through to the maximum we have seen in the species. Given the relationship between food intake and foliar sideroxylonal concentrations described above (Fig. 3) (Lawler *et al.* 2000), we believe that any future survey of foraging of arboreal folivores must account for intraspecific variation in leaf chemistry.

In the third survey (Moore *et al.* 2004b) we sampled 12 tallowwood trees (*E. microcorys*) at each of 42 sites, from just north of Newcastle to the Queensland border. The sites, which were described extensively, ranged from sea level to almost 1 000 m a.s.l. and from areas of low fertility (coastal sands) to highly fertile basaltic plateaux. The fourth survey was of 150 *E. melliodora* at Mulligans Flat in the Australian Capital Territory (Fig. 5) (Wallis *et al.* 2002). In neither of these surveys did we find that simple morphological attributes of the tree (d.b.h. or height) influenced formylated phloroglucinol compound concentration. The wide variation in formylated phloroglucinol compound concentration between trees, within sites, suggests variation due to genetic differences that we are now investigating in the *E. melliodora* population at Mulligans Flat.
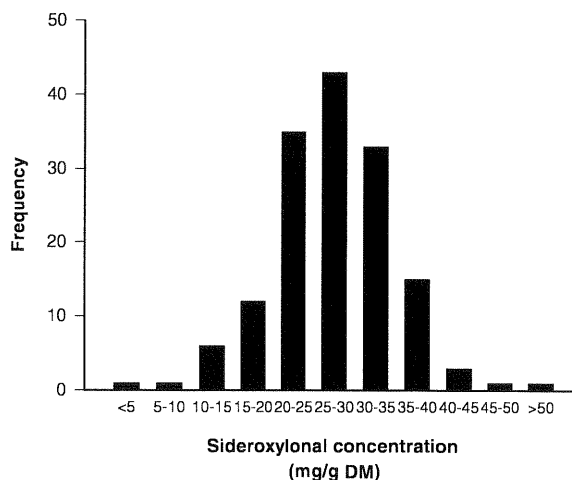

Fig. 5. Frequency distribution of sideroxylonal concentrations within a population of *E. melliodora* at Mulligans Flat, Australian Capital Territory (after Wallis *et al.*, submitted).

FUTURE DIRECTIONS

General activity of formylated phloroglucinol compounds

Although our understanding of formylated phloroglucinol compounds is in its infancy, it is clear that this is a diverse group of secondary plant compounds. More than 40 different formylated phloroglucinol compounds have been identified from less than 5% of eucalypt species and doubtless more remain to be discovered. There are also strong indications

that the formylated phloroglucinol compounds vary in their effectiveness against marsupial folivores (Lawler et al. 1999b). Further study is needed to identify trends in activity related to the structural features of formylated phloroglucinol compounds molecules. It is likely that significant differences in activity exist at least between macrocarpals, sideroxylonals and euglobals (Fig. 1), but it is unclear whether differences exist within groups. Studies of chemical structure in relation to biological activity in microbial systems suggest that the nature of the terpene moiety is of minor importance in determining the biological activity of these compounds (Takasaki et al. 1990). It is possible to isolate and purify enough sideroxylonal for small feeding studies, although the process is slow and costly. In contrast, it has not been possible to isolate such quantities of macrocarpals and euglobals and this limits our understanding of the significance of structural variation in formylated phloroglucinol compounds. New approaches to both isolation of the natural products and production of synthetic derivatives may solve this problem.

Re-evaluating the role of tannins in marsupial-Eucalyptus interactions

As discussed previously, tannins have always been regarded as important components in folivore-eucalypt interactions. Nevertheless, recent research suggests that formylated phloroglucinol compounds probably confounded some of the conclusions from earlier feeding studies with marsupial folivores that focused on tannins. For example, in research preceding the discovery of formylated phloroglucinol compounds, both Foley and Hume (1987c) and McArthur and Sanson (1991) used polyethylene glycol to block the effects of tannins and obtained conflicting results. In a stroke of fortune, Foley and Hume (1987c) located a single E. melliodora favoured by common brushtail possums for their study, while McArthur and Sanson (1991) fed several E. ovata to common ringtails. We now know that E. melliodora contains a single formylated phloroglucinol compound, sideroxylonal, and that favoured trees contain low concentrations. In contrast, E. ovata contains many formylated phloroglucinol compounds and their type and concentrations often vary widely between individual trees in a way that may mask the effects of tannins.

The discovery of the importance of formylated phloroglucinol compounds to marsupials makes it pertinent to re-evaluate the defensive role of eucalypt tannins. Two areas of research are particularly appealing. First, how do Monocalyptus species deter herbivores? These species appear to lack formylated phloroglucinol compounds and yet generally suffer less leaf loss

and damage from herbivores than Symphyomyrtus species (Noble 1989). Secondly, do tannins and formylated phloroglucinol compounds interact to determine food intake? Although common ringtail possums avidly feed on many monocalypt species (e.g., E. rossii, E. andrewsii, E. haemostoma, E. dives, E. radiata, E. regnans and E. consideniana), common brushtail possums tend to avoid them. Recent research suggests that common ringtail possums show a small but significant increase in the amount of E. rossii and E. consideniana foliage they are willing to eat after the foliage has been coated with polyethylene glycol (Marsh et al. 2003a). In contrast, common brushtail possums, although willing to eat only a small amount of the same foliage initially, will almost double their intake if it is coated with polyethylene glycol (Marsh et al. 2003a). However, intakes were still lower than that required for maintenance, possibly because dipping the leaf supplied only enough polyethylene glycol to bind 20–35% of the tannin pool. Further research is required to increase the dose of polyethylene glycol and to determine whether other compounds deter common brushtails from feeding on these monocalypt species.

In our research with formylated phloroglucinol compounds, we have often found wide variation in food intake at a given concentration of formylated phloroglucinol compounds (e.g., Wallis et al. 2002). Now that it is possible to account for variation in food intake attributable to formylated phloroglucinol compounds, we can examine the causes of the residual variation. Our preliminary research (Marsh et al. 2003b) suggested that, in the presence of formylated phloroglucinol compounds, tannins play little role in determining how much common ringtail possums eat. Common ringtails appear particularly susceptible to the effects of formylated phloroglucinol compounds with small doses significantly reducing how much they eat and thus overwhelming any tannin effect. In contrast, when common brushtail possums are fed foliage containing varying concentrations of sideroxylonal from low (<1% of dry matter) to extreme (>5% of dry matter), they can eat more foliage, and hence sideroxylonal, when the foliage is coated with polyethylene glycol. Common brushtail possums are widely regarded as generalist herbivores and common ringtails as specialists. However, the difference between the reactions of the two possum species to tannins and formylated phloroglucinol compounds suggests not only the generalist/specialist paradigm, but also a specialist/specialist paradigm. In other words, common ringtail possums may specialize by eating Eucalyptus foliage that is rich in tannins but poor in formylated phloroglucinol compounds, while common brushtail possums

are able to ingest foliage with large concentrations of formylated phloroglucinol compounds, but show a poor tolerance of some tannins. The preference shown by greater gliders for the foliage of many monocalypts implies that they show greater affinity to common ringtail possums. The foliage of symphyomyrts, apart from containing formylated phloroglucinol compounds, also contains tannins. This invites the question of how these tannins differ qualitatively and quantitatively from those of the monocalypts that common brushtail possums are so reluctant to eat.

Detoxification as a limit to feeding

Although we have described one role for terpenes in conditioning food aversions, they may also limit intake through kinetic mechanisms. Folivorous marsupials consume large quantities of both phenolics and terpenes that, in contrast to formylated phloroglucinol compounds, do not appear to acutely limit food intake (McLean *et al.* 1993; McLean and Foley 1997). Several recent studies (McLean *et al.* 1993; Boyle *et al.* 1999, 2000, 2001) have quantified the urinary metabolites of 1,8 cineole and *p*-cymene, with consideration of whether the rate at which marsupials can detoxify and eliminate terpenes and phenolics might limit the rate at which they consume foliage.

These metabolic studies have shown that different marsupials produce different patterns of metabolites. Generally, the more specialized species such as koalas and greater gliders excrete highly oxygenated non-conjugated metabolites of terpenes (Boyle *et al.* 1999, 2000, 2001). In contrast, common brushtails excrete mono or di-oxygenated compounds. The significance of this is that the limited-capacity conjugation pathways in the specialist folivores are reserved for other compounds, possibly the phenolics (McLean *et al.* 1993). Whether these differences explain the different reliance on *Eucalyptus* foliage among species remains to be seen but is an exciting area for future research.

Questions of scale

One of the difficulties with our current understanding of how plant secondary metabolites affect *Eucalyptus* folivores is the question of temporal scale. Put simply, how does measurement of short-term food intake relate to the life histories of free-ranging animals? This can be assessed only with a detailed field study, but we believe that the constraints on feeding in the captive experiments will be as strong in the field. In a long series of experiments measuring food intake by captive common ringtail possums, common brushtail possums and koalas offered formylated phloroglucinol compound-rich foliage or equivalent amounts of pure formylated phloroglucinol compound, we consistently found intakes that were remarkably low (e.g., Lawler *et al.* 1998a, 2000; Stapley *et al.* 2000; Wallis *et al.* 2002). The sheer volume of data from both short and long experiments convinced us that animals were unable to feed, rather than simply choosing to avoid an unpleasant sensation. The threshold tolerances of wild animals for these compounds are likely to vary, decreasing as the availability of more palatable alternatives increases, but always remaining at or below the threshold established in our experiments. Common brushtail possums in negative nitrogen balance, or with increased energy requirements (cold exposure) were unable to increase their intakes of an otherwise adequate artificial diet containing jensenone (Stapley *et al.* 2000). A major task should be to define the foliar concentration of formylated phloroglucinol compounds that will always deter animals from feeding. Afterwards, we can establish the proportion of individual trees of a food species that are edible in habitats supporting different numbers of marsupial folivores.

Ultimately, assessment of habitat using leaf chemistry will only be useful for wildlife management if it covers the landscape scale. Previous studies have been extended to the landscape scale by sampling just tree species and have highlighted the importance of nutrients and nutrient: phenolic ratios (Braithwaite *et al.* 1983; Norton 1987; Cork 1992; Pausas *et al.* 1995). However, this level of sampling ignores large differences in formylated phloroglucinol compound concentrations found among trees of a single eucalypt species growing close together (e.g., Lawler *et al.* 2000). If food availability for marsupial folivores is to be accurately assessed, sampling regimes must be intense.

Near infrared spectroscopes enables intensive sampling of foliage at two scales. Near infrared spectroscopes can be used to substantially increase sample sizes in studies directed at identifying trends relating foliar chemistry to folivore abundance. If the lessons learnt from these sorts of studies are to be applied to conservation management, foliar measurements must be made across entire landscapes. This logistical challenge may be met by applying the principles used in laboratory near infrared spectroscopes to a remote-sensing platform. Just as laboratory instruments capture the spectrum resulting from reflectance or transmission of near infrared light from a sample, remote instruments can capture the spectrum in the near infrared region resulting from reflectance of sunlight. With the increasing power of modern equipment it has proven possible to measure leaf nitrogen, cellulose and even

susceptibility to insect attack for individual tree canopies across whole forests in the Northern Hemisphere (Gastelluetchegorry *et al.*, 1995; Curran *et al.*, 1997; Martin and Aber 1997). Current research directed to these questions in *Eucalyptus* forests (Ebbers *et al.* 2002) promise to deliver the same landscape scale data.

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REFERENCES

Ayres, M. P., Clausen, T. P., Maclean, S. F., Redman, A. M. and Reichardt, P. B., 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78:** 1696–712.

Barry, K. M., Davies, N. W. and Mohammed, C. L., 2001. Identification of hydrolysable tannins in the reaction zone of *Eucalyptus nitens* wood by high performance liquid chromatography-electrospray ionisation mass spectrometry. *Phytochem. Anal.* **12:** 120–27.

Bednarik, R., 1996. Habitat use by koalas at the Koala Conservation Centre, Phillip Island. Hons. Thesis, University of Melbourne, Melbourne.

Betts, T. J., 1978. Koala acceptance of *Eucalyptus globulus* Labill. as food in relation to the proportion of sesquiter-penoids in the leaves. Pp. 75–85 *in* The Koala ed by T. J. Bergin. Zoological Parks Board of NSW, Sydney.

Boland, D. J., Brophy, J. and House, A. P. N., 1991. Eucalyptus Leaf Oils: Use, chemistry, distillation and marketing. Inkata Press, Sydney.

Boyle, R., McLean, S., Foley, W. J. and Davies, N., 1999. Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. *J. Chem. Ecol.* **25:** 2109–126.

Boyle, R., McLean, S., Foley, W. J., Moore, B. D., Davies, N. W. and Brandon, S., 2000. Fate of the dietary terpene, p-cymene in the koala. *J. Chem. Ecol.* **26:** 1095–111.

Boyle, R., McLean, S., Foley, W. J., Davies, N. W., Peacock, E. and Moore, B., 2001. Investigation of urinary 1,8-cineole metabolites in the male koala (*Phascolarctos cinereus*). *Comp. Biochem. Physiol.* C **129:** 385–95.

Braithwaite, L., 1996. Conservation of arboreal herbivores — the Australian scene. *Austral. J. Ecol.* **21:** 21–30.

Braithwaite, L., Dudzinski, M. L. and Turner, J., 1983. Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, N.S.W. II. Relationship between fauna density, richness and diversity, and measured variables of the habitat. *Aust. Wildl. Res.* **10:** 231–47.

Brophy, J. J., Forster, P. I., Goldsack, R. J. and Hibbert, D. B., 1998. The essential oils of the yellow bloodwood Eucalypts (Corymbia, Section Ochraria, Myrtaceae). *Biochem. System. Ecol.* **26:** 239–49.

Bryant, J. P., Wieland, G. D., Reichardt, P. B., Lewis, V. E. and McCarthy, M. C., 1983. Pinosylvin methyl ether deters snowshoe hare feeding on green alder. *Science* **222:** 1023–025.

Clausen, T. P., Provenza, F. D., Burritt, E. A., Reichardt, P. B. and Bryant, J. P., 1990. Ecological implications of condensed tannin structure: a case study. *J. Chem. Ecol.* **16:** 2381–392.

Clausen, T. P., Reichardt, P. R., Bryant, J. P. and Provenza, F. D., 1992. Condensed tannins in plant defense: a perspective on classical theories. Pp. 639–52 *in* Plant Polyphenols: Synthesis, Properties, Significance ed by R. W. Hemmingway. Plenum Press, New York.

Comport, S. S., Ward, S. J. and Foley, W. J., 1996. Home ranges, time budgets and food-tree use in a high-density tropical population of greater gliders, *Petauroides volans minor* (Pseudocheiridae, Marsupialia). *Wildl. Res.* **23:** 401–19.

Cork, S. J., 1986. Foliage of *Eucalyptus punctata* and the maintenance nitrogen requirements of koalas *Phascolarctos cinereus*. *Aust. J. Zool.* **34:** 17–24.

Cork, S. J., 1992. Polyphenols and the distribution of arboreal folivorous marsupials in *Eucalyptus* forests of Australia. Pp. 653–63 *in* Plant Polyphenols: Synthesis, Properties, Significance ed by R. W. Hemmingway. Plenum Press, New York.

Cork, S. J. and Catling, P. C., 1996. Modelling distributions of arboreal and ground-dwelling mammals in relation to climate, nutrients, plant chemical defences and vegetation structure in the eucalypt forests of Southeastern Australia. *Forest Ecol. Manag.* **85:** 163–75.

Cork, S. J. and Foley, W. J., 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. Pp. 133–66 *in* Plant Defenses Against Mammalian Herbivory ed by R. T. Palo and C. T. Robins. CRC Press Incorporated, Boca, Raton.

Cork, S. J. and Krockenberger, A. K., 1991. Methods and pitfalls of extracting condensed tannins and other phenolics from plants: insights from investigations on *Eucalyptus* leaves. *J. Chem. Ecol.* **17:** 123–34.

Cork, S. J. and Pahl, L., 1984. The possible influence of nutritional factors on diet and habitat selection by the ringtail possum *Pseudocheirus peregrinus*. Pp. 269–76 *in* Possums and Gliders ed by A. P. Smith and I. D. Hume. Surrey Beatty & Sons, Chipping Norton.

Cork, S. J. and Sanson, G. D., 1990. Digestion and nutrition in the koala: a review. Pp. 129–44 *in* Biology of the Koala ed by A. K. Lee, K. A. Handasyde and G. D. Sanson. Surrey Beatty & Sons, Chipping Norton.

Curran, P. J., Kupiec, J. A. and Smith, G. M., 1997. Remote sensing the biochemical composition of a slash pine canopy. *IEEE Trans. Geosci. Remote Sens.* **35:** 415–20.

Degabriele, R., 1981. A relative shortage of nitrogenous food in the ecology of the koala (*Phascolarctos cinereus*). *Aust. J. Ecol.* **6:** 139–41.

Ebbers, M. J. H., Wallis, I. R., Dury, S., Floyd, R. and Foley, W. J., 2002. Spectrometric prediction of secondary meta-bolites and nitrogen in fresh *Eucalyptus* foliage: towards remote sensing of the nutritional quality of foliage for leaf-eating marsupials. *Aust. J. Bot.* **50:** 761–68.

Eberhard, I. H., 1978. Ecology of the koala, *Phascolarctos cinereus* (Goldfuss) (Marsupialia: Phascolarctidae) in Australia. Pp. 315–28 *in* Ecology of Arboreal Folivores ed by G. G. Montgomery. Smithsonian Institution Press, Washington, D.C.

Eschler, B. M., Pass, D. M., Willis, R. and Foley, W. J., 2000. Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochem. System. Ecol.* **28:** 813–24.

Fleay, D., 1937. Observations on the koala in captivity. *Aust. J. Zool.* **9:** 68–80.

Foley, W. J., 1992. Nitrogen and energy retention and acid-base status in the common ringtail possum (*Pseudocheirus peregrinus*): evidence of the effects of absorbed allelochemicals. *Physiol. Zool.* **65:** 403–21.

Foley, W. J. and Hume, I. D., 1987a. Nitrogen requirements and urea metabolism in two arboreal marsupials, the Greater Glider (*Petauroides volans*) and the Brushtail Possum fed *Eucalyptus* foliage. *Physiol. Zool.* **60:** 241–50.

Foley, W. J. and Hume, I. D., 1987b. Passage of digesta markers in two species of arboreal folivorous marsupials — the Greater Glider (*Petauroides volans*) and the Brushtail Possum (*Trichosurus vulpecula*). *Physiol. Zool.* **60:** 103–13.

Foley, W. J. and Hume, I. D., 1987c. Digestion and metabolism of high-tannin *Eucalyptus* foliage by the brushtail possum, *Trichosurus vulpecula* (Marsupialia: Phalangeridae). *J. Comp. Physiol.* **B157:** 67–76.

Foley, W. J. and McArthur, C., 1994. The effects and costs of ingested allelochemicals in mammals: an ecological perspective. Pp. 370–91 *in* The Digestive System in Mammals: Food, Form and Function ed by D. J. Chivers and P. Langer. Cambridge University Press, Cambridge.

Foley, W. J., Iason, G. and McArthur, C., 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores — how far have we come in 25 years? Pp. 203–74 *in* Fifth International Symposium on the Nutrition of Herbivores ed by H-J. G. Jung and G. C. Fahey Jr. American Society of Animal Science, Savoy, Illinois.

Foley, W. J., Lassak, E. V. and Brophy, J., 1987. Digestion and absorption of *Eucalyptus* essential oils in greater glider (*Petauroides volans*) and brushtail possum (*Trichosurus vulpecula*). *J. Chem. Ecol.* **13:** 2115–130.

Foley, W. J., McIlwee, A. P., Lawler, I. R., Aragones, L., Woolnough, A. P. and Berding, N., 1998. Ecological applications of near infrared spectroscopy — a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia* **116:** 293–305.

Foley, W. J., McLean, S. and Cork, S. J., 1995. Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals — a final common pathway. *J. Chem. Ecol.* **21:** 721–43.

Gastelluetchegorry, J. P., Zagolski, F., Mougin, E., Marty, G. and Giordano, G., 1995. An assessment of canopy chemistry with AVARIS — a case study in the Landes forest, south-west France. *Inter. J. Remote Sensing* **16:** 487–501.

Geritz, R., 1987. Diet selection in the common ringtail possum, *Pseudocheirus peregrinus*. Hons. Thesis, Monash University, Melbourne, Australia.

Ghisalberti, E. L., 1996. Bioactive acylphloroglucinol derivatives from *Eucalyptus* species. *Phytochem.* **41:** 7–22.

Grant, V. L., 1987. Do conditioned taste aversions result from activation of emetic mechanisms? *Psychopharmacol.* **93:** 405–15.

Hartzfeld, P. W., Forkner, R., Hunter, M. D. and Hagerman, A. E., 2002. Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *J. Agric. Food Chem.* **50:** 1785–790.

Hillis, W. E., 1966. Variation in polyphenol composition within species of *Eucalyptus*. *Phytochem.* **5:** 541–56.

Hindell, M. A. and Lee, A. K., 1987. Habitat use and tree preferences of koalas in a mixed eucalypt forest. *Aust. Wildl. Res.* **14:** 349–60.

Hindell, M. A. and Lee, A. K., 1988. Tree use by individual koalas in a natural forest. *Aust. Wildl. Res.* **15:** 1–7.

Hindell, M. A. and Lee, A. K., 1990. Tree preferences of the koala. Pp. 117–21 *in* Biology of the Koala ed by A. K. Lee, K. A. Handasyde and G. D. Sanson. Surrey Beatty & Sons, Chipping Norton.

Hindell, M. A., Handasyde, K. A. and Lee, A. K., 1985. Tree species selection by free-ranging koala populations in Victoria. *Aust. Wildl. Res.* **12:** 137–44.

Hume, I. D., Bladon, R. V. and Soran, N., 1996. Seasonal changes in digestive performance of common ringtail possums (*Pseudocheirus peregrinus*) fed *Eucalyptus* foliage. *Aust. J. Zool.* **44:** 327–36.

Hume, I. D. and Esson, C., 1993. Nutrients, antinutrients and leaf selection by captive koalas (*Phascolarctos cinereus*). *Aust. J. Zool.* **41:** 379–92.

Kavanagh, R. P. and Lambert, M. J., 1990. Food selection by the greater glider, *Petauroides volans*: Is foliar nitrogen a determinant of habitat quality? *Aust. Wildl. Res.* **17:** 285–99.

Lawler, I. R. and Foley, W. J., 1999. Swamp wallabies and Tasmanian pademelons show intraspecific preferences for foliage of *Eucalyptus ovata*. *Aust. For.* **62:** 17–20.

Lawler, I. R., Foley, W. J. and Eschler, B. M., 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81:** 1327–338.

Lawler, I. R., Foley, W. J., Eschler, B. M., Pass, D. M. and Handasyde, K., 1998a. Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116:** 160–69.

Lawler, I. R., Foley, W. J., Pass, G. J. and Eschler, B. M., 1998b. Administration of a 5HT$_3$ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials. *J. Comp. Physiol.* **B168:** 611–18.

Lawler, I. R., Schliebs, D. M., Eschler, B. M. and Foley, W. J., 1999a. Relationship between chemical functional groups on *Eucalyptus* secondary metabolites and their effectiveness as marsupial antifeedants. *J. Chem. Ecol.* **25:** 2561–573.

Lawler, I. R., Stapley, J., Foley, W. J. and Eschler, B. M., 1999b. Ecological example of conditioned flavor aversion in plant-herbivore interactions: effect of terpenes of *Eucalyptus* on feeding by common ringtail and brushtail possums. *J. Chem. Ecol.* **25:** 401–15.

Marsh, K. J., Wallis, I. R. and Foley, W. J., 2003a. The effect of inactivating tannins on the intake of *Eucalyptus* foliage by a specialist *Eucalyptus* folivore (*Pseudocheirus peregrinus*) and a generalist herbivore (*Trichosurus vulpecula*). *Aust. J. Zool.* **51:** 31–42.

Marsh, K. J., Foley, W. J., Cowling, A. and Wallis, I. R., 2003b. Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol.* B **173:** 69–78.

Martin, M. E. and Aber, J. D., 1997. High spectral resolution remote sensing of forest canopy lignin, nitrogen, and ecosystem processes. *Ecol. Appli.* **7:** 431–43.

Martin, R. W., 1985. Overbrowsing, and decline of a population of the koala, *Phascolarctos cinereus*, in Victoria. I. Food preference and food tree defoliation. *Aust. Wildl. Res.* **12:** 355–65.

McArthur, C. and Sanson, G. D., 1991. Effects of tannins on digestion in the common ringtail possum (*Pseudocheirus peregrinus*), a specialized marsupial folivore. *J. Zool.* **225:** 233–51.

McArthur, C. and Sanson, G. D., 1993. Nutritional effects and costs of a tannin in two marsupial arboreal folivores. *Funct. Ecol.* **7:** 697–703.

McArthur, C., Hagerman, A. E. and Robbins, C. T., 1991. Physiological strategies of mammalian herbivores against plant defenses. Pp. 103–14 *in* Plant Defenses Against Mammalian Herbivory ed by R. T. Palo and C. T. Robbins. CRC Press Inc., Florida.

McDowell, A., 1999. Effect of carbon dioxide concentration on *Eucalyptus ovata* foliage and its utilization by the common ringtail possum, (*Pseudocheirus peregrinus*). MSc thesis, School of Botany, University of Melbourne, Melbourne, Australia.

McIlwee, A. M., Lawler, I. R., Cork, S. J. and Foley, W. J., 2001. Near infrared spectroscopy as a predictor of *Eucalyptus* foliar nutrients and of the feeding rates of folivorous marsupials. *Oecologia* **128:** 539–48.

McLean, S. and Foley, W. J., 1997. Metabolism of *Eucalyptus* terpenes by herbivorous marsupials. *Drug Metab. Rev.* **29:** 213–18.

McLean, S., Foley, W. J., Davies, N. W., Brandon, S., Duo, L. and Blackman, A. J., 1993. The metabolic fate of dietary terpenes from *Eucalyptus radiata* in the common ringtail possum (*Pseudocheirus peregrinus*) *J. Chem. Ecol.* **19:** 1625–643.

McLean, S., Brandon, S., Davies, N. W., Foley, W. J. and Muller, H. K., 2004. Jensenone: Biological reactivity of a marsupial antifeedant from *Eucalyptus. J. Chem. Ecol.* **30:** 19–36.

Melzer, A., 1994. Aspects of the ecology of the koala, *Phascolarctos cinereus* (Goldfuss, 1817), in the sub-humid woodlands of central Queensland. Ph.D. Thesis, University of Queensland, Brisbane, Australia.

Montague, T. L., 1994. Wallaby browsing and seedling palatability. *Aust. For.* **57:** 171–75.

Montague, T. L., 1996. The extent, timing and economics of browsing damage in eucalypt and pine plantations of Gippsland, Victoria. *Aust. For.* **59:** 120–29.

Moore, B. D. and Foley, W. J., 2000. A review of feeding and diet selection in koalas (*Phascolarctos cinereus*). *Aust. J. Zool.* **48:** 317–33.

Moore, B. D., Wallis, I. R., Pala-Paul, J., Brophy, J. J., Willis, R. H. and Foley, W. J., 2004a. Anti-herbivore chemistry of *Eucalyptus* — cues and deterrents for marsupial folivores. *J. Chem. Ecol.* (in press).

Moore, B. D., Wallis, I. R., Wood, J. T. and Foley, W. J., 2004b. Foliar nutrition, site quality and temperature influence foliar chemistry of tallowwood (*Eucalyptus microcorys*). *Ecol. Monogr.* (in press).

Morrison, R. T. and Boyd, R. N., 1987. Organic Chemistry. 5th ed. Allyn and Bacon, Incorporated: Sydney.

Murata, M., Yamakoshi, Y., Homma, S., Aida, K., Hori, K. and Ohashi, Y., 1990. Macrocarpal A, a novel antibacterial compound from *Eucalyptus macrocarpa*. *Agric. Biol. Chem.* **54:** 3221–226.

Nagy, J. G., Steinhoff, H. W. and Ward, G. M., 1964. Effects of essential oils of sagebrush on deer rumen microbial function. *J. Wildl. Man.* **28:** 785–90.

Noble, I. R., 1989. Ecological traits of the *Eucalyptus* L'Herit. subgenera *Monocalyptus* and *Symphomyrtus*. *Aust. J. Bot.* **37:** 207–24.

Norton, T. W., 1987. Ecology of greater gliders, *Petauroides volans* Kerr 1792, in relation to variations in habitat quality in eucalypt forests in south-east New South Wales. Ph.D. Thesis, Australian National University, Canberra, Australia.

Osawa, K., Yasuda, H., Morita, H., Takeya, K. and Itokawa, H., 1997. Configurational and conformational analysis of macrocarpals H, I, and J from *Eucal. globulus*. *Chem. Pharm. Bull.* **45:** 1216–217.

Pahl, L. I., 1984. Diet preference, diet composition and population density of the ringtail possum (*Pseudocheirus peregrinus cooki*) in several plant communities in southern Victoria. Pp. 252–60 *in* Possums and Gliders ed by A. P. Smith and I. D. Hume. Surrey Beatty & Sons, Chipping Norton.

Pahl, L. I., 1987. Feeding behaviour and diet of the common ringtail possum, *Pseudocheirus peregrinus*, in *Eucalyptus* woodlands and *Leptospermum* thickets in Southern Victoria. *Aust. J. Zool.* **35:** 487–506.

Pahl, L. I. and Hume, I. D., 1990. Preferences for *Eucalyptus* species of the New England Tablelands and initial development of an artificial diet for koalas. Pp. 123–28 *in* Biology of the Koala ed by A. K. Lee, K. A. Handasyde and G. D. Sanson. Surrey Beatty & Sons, Chipping Norton.

Pass, D. M., Foley, W. J. and Bowden, B., 1998. Vertebrate herbivory on *Eucalyptus* — identification of specific feeding deterrents for common ringtail possums (*Pseudocheirus peregrinus*) by bioassay-guided fractionation of *Eucalyptus ovata* foliage. *J. Chem. Ecol.* **24:** 1513–527.

Pausas, J. G., Braithwaite, L. W. and Austin, M. P., 1995. Modelling habitat quality for arboreal marsupials in the south coastal forests of New South Wales, Australia. *For. Ecol. Manag.* **78:** 39–49.

Pratt, A., 1937. The Call of the Koala. Robertson and Mullens, Melbourne.

Provenza, F. D., Burritt, E. A., Clausen, T. P., Bryant, J. P., Reichardt, P. B. and Distel, R. A., 1990. Conditioned food aversion: a mechanism for goats to avoid condensed tannins in blackbrush. *Am. Nat.* **136:** 810–28.

Reichardt, P. B., Bryant, J. P., Clausen, T. P. and Wieland, G. D., 1984. Defence of winter-dormant Alaska paper birch against snowshoe hares. *Oecologia* **65:** 58–69.

Robbins, R. and Russell, E., 1978. Observations on movements and feeding activity of the koala in a semi-natural situation. Pp. 29–41 *in* The Koala ed by T. J. Bergin. Zoological Parks Board of NSW, Sydney.

Silanikove, N., Shinder, D., Gilboa, N., Eyal, M. and Nitsan, Z., 1996. Binding of poly(ethylene glycol) to samples of forage plants as an assay of tannins and their negative effects on ruminal degradation. *J. Agric. Food Chem.* **44:** 3230–234.

Singh, I. P., Takahashi, K. and Etoh, H., 1996. Potent attachment-inhibiting and -promoting substances for the blue mussel, *Mytilus edulis galloprovincialis*, from two species of *Eucalyptus*. *Biosci. Biotech. Biochem.* **60:** 1522–523.

Southwell, I. A., 1978. Essential oil content of koala food trees. Pp. 62–74 *in* The Koala ed by T. J. Bergin. Zoological Parks Board of NSW, Sydney.

Southwell, I. A., Flynn, T. M. and Degabriele, R., 1980. Metabolism of a- and b-pinene, *p*-cymene and 1,8-cineole in the brushtail possum, *Trichosurus vulpecula*. *Xenobiotica* **10:** 17–23.

Stapley, J., Foley, W. J., Cunningham, R. and Eschler, B., 2000. How well can common brushtail possums regulate their intake of *Eucalyptus* toxins? *J. Comp. Physiol.* **170:** 211–18.

Statham, H. L., 1984. The diet of *Trichosurus vulpecula* (Kerr) in four Tasmanian forest locations. Pp. 213–19 *in* Possums and Gliders ed by A. P. Smith and I. D. Hume. Surrey Beatty & Sons, Chipping Norton.

Takasaki, M., Konoshima, T., Fujitani, K., Yoshida, S., Nishimura, H., Tokuda, H., Nishino, H., Iwashima, A. and Kozuka, M., 1990. Inhibitors of skin-tumor promotion. VIII. Inhibitory effects of euglobals and their related compounds on Epstein-Bar virus activation. *Chem. Pharm. Bull.* **38:** 2737–739.

Ullrey, D. E., Robinson, P. T. and Whetter, P. A., 1981. Composition of preferred and rejected *Eucalyptus* browse offered to captive koalas, *Phascolarctos cinereus* (Marsupialia). *Aust. J. Zool.* **29:** 839–46.

Wallis, I. R., Watson, M. L. and Foley, W. J., 2002. Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Aust. J. Zool.* **50:** 507–19.

Watson, M. L., 1998. Interactions between the common brushtail possum (*Trichosurus vulpecula*) and *Eucalyptus*: evaluating natural resistance as a method of pest control in *Eucalyptus* plantations. Hons. Thesis, Australian National University, Canberra.

Welch, B. L., Narjisse, H. and McArthur, E. D., 1982. *Artemisia tridentata* monoterpenoid effect on ruminant digestion and forage selection. Pp. 73–84 *in* Aromatic Plants: Basic and Applied Aspects ed by N. Margaris, A. Koedam and D. Vokou. Martinus Nijhoff, Boston.

Williams, J. E. and Woinarski, J. C. Z., 1997. Eucalypt Ecology: Individuals to Ecosystems. Cambridge University Press: Cambridge.

Yamakoshi, Y., Murata, M., Shimizu, A. and Homma, S., 1992. Isolation and characterization of macrocarpals B-G, antibacterial compounds from *Eucalyptus macrocarpa*. *Biosci. Biotech. Biochem.* **56:** 1570–576.

Zoidis, A. M. and Markowitz, H., 1992. Findings from a feeding study of the koala (*Phascolarctos cinereus*) at the San Francisco Zoo. *Zoo Biol.* **11:** 417–31.