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Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum

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Abstract

We studied the influence of a group of plant secondary compounds, the sideroxylonals, on feeding by the common brushtail possum (*Trichosurus vulpecula*), a generalist herbivore. Possums were offered synthetic diets either with or without sideroxylonals or, in separate experiments, foliage from 28 individual *Eucalyptus melliodora* trees. Possums ate less of the synthetic diet at sideroxylonal concentrations of 4 and 7 mg g^{-1} when offered a choice or no choice, respectively. Possums fed foliage in no-choice protocols ate 12–61 g of dry matter per day. Sideroxylonal concentration was an essential determinant of feeding on foliage but the wide variation, particularly at intermediate sideroxylonal concentrations, suggests that other secondary plant chemicals, e.g. tannins, are important also. The normal distribution of sideroxylonal concentrations (mean = 27.7, s.d. = 7.85 mg sideroxylonals per g dry leaf) in a natural population of 150 *E. melliodora* trees shows that brushtail possums will rarely encounter highly palatable trees (<10 mg sideroxylonals per g dry leaf) nor highly unpalatable foliage (>40 mg sideroxylonals per g dry leaf). When foraging on *E. melliodora*, brushtail possums must contend with intermediate sideroxylonal concentrations (20–30 mg per g dry leaf), where variability in food intake is most noticeable.

Introduction

Although plant secondary metabolites are widely recognised as contributing to diet choice by folivores (Freeland and Janzen 1974; Palo and Robbins 1991; Foley *et al.* 1999), few studies have shown how particular compounds influence feeding and how those compounds are distributed in natural forests. This is essential information for understanding how much of an abundant foliage resource is of value and how guilds of herbivores partition these resources (Ganzhorn 1992). In this line of research much has been made of the differences between specialist and generalist herbivores, mainly focussed on understanding how differences in nutrient acquisition or detoxication capacity might contribute to differences in dietary niches (Freeland 1991; Dearing and Cork 1999; Dearing *et al.* 2000).

Despite the vast number of eucalypts and related species, only four arboreal species eat eucalypt foliage. These marsupial folivores span the range from extreme dietary specialists, such as the koala (*Phascolarctos cinereus*) (Cork and Sanson 1990) and the greater glider (*Petauroides volans*) (Marples 1973; McKay 1995), through the intermediate ringtail possum (*Pseudocheirus peregrinus*) (Pahl 1987) to the generalist herbivore, the common brushtail possum (*Trichosurus vulpecula*) (Kerle 1984; MacLennan 1984; Statham 1984). The characterisation of the brushtail possum as a generalist feeder, with a low reliance on eucalypt foliage, is supported by studies of feeding in captivity (Freeland and Winter 1975), digestive physiology (Foley and Hume 1987) and the metabolism of plant secondary metabolites (Boyle *et al.* 1999). Also, there is indirect evidence for plasticity in the diet of the brushtail possum. For example, possums frequently feed on the ground but spend less

time on the ground when foxes are present (C. R. Dickman, personal communication). Secondly, the success of the species in New Zealand is frequently attributed to the abundance of supplemental foods, like fruit, in some forests (Nugent *et al.* 2001). While brushtails rarely feed solely on eucalypt foliage the few cases documented show that there is no inherent barrier to their doing so (e.g Foley and Hume 1987). Instead, their low reliance on eucalypt foliage is likely due to some widespread aspect of foliar chemistry.

The recent identification of formylated phloroglucinol compounds (FPCs) in eucalypt foliage as antifeedants against ringtail possums (Lawler *et al.* 1998, 2000) has given new impetus to understanding the dietary niches of folivorous marsupials. As generalist feeders, brushtail possums might be expected to be more susceptible to FPCs than are the previously studied specialist feeders, the common ringtail possum and the koala. Indeed, Lawler *et al.* (1999) showed that brushtail possums were more susceptible to the simple FPC, jensenone, than are ringtail possums.

This paper examines whether the foliar concentration of one group of FPCs, the sideroxylonals, explains feeding by brushtail possums on yellow box (*Eucalyptus melliodora*). Convincing evidence requires information on food intake from trees covering a wide range of sideroxylonal concentrations. Thus, the distribution of sideroxylonal concentrations used in experiments may bear little resemblance to the distributions in a natural stand of the eucalypt species.

We tackled this problem in four ways. First, we examined the impact of purified sideroxylonals on food intake when animals had either no choice or a choice amongst alternative foods. Second, we established the relationship between feeding and sideroxylonal concentration using trees chosen for their wide variation in sideroxylonal concentrations and compared the results with those obtained previously for the more specific eucalypt feeder, the common ringtail possum. We then repeated the study with trees from a much narrower range of sideroxylonal concentrations to examine variation in feeding. In both of these studies with foliage, we examined whether any relationship existed between nitrogen (an indicator of nutritional value) and the secondary chemicals, sideroxylonals and cineole. Finally, we placed the findings into a landscape context by measuring the distribution of foliar sideroxylonal concentrations in a natural stand of 150 *E. melliodora* trees.

Materials and Methods

Animals and housing

We captured common brushtail possums in cage traps on the campus of the Australian National University. They were placed in metabolism cages measuring 130 cm long × 60 cm wide × 85 cm high, fitted with a nesting box (32 cm long × 27 cm wide × 18 cm high), a tree branch for a perch, a water container and a polythene pipe (30 × 10 cm) filled with water in which to stand foliage. These cages were housed in a room with a 12 : 12 hour light : dark cycle (lights on at 0600 hours) and a temperature of 18°C (range 16–20°C). Four 60-W incandescent bulbs provided the daytime lighting while the minimal lighting at night came from a single 40-W red bulb. In the wild, brushtail possums are strictly nocturnal so food was offered in the evening (*c*. 1700 hours) and removed at 0900 hours the next day.

Upon capture we fed the possums a mixed diet of apple, carrot, banana and *E. melliodora* cut from a tree that possums are known to eat. Three to four weeks before data collection commenced, the experimental diets were introduced. For experiments with purified sideroxylonals we gradually introduced the animals to the synthetic diet of Lawler *et al.* (1998). This diet consisted of: apple (55.5% wet matter), banana and carrot (15.0), sugar (5.35), ground rice-hulls (5.0), ground Weetbix (a breakfast cereal, 3.0), ground lucerne hay (1.0) and acid casein (0.15). It contained, on average, 26% dry matter (DM) and 4.7% crude protein. For experiments with foliage we gradually removed the fruit and increased the amount of foliage until the possums were feeding exclusively on foliage.

Effect of purified sideroxylonals on intake and diet choice by common brushtail possums

Sideroxylonals were extracted and purified from *E. melliodora* foliage as described by Eschler and Foley (1999). It is difficult and costly to purify large quantities of sideroxylonals so the amounts available limited the extent of these experiments. We dissolved sideroxylonals in minimal acetone and added it to the dry ingredients of the synthetic diet. Diets lacking sideroxylonals were treated with acetone alone. When the acetone had evaporated we mixed the dry ingredients with the fruit and vegetables and presented the whole diet as a wet mash. The DM contents of the food offered and the food refusals were measured by drying a portion of each to constant mass at 80°C.

No-choice protocol

Four brushtail possums (2.0 ± 0.2 kg) were offered diets containing varying concentrations of sideroxylonals (0, 4, 7 and 11 mg per g DM). The experiment was designed as a 4 × 4 Latin Square and each observation was preceded by a rest day on which animals were offered only the synthetic diet. This was done to minimise carry-over effects from treatments that depressed feeding. We measured food intake as described by Stapley *et al.* (2000).

Choice protocol

Five brushtail possums (2.0 ± 0.2 kg) were offered a choice between the synthetic diet alone and the same diet containing sideroxylonals at concentrations of 0, 2, 4, 7 and 11 mg per g DM. The experiment was designed as a 5 × 5 Latin Square. Each animal was offered two dishes of food that each contained enough to meet voluntary food intake. The location of the dishes was randomised each night. We measured the total eaten and calculated the proportion of the total that came from the treated diet.

Effect of foliar sideroxylonals on feeding in common brushtail possums

The two experiments were of similar design and gave the animals no choice of what they ate. In the first experiment (March/April 1999) we used a 6 × 6 Latin Square design run twice and in the second (November/December 2000), an 8 × 8 Latin Square run twice. Thus, in Experiment 1, each of six animals was offered foliage from each of six *E. melliodora* trees in the first period and from each of another six trees in the second period. Likewise, in each period, every tree was fed to a possum on every night. The experimental trees were chosen by collecting samples of leaf from 30 individual mature trees, with heights between 10 and 20 m, and then predicting the sideroxylonal content using near infrared reflectance spectroscopy (NIRS) (see below) and a previously determined model. We then chose 12 of these trees to give a range of concentrations of sideroxylonals (0.50–51.5 mg per g DM, mean = 24.6, s.d. = 12.2), which in ringtail possums, determines largely how much food they eat. The risk in experiments like this is that a possum might be offered unpalatable foliage on consecutive nights to the point of fasting. To avoid this situation we interspersed each experimental day with a 'rest' day when we offered the possums *E. melliodora* foliage from a tree known to be palatable (sideroxylonals = 0.5 mg per g DM). In Experiment 2 we chose 16 trees over a narrower range of sideroxylonal concentration (5.6–35.6 mg per g DM, mean = 17.5, s.d. = 6.92), and again included the rest day between experimental days.

Branches of foliage, of equivalent maturity, were cut from the tree and immediately placed in a large plastic bag with the stems protruding. Upon returning to the laboratory (*c.* 2–3 h) these bags were stored in a cool room (~5°C) with the stems of the branches standing in water. When stored like this, foliage stays fresh for 8–10 days so we made two collections from each tree during the course of each experiment.

On the experimental days, a bundle of foliage weighing 350–500 g was placed in the feeding tube at 1700 hours. A smaller (150 g) but otherwise identical bundle of 'control' foliage was placed in a similar tube in front of each cage. Starting at 0900 hours the following day, we weighed the uneaten intact foliage and collected the spilled foliage into a paper bag. This was dried at 40°C to constant mass. The control foliage was used to monitor changes in hydration during the feeding period but was also used for sampling purposes in the following way. A 10-g sample was placed in a paper bag and dried in a forced draught oven at 40°C for 48 h to determine the DM of the foliage offered and the dry mass of the foliage refused. These data, together with the dry mass of spilt foliage, enabled us to calculate the dry matter intake (DMI) of the possums. A separate sample (~20 g) of the control leaves was frozen in a plastic bag for later freeze-drying and chemical analysis. Another sample of the control leaves (~5 g) was frozen for cineole analysis. The mass of the control foliage never changed by more than 1% during a feeding period so we assumed that the foliage refused contained the same proportion of water as that offered.

Field survey of sideroxylonal concentrations in Eucalyptus melliodora foliage

We surveyed sideroxylonal concentrations in foliage from individual *E. melliodora* trees using NIRS to relate observations on captive possums to the situation facing free-living brushtail possums. Specifically, we cut a small branch from mid-canopy height on the northern side of each tree and stripped about 80 g of leaf, which we sealed in a plastic bag and placed on ice. In this way we sampled two groups of *E. melliodora* at Mulligan's Flat, part of Canberra Nature Park, on the north-east boundary of the Australian Capital territory and New South Wales (35°10S, 149°10E; 600 m above sea level). The first group consisted of 64 trees along a transect 250 m long and 70–130 m wide; the second, 86 trees, was sampled along a transect 390 m long and 70–100 m wide. The site, a property that was grazed from the 1850s to the 1990s, has a vegetation structure that is mainly forest (50%), woodland (25%), forest–woodland (12.5%) and grassland (12.5%), with the woodland components dominated by *E. melliodora* while *E. blakelyii* is common also (Fischer 1999).

Chemical analyses

Leaf samples were freeze-dried and then ground to pass a 1-mm sieve using a Cyclotec 1093 Mill (Tecator, Sweden). The resulting powder was stored in the dark in clear plastic 50-mL specimen containers. All samples were analysed for sideroxylonals and nitrogen but samples from Experiment 1 only were analysed for cineole.

Nitrogen

The nitrogen content of freeze-dried, ground foliage from Experiment 1 was determined on duplicate samples (250 ± 10 mg) using a semi-micro Kjeldahl technique with a Tecator 2012 digester, selenium catalyst and a Gerhardt Vapodest-5 distillation and titration apparatus. The method was standardised using ammonium sulfate. The nitrogen content of foliage samples from Experiment 2 was predicted with NIRS.

Sideroxylonals

We determined the sideroxylonal content of the foliage according to the methods of Wallis *et al.* (2002). All results refer to the combined concentrations of sideroxylonals A and C.

Cineole

The analysis of cineole followed the method used by Edwards *et al.* (1993).

Near infrared reflectance spectroscopy analysis

The use of NIRS for ecological applications has been reviewed by Foley *et al.* (1998). In summary, the light reflected when a sample is exposed to light in the near-infrared spectrum depicts the chemical bonds in the sample. These spectra can then be calibrated against reference values obtained by analysing a portion of the samples using traditional analyses. We used NIRS to choose trees for the possum experiments and to predict the foliar concentration of sideroxylonals in foliage collected from the trees at Mulligan's Flat. Samples of freeze-dried, whole leaf were stored in an oven (40°C) overnight to equilibrate sample moisture. After cooling the samples in a desiccator, we made spectral measurements (400–2500 nm) with an NIR Systems Model 6500 scanning Spectrophotometer fitted with a transport module and a rectangular cell (202×47 mm). The reflectance (R) reading of each spectrum was converted to absorbance (A) values using $A = \log(1/R)$. Further, we converted each spectrum to a first-derivative spectrum in order to emphasise subtle changes in slope.

Sideroxylonal concentrations were estimated using calibration equations derived from the 'whole-leaf' spectra and analysis of foliage from 77 independent *E. melliodora* trees. The development of the equations used modified partial least-squares regression with cross-validation to avoid overfitting of the model (Shenk and Westerhaus 1991). The raw spectra, stored as $\log(1/R)$, were treated using normal variate and detrend transformations to reduce the influence of particle size (Barnes *et al.* 1989). Optimal calibration was obtained using the second derivative of the spectra with a spectral gap size of 10 nm within a range spanning 1100–2500 nm. These prediction equations for sideroxylonal concentrations were examined by selecting 14 samples for chemical analysis. Regression analysis of predicted versus analysed sideroxylonal concentration showed a close relationship ($r^2 = 0.92$, $F_{1,12} = 132$, $P < 0.0001$).

Statistical analyses

Relationships between the foliar concentrations of cineole, nitrogen and the independent variable, sideroxylonal A, were investigated with stepwise linear regression with a rejection level of $\alpha = 0.05$. A strong relationship between sideroxylonals and cineole has been reported previously by Lawler *et al.* (2000) but they showed separately that, of the two compounds, only sideroxylonal deters feeding (Lawler *et al.* 1998).

This information formed the basis of our statistical analysis. In the first instance, we used the restricted maximum-likelihood algorithm to analyse each experiment individually by testing the following model, including second-order interaction terms:

Response: Dry matter intake
Fixed model: Constant + Tree + Experimental day + Food offered + Body mass
Random model: Possum + Possum × Experimental day

In both animal experiments the analysis revealed a significant effect on DMI of 'tree' so at this point we replaced 'tree' in the model by various attributes of the trees, namely the concentrations of sideroxylonals and nitrogen and the interaction of the two. Terms that were non-significant were progressively dropped from the model, leaving a reduced model on which the results are based. Further analysis combined all of the data and introduced 'experiment' and its interactions as terms in the random model. In all cases, significance was measured using a submodel, whereby the term of interest is dropped from the full model to enable deviance to be measured between the full model and the submodel. This deviance is attributable to the term of interest and its significance is calculated using a Chi-squared test.

Results

Effect of purified sideroxylonals on intake and choice of a synthetic diet.

There was a significant difference in the amount of food eaten when animals were offered diets varying in their sideroxylonal concentration (Fig. 1*a*). Intake was depressed at concentrations of 7 and 11 mg per g DM. When offered a choice, brushtail possums preferred to eat the control diet when the concentrations of sideroxylonals were ≥4 mg per g DM (Fig. 1*b*).

Effect of foliar sideroxylonals on intake of E. melliodora leaves

Experiment 1

The foliage from the twelve *E. melliodora* trees varied substantially in concentrations of both sideroxylonals (~1–52 mg per g DM) and cineole (~1–31 mg per g DM). There was a strong positive correlation between the concentrations of these substances ($r^2 = 0.88$, $F_{1,10} = 74$, $P < 0.0001$). One tree had extremely high concentrations of both sideroxylonals (52 mg per g dry leaf) and cineole (31 mg per g dry leaf) whereas two trees contained negligible amounts of these substances. The remaining trees had intermediate concentrations of sideroxylonals (15–30 mg per g dry leaf) and of cineole (14–25 mg per g dry leaf). The nitrogen content of the leaf varied from 10 to 16.5 mg per g dry leaf but these values were unrelated to those for sideroxylonals and cineole.

The possums, which ranged in mean body mass from 1300 to 2100 g, all gained mass during the experiment (2–13%). Differences in body mass did not explain the wide variation in the amount that each possum ate (19.7–38.2 g DM per animal per day averaged over all trees) so results are expressed as grams per animal. The amount eaten was not affected by the amount offered ($P = 0.99$) and did not differ between experimental days ($P = 0.28$) but it did differ substantially between individual trees (12.1–61.0 g DM per animal). This variation was explained overwhelmingly by foliar sideroxylonal concentration ($\chi^2_{10} = 81.2$, $P < 0.001$).
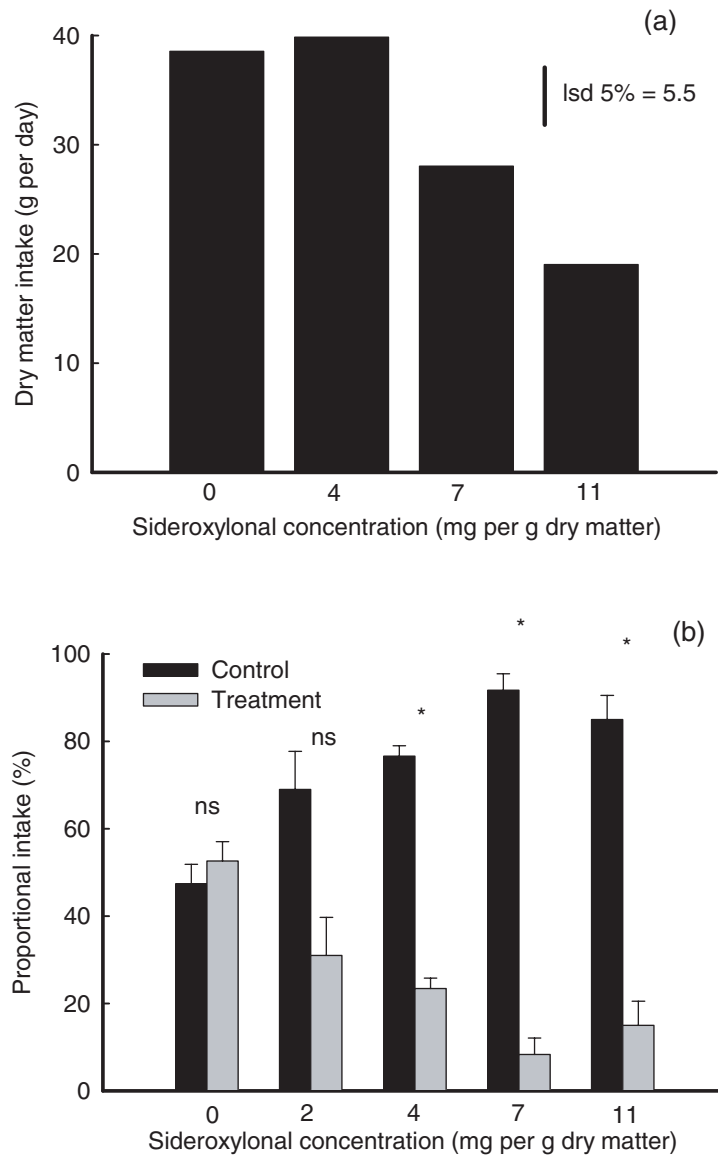
Fig. 1. (*a*) The mean DMI of common brushtail possums fed a basal diet containing graded concentrations of sideroxylonal. (*b*) The proportional intake (±s.e.) by common brushtail possums given a choice between a basal diet containing graded concentrations of sideroxylonal or the basal diet alone.

Experiment 2

The possums in Experiment 2 covered a wider range of body masses (2000–3400 g) than did those in Experiment 1 but again this did not explain ($P = 0.32$) the wide variation in feeding (30.0–46.9 g DM per animal per day averaged over all trees). The amount eaten was influenced by the amount of food offered ($\chi^2_1 = 8.64$, $P = 0.003$) and by the concentration of foliar sideroxylonals ($\chi^2_{14} = 41.0$, $P < 0.001$). Interestingly, food intake depended also

on experimental day (χ^2_{14} = 44.7, P < 0.001), with possums tending to eat more as the experiment proceeded.

Experiments 1 and 2 combined

We combined the data from both experiments in the following model:

Response: Dry matter intake
Fixed model: Constant + Sideroxylonal + Nitrogen + Food offered + Body mass + Nitrogen $\times$ Sideroxylonal + Body mass $\times$ Sideroxylonal
Random model: Experiment + Experiment $\times$ Possum + Experiment $\times$ Experimental day + Experiment $\times$ Experimental day $\times$ Possum

In keeping with the results for the individual experiments, body mass (P = 0.80) and its interaction with foliar sideroxylonal concentration (P = 0.245), did not influence food intake so these terms were dropped from the model. The amount of food offered significantly affected the amount that possums ate (χ^2_1 = 7.8, P = 0.005), but there was no interaction between the amount offered and sideroxylonal concentration (P = 0.62). Feeding was significantly influenced by the nitrogen content of the leaf (χ^2_1 = 5.5, P = 0.02) but, again, the term explaining most of the variation was foliar sideroxylonal concentration (χ^2_{22} = 186, P < 0.0001). The DMIs, adjusted for nitrogen concentration and the amount of food offered, are plotted against sideroxylonal concentration in Fig. 2. Although there is a sharp reduction in DMI over the entire range of sideroxylonal concentrations, there is much variation in DMI over the intermediate range of sideroxylonal concentrations (15–30 mg per g DM). A similar analysis showed that sideroxylonal intake was influenced by foliar sideroxylonal content (χ^2_{22} = 147, P < 0.0001) and the amount of foliage offered (χ^2_1 = 10.7, P = 0.001), but not by foliar nitrogen (χ^2_1 = 2.75, P = 0.097). Possums were willing to ingest roughly 700 mg of sideroxylonal per day (Fig. 3) but, again, there was wide variation about this ceiling. For instance, possums were willing to eat relatively more
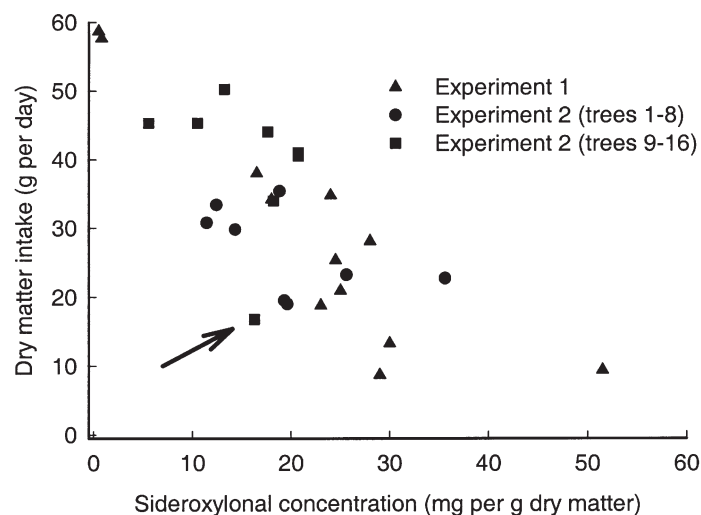

Fig. 2.   The relationship between DMI and foliar sideroxylonal concentration in common brushtail possums each fed leaves from 28 *E. melliodora* trees (combined data from Experiments 1 and 2).
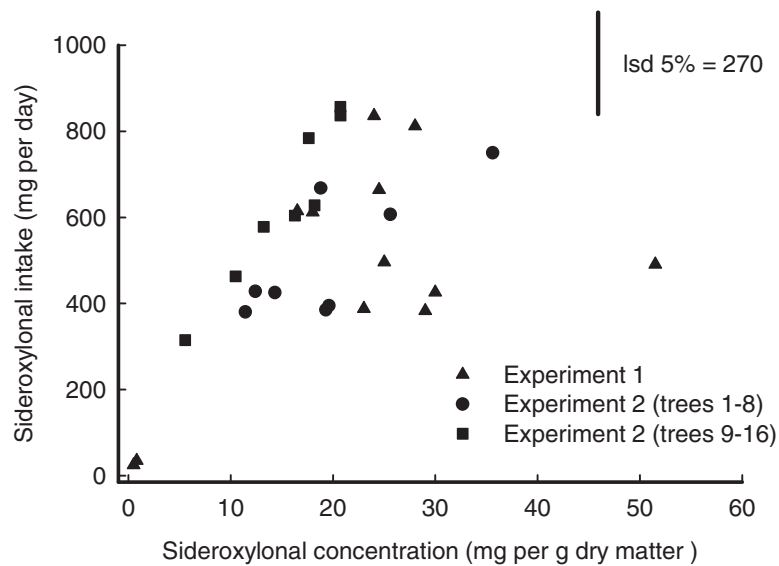
Fig. 3. The sideroxylonal intake by possums in relation to the sideroxylonal concentrations of 28 *E. melliodora* trees fed in Experiments 1 and 2.
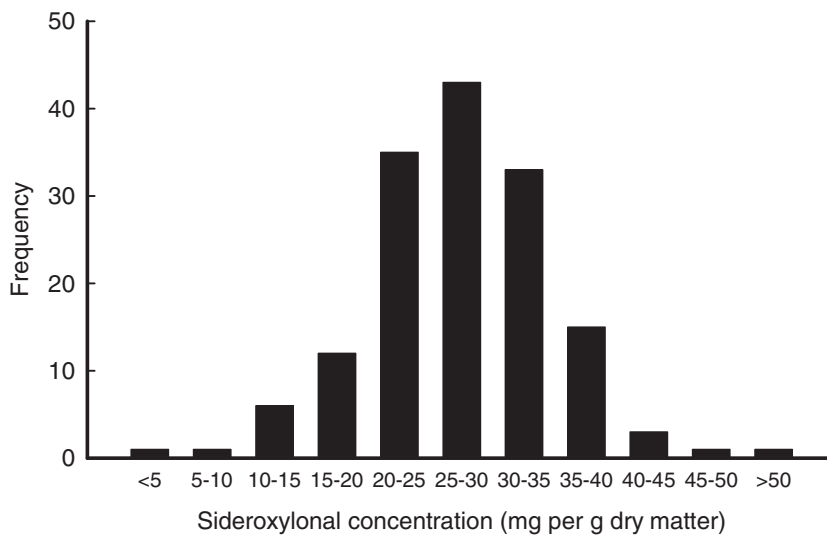

Fig. 4. The distribution of sideroxylonal concentrations in foliage sampled from 150 *E. melliodora* trees at Mulligan's Flat.

foliage from several trees with sideroxylonal concentrations of 20–30 mg per g DM, in which cases the animals' consumption of sideroxylonals rose to over 800 mg per day. In contrast, the possums were reluctant to eat foliage with a sideroxylonal concentration of only 16 mg per g DM.

Field sampling at Mulligan's Flat

A one-way ANOVA showed that mean foliar sideroxylonal concentrations did not differ between the two areas surveyed at Mulligan's Flat (Area 1: mean = 27.4, s.d. = 7.5, n = 64; Area 2: mean = 28.0, s.d. = 7.9, n = 76; $F_{1,148}$ = 0.24, P = 0.62). The combined data (mean = 27.7, s.d. = 7.85, n = 140) shows that foliar sideroxylonal concentrations are normally distributed, with 75% of values falling between 20 and 35 mg per g of dry foliage (Fig. 4). Only one tree contained less than 10 and only one more than 50 mg of sideroxylonals per g DM.

Discussion

This study shows clearly that sideroxylonals are a potent feeding inhibitor for brushtail possums and that intraspecific variation in the susceptibility of *E. melliodora* to herbivory by brushtail possums is explained, in part, by the 100-fold range in the foliar concentration of sideroxylonals. The potency of synthetic sideroxylonals is evident from the reduction in feeding by possums offered synthetic diets with as little as 4 mg sideroxylonals per g DM (when a choice was available) and 7 mg per g DM when no choice was offered (Fig. 1). The results of the feeding experiments with foliage also suggested a severe reduction in feeding even at low concentrations of sideroxylonals but the paucity of trees (~0.5%) with less than 10 mg per g of sideroxylonals prevented us describing the relationship adequately.

It is remarkable that brushtail possums will eat some foliage even when it contains extremely high concentrations of sideroxylonals. Unfortunately, it is still difficult to isolate enough sideroxylonals for feeding studies using synthetic diets that preclude other secondary chemicals. Even so, past work with brushtails fed FPCs in synthetic diets suggests that the speed with which animals receive feedback differs between purified and foliage diets (Stapley *et al.* 2000). The synthetic diet is highly palatable, possums eat it quickly, the sideroxylonals are released quickly and animals stop feeding quickly. In contrast, foliage takes longer to ingest and the sideroxylonals are probably released relatively slowly. Additionally, feedback mechanisms to limit consumption of FPCs involve flavour aversions that are conditioned by volatile terpenes such as cineole, which occur in foliage but were not part of the synthetic diets fed by Stapley *et al.* (2000).

The strong relationship between the concentrations of sideroxylonals and cineole for the 12 trees fed in the first foliage experiment reinforces the finding of Lawler *et al.* (1998) of a similar relationship between the concentrations of cineole and that of another FPC, macrocarpal-G. The difficulty in purifying large quantities of sideroxylonals or macrocarpals led Lawler *et al.* (1998) to experiment further using the simplest FPC, jensenone, which is readily extracted from *E. jensenii*. They fed both ringtail and brushtail possums a synthetic diet containing various combinations of cineole and jensenone and showed, unequivocally, that jensenone determines how much the animal eats, while the concentration of cineole provides the animal with a cue about the concentration of jensenone. We assume that the brushtail possum uses this same conditioned aversion system to detect foliar sideroxylonal concentrations because, first, sideroxylonals are dimers of jensenone and the two compounds presumably have similar biological actions. Secondly, like the ringtail possum (Lawler *et al.* 1998) and the koala (Zoidis and Markowitz 1992), brushtail possums smell leaves carefully before ingesting them (IRW, personal observation).

A notable finding in the first foliage experiment was the variability (a two-fold range) in the amount possums were willing to eat from different trees with the same concentrations of sideroxylonals. Indeed, demonstrating the strong relationship between DMI and the

concentration of sideroxylonals in this experiment would have been difficult without the inclusion of trees with remarkably low and remarkably high concentrations of sideroxylonals. These findings invite many questions. For instance, what range of FPCs confronts free-living brushtail possums? What is the distribution of sideroxylonals such that trees with less than 15 or more than 30 mg per g DM of sideroxylonals are few? What explains the variability in food intake over a narrow range of sideroxylonal concentrations? Finally, what is the shape of the relationship between DMI and sideroxylonal concentration? If possums can closely regulate their intake of sideroxylonals then we might expect to see a curvilinear relationship, with possums eating until they ingest a threshold dose of the FPC.

The feeding experiments showed that possums select foliage with low concentrations of sideroxylonals. Thus, the number of potential food trees depends on the density of trees, the distribution of their chemistry and on the size of a possum's home range. The sampling of 150 trees at Mulligan's Flat shows that sideroxylonal concentrations are distributed normally. The probabilities of trees containing less than 10 or more than 50 mg of sideroxylonals per g DM are 0.0122 and 0.0023, respectively and roughly 80% of trees have sideroxylonal concentrations of 20–40 mg per g DM. Brushtail possums reside at Mulligan's Flat but we do not have information on the sizes of their home ranges. We sampled every *E. melliodora* within a grid of roughly 6 ha at the site. If the population density is similar to that (0.2–0.3 animals per hectare) measured by Dunnett (1964) for possums inhabiting eucalypt woodland around Canberra, it is unlikely that an animal at Mulligan's Flat will have access to trees of low sideroxylonal concentration. Thus, the trees of relevance to possums are those in the middle of the sideroxylonal distribution, where variability in DMI is most noticeable.

We examined this variability in Experiment 2, by repeating the feeding study with 16 trees of predominantly intermediate sideroxylonal concentrations. While sideroxylonals were still an important determinant of feeding, these data show a clear dichotomy in feeding that is most likely attributable to the timing of the study. Regardless of sideroxylonal concentration, possums tended to eat less of the first eight trees (squares in Fig. 2) fed from 23 November to 7 December 2000 than they did of the second eight (circles), fed from 9 to 23 December 2000. There was one exceptional tree, from the second eight, that is marked with an arrow in Fig. 2, of which possums ate much less than expected. The first part of the study coincided with the appearance of new foliage on many *E. melliodora* but, by the second part, most trees were showing a flush of new growth. Even so, it was still easy to cut branches containing predominantly mature foliage and to remove any young growth before offering the branch to possums. Nevertheless, the data indicate that while leaves may look homogeneous the dichotomy suggests that changes in palatability occur at this time that are unrelated to the concentration of sideroxylonals. Reinforcing this argument is the significant positive effect on DMI of the amount of food offered. Possums offered more food tended to eat more, suggesting that they found more leaves worth eating. While it may appear flawed to offer possums varying amounts of food, this is largely unavoidable because branches contain different amounts of foliage relative to wood and individual possums eat different amounts of food. Thus, we offered possums branches that weighed between 350 and 500 g. These were judged to provide an animal with as much foliage as it wished to eat from that particular tree. The results of Experiment 1 support the hypothesis that phenological changes explain the dichotomy. Experiment 1 was done in March 1999 when there was no evidence of young foliage and, in this case, the amount of food offered did not influence feeding. We contend that physiological changes occur when trees begin to

produce young foliage, leaving mature leaves less protected against herbivory. If so, changes occur in substances other than sideroxylonals because the palatable and less palatable foliage contained equal amounts of the compound. Likewise, the data are not explained by a simple nitrogen-to-sideroxylonals ratio, as suggested by Cork (1992), although both factors influenced feeding.

Perhaps the best evidence for the involvement of secondary plant chemicals other than FPCs in protecting eucalypts from herbivory comes from the data of sideroxylonal consumption relative to sideroxylonal concentration in foliage (Fig. 3). If sideroxylonals were acting alone we would expect possums to stop eating after consuming a threshold dose. While there is a trend towards a threshold there is wide variability in the data.

Further evidence for protection from other secondary chemicals comes from comparisons with animals fed fruit-based synthetic diets containing graded concentrations of FPC. Our experiments with synthetic diets along with those by Lawler *et al.* (1999) (on ringtail possums) and Stapley *et al.* (2000) (on brushtail possums) show minimal variation in DMI at any concentration of FPC compared with the variation observed in the present experiments with possums fed foliage. Of course, other factors increase variation in animals fed foliage. For instance, different trees vary in their ratios of FPCs to nutrients while, in synthetic diets, nutrient concentrations are held constant while FPC concentrations change. Secondly, accurate measures of chemical compounds, like FPCs, require us to sample leaves in the same way that possums do. Even though preliminary NIR studies suggest that the leaves refused by possums contain similar amounts of sideroxylonals as the leaves offered, the results of this study suggest that trying to sample leaves as possums do is fraught with error. Even so, it is unlikely that these factors explain the variability in DMI at intermediate concentrations of sideroxylonals. Instead, it seems plausible that eucalypts possess defence mechanisms other than FPCs because sideroxylonals are the only FPCs in *E. melliodora*.

What other secondary plant chemicals might be protecting eucalypts? Before the discovery of FPCs as potent anti-feeding chemicals in eucalypts (Pass *et al.* 1998), attention focused on total phenolics and tannins as probable defence mechanisms (e.g. Foley and Hume 1987; Cork 1992; Cork and Catling 1996). While the latter studies are correlative, that of Foley and Hume (1987) showed that supplementation of *E. melliodora* diets with polyethylene glycol 4000, to bind tannins, resulted in brushtail possums eating more and digesting more of the energy-yielding nutrients in the diet. Thus, two groups of plant secondary chemicals have now been shown to influence feeding by brushtail possums. It now seems appropriate to examine interactions between the two groups in determining feeding strategies of folivorous marsupials.

In this research we were keen to examine how the tolerance for sideroxylonals of a generalist herbivore, the brushtail possum, compares with the tolerance shown by a specialist eucalypt herbivore, the common ringtail possum. Direct comparisons are difficult because the two species show preferences for different eucalypts. However, Lawler *et al.* (2000) showed that ringtail possums were unwilling to eat foliage with sideroxylonal concentrations exceeding 15 mg per g dry leaf. In contrast, brushtail possums will still eat some foliage even when the sideroxylonal concentrations exceed 50 mg per g DM.

Conclusion

Lewontin (1993) suggests that one of the major prejudices in modern biology concerns the nature of causes and the desire to split them into major and subsidiary causes. It is tempting to make this error with the present data. While there is substantial unexplained variation, it

seems fair to conclude that FPCs in eucalypt foliage are important determinants of feeding by the common brushtail possum. This conclusion concurs with similar findings for the common ringtail possum and the koala.

Acknowledgments

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