INTRASPECIFIC VARIATION IN NUTRITIONAL COMPOSITION AFFECTS THE LEAF AGE PREFERENCES OF A MAMMALIAN HERBIVORE

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Abstract - Ecologists have long been interested in how the nutritional composition of leaves change as they age, and whether this affects herbivore feeding preferences. As a consequence, the literature abounds with reports that younger leaves contain higher concentrations of nitrogen (N) and plant secondary metabolites (PSMs) than do older leaves. Most of these studies, however, base their conclusions on average values that often mean little to herbivores. We examined this issue in the well-studied marsupial-eucalypt system using *Eucalyptus melliodora* and captive common brushtail possums (*Trichosurus vulpecula*) offered branches from individual trees containing both young and mature leaves. Like many plants, the concentrations of N and PSMs differ between individual *E. melliodora*. We found that although young leaves were, on average, "better defended" by the PSM, sideroxylonal, than were mature leaves, some trees produced leaves that were relatively undefended at both ages. In response, possums chose different proportions of young and mature leaves depending on the chemistry of the individual tree. They did not always prefer the leaves with lower concentrations of sideroxylonal (mature leaves), nor those with higher concentrations of available N (young leaves). Instead, the sideroxylonal concentration of young leaves dictated their choice; they preferred young leaves at low sideroxylonal concentrations, but not at high concentrations. By skewing their feeding towards trees producing young leaves with low concentrations of PSMs, possums may influence plant fitness. Researchers will detect these potentially important interactions only if they are aware that measuring variation between plants discloses more information than do average relationships.

Key Words - Plant secondary metabolite, available N, herbivory, feeding decision, trade-off.

INTRODUCTION

Optimal plant defense theories suggest that younger leaves are more valuable to plants than are older leaves (McKey 1979; Rhoades 1979). Thus, plants should allocate more resources to defend younger leaves against herbivory (McKey 1979; Rhoades 1979). Plants generally conform to this pattern – young leaves typically contain higher concentrations of protein (measured as nitrogen; N) and plant secondary metabolites (PSMs) than do older leaves (McCall and Fordyce 2010). However, whether or not elevated levels of PSMs protect young leaves from herbivory depends on the particular plant and herbivore.

The trade-off between acquiring nutrients, while minimising the ingestion of PSMs, may determine whether herbivores select young or old leaves. Importantly, the size of this trade-off differs between herbivore species. For example, specialist herbivores – those that feed on particular groups of plants, may tolerate elevated PSM concentrations in young leaves that generalists – those herbivores that eat a wider variety of foods, will not (Gutbrodt et al. 2012; Jensen et al. 2014; Lambdon and Hassall 2005). Thus, specialist herbivores may prefer young leaves, while generalists may prefer to eat older leaves (Gutbrodt et al. 2012).

One aspect, however, that researchers often disregard is that the size of the trade-off may also differ between conspecific plants. The concentrations of both PSMs and N in mature foliage can differ greatly between neighboring conspecifics (Moore et al. 2010). Some plants contain high concentrations of PSMs that deter herbivores, while others of the same species are less defended (Marsh et al. 2014; Villalba et al. 2014; Vourc'h et al. 2002). Thus, it may be more costly (or beneficial) to eat the young leaves from some plants than others. They may, for instance, prefer the higher protein concentrations in young leaves that contain low concentrations of PSMs, but may prefer to minimize their ingestion of PSMs in highly defended young leaves of conspecifics by selecting the less nutritious older leaves. These sorts of responses will be hidden if researchers document only the average composition of leaves from each age class and the average preferences of herbivores, rather than identifying variation in these parameters.

The aim of our study was to investigate whether differences in nutritional composition between individual plants alters the relative palatability of younger and older foliage. We used *Eucalyptus melliodora* (yellow box) as our model plant because it contains variable concentrations of a PSM called sideroxylonal (Wallis et al. 2002). This PSM influences the feeding decisions of a variety of marsupial and insect herbivores (Jensen et al. 2014; Matsuki et al. 2011), including common brushtail possums (*Trichosurus vulpecula*). Like most eucalypts, *E. melliodora* always has mature leaves, while flushes of new growth are irregular so that young leaves are present only for short periods (Landsberg and Cork 1997). We analysed the composition of newly emerging, young, and mature adult phase leaves present concurrently in order to confirm that a) younger foliage contains more available N than does mature foliage, b) younger foliage contains more sideroxylonal than does mature foliage, and c) sideroxylonal concentrations are correlated in young and mature leaves from the same tree.

We then investigated how the composition of foliage affects the feeding preferences of common brushtail possums by offering them young and mature *E. melliodora* foliage in two ways. In the first experiment, we did not give possums a choice. We offered them either young or mature foliage from a variety of trees in order to establish the absolute amount that they were willing to consume relative to the leaf age class and the concentrations of sideroxylonal and available N. In the second experiment we offered them choices between young and mature leaves from the same trees. In this case we wanted to know whether possums consistently preferred leaves of a particular age class, or whether their preferences differed between trees due to variation in PSM concentrations. We envisaged three potential outcomes: 1) possums always prefer younger leaves (higher concentrations of available N); 2) possums always prefer older leaves (lower concentrations of sideroxylonal); or 3) possums prefer younger leaves from trees with low sideroxylonal concentrations, but prefer older leaves from trees with higher concentrations of sideroxylonal. This latter scenario could occur if both the young and mature leaves from some trees contain sideroxylonal concentrations that are relatively non-deterrent to possums (in which case they may prefer the more nutritious young leaves), while other trees contain highly deterrent concentrations (in which case they may seek to minimize their ingestion of sideroxylonal by choosing the less defended older leaves).

METHODS

Compositional Analysis. Bulked leaf samples (approx. 10 g of each age class from each tree) were freeze-dried and ground in a Cyclotec 1093 Mill (Tecator, Höganäs, Sweden) to pass a 1mm sieve.

Quantification of Sideroxylonal. Ground leaves from the bulk samples (50.0 ± 0.5 mg) were extracted by sonication for 10 mins in a known mass (approximately 5 g) of 7 % water in acetonitrile with 0.1 % trifluoroacetic acid (Wallis and Foley 2005). Total sideroxylonal concentrations in the extract were quantified by HPLC following the methods of Wallis and Foley (2005).

Analysis of Total and Available N, and Dry Matter Digestibility. Total N was measured for each sample on 205.0 ± 5.0 mg ground leaves using the Dumas combustion procedure in a Leco Truspec CN analyser. The same procedure, but with 105.0 ± 5.0 g (or all remaining sample if less than this was available), was used to measure the N remaining in duplicates of each sample, after a two-step digestion in Ankom F57 filter bags, as described in DeGabriel et al. (2008). These values were used to calculate the *in vitro* available (digestible) N for each sample. Dry matter digestibility was likewise calculated from the residue remaining in the bags after the digestion procedure (DeGabriel et al. 2008).

Comparison of Leaf Composition From Different Age Classes. Leaves were collected from each of 19 mature *Eucalyptus melliodora* trees growing near Black Mountain, Canberra, Australia, and were sorted into batches of unexpanded leaves, young leaves (fully expanded leaves that were still soft), and mature leaves (all other leaves) from each tree. They were immediately frozen at -20°C in paper bags pending compositional analysis.

A one-way ANOVA (Genstat v17.1, VSN International Ltd, UK) was used to test whether foliage of different ages differed in dry matter digestibility and the concentrations of total N, available N and sideroxylonal.

Possum capture and housing. Six male common brushtail possums (*Trichosurus vulpecula*; mean body mass ± SE = 2918 ± 170 g) were captured on the campus of The Australian National

University, Canberra, Australia in cage traps baited with apple. Possums were kept in sheltered pens measuring ~ 2 x 3 x 4 m. During acclimation to captivity, possums were fed chopped apples and carrots, and branches of leaves from a variety of native and non-native tree species. Possums had access to drinking water *ad libitum*.

Experiment 1 – Consumption of Leaves From Different Age Classes ("No-Choice"). Possums were fed one of eighteen possible "treatments" on each night of the experiment. They were offered a different "treatment" on each night. The eighteen treatments consisted of mature leaves from each of seven *E. melliodora* trees and young leaves from each of 11 trees. We did not offer unexpanded foliage to possums because there was not enough on each tree to provide it *ad libitum*.

The experiment was organised into three rounds. Each treatment was allocated to one of the rounds (i.e. six treatments per round) to enable us to collect all leaves required for that treatment at one time, eliminating the risk that leaf age and leaf chemistry within a tree would change between collections. Branches were collected at least 24 h before the start of a round, placed in plastic bags and stored with their cut ends in water in the dark at 4 °C. The experiment was designed as a Youden square so that 1) each possum received a different treatment on each night, 2) no two possums received the same treatment on the same night, and 3) no possum received the same treatment twice. Each round lasted for four nights, which meant that, during the whole experiment, each of the six possums received 12 of the possible 18 treatments. Possums were fed only leaves throughout the experiment.

At 1700 h each day, possums were provided with a bunch (> 400 g) of their allocated foliage, with the stems immersed in water. At the same time, “control” bunches of the same foliage were placed in water outside the pens, enabling us to confirm that bunches remained within 1-2 g of their initial weight in the absence of herbivory. At 0830 h the next day we removed the foliage from the pens and weighed them to determine wet matter intake. Any leaves that had been detached from stems during the night were collected and dried to constant mass at 60 °C. Samples of leaves similar to those eaten by possums were removed from control bunches (approximately 20 g) and separated into two bags. One was frozen at -20 °C pending chemical analysis (see “compositional analysis” section). The other was dried at 60 °C to constant mass, and used to calculate % dry matter (DM) of the leaves. Dry matter intake (DMI) was calculated by multiplying % DM by apparent wet matter intake, and then subtracting the dry mass of leaves that had detached from the stems.

Experiment 1 was analysed using the residual maximum likelihood (REML) linear mixed model function in Genstat. The response variate was DMI. The confounding of leaf age and available N concentrations prompted us to analyze the data with two models that included either leaf age or the available N concentration. The fixed model included the concentration of sideroxylonal, the available N concentration or leaf age, and all interaction terms, while the random model included the possum identity and the experimental day. We sequentially removed any non-significant terms ($P > 0.05$) to leave a final model with only significant terms ($P < 0.05$). Non-significant results are reported from the full models, whereas significant results are from the final models.

Experiment 2 – Consumption of Leaves When Offered a Choice Between Young and Mature Foliage From The Same Tree. The design of experiment 2 resembled that of experiment 1, and we used "control" bunches in the same way in both experiments. Eighteen *E. melliodora* trees were allocated to one of three, four-night periods (six trees per period) so that all leaves for a given treatment could be collected at the same time. Within each period, a Youden square design was used to determine which possum was offered which tree each night. There were four observations per tree and all six possums received 12 of the possible 18 trees. At 1700 h, possums were provided with two bunches of foliage from their allocated tree; one containing only young leaves and the other only mature leaves, each weighing at least 300 g. The two bunches were placed at least 2 m apart with their stems in tubes of water; the positions of the young and mature bunches were swapped each night. At 2100 h experimental and control bunches were removed and reweighed. We offered the foliage over a shorter time than in experiment 1 to ensure that there were enough young and mature leaves from all trees to complete the experiment. From 2100 h until 0900 h, possums were offered chopped apples and carrots, and leaves from a variety of tree species. DMI was determined separately for young and mature leaves, and samples of leaves from control bunches were frozen for later chemical analysis. A leaf age class "preference index" was calculated by subtracting the amount of mature leaves consumed from the amount of young leaves consumed.

The ANOVA function in Genstat was used to test a) for differences in total DMI between individual trees, b) whether the young leaf proportion of DMI differed between trees, and c) whether individual possums differed in the leaf age class they preferred. A paired sample (two-sided) t-test was used to test whether possums ate more young or mature leaves. All other analyses were performed using REML linear mixed models. Using separate models for young and mature leaves, we tested whether the sideroxylonal concentration, available N concentration, or the interaction between sideroxylonal and available N affected the DMI of each foliage age class. We also tested whether the composition of young foliage (chemical concentrations were strongly correlated between age classes; *P*<0.001 for both sideroxylonal and available N), or the difference in composition between young and mature foliage, influenced foliage age class preference. In all models, possum and day were included as random effects.

We also used the geometric framework of Raubenheimer and Simpson (1993) to plot the relative available N and FPC content of young and mature leaves, and the amount of those constituents that possums chose to ingest, for each choice that they were offered. This method allows the visualization of whether herbivores prioritize the ingestion of one of the plotted constituents over the other. In addition, it is possible to see the scope that herbivores have to alter their nutritional trajectory if they switch between the two food items that are offered.

RESULTS

Composition of Leaves From Different Age Classes. Unexpanded leaves had the highest *in vitro* DM digestibility and the highest concentrations of total and available N, followed by young and then mature leaves (Table 1). The highest concentrations of sideroxylonal occurred in young leaves, with the lowest concentrations in unexpanded leaves (Table 1).

Sideroxylonal concentrations were positively correlated in young and mature leaves within trees ($F_{1,17}$ = 17.19, P < 0.001; Figure 1a), but not in unexpanded and mature leaves ($F_{1,15}$ = 2.20, P =

0.159). The same was true for available N concentration (young compared to mature leaves: $F_{1,17}$ = 4.69, P = 0.045; unexpanded compared to mature leaves: $F_{1,15}$ = 1.51, P = 0.238; Figure 1b). There was no relationship between sideroxylonal and available N concentrations in mature ($F_{1,18}$ = 0.04, P = 0.843) or young leaves ($F_{1,18}$ = 3.46, P = 0.081). There was, however, a slight negative correlation (slope = -0.07, r^2 = 0.25) between the concentrations of sideroxylonal and available N in unexpanded leaves ($F_{1,18}$ = 6.19, P = 0.025).

Experiment 1 – Consumption of Leaves From Different Age Classes ("No-Choice"). Possums ate less as sideroxylonal concentrations in foliage increased ($F_{1,60}$=37.00, P<0.001; Figure 2a) and ate more as available N concentrations increased ($F_{1,64}$=9.48, P=0.003). However, this apparent response to available N was because they preferred eating young rather than mature leaves ($F_{1,62.7}$=11.88, P<0.001; Figure 2a); the available N concentration did not affect intake within either the young ($F_{1,5.6}$=2.46, P=0.171) or mature ($F_{1,19.8}$=2.43, P=0.135) foliage age class (Figure 2b). The sideroxylonal concentration in foliage depressed feeding to a similar degree in possums fed young and mature foliage (Figure 2a), and this was not influenced by the available N content of the leaves (all interactions P>0.05). However, because possums ate more young than mature leaves, they ingested more sideroxylonal when eating young leaves (Figure 2c).

Experiment 2 – Consumption of Leaves When Offered a Choice Between Young and Mature Foliage. The amount of DM (total of young and mature foliage) that possums ate varied substantially between trees ($F_{17,54}$=13.24, P<0.001; Figure 3). Possums usually ate a mixture of young and mature leaves, with the proportions differing between trees ($F_{17,54}$=2.36, P=0.009; Figure 3). There was a trend for possums to eat more young than mature leaves when all trees were considered (mean difference in DMI between young and mature leaves = 2.7 ± 1.6 g; $t(71)=1.72$, $P=0.091$). However, preferences also differed between individual possums ($F_{5,66}=3.05$, $P=0.016$). The possum that least preferred young leaves selected a diet with 37 ± 6 % young leaves – less than half that of the possum that most preferred them (77 ± 8 %).

As in experiment 1, possums ate less of both young and mature foliage as sideroxylonal concentrations increased (young: $F_{1,66.3}=35.07$, $P<0.001$; mature: $F_{1,65.5}=7.10$, $P=0.01$). Available N concentrations had no bearing on the possums' consumption of leaves from either age class (young: $F_{1,62.6}=0.29$, $P=0.593$; mature: $F_{1,65}=0.13$, $P=0.718$).

The difference between young and mature foliage in the concentration of either sideroxylonal ($F_{1,59.1}=0.44$, $P=0.508$) or available N ($F_{1,62.9}=1.52$, $P=0.222$) did not explain why possums chose to eat young or mature foliage (i.e. the "preference index"). However, as concentrations of sideroxylonal increased in young leaves possums ate relatively more mature leaves – i.e. the preference index decreased ($F_{1,65}=4.27$, $P=0.043$; Figure 4a). The available N concentration of young foliage did not influence the preference index ($F_{1,64} = 0.46$, $P = 0.500$; Figure 4b).

A geometric framework plot showed that, regardless of which leaf age class they consumed, possums offered a choice between young and mature leaves from the same tree remained on a similar nutritional trajectory (Figure 5). Interestingly, however, the trajectories differed substantially between individual trees (Figure 5). Possums did not appear to prioritize the ingestion of a set amount of available N (Figure 5) but, instead, may have been limiting their ingestion of sideroxylonal.

DISCUSSION

Our study demonstrates that it is important to look beyond the average composition of leaf age classes when considering the trade-offs faced by herbivores attempting to maximize their ingestion of nutrients while minimizing their ingestion of PSMs. The two main classes of leaves we used in this study differed widely in their chemical composition. Even though young, fully expanded *E. melliodora* leaves contained higher average concentrations of available N than did mature leaves, they also contained higher concentrations of the PSM, sideroxylonal. In both the young and the mature leaves, the concentration of sideroxylonal ranged from negligible and of no physiological importance, to highly deterrent. In response to this conundrum, possums varied the amounts and the proportions they ate of young and mature leaves depending on the chemical characteristics of the individual tree.

As expected, the concentrations of sideroxylonal were closely correlated in the young and mature leaves of individual *E. melliodora*. There is no evidence that eucalypts can induce the production of sideroxylonal (Henery et al. 2008), so presumably the correlation indicates the strong genetic control that production of this compound is under (Andrew et al. 2005; Andrew et al. 2007). Thus, the factors that determine sideroxylonal concentrations in mature leaves, which are unknown, but could include, for example, the level of gene expression (Padovan et al. 2013), the regulation of biosynthetic pathways (Padovan et al. 2015), or the availability of precursors, presumably also operate in young leaves. This genetic regulation leads to an important consequence: although young leaves were “better defended” by sideroxylonal than were mature leaves from the same tree, young leaves from some trees were less defended than mature leaves from other trees. This indicates that in order to meet their nutrient requirements while limiting their ingestion of PSMs, possums must make complex decisions based on the trees available from which they can feed, the age classes of leaves on those trees and the chemical composition of those leaves.

Brushtail possums reduce their intake of mature *E. melliodora* foliage as sideroxylonal concentrations increase (Marsh et al. 2003; Wallis et al. 2002). We found the same when possums were offered both mature and young leaves in the current study, resulting in large variation in DMI between trees for both leaf age classes. Thus, some trees provided better food than did others, regardless of the age of the leaves or whether they were offered separately or as a choice. It was evident from the no-choice study, however, that possums preferred eating young leaves over mature leaves at equivalent sideroxylonal concentrations. Consequently, they ingested more sideroxylonal from young than from mature leaves. This is interesting because, in previous studies, brushtail possums (and other marsupial folivores) regulated their intake to remain below a threshold dose of sideroxylonal and related compounds (Lawler et al. 1998; Stapley et al. 2000; Wallis et al. 2002). For example, possums offered mature *E. melliodora* foliage limited their ingestion of sideroxylonal to around 800 mg per day (Wallis et al. 2002). This is similar to possums offered mature leaves in our study, but the threshold was closer to 1200 mg per day when they were eating young foliage. Possums detect their threshold through the emetic pathway (DeGabriel et al. 2010; Lawler et al. 1998). A variety of other herbivores also select their diets to keep PSM ingestion below a threshold (e.g. woodrats: Mangione et al. 2000; cattle: Pfister et al. 1997; sheep: Wang and Provenza 1997), probably because there are physiological limitations to PSM metabolism (Marsh et al. 2006). Our study suggests that the conditions under which possums encounter sideroxylonal may influence how much they can ingest.

Young leaves contain higher concentrations of available N, and potentially other nutrients. For example, young water hyacinth (*Eichhornia crassipes*) leaves contain higher concentrations of phosphorous, potassium and magnesium, in addition to N, than do older leaves (Center and Wright 1991). A higher availability of nutrients may allow animals that eat young leaves to ingest more sideroxylonal, but there are arguments both for and against this idea. In support, metabolising PSMs requires an animal to expend protein (Au et al. 2013). Thus brushtail possums ingest more of two PSMs, 1,8-cineole and benzoic acid, when offered artificial diets containing more N (Au et al. 2013; Nersesian et al. 2012). Similarly, supplementary protein allows sheep and goats to increase their intake of various PSMs, or plants containing PSMs (Utsumi et al. 2009; Villalba and Provenza 2005; Villalba et al. 2002). The counter argument to protein enrichment enabling possums to ingest more sideroxylonal comes from the current study. If this were so we would expect animals to eat more as available N concentrations in leaves increased. This did not happen. Available N concentration did not influence DMI within leaf age classes in either the choice or no-choice trials. It is possible, however, that other nutrients limit intake of sideroxylonal by possums. Plants contain complex mixtures of nutrients and PSMs, and we do not have a good understanding in any plant-herbivore system of how the various components interact to influence herbivory.

Even though possums tolerated more sideroxylonal when feeding on young leaves, they did not necessarily prefer to eat those leaves. Instead, they ate similar amounts of both young and mature leaves from 10 of the 18 trees, preferring young leaves from only five trees and mature leaves from three. This variation in preference reflected the variation in nutritional composition; possums turned away from young leaves as the sideroxylonal concentration in those leaves increased. This implies that for some trees the benefit of eating young leaves outweighed the metabolic costs associated with ingesting more sideroxylonal. For other trees, however, it did not. One important consequence of this is that some individual plants may lose most or all of their young foliage to herbivory while others go largely untouched. For example, in this study possums ate seven-fold more from relatively undefended trees than they did from highly defended trees. Thus, young leaves from relatively undefended plants are not only preferentially consumed over older leaves, but more is also eaten from those plants.

This differential herbivory invites the question of how relatively undefended trees survive in the landscape. Marsupial folivores occur at very low densities compared with those of invertebrate herbivores, such as scarab beetles (*Anoplognathus* sp.), that feed on the same eucalypts and react similarly to sideroxylonal (Matsuki et al. 2011). Presumably these species apply much of the selection pressure. Scarabs, however, tend to be outbreak species so that years may pass when their numbers are low and poorly defended trees remain little affected.

The deterrent effects of sideroxylonal also meant that possums in the choice experiment did not appear to meet any particular protein target while feeding. One of the useful features of the geometric framework, introduced by Raubenheimer and Simpson (1993), is that it allows researchers to visualize whether herbivores prioritize the ingestion of particular nutrients (e.g. protein; Felton et al. 2009), or whether they make compromises when foods are imbalanced, or, for example, contain PSMs (Behmer et al. 2002). We were able to infer two things by plotting the ingestion of sideroxylonal and available N by possums in the choice experiment. First, possums appeared to prioritize minimizing the ingestion of sideroxylonal over meeting a protein target. Second, the experimental design whereby possums could choose only between young and mature leaves from the same tree essentially constrained them to a single “nutritional rail” (with respect to sideroxylonal and available N). In other words, sideroxylonal and available N essentially occurred in the same ratio in young and mature leaves within a single tree. The trajectories of the nutritional rails, however, differed substantially between individual trees. This suggests that a wild possum behaving typically by feeding on a variety of foliage from a variety of trees (Freeland and Winter 1975) could alter their nutritional trajectory .

Several authors have suggested that forests containing a mixture of eucalypt species and a steady flow of young foliage throughout the year are an important resource for marsupial folivores (e.g. see Moore and Foley 2000). Our results suggest that the presence of trees with low FPC concentrations may be at least as important. This complicates the widely held belief that marsupial folivores prefer new foliage to old due to the extra protein that it provides (Degabriele 1981; Hume et al. 1996; Kavanagh and Lambert 1990; Krockenberger et al. 1998; Landsberg and Cork 1997; Moore and Foley 2000; Pahl 1987). Nevertheless, it is worth noting that possums would receive more available N from eating young foliage than from eating equivalent amounts of mature foliage. The caveat is that the benefits of extra protein depend on the protein concentration of the diet. If essential amino acids are the limiting nutrient then the additional nitrogen is probably beneficial. This was the case in the eucalypt forests in which DeGabriel et al. (2009) demonstrated that female brushtail possums living in home ranges containing eucalypts with higher foliar available N concentrations had greater breeding success, and their pouch young grew faster. Thus, additional available N from consuming young or unexpanded leaves may have benefits beyond any effects on PSM tolerance or feeding preferences.

CONCLUSIONS

Although many studies demonstrate that young leaves contain higher nutrient and PSM concentrations than do older leaves, these average differences may not adequately identify the nutritional trade-offs faced by herbivores. First, the amount of PSM that herbivores can ingest may depend on the nutritional context in which the PSM is presented. For example, in some cases, higher concentrations of N or other nutrients may allow herbivores to tolerate higher PSM intakes. Thus, the same concentration of PSM may be less deterrent in young than in mature leaves.

Secondly, PSM and nutrient concentrations can differ widely between conspecifics. This means that some individual plants may not produce enough PSMs to deter herbivores effectively, even if young leaves contain higher concentrations of PSMs than mature leaves from the same tree. The reciprocal of this is that animals may prefer the higher nutrient concentrations in young leaves only when PSM concentrations are low. Either way, herbivory on young leaves may be more prevalent on individual plants with low concentrations of PSMs. These responses may affect plant fitness, but this likely depends on identifying the selective force. Researchers should therefore be aware that measuring variation in both plant composition and herbivore responses can provide important ecological information that may differ from the conclusions obtained from measuring average relationships.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Mean [range] nutritional composition of unexpanded, young and mature foliage from 19 mature *Eucalyptus melliodora* trees. Within each row, means with the same superscript are not significantly different from one another. DM = dry matter; lsd = 5% least significant difference.

	Unexpanded	**Young**	**Mature**	***F*-value**	***P*-value**	**lsd**
In vitro DM digestibility (%)	87^{a} [82-93]	80^{b} [72-85]	67^{c} [56-73]	179.07	<0.001	2
Total N (% DM)	2.8^{a} [1.9-3.4]	2.3^{b} [1.4-3.2]	1.7^{c} [1.4-2.1]	35.28	<0.001	0.3
In vitro available N (% DM)	2.5^{a} [1.6-3.1]	1.9^{b} [1.1-2.9]	1.2^{c} [0.6-1.6]	48.17	<0.001	0.3
Sideroxylonal (mg/g DM)	8.7^{a} [0.9-14.4]	22.2^{c} [1.5-40.6]	15.8^{b} [2.2-39.9]	16.52	<0.001	4.7

FIGURE LEGENDS

Figure 1: The relationship between concentrations of a) sideroxylonal, and b) available N in mature compared to young *E. melliodora* leaves from the same tree.

Figure 2. The effect of the sideroxylonal or available N concentration in young or mature *E. melliodora* leaves on the intake of a), b) dry matter, and c) sideroxylonal by brushtail possums in a no-choice experiment.

Figure 3. The mean amount of *E. melliodora* foliage eaten by brushtail possums offered a choice between young and mature foliage from 18 trees. Asterisks indicate a significant difference between the amount of young and mature leaves eaten from that tree.

Figure 4. The effect of a) sideroxylonal concentration, and b) available N concentration in young leaves on the mean preference index of brushtail possums offered a choice between young and mature *E. melliodora* foliage. The line shows the fitted relationship from the statistical model when the effect was significant.

Figure 5. The nutritional rails of leaves from five of the *E. melliodora* trees offered to possums during the choice experiment. Young and mature leaves from the same tree are shown with the same line pattern. The nutritional rails from other trees were excluded to reduce overcrowding on the figure. The mean (± SE) amount of FPCs and available N ingested by possums is shown as filled circles for the trees for which the nutritional rails are shown, and as filled squares for all other trees.

Figure 1.

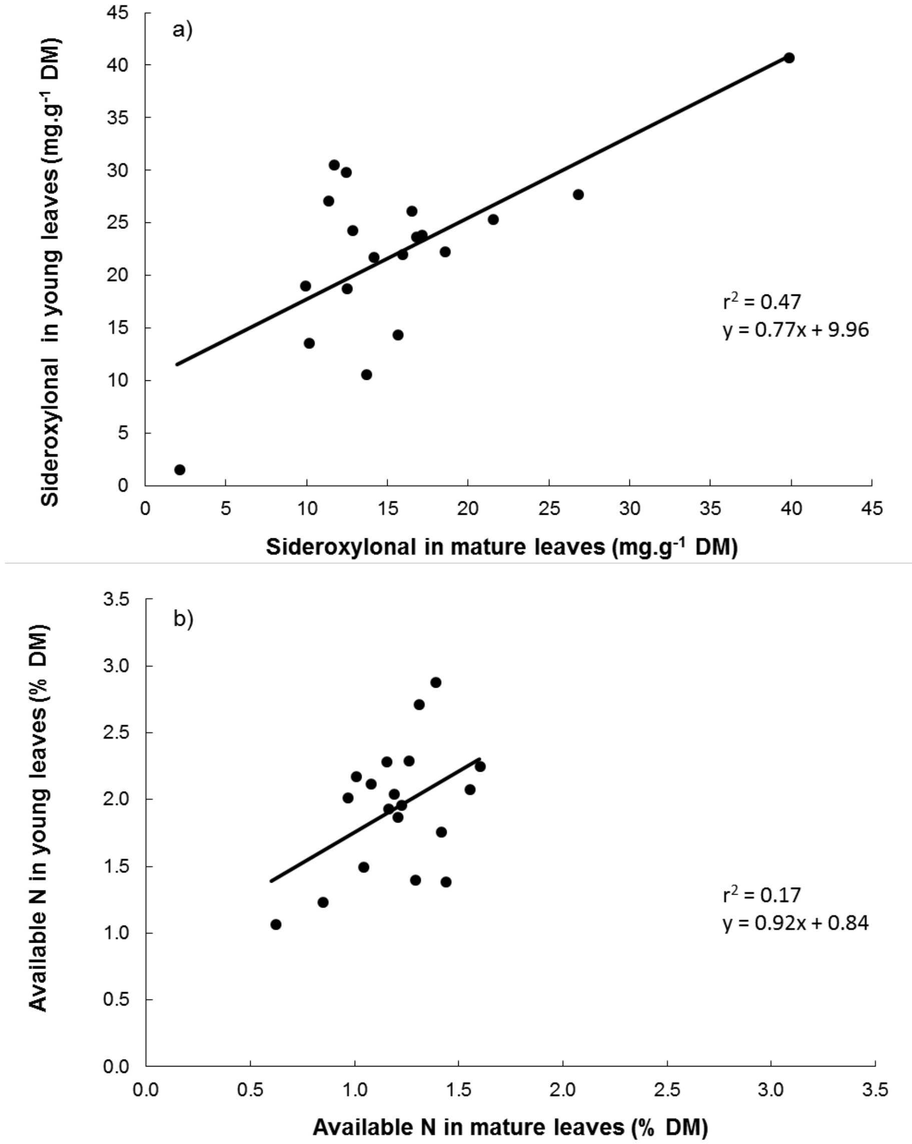
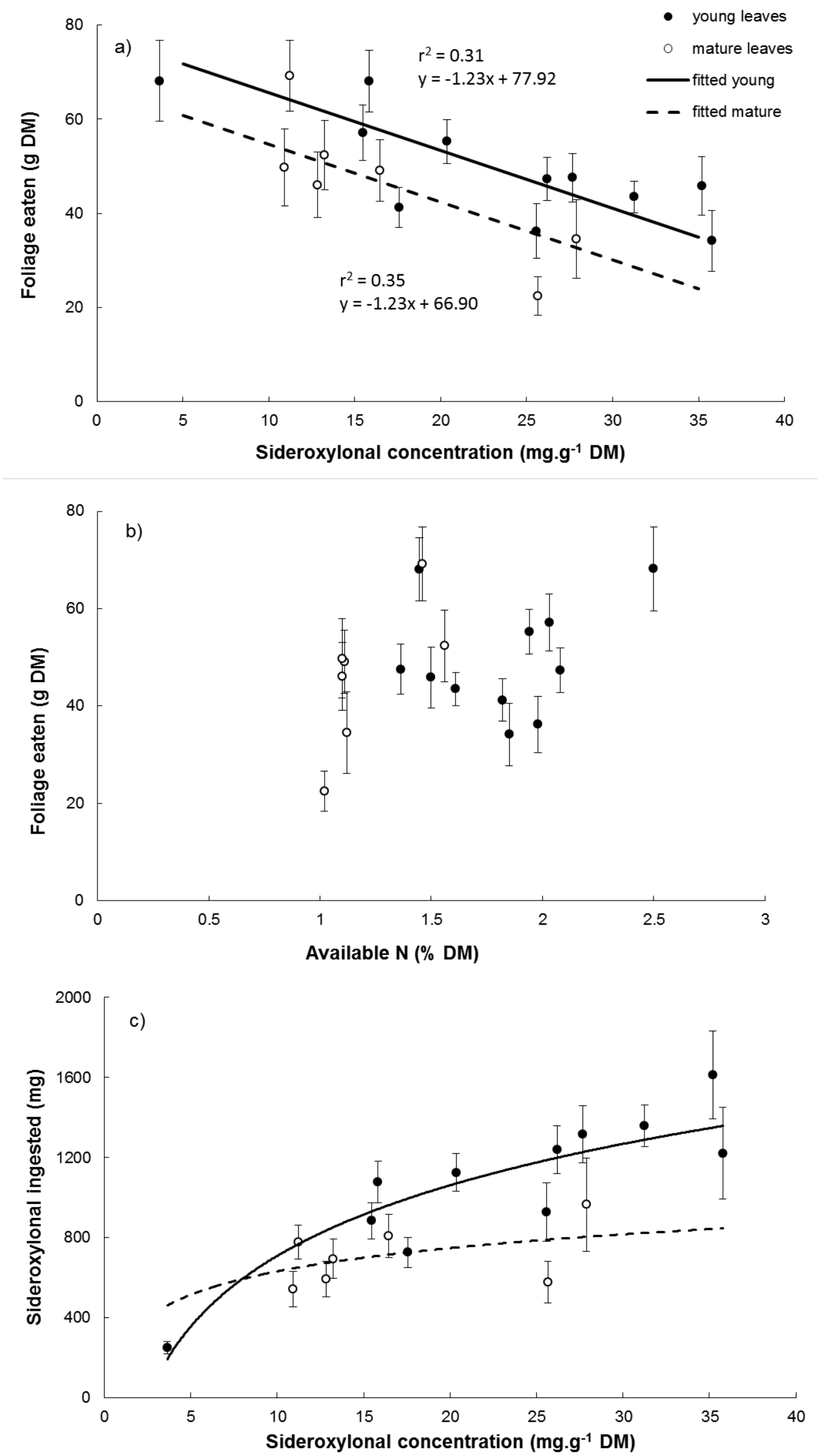

a)
Sideroxylonal in young leaves (mg.g-1 DM)
Sideroxylonal in mature leaves (mg.g-1 DM)
r2 = 0.47
y = 0.77x + 9.96
0
5
10
15
20
25
30
35
40
45
b)
Available N in young leaves (% DM)
Available N in mature leaves (% DM)
r2 = 0.17
y = 0.92x + 0.84
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Figure 2.



a)
young leaves
mature leaves
fitted young
fitted mature
r² = 0.31
y = -1.23x + 77.92
r² = 0.35
y = -1.23x + 66.90
Foliage eaten (g DM)
Sideroxylonal concentration (mg.g⁻¹ DM)
b)
Foliage eaten (g DM)
Available N (% DM)
c)
Sideroxylonal ingested (mg)
Sideroxylonal concentration (mg.g⁻¹ DM)

Figure 3.

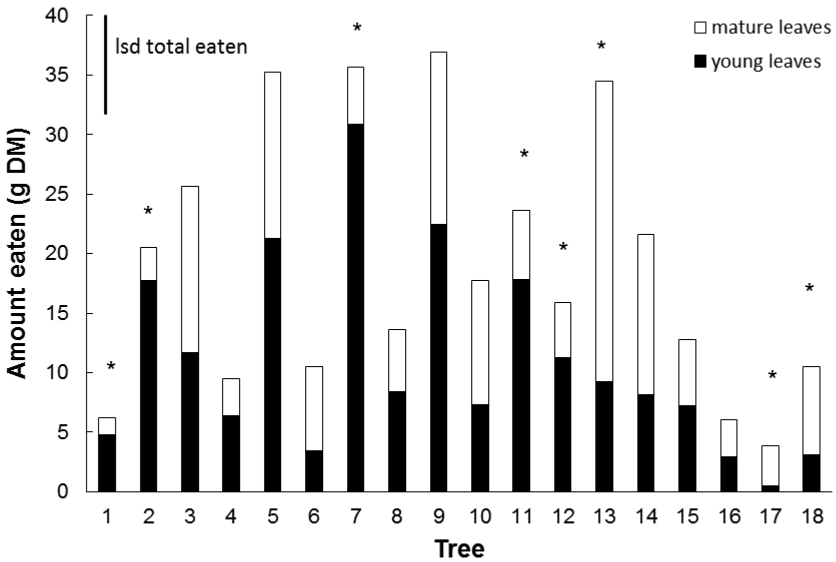
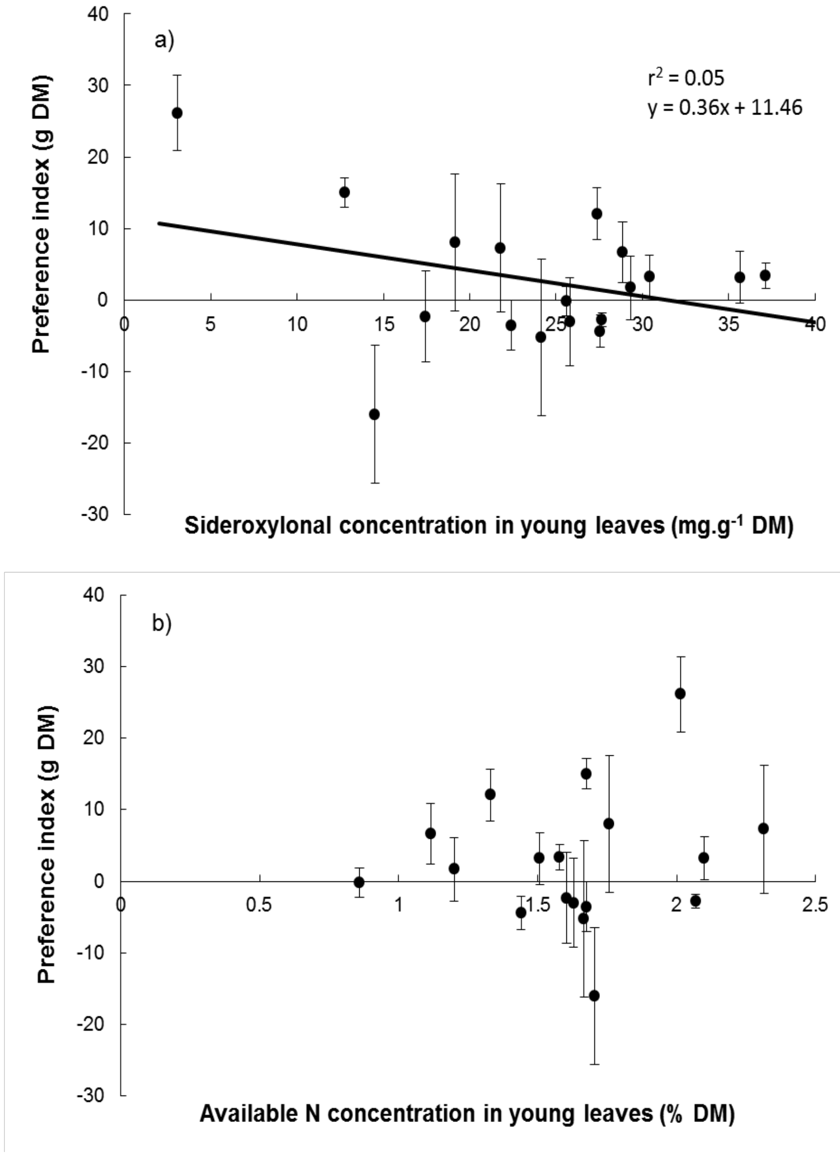
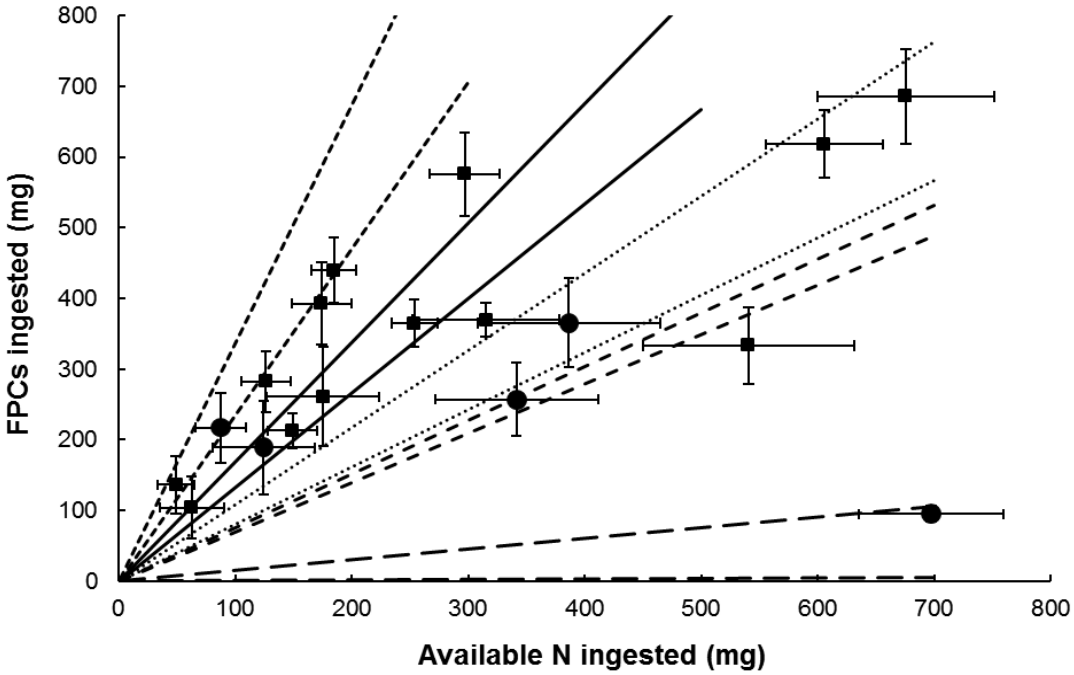

lsd total eaten
mature leaves
young leaves
Amount eaten (g DM)
0
5
10
15
20
25
30
35
40
Tree
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
*

Figure 4.



a)
r2 = 0.05
y = 0.36x + 11.46
Preference index (g DM)
Sideroxylonal concentration in young leaves (mg.g-1 DM)
b)
Preference index (g DM)
Available N concentration in young leaves (% DM)

Figure 5.


FPCs ingested (mg)
800
700
600
500
400
300
200
100
0
0
100
200
300
400
500
600
700
800
Available N ingested (mg)