

Quantifying the response of free-ranging mammalian herbivores to the interplay between plant defense and nutrient concentrations

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Abstract While trying to achieve their nutritional requirements, foraging herbivores face the costs of plant defenses, such as toxins. Teasing apart the costs and benefits of various chemical constituents in plants is difficult because their chemical defenses and nutrient concentrations often co-vary. We used an approach derived from predator–prey studies to quantitatively compare the foraging response of a free-ranging mammalian herbivore, the swamp wallaby (*Wallabia bicolor*), through three feeding trials with artificial diets that differed in their concentrations of (1) the terpene 1,8-cineole, (2) primary constituents (including nitrogen and fiber), and (3) both the terpene and the primary constituents. Applying the giving-up density (GUD) framework, we demonstrated that the foraging cost of food patches increases with higher dietary cineole concentration and decreases with higher dietary nutrient concentration. The effect of combined differences in nutrients and cineole concentrations on GUD was interactive, and high nutrient food required more cineole to achieve the same patch value as low nutrient food. Our results indicate that swamp wallabies equate low nutrient, poorly defended food with high nutrient, highly defended food, providing two contrasting diets with similar cost–benefit outcomes. This behavior suggests that equal concentrations of chemical defenses provide nutrient-poor plants with relatively greater protection as nutrient-rich plants. Nutrient-rich plants may therefore face the exacerbated problem of being preferred by herbivores and therefore need to produce more

defense compounds to achieve the same level of defense as nutrient-poor plants. Our findings help explain the difference in anti-herbivore strategy of nutrient-poor and rich plants, i.e., tolerance versus defense.

Keywords Mammalian herbivore · Nutrient–toxin interactions · Foraging response · Giving-up density · Plant chemistry

Introduction

Many plants produce secondary metabolites (PSMs), argued to be an evolutionary response to herbivory (Fraenkel 1959; Jones et al. 1991; Stamp 2003). PSMs vary within a backdrop of primary plant constituents, including nitrogenous compounds such as proteins and amino acids (Coley et al. 1985). The variation in levels of primary and secondary compounds has both a genetic (Bailey et al. 2004; O'Reilly-Wapstra et al. 2004, 2005b) and an environmental basis, the latter arising from factors such as shade, soil nutrient, or wind conditions (Close et al. 2003; Loney et al. 2006b; McArthur et al. 2010).

Just how plant characteristics affect mammalian consumers (i.e., herbivores) has been explored extensively—usually in experiments with captive animals to simplify the complexity of natural ecosystems. These experiments have shown that PSMs can act as toxins, digestibility-reducers, or feeding deterrents (McArthur et al. 1991; Bennett and Wallsgrave 1994), affecting intake (Boyle and McLean 2004; Loney et al. 2006b; Marsh et al. 2006), feeding behavior (Wiggins et al. 2003; Sorensen et al. 2005; Nersesian et al. 2011), physiology, and diet choice (O'Reilly-Wapstra et al. 2005a; Wiggins et al. 2006; Dziba and Provenza 2008; Torregrossa and Dearing 2009). Data

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obtained from captive animal trials demonstrate that particular nutrients can help herbivores counteract the effects of specific PSMs (Villalba et al. 2002b; Villalba and Provenza 2005) and that some PSMs interact with other PSMs, neutralizing each other (Lyman et al. 2008). Such interactions among nutrients and PSMs ultimately change the way that herbivores perceive the costs and benefits of eating certain plants, altering intake and diet choice (Wang and Provenza 1997; Behmer et al. 2002; Mote et al. 2007).

Field studies show that free-ranging herbivores collectively consume more high quality plants than low quality ones—defined loosely by the plant chemical profile, as a combination of nutrients and PSMs, and mechanical defenses—irrespective of whether this variation in plant quality occurs among plant species, individual plants, or plant parts (Bryant and Kuropat 1980; Bryant et al. 1989; Miller et al. 2011). How free-ranging herbivores respond to specific quantitative combinations of nutrients and PSMs, however, is less clear, but this behavior is ecologically and evolutionarily significant to both herbivores and plants. The responses of herbivores to PSMs and nutrients help define their spatial and temporal foraging patterns (Duncan and Gordon 1999; Duncan and Poppi 2008) and are important because they can influence herbivore fitness (DeGabriel et al. 2009). These responses also have wider ecological ramifications if they alter the distribution and abundance of plant species (Wolff 1980; Krebs et al. 1995; Moore and Foley 2005).

The practical difficulty of distinguishing the individual and combined effects of PSMs and nutrients on foraging herbivores, using plants themselves, is that primary and secondary constituents often co-vary (McArthur et al. 2003; Close et al. 2005). The problem of plant chemical co-variance can be avoided, however, by adopting the giving-up density framework (GUD) (Brown 1988). This is an experimental approach used in field studies, primarily in predator–prey studies (Bedoya-Pérez et al. 2013). Using this approach for studying plant–herbivore interactions can provide a valuable complement to studies with plants, with the potential to deliver new insights into how herbivores interact with plant chemistry.

The GUD framework (Brown 1988) imposes an exponentially increasing harvesting cost as food is consumed at patches. The amount of food that a forager leaves in a patch (GUD) is a reflection of the cost of foraging at that particular patch: lower GUD indicates a lower net cost. The GUD methodology has been used to compare the relative costs and benefits of foraging in response to predation risk and PSMs, but only as dichotomous variables (Schmidt 2000; Fedriani and Boulay 2006; Kirmani et al. 2010) or as a gradient in the concentration of a single plant toxin (McArthur et al. 2012; Bedoya-Pérez et al. 2014). It has yet to be used to explore the effects of two factors,

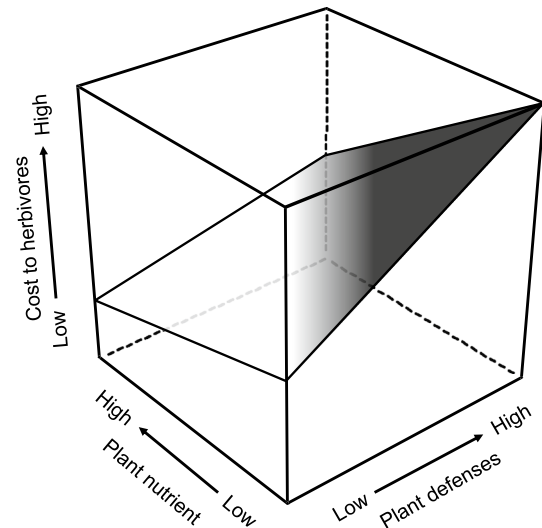


Fig. 1 Conceptual framework showing the perceived cost of plants to herbivores as a function of the concentration of plant defense compounds and nutrients. *White areas* represent the “best” food patch for herbivores, *black areas* represent the most costly choice of food. Similar benefit from foods that are either low in plant defense compounds and low in nutrients, or high in nutrients and high in plant defense compounds are shown in *gray*

such as nutrients and PSMs, as continuous variables along quantitative gradients.

Here, our aim was to test whether free-ranging generalist herbivores alter their feeding behavior among food patches, along a gradient of profitability based on food chemistry. Our conceptual model (Fig. 1) is based on the predicted net cost to herbivores of both plant defense compounds and nutrients. In this model, the “best” food patch for herbivores has both low levels of plant defense compounds and a high nutrient content, while the most costly choice is food that has high levels of plant defense compounds and low nutrient content. However, our model indicates that herbivores can obtain an intermediate—and similar—benefit from foods that are either low in plant defense compounds and low in nutrients, or high in nutrients and high in plant defense compounds. Thus, as the concentration of the dietary defense compound increases, a concomitant increase in nutrient level may offset its cost to the herbivore.

We used the GUD framework to quantify the impact of a plant toxin (i.e., 1,8-cineole) and nutrient concentrations (i.e., nitrogen) on the foraging patterns of a free-ranging mammalian herbivore using a browser, the swamp wallaby (*Wallabia bicolor*), as a model. Swamp wallabies are medium-sized (10–23 kg) ground-dwelling browsers with a foregut fermenting digestive system that occur in open dry sclerophyll forest in many parts of temperate eastern Australia (Hume 1999; Menkhorst and Knight 2009; Di Stefano et al. 2010). They encounter a range of PSMs in their diet which consists of the foliage of trees (including *Eucalyptus*

seedlings/saplings) and shrubs (Davis et al. 2008; Di Stefano and Newell 2008). A common and major component of the terpene profile of leaves of the genus *Eucalyptus* and in a series of related genera in Australia is 1,8-cineole (here after cineole) (Boland and Brophy 1993).

Terpenes, such as cineole, are considered to be toxic when ingested in high quantities (Johnson et al. 1976). They may cause microbial suppression in the rumen of sheep (foregut fermenters), resulting in reduced feeding (Ngugi et al. 1995). Cineole, when inoculated both intravenously and into the rumen, causes lambs to reduce feeding (Dziba et al. 2006). This terpene also reduces feeding in hindgut fermenters, such as brushtail possums (*Trichosurus vulpecula*) (Wiggins et al. 2003; Marsh et al. 2006), due to constraints on detoxification (Boyle et al. 2000, 2005; Boyle and McLean 2004). We have recently demonstrated a secondary effect of cineole on swamp wallabies (Bedoya-Pérez et al. 2014): wallabies can use its odor to detect food in patches, increasing patch value at low concentrations. Nevertheless, at high concentrations any benefit of the cineole odor is more than offset by its toxicity (Bedoya-Pérez et al. 2014). Nitrogen is particularly pertinent to mammalian herbivores because plants, as a food source, are relatively low in nitrogen. Further, there is some evidence that foregut fermenters can, to some extent, overcome the effect of several PSMs (Villalba et al. 2002b, c), and particularly terpenes (Villalba et al. 2002a), by increasing their nitrogen intake.

The aim of our study was to test and quantify the effects complex plant chemical profiles on swamp wallabies foraging by manipulating the concentration of cineole and nutrients in artificial diets. We do this using a novel experimental approach in a natural scenario which allows comparisons and fundamental links between cafeteria experiments with captive animals and correlative studies with plants.

Materials and methods

Study area

Experiments were run in Ku-ring-gai Chase National Park (KCNP), New South Wales, Australia, between December 2009 and January 2012, at two sites situated 1 km apart: one along Murrua (33°41'S, 151°08'E) and another along Bobbin Head (33°40'S, 151°09'E) fire tracks, i.e., permanent tracks cleared through the bush to provide access for firefighting. Both sites are located in an area of the National Park dominated by eucalyptus woodland, including scribbly gum *Eucalyptus haemastoma*, red bloodwood *Corymbia gummifera*, and yellow bloodwood *Corymbia eximia*, with old man banksia *Banksia serrata* and black she-oak *Allocasuarina littoralis* (Thomas and Benson 1985).

The study is a compilation of four GUD experiments. The details of methodology (design of feeders, inedible matrix, and size of pellets) varied slightly across experiments, as extracting the most out of GUD trials requires some finessing (Bedoya-Pérez et al. 2013). Wallabies were attracted and familiarized with the experimental designs by placing commercial rabbit pellets (100 g of rabbit pellets YSFEEDS; D & R Stockfeeds, Narellan, Australia; 0.27 g/g dry matter nitrogen measured using Leco® FP 428; Leco® Corporation, St. Joseph, MI, USA) in feeders at the experimental sites. Food was replaced daily until all feeders were visited for at least 3 consecutive days.

During all experiments, infrared motion-triggered cameras (Scout Guard SG550; Hunting Cam Online, Gadsden, SC) were placed 1.5 m from each feeder to provide information on visits by wallabies and identify any non-target species. The cameras were active throughout the experiments and set to record consecutive 60-s videos as long as movement was detected. From the camera videos, we estimated that, in all experiments, at least three different individuals visited each experimental block per night, and no individual visited more than one block. Videos also showed that rats (the native *Rattus fuscipes* and the invasive *R. rattus*) were the only non-target species visiting the feeders (60 % of feeders were visited at least once by rats). For all experiments the number of rat scats at each feeder was recorded daily. As any feeder confirmed by video to have been visited by rats also had scats, we chose the latter as a proxy for their effect on GUD and calculated that the average effect of rats on GUD, for all experiments, was between 0.9 to 1.0 % of the average GUD per feeder (see “Results”). Thus, we are confident that the GUD we measured was largely due to wallabies. A pilot study excluding swamp wallabies from feeders also revealed that, even in the absence of competition from wallabies, food removal by rats was minimal as a proportion of the food offered (6 %).

Effect of cineole on GUD

In Experiment 1 we tested the effect of dietary cineole concentration at food patches dispersed in the landscape at a relatively large spatial scale using a repeated measures design. Both fire tracks were divided into three 250 × 60-m areas along the track. Within each area, ten feeders were placed randomly within a grid of 6 × 6 stations that were spaced at 50-m intervals parallel to the track and at 5, 15 and 25 m either side of and perpendicular to the track. Feeders ($n = 60$) comprised a 1-L bucket attached to a 90-cm-long wooden stake hammered into the ground, with the mouth of the bucket at a height of 50 cm. A plywood sheet (4 × 1,200 × 900 mm) was nailed to the stake, forming a roof to exclude rain. The inedible matrix consisted of 700 g (± 5.0 g) of pebbles (diameter 5–7 mm).

We offered five different diets (pelleted feed) of 0, 0.01, 0.02, 0.05, and 0.10 g cineole per gram of dry matter of food (g/gDM) prepared from five solutions of cineole (1,8 cineole, purity 99 %; Felton Grimwade & Bickford, Oakleigh South, Australia) and peanut oil (5 g oil, 0 g/gDM nitrogen, per 100 g rabbit pellets). The five solutions were measured separately into buckets (14 per treatment), and rabbit pellets were added to each bucket, stirred, and left overnight to absorb the solution. Cineole treatments were allocated randomly within each block of the study area and left overnight. The remaining food and matrix (pebbles) were then separated manually and their weights recorded. This process was repeated for 5 experimental days, with a non-experimental day between each experimental set for logistic reasons.

Experiment 2 was similar to Experiment 1 but tested the effect of dietary cineole concentration at food patches dispersed in the landscape at a small spatial scale and used a randomized block, cross-over design (Ratkowsky et al. 1993). Few studies test the influence of lay-out of experiments on results, but for foraging animals the effect of experimental lay-out could be important. Thus, we ran this experiment, along with the first, to test the robustness of the impact of plant chemistry on foraging under different spatial and temporal patterning. As the results were consistent (see “Results”), we used a block design at the small spatial scale in subsequent experiments. The feeders were placed along each track. Three blocks spaced at least 200 m apart were chosen at random, and five feeders were placed within 4–7 m of one another at each block. Feeders ($n = 30$) comprised a 10-L plastic container (12 × 27 × 33 cm) attached to two 90-cm-long wooden stakes anchored in the ground, with the mouth of the bucket at a height of 50 cm; a plywood sheet (4 × 1,200 × 900 mm) nailed to the stakes at a height of 60 cm served as a roof. A lid with a hole in the middle (diameter 13 cm) was secured on top of the container to allow wallabies to search through the matrix for food without removing any matrix. The inedible matrix consisted of plastic tubing (19 mm diameter × 1 cm length), modified from Hochman and Kotler (2006).

Five diets (pelleted feed) of 0, 0.01, 0.02, 0.05, and 0.10 g/gDM cineole were prepared by grinding commercial rabbit pellets through a 2-mm die, then reconstituting them with appropriate amounts of cineole, plus 6 % water, into cylindrical pellets using a 3-mm die in a stock feed pelleting machine (Pellet Mill PM600; Buskirk Engineering, Ossian, IN). The food was placed in airtight containers and kept in a refrigerator (4 °C) until use to prevent cineole evaporation. During the trial, each feeder was provided with 25 g (± 0.01 g) of the treatment diet in 700 g (± 0.1 g) of matrix [plastic tubing, 19 mm diameter × 1 cm length, modified from Hochman and Kotler (2006)] and left overnight. The feeder contents were sieved the following day,

and the leftover food weight was recorded (GUD). Food was replaced daily at each feeder over 5 days, with treatments switched daily. Each block (five feeders) received all treatments at any one night, and each feeder received all treatments over the 5 days.

Effect of nutrients on GUD

In Experiment 3 we tested the effect of dietary nutrient level at food patches, dispersed at the small spatial scale as in Experiment 2 in a randomized block design. The experiment was run as repeated measures over 6 days. Along each track, three blocks were located at least 400 m apart, and at each block six feeders were installed at least 15 m apart. Feeders ($n = 36$) consisted of a 10-L plastic container (as per Experiment 2) fixed to the base of a 2.40-m-long metal fencing picket at ground level; a clear PVC sheet (3 × 600 × 600 mm) was fixed to the picket 60 cm above the feeder and used as a roof. Ground sawdust (passed through a 2-mm die) was used as the matrix to minimize the chance of GUD reaching zero.

We offered six diets (pelleted feed), with nitrogen concentrations of 0.01, 0.14, 0.21, 0.24, 0.27, and 0.29 g/gDM. These levels of nitrogen reflect the range found in *Eucalyptus* and *Acacia* species (0.05–0.23 g/gDM) (Lindenmayer et al. 1994; Loney et al. 2006a, b), plant species which form part of the swamp wallaby diet (Davis et al. 2008; Di Stefano and Newell 2008). The diets were prepared by grinding barley hay (0.01 g/gDM nitrogen) and commercial rabbit pellets (0.27 g/gDM nitrogen) separately through a 2-mm die, then combining them in the ratios of 0:1, 1:4; 2:3, 3:2, 4:1, and 1:0. The dietary combination was reconstituted with 6 % water into cylindrical pellets using an 8-mm die in a stock feed pelleting machine. Levels of nitrogen and neutral detergent fiber [modified procedure based on Goering and Van Soest (1970) and Van Soest et al. (1991)] were negatively correlated ($P = 0.007$, Pearson's correlation $r = -0.93$). Given the importance of dietary nitrogen to herbivores and because the food was ground up, thus avoiding potential food processing constraints of the fiber, we hereafter refer to the diets by their nitrogen content. During the experiment, 30 pellets of each of the six diets were randomly allocated to one feeder per block in a sawdust matrix. The mix was left at the feeders overnight, and then the leftover pellets were sieved from the matrix and counted (GUD).

Combined effect of cineole and nutrient on GUD

The combined effect of dietary cineole and nitrogen at food patches was tested in Experiment 4, using the same setting as for Experiment 3, but in a randomized block, cross-over design and with two extra feeders at each of the six blocks

($n = 48$). We combined the dietary cineole and nitrogen as two factors, each with three levels (cineole: 0.01, 0.05, and 0.10 g/gDM; nitrogen: 0.01, 0.21, and 0.29 g/gDM), except that one diet (highest cineole: 0.10 g/gDM, lowest nitrogen: 0.01 g/gDM) could not be pelleted and so was excluded. Hence, there were eight treatment combinations, eight feeders per block, and the experiment ran for 8 days. These eight diets were prepared as in Experiment 3 by combining ground commercial rabbit pellets and barley hay as required, adding the appropriate amount of cineole, and then reconstituting the ground mixture with 6 % water into pellets (diameter 8 mm) using a stock feed pelleting machine; the reconstituted pellets were stored in airtight containers at 4 °C until use.

Due to the volatile nature of cineole, evaporation from artificial diets was estimated by measuring the weight loss of three sets of 100 g of each diet over 12 and 24 h and comparing this loss to the weight loss in replicated 100-g sets dried in an oven (60 °C, 48 h). Our results showed a maximum loss of cineole of approximately 40 % (Experiments 1 and 2) or approximately 20 % (Experiment 4) over 24 h. However, the ranking of the diets, based on their relative concentrations, persisted overnight. Moreover, from video data, wallabies visited feeders within 1 h of the diets being offered, and their last visit was approximately 14 h later. Thus, we are confident that the GUD we measured reflects an accurate response by wallabies to the initial concentrations of cineole in the diets.

Statistical analysis

The effects of the independent variables (described below) on GUD for all experiments were analyzed using the mixed model procedure in SAS ver. 2003 (PROC MIXED; SAS Institute Inc., Cary, NC). To account for different natural rates of feeding in different locations, the GUD obtained during the pre-trial periods in Experiments 2, 3, and 4 were averaged by feeder and included in the model as a covariate (XGUD). We generated two variables for signs of rats, i.e., (1) the absolute number of rat scats and (2) the presence (1) or absence (0) of rat scats, and tested each separately in the initial models. The number of rat scats provided the greatest explanatory power of the two variables and so was used as the best variable representing the effect of non-target species. In order to simplify the models tested, any effect with $P > 0.3$ was excluded from the final model.

For Experiment 1, the independent variables cineole concentration and day (as a repeated measure, with feeder as the subject) were included as fixed effects, weight of the remaining pebbles as a covariate, and site as a random factor. Two samples were excluded due to low weight of the remaining pebbles (<500 g). No transformation was needed based on normal probability and residuals plots. We tested the linear relationship between GUD and cineole

concentration using cineole concentration as a continuous variable and including the same variables as before. However, we excluded the cineole-free diet from this analysis in order to compare the effects of diets that all contained the odor cue of cineole (Bedoya-Pérez et al. 2014).

For Experiment 2, the independent variables of cineole concentration, number of rat scats, and XGUD were included as fixed effects; site and the interaction between block and day were included as random factors. XGUD was excluded from the final model. A $\log_{10}(\text{GUD} + 1)$ transformation was used, based on normal probability and residuals plots. As in Experiment 1, our results indicated a positive linear relationship between GUD and cineole concentration, excluding the no-cineole treatment. This relationship was again tested using cineole concentration as a continuous variable, including cineole concentration, carryover (treatment of that feeder on the previous day) and XGUD as fixed effects, and the three interactions of block with day, cineole concentration, and carryover as random factors. XGUD was excluded from the final model.

For Experiment 3, the independent variables, namely, diet treatment, number of rat scats, day (as a repeated measure with feeder as the subject), and XGUD, were included as fixed effects; site, block, and feeder within block were included as random factors. A square root ($\sqrt{\text{GUD}}$) transformation was needed, based on normal probability and residuals plots. Our results indicated a negative linear relationship between GUD and dietary nitrogen. This relationship was tested using dietary nitrogen as a continuous variable, including the same variables as in preceding experiments. Site was excluded from the final model.

For Experiment 4, the various diets were tested in three stages due to the lack of a fully factorial design (one diet was missing). First, the different combinations of dietary cineole and nitrogen were tested as eight levels of a single treatment (Diet). Diet, carryover, day, XGUD, and number of rat scats were included as fixed effects; site, block, the three interactions between block with day, diet, and carryover were included as random factors. XGUD and site were excluded from the final model. A square root ($\sqrt{\text{GUD}}$) transformation was used, based on normal probability and residuals plots. We then performed two further analyses: the first excluded the highest cineole (0.10 g/gDM) diet, and the second excluded the lowest nitrogen (0.01 g/gDM) diets, to allow a test of dietary cineole and nitrogen as separate fixed factors, with two levels each, and their interaction. For both of these analyses, dietary cineole and nitrogen, and their interaction, day, XGUD, and number of rat scats were included as fixed effects; site, block, and the block-by-day interaction were included as random factors. For both cases, XGUD and site were excluded from the final model. A square root ($\sqrt{\text{GUD}}$) transformation was used, based on normal probability and residuals plots.

Table 1 Results of the mixed model analysis in Experiments 1 and 2

Experiments 1 and 2	Factor	Num <i>df</i>	Den <i>df</i>	<i>F</i> value	<i>P</i>
Experiment 1 ^a					
(a)	Dietary cineole (g/gDM)	4	283	13.00	<0.001
	Remaining pebbles (g)	1	283	91.69	<0.001
	Day	4	283	4.44	0.002
(b)	Dietary cineole (g/gDM)	1	227	51.79	<0.001
	Remaining pebbles (g)	1	227	71.18	<0.001
	Day	4	227	4.89	0.001
Experiment 2 ^b					
(c)	Dietary cineole (g/gDM)	4	115	3.42	0.011
	Rats (number of scats)	1	81	0.02	0.902
(d)	Dietary cineole (g/gDM)	1	3	35.93	0.013
	Carryover	4	4	2.23	0.230

df Degrees of freedom, *DM* dry matter

^a Experiment 1 tested: (a) the fixed effect of dietary cineole (as a class variable), with the remaining pebbles as a covariate and day as a repeated measure; (b) the fixed effect of dietary cineole (as a continuous variable) excluding data for dietary cineole of 0 g/gDM

^b Experiment 2 tested: (c) the fixed effect of dietary cineole (as a class variable) and number of rat scats as a covariate; (d) the fixed effect of dietary cineole (as a continuous variable), and carryover (previous day treatment) as a covariant, excluding data for dietary cineole of 0 g/gDM

Results

Effect of cineole on GUD

Results for Experiment 1 and 2 both showed a significant effect of cineole concentration on GUD (Table 1). In both experiments, GUD dropped from the toxin-free diet to the lowest cineole concentration (0.01 g/gDM) diet, then increased linearly with increasing cineole concentration to ultimately reach similar GUD values as the cineole-free diet (Table 1; Fig. 2a, b). In Experiment 1, feeders with lower remaining pebbles showed significantly lower GUD, and there was a significant decrease of GUD across days (Table 1; patterns not shown).

Effect of nutrient on GUD

The effect of dietary nitrogen on GUD was significant in Experiment 3 (Table 2). GUD decreased from the lowest dietary nitrogen concentration diet to the highest (Table 2; Fig. 3). It also fluctuated significantly across day, feeders

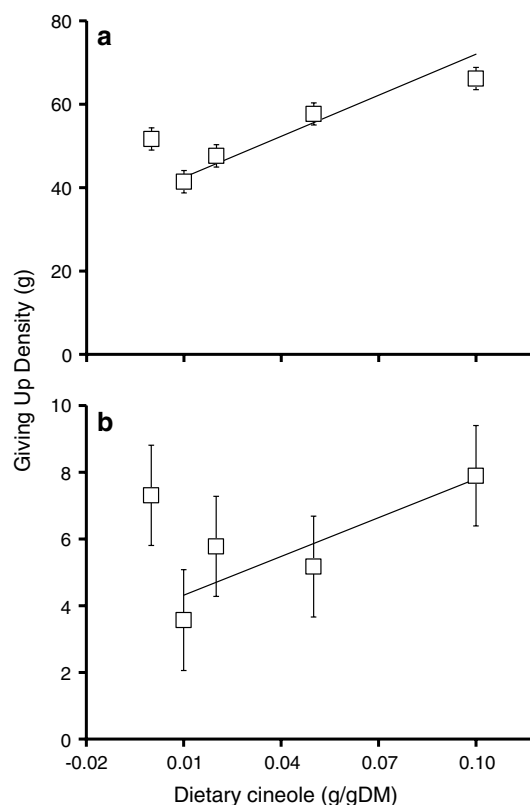


Fig. 2 Results for feeding Experiments 1 and 2 showing the giving-up density (GUD) as the weight (g) of pellets remaining [(least-squares mean \pm standard error (SE))] as a function of dietary cineole [g/g dry matter (*DM*)] in Experiment 1 (**a**) and Experiment 2 (**b**). *Trend lines* represent linear regression analysis using dietary cineole as a continuous variable (excluding zero cineole)

with higher XGUD consistently showed higher GUD, and there was a small but negative effect of incidence of rats (number of rat scats) on GUD (Table 2, patterns not shown; rat effect: 0.9–1.0 % of the average GUD per feeder).

Combined effect of cineole and nutrient on GUD

In Experiment 4, the effect of diet (the combination of dietary cineole and nitrogen) on GUD was significant (Table 3; Fig. 4), and the GUD fluctuated significantly across days (Table 3). When the highest cineole (0.10 g/gDM) diets were excluded, the main effects of dietary cineole and nitrogen were significant, but their interaction was not (Table 3). When the lowest nitrogen (0.01 g/gDM) diets were excluded, dietary cineole and nitrogen were significant, as was their interaction (Table 3; Fig. 4). Consistent with the results from Experiments 1–3, GUD increased with increasing concentration of cineole and with decreasing nitrogen concentration (Fig. 4), but the slope from medium to high nutrient levels was greatest at the highest cineole concentration tested (Fig. 4). GUDs

Table 2 Results of the mixed model analysis in Experiment 3

Experiment 3 ^a	Factor	Num df	Den df	F value	P
(a)	Dietary nitrogen (g/gDM)	5	58	13.67	<0.001
	XGUD (average pellets)	1	11	13.39	0.004
	Day	5	157	3.28	0.008
	Rats (number of scats)	1	147	6.14	0.014
(b)	Dietary nitrogen (g/gDM)	1	63	51.37	<0.001
	XGUD (Average pellets)	1	8	19.85	0.002
	Day	5	161	3.31	0.007
	Rats (number of scats)	1	128	5.58	0.020

GUD, Giving-up density; XGUD is the GUD obtained during the pre-trial periods in Experiments 2, 3, and 4 and averaged by feeder, to be included in the model as a covariate

^a Experiment 3: (a) the fixed effects of dietary nitrogen (g/gDM) and number of rat scats were tested, with average GUDs during pre-assay period (XGUD) as a covariate, and day as a repeated measure; (b) linear regression analysis testing the fixed effect of dietary nitrogen (g/gDM) as a continuous variable, number of rat scats, average GUDs during pre-assay period (XGUD) as a covariate, and day (as a repeated measure)

were intermediate and equivalent for diets that were either (1) lowest in both nitrogen and cineole (0.01 g/gDM nitrogen and 0.01 g/gDM cineole) or (2) highest in nitrogen (0.029 g/gDM) with both medium and high cineole concentration (0.05 g/gDM and 0.1 g/gDM) (Fig. 4).

Discussion

By making novel use of the GUD framework in plant-herbivore research, we show that the plant terpene cineole and primary nutrients both alter foraging by free-ranging swamp wallabies in a dose-dependent manner. More importantly, our study demonstrates that the effectiveness of the terpene in shaping foraging decisions of free-ranging herbivores depends on the nutritional content of the food, and vice versa.

Effect of cineole on GUD

Our result of an increasing GUD with increasing cineole concentration (above zero) is consistent with the known toxic characteristics of cineole (Boyle et al. 2000, 2005; Boyle and McLean 2004). While its strong odor at high concentrations may deter feeding, we believe that the post-consumptive toxic effects were more influential for two reasons. First, when we masked the smell of cineole in food

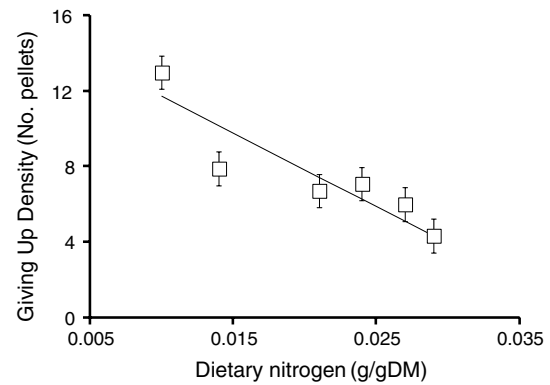


Fig. 3 Results for feeding Experiment 3 showing GUD as the number of pellets remaining (least-squares mean \pm SE) as a function of dietary nitrogen (g/gDM). Trend line shows the linear regression analysis using dietary nitrogen as a continuous variable (excluding zero cineole)

by adding cineole to the matrix in a separate GUD experiment (Bedoya-Pérez et al. 2014), GUD remained the same at the highest (10 %) dietary cineole, rather than increasing. Second, swamp wallabies are used to consuming highly pungent leaves, which are common to many Australian plants (Penfold 1948), and so this pungency is unlikely to be a deterrent. Wallabies also showed higher GUD for the zero-cineole treatments than for the low cineole treatments in Experiments 1 and 2, which appears to be counterintuitive. We argue that this behavior is likely due to an odor effect because wallabies do use cineole as a volatile odor cue to find food items (Bedoya-Pérez et al. 2014). It is also possible that swamp wallabies have an intrinsic preference for low concentrations of cineole, possibly seeking physiological benefits (Forbey and Foley 2009; Forbey et al. 2009). Other differences in the characteristics of the food items (e.g., size, storability) may also contribute to differences in GUD (Kotler et al. 1999; Garb et al. 2000; Hochman and Kotler 2006), but these were all held constant in each of our experiments.

The negative impact of increasing cineole concentration on foraging and food intake by swamp wallabies is also seen in the free-ranging frugivore, the African thick-tailed bushbaby (*Otolemur crassicaudatus*) (McArthur et al. 2012) and with captive herbivorous brushtail possums (Wiggins et al. 2003; Marsh et al. 2006). The consistency in results for both free-ranging and captive animals establishes the effectiveness of plant terpenes to reduce the use of food patches, even when patches are embedded in a landscape with a large variety of other foods on offer, a typical characteristic of natural systems, and when more than one individual feeds at such patches.

It is significant that the response of free-ranging swamp wallabies to cineole was the same, regardless of the spatial and temporal variation we imposed on the food patches.

Table 3 Results of the mixed model analysis in Experiment 4

Experiment 4 ^a	Factor	Num <i>df</i>	Den <i>df</i>	<i>F</i> value	<i>P</i>
(a)	Diet (cineole and nitrogen)	7	283	17.93	<0.001
	Day	7	37	2.48	0.034
	Rats (number of scats)	10	325	1.37	0.193
	Carryover	7	44	1.39	0.232
(b)	Dietary cineole (g/gDM)	1	224	26.51	<0.001
	Nitrogen (g/gDM)	2	225	18.89	<0.001
	Cineole × nitrogen	2	225	1.94	0.147
	Day	7	33	1.7	0.143
	Rats (number of scats)	9	251	1.32	0.225
(c)	Cineole (g/gDM)	2	192	40.16	<0.001
	Nitrogen (g/gDM)	1	192	13.18	0.001
	Cineole × nitrogen	2	194	4.77	0.010
	Day	7	34	1.78	0.123
	Rats (number of scats)	9	241	1.52	0.140

^a Experiment 4 tested: (a) the fixed effects of diet (combination of cineole and nitrogen content, as a class variable), with carryover (previous day treatment), day, and number of rat scats as covariates; (b) the fixed effects of dietary nitrogen (g/gDM) dietary cineole (g/gDM), and their interaction, excluding the highest (0.10 g/gDM) cineole diets, with day and number of rat scats included as covariates; (c) the fixed effects of dietary nitrogen (g/gDM), dietary cineole (g/gDM), and their interaction, excluding the lowest (0.01 g/gDM) nitrogen diets, with day and number of rat scats included as covariates

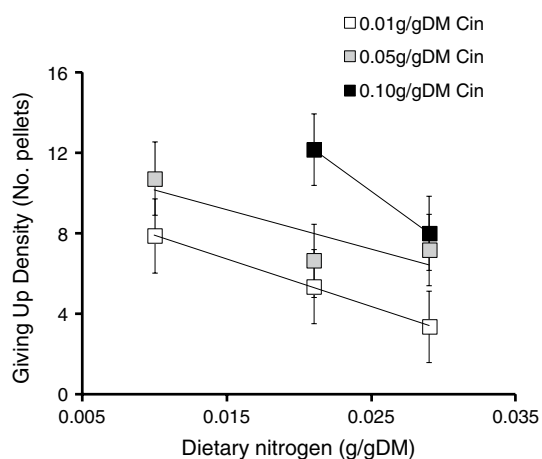


Fig. 4 Results for feeding Experiment 4 showing GUD as the number of pellets remaining (least-squares mean \pm SE) as a function of dietary nitrogen (g/gDM) (*X*-axis) and cineole (*Cin*) (*white squares*: 0.01 g/gDM cineole; *grey squares*: 0.05 g/gDM cineole; and *black squares*: 0.10 g/gDM cineole)

That is, the pattern was consistent when the food types were offered at both relatively large (Experiment 1) and small (Experiment 2) spatial scales, and whether consistent in a given location across time (repeated measures, Experiment 1) or not (cross-over design, Experiment 2). Thus, while other extrinsic factors, such as neighboring vegetation, may modify the foraging patterns of herbivores (Bergman et al. 2005; Bergvall et al. 2006; Miller et al. 2007), our results show that the foraging decisions of wallabies towards the chemical characteristics of food are nonetheless robust.

Effect of nutrients on GUD

We confirmed the relevance of nitrogen as a macronutrient to the foraging decisions of herbivores (Experiment 3). Wallabies equated higher nitrogen diets with greater patch value (lower GUD), as has been shown for other mammalian herbivores (Kavanagh and Lambert 1990; Wang and Provenza 1997; Hochman and Kotler 2006). In this experiment, rats also responded positively to higher nitrogen diets—although the absolute effect on GUD was small—confirming the general value of food nitrogen to animals in the landscape.

Effect of cineole and nutrients on GUD

Our results showing the interplay between plant defense compounds (represented here as the terpene cineole) and nutrients (Fig. 4) support the conceptual model presented in Fig. 1, in which the relative cost to herbivores equates to the GUD. The “best” food source for herbivores had low levels of plant defense compounds and a high nutrient content, while food with high levels of plant defense compounds and a low nutrient content was the most costly choice (Figs. 1, 4). Our results also support the hypothesis that herbivores can obtain an intermediate benefit from foods that are either low in plant defense compounds/low in nutrients, or high in nutrients/high in plant defense compounds (Figs. 1, 4). The interaction between nutrients and plant defense compounds shown here has been previously qualitatively demonstrated in cafeteria experiments with captive animals (Villalba et al. 2002c; Ginane et al. 2005; Papachristou et al. 2007), but here we provide quantitative evidence using a field-based approach with free-ranging foraging herbivores. Given that the swamp wallaby is a foregut fermenter, we predict a similar response in other foregut fermenters, such as ruminants.

Broader ecological implications

Our results also demonstrate the value of using the GUD framework to explore realistic variations in food quality,

including detailed and interacting effects of food constituents, thus extending the use of GUD beyond its common application for assessing predation risk. In future studies, GUD can be used to tease apart the main and interactive effects of primary and secondary chemistry of plants in an efficient and meaningful way with other free-ranging animals.

Given that the foraging patterns identified in our study revealed that using the GUD approach should map closely to foraging amongst plants, our findings have important ecological implications not only for herbivores but also for plants. They imply that plants with lower nutrient levels gain relatively greater protection from the same concentration of terpene as those with higher nutrient content. Nutrient-rich plants therefore face the exacerbated problem of not only being preferred by herbivores, but also of needing to produce comparatively more terpene to achieve the same level of defense as low nutrient plants. These findings suggest a new explanation for the contrasting strategies of plants to either defend against herbivory or tolerate it (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999), namely, that high nutrient plants are not only able to grow quickly (herbivory tolerate strategy), but that this strategy may in fact be more efficacious than the use of defense compounds.

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