J Comp Physiol B (2003) 173: 69–78
DOI 10.1007/s00360-002-0318-4

ORIGINAL PAPER

K.J. Marsh · W.J. Foley · A. Cowling · I.R. Wallis

Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*)

Accepted: 16 November 2002 / Published online: 18 January 2003

Abstract The effect of two plant secondary metabolites, tannins and formylated phloroglucinol compounds (FPCs), on the intake of *Eucalyptus* foliage by common ringtail (*Pseudocheirus peregrinus*) and common brushtail possums (*Trichosurus vulpecula*) was studied. We manipulated the amount of tannin that was free to bind with protein by coating foliage with polyethylene glycol 4000 (PEG) and relied on natural intraspecific variation in FPC concentrations. In contrast to ringtail possums, brushtail possums showed a greater tolerance to FPCs and ate more foliage when it was coated with PEG, suggesting that tannins limited their food intake. Brushtails detected the effects of tannins through immediate oral sensations rather than through systemic effects. Ringtail possums appeared highly tolerant of foliar tannins yet susceptible to low concentrations of FPCs. We could not detect any interaction between tannins and FPCs that affected the intake of *Eucalyptus* foliage by either species of possum. Although ringtail and brushtail possums are widely regarded as specialist and generalist folivores, respectively, their differential susceptibility to co-occurring secondary metabolites suggests greater complexity. Each possum species appears to be a specialist in its own right, which leads to a partitioning of available foliage. Brushtails avoid tannins and ringtails avoid FPCs.

Communicated by I.D. Hume

K.J. Marsh (✉) · W.J. Foley · I.R. Wallis
School of Botany and Zoology,
Australian National University,
0200 Canberra, ACT, Australia
E-mail: Karen.Marsh@anu.edu.au
Tel.: +61-2-61252533
Fax: +61-2-61255573

A. Cowling
Statistical Consulting Unit,
Australian National University,
0200 Canberra, ACT, Australia

Keywords Folivore · FPC · Sideroxylonal · Specialist · Tannins

Abbreviations *DM* dry matter · *DMI* dry matter intake · *FPC* formylated phloroglucinol compound · *PEG* polyethylene glycol · *PSM* plant secondary metabolite · *SED* standard error of a difference between means

Introduction

Many researchers have investigated how individual plant secondary metabolites (PSMs) influence feeding by herbivores, but few have considered interactions between different classes of PSMs. This is surprising because plants rarely contain a single PSM and some PSMs can interact in ways that reduce or enhance their individual effects (Burritt and Provenza 2000). Eucalypt foliage is renowned for its variety of PSMs that makes for some potentially interesting interactions.

Common ringtail (*Pseudocheirus peregrinus*) and common brushtail possums (*Trichosurus vulpecula*) are marsupial folivores that feed heavily on *Eucalyptus*. Research with captive animals shows that both species closely regulate their intake of formylated phloroglucinol compounds (FPCs) when these are isolated from eucalypts and added to artificial diets (Lawler et al. 1998a, 2000; Stapley et al. 2000). However, when foliage is offered to possums, particularly brushtails, variation in food intake occurs that is not attributable to FPCs (Lawler et al. 2000; Wallis et al. 2002). This implies that other factors in eucalypt foliage also influence feeding. One such factor may be the concentration of tannins.

Tannins are often implicated as feeding deterrents and, indeed, Foley and Hume (1987) showed that brushtail possums ate more *Eucalyptus melliodora* foliage when their diet was supplemented with polyethylene glycol (PEG) 4000 to inactivate tannins. Ringtail possums, however, did not eat more *Eucalyptus ovata* foliage coated with PEG 20000 (McArthur and Sanson

1991). These two studies were conducted before the discovery of FPCs. Foley and Hume (1987) fed possums foliage from a single *E. melliodora* tree that they willingly ate. Our experience suggests that the foliage from this tree almost certainly contained low concentrations of FPCs. In contrast, McArthur and Sanson (1991) fed foliage from several trees and subsequent analysis by Lawler et al. (1998a) revealed variable concentrations of the FPC, macrocarpal G, which may have confounded results. It is possible that the ringtail possums used by McArthur and Sanson (1991) were limited first by the macrocarpal G content of their food and could not respond to the inactivation of tannins by PEG. It follows that tannin effects may be detected only when FPC concentrations are low.

PEG precipitates tannins through hydrogen bonding with the hydroxyl group of the tannin side-chain (Fig. 1c; Jones and Mangan 1977). When fed to animals, PEG is able to neutralise the negative effects of tannins, such as the binding of protein, reduced digestibility and the subsequent diminished appetite (Silanikove et al. 1996a). In this way it provides a direct method of measuring the biological effects of tannins without needing to classify them into hydrolysable or condensed tannins and then into individual compounds. Furthermore, measures of PEG-binding from in vitro assays with dried, ground plant material provide excellent predictions of the negative effects of tannins on digestibility (Silanikove et al. 1996b).

Apart from binding tannins, PEG may also bind non-tannin phenolics (Foley et al. 1999; A.E. Hagerman, personal communication). Although tannins and FPCs differ considerably in their chemical structure, they both have phenolic groups. Thus, coating the leaves of an FPC-containing eucalypt with PEG might allow an animal to eat more due to inactivation of tannins, FPCs, or both. To adequately test our hypotheses it was important that we first describe the effects of PEG on FPC intake in the absence of tannins.

This paper addresses three important issues in plant-herbivore interactions. First, it examines how *Eucalyptus* tannins and the FPC, sideroxylonal, contribute to defence against herbivory by ringtail and brushtail possums. We hypothesised that sideroxylonals and tannins both influenced feeding, but that sideroxylonals were the dominant regulators. Thus, we predicted that both brushtail and ringtail possums ingesting *Eucalyptus* foliage containing low concentrations of sideroxylonals would eat more PEG-coated than untreated foliage. In contrast, inactivating the tannins with PEG would not be expected to affect intake when possums are fed foliage with high concentrations of sideroxylonals (Fig. 2).

Second, by examining the degree to which PSMs influence feeding and digestibility, we address the widespread assumption that the common brushtail possum, a generalist feeder (Kerle 1984), is more susceptible to the effects of PSMs than is the more specialised common ringtail possum (Pahl 1984).

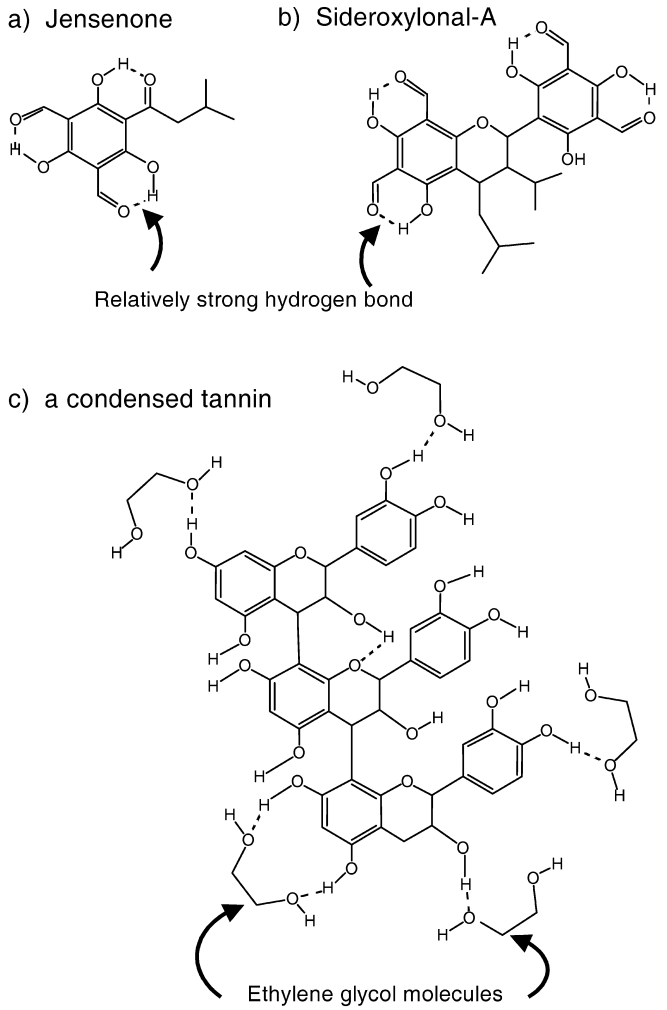

Fig. 1a–c The structures of the formylated phloroglucinol compounds (FPCs) jensenone (**a**) and sideroxylonal-A (**b**), and of a condensed tannin (**c**). FPCs show relatively strong intra-molecular hydrogen bonding (O–H) that prevents bonding by polyethylene glycol (PEG). Tannins have only weak potential for intra-molecular hydrogen bonding, and inter-molecular hydrogen bonding with PEG inactivates the tannin

Last, we try to distinguish the systemic and oral effects of tannins in brushtail possums feeding on *Eucalyptus* foliage. It is believed that FPCs damage enterochromaffin cells in the gut, releasing serotonin that is subsequently detected by the emetic system (Lawler et al. 1998b). In contrast, there are many suggested actions of tannins. These depend on the type of tannins and adaptations possessed by the consumer, but include both oral and post-ingestive or systemic effects. Oral effects include astringent taste (Landau et al. 2000). Post-ingestive effects include reduced digestibility, particularly of protein (Silanikove et al. 1996a), lesions in the gut (Steinly and Berenbaum 1985; Reed 1995), and toxicity from absorbed hydrolysable tannins (Reed 1995). If post-ingestive effects govern the reaction of possums to eucalypt tannins, they should show the same feeding response when PEG is provided directly into the stomach or when it is eaten with foliage.

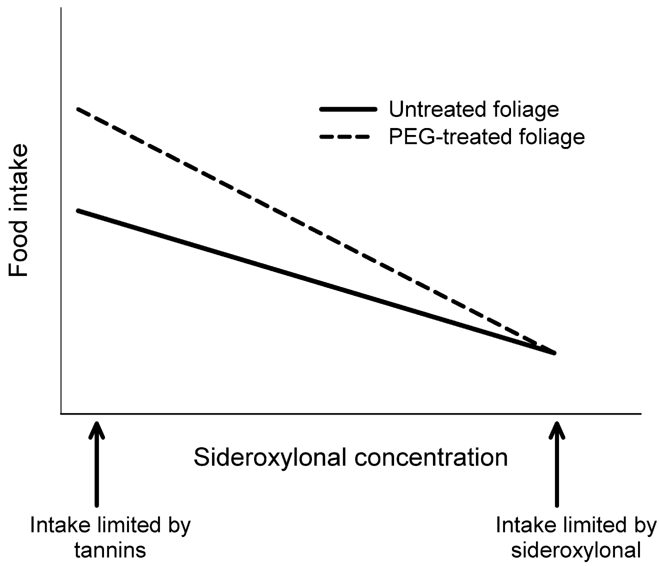

Fig. 2 Postulated impact of PEG on the consumption of eucalypt foliage by brushtail and ringtail possums. Tannins are expected to limit feeding at low concentrations of sideroxylonals. Treating such foliage with PEG should elicit an increase in food intake. As sideroxylonal concentrations increase, feeding should be limited by the amount of sideroxylonals that possums can ingest before suffering toxic effects. Thus, PEG is expected to have a diminishing effect on food intake with increasing concentrations of sideroxylonals

Alternatively, if the oral effects of tannins are important then possums should not respond to PEG that is placed directly in their stomachs.

Materials and methods

Animal capture and housing

One female and five male common ringtail possums (mean body mass 739 ± 128 g SD) and twelve common brushtail possums (mean body mass 2264 ± 491 g SD) were captured near Canberra. The adaptation of the animals to captivity and all aspects of their husbandry followed the procedures described by Lawler et al. (2000) and Wallis et al. (2002). The brushtails lost ca. 5% and the ringtails gained ca. 3% of their starting body mass during the study.

Leaf collection, storage and feeding

Enough foliage for each experiment (normally six nights) was cut from the appropriate trees at least 1 day before the beginning of experiments and stored standing in water in a room maintained at 5 °C.

Bunches of foliage were weighed each morning at 1000 h and, when necessary, were dipped in a 20% (w/v) aqueous solution of PEG (molecular weight 4000). After dipping, all bunches were left with their stems in water until 1700 h. By this time the PEG solution had dried, leaving an even coating of PEG on the leaves. We reweighed each bunch and placed it in a container of water in the possum cage. Control bunches were used to monitor mass changes during the feeding period. These changes were always minimal so they were ignored in calculations of food intake.

The measurement of food intake and the sampling and preparation of leaf for analysis followed the procedures described by Wallis et al. (2002).

Laboratory analyses

All analytical results were expressed on a dry matter (DM) basis determined by drying 1.00 ± 0.01 g of freeze-dried, ground eucalypt leaf to constant mass at 60 °C.

Sideroxylonals were extracted by refluxing the ground eucalypt leaf with 4:1 light petroleum spirit: acetone in a soxhlet extractor and then separated by high performance liquid chromatography (HPLC). Wallis et al. (2003) described all aspects of the analysis.

The nitrogen content of freeze-dried, ground foliage was determined on duplicate 250 ± 10 mg samples 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 sulphate.

We used a modified version of the PEG-binding assay of Silanikove et al. (1996b) to quantify the amount of material present in foliage that binds to PEG. Briefly, 0.50 ± 0.01 g of freeze-dried, ground eucalypt leaf was weighed into a scintillation vial and incubated with 7.5 ml reagent (4.6 kBq ^{14}C-PEG and 83 mg PEG 4000) at 37 °C for 24 h with occasional mixing. Immediately following incubation, 2 ml fluid was centrifuged at 12,000 rpm for 15 min. Duplicate 40 μl samples of supernatant were decanted into 4 ml scintillant and counted to a precision of 1%. PEG-binding, expressed as percentage of plant DM, was calculated using the equation of Silanikove et al. (1996b).

Animal experiments

Before conducting the main experiments it was necessary to test the suitability of short-term feeding and to test whether or not PEG inactivates a typical FPC.

Long-term versus short-term feeding

We measured the efficacy of short-term treatments by comparing the average intake for eight brushtail possums over one night with their average intake over the subsequent week. This showed that PEG provided on the first treatment day (dry matter intake, DMI = 58.8 g) had the same effect as on subsequent days (mean DMI for 7 days = 58.0; SED = 4.4 g; $P = 0.864$). Therefore, we used experimental protocols in which individual treatments were offered to possums for 1–2 days only.

The effect of PEG on the intake of a jensenone-rich diet by common brushtail possums

Sideroxylonals are the main FPCs in *E. melliodora* and *Eucalyptus polyanthemos* (Eschler et al. 2000) but it is difficult to isolate enough of these compounds for feeding studies (Lawler et al. 2000). Therefore, we measured whether PEG binds FPCs using jensenone, a monomer of sideroxylonal (Fig. 1). Jensenone, which is easily isolated from *Eucalyptus jensenii*, has the same antifeedant properties per mole as sideroxylonal (Lawler et al. 1999, 2000).

Four male brushtail possums were introduced to the artificial diet described by Stapley et al. (2000). This diet consisted of (percentage wet matter): grated apple (55.5), grated banana and carrot (15.0), sucrose (5.35), commercial breakfast cereal (5.0), and casein (0.15), and contained, on average, 26% dry matter and 4.7% crude protein. After about 2 weeks, when each animal's intake of the diet had stabilised, we added jensenone (0.4% DM) to the diet for 2 days to induce cautious feeding behaviour. We then conducted a 4×4 Latin square experiment with the treatments being: basal diet, basal diet + 6% PEG, basal diet + 0.4% jensenone, basal diet + 6% PEG + 0.4% jensenone, all on a DM basis. The concentration of

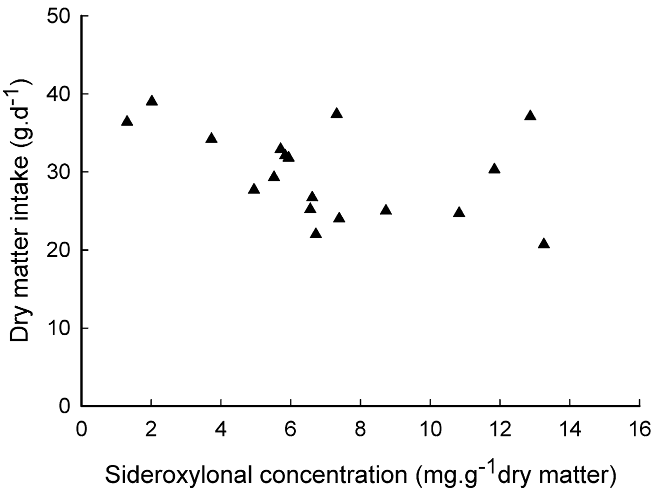

Fig. 3 Food intake by brushtail possums offered *Eucalyptus polyanthemos* with varying concentrations of sideroxylonals

PEG mimicked the amount of PEG that animals ate when feeding on leaf dipped in a 20% PEG solution, while the concentration of jensenone (0.4% DM) was previously shown to reduce DMI significantly (Stapley et al. 2000). Diets were prepared as described by Stapley et al. (2000) and each brushtail possum received each treatment for two consecutive nights.

The effect of PEG on the intake of *E. polyanthemos* foliage by common ringtail possums and of *E. melliodora* foliage by common brushtail possums

It was not possible to feed the same eucalypt species to both ringtail and brushtail possums, partly because ringtail possums do not tolerate the high sideroxylonal concentrations (usually >20 mg.g^{-1} DM) found in *E. melliodora*. In contrast, preliminary work with a different group of 6 brushtail possums fed 18 individual *E. polyanthemos* showed that they eat relatively little and do not alter their intake significantly over the narrow range of sideroxylonal concentrations found in the species (Fig. 3). Thus, in three six by six Latin squares, one female and five male ringtail possums were fed *E. polyanthemos* foliage from nine trees known to differ in sideroxylonal concentration (1–20 mg.g^{-1} DM). Likewise, six male brushtail possums were fed foliage from nine *E. melliodora* trees with sideroxylonal concentrations covering almost the full range known for the species (0–60 mg.g^{-1} DM). Each night, we measured DMI by possums receiving untreated or PEG-coated foliage. To ensure that possums remained well fed we interspersed experimental nights with rest nights: the ringtail possums were fed *Eucalyptus rossii* foliage while the brushtail possums received foliage from a favoured *E. melliodora* with low concentrations of sideroxylonals.

Dry matter and nitrogen digestibility of *Eucalyptus* foliage for brushtail and ringtail possums in the presence and absence of PEG

Eight male brushtail possums were used in a series of 2×2 crossover experiments so that every possum received untreated and PEG-coated foliage from two of the four *E. melliodora*. We measured the digestibility of dry matter and nitrogen by collecting all faeces produced over the final 5 nights of each 8-night period. Faeces were dried to constant mass at 60 °C. PEG is not absorbed from the digestive tract so the amount of PEG ingested was subtracted from the mass of faeces produced when calculating dry matter digestibility.

We were unable to maintain ringtail possums on *E. polyanthemos* foliage long enough for digestibility studies. Instead, we measured the influence of PEG on digestibility using *E. rossii* foliage. Six male ringtail possums were randomly allocated to two groups. One group was fed *E. rossii* foliage coated with PEG for 10 days, while the other received untreated foliage from the same tree. Treatments were then crossed and measurements resumed after 5 days, by which time the PEG reingested during caecotrophy would have passed through the digestive tract. Faeces were collected over the last 5 days of each period and treated as described above.

The responses of common brushtail possums to PEG administered on the foliage or by gavage

On two occasions in the week preceding the experiment, we gavaged all possums with water to familiarize them with the routine. Then, six male brushtail possums were randomly allocated to two groups: one group received *E. melliodora* foliage coated with PEG and the other group received untreated foliage from the same tree. The treatments were crossed after 3 nights. Additionally, at 1600 h each day, all possums received a small amount (10–20 g DM) of untreated *E. melliodora* foliage so that on treatment nights the PEG and the water were gavaged into a stomach containing digesta. In general, possums ate all of this foliage within 1 h, leaving just stems, which were removed at 1700 h and replaced with the appropriate foliage for the night. At about 1700 h on the second day of each period, possums receiving foliage coated with PEG were gavaged with 10 ml water while those receiving untreated foliage were gavaged with 10 ml 30% PEG (w/v) solution. This ensured that all possums received approximately 3 g PEG, whether by gavage or through ingestion of PEG-coated leaf.

Statistical analysis

Experiments were designed either as crossovers or as digram balanced Latin squares (Ratkowsky et al. 1993) so that all possums received all treatments. These designs were appropriate because we had few animals available and intake varied widely between animals, regardless of differences in body mass. Carryover effects were never significant and were deleted from statistical models.

Experiments were analysed using the residual maximum likelihood (REML) algorithm in Genstat 5 (4th edn). The fixed effects were the identifiable sources of variation (e.g. treatments or individual tree); the random effects were sources of variation such as possums, where the animals used were a random sample of the possum population.

Full models tested all possible combinations of effects, and non-significant effects were sequentially deleted to obtain a reduced model containing only the statistically significant effects. Any non-significant terms reported in the results came from the full model, while significant terms use values from the reduced model. In all cases, this significance was measured using a sub-model, whereby the term of interest is dropped from the full model so that deviance can be measured between the full model and the sub-model. This deviance is attributable to the term of interest and its significance is calculated using a chi-squared test. A bar depicting the least significant difference (lsd; $P=0.05$) is shown on graphs as a measure of experimental variance.

Results

The effect of PEG on the intake of a jensenone-rich diet by common brushtail possums

Possums ate similar amounts of the basal diet with and without PEG ($P=0.49$) (Fig. 4). Regardless of the PEG treatment, brushtail possums offered diets containing

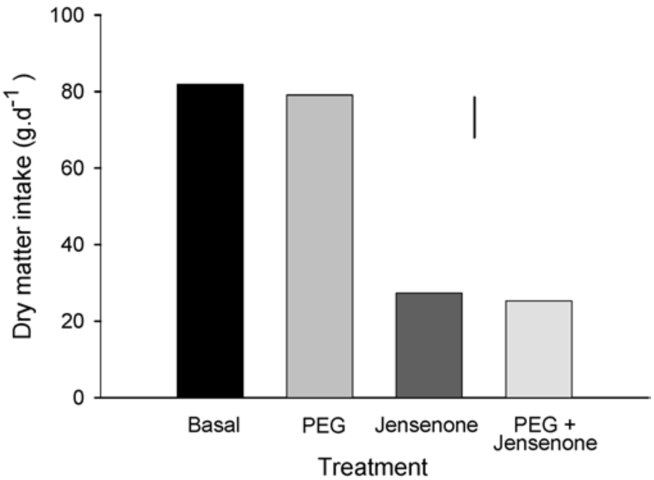

Fig. 4 Food intake by brushtail possums offered a synthetic basal diet or the basal diet supplemented with PEG, jensenone or jensenone plus PEG. The bar represents the 5% least significant difference. $P_{\text{treatment}} < 0.001$

0.4% jensenone ate about 60% less than did those offered diets without jensenone ($P < 0.001$; Fig. 4). This indicates that PEG does not bind with jensenone in vivo and is thus unlikely to inactivate other FPCs.

The effect of PEG on the intake of *E. polyanthemos* foliage by common ringtail possums

Ringtail possums preferred the untreated foliage from some *E. polyanthemos* trees to others; the average amount eaten ranged from 15.6 g DM.day^{-1} for the least preferred foliage to 38.1 g DM.day^{-1} for the most preferred. This variation in DMI could be attributed to two factors: increasing concentrations of sideroxylonal depressed DMI ($P < 0.0001$; Fig. 5b) while increasing concentrations of material able to bind PEG increased DMI ($P < 0.0001$). This is largely explained by the negative relationship, a confounding, between the sideroxylonal concentration of the foliage and its PEG-binding capacity. This, in turn, explains the interaction between sideroxylonal concentration and in vitro PEG-binding that reduced DMI ($P < 0.0001$).

Treating the fresh foliage with PEG did not influence DMI ($P = 0.90$), which suggests that in vitro PEG-binding is probably less important than sideroxylonal in explaining DMI.

As the concentration of sideroxylonal in the foliage increased, ringtail possums ingested more sideroxylonal (Fig. 6) until their intake of sideroxylonal reached almost 400 mg.kg$^{-0.75}$.day^{-1}.

The effect of PEG on the intake of *E. melliodora* foliage by common brushtail possums

The amount of untreated foliage brushtail possums ate varied enormously among the nine *E. melliodora*

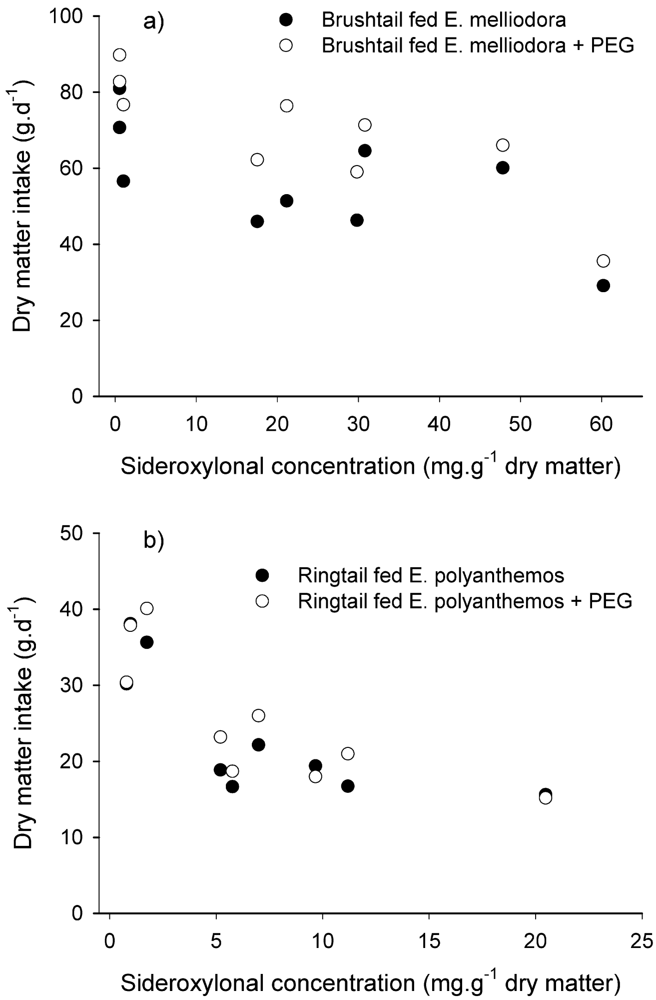

Fig. 5a–b Food intake by **a** brushtail possums and **b** ringtail possums offered foliage from nine *Eucalyptus melliodora* or from nine *E. polyanthemos*, respectively, that varied in their concentration of sideroxylonals. The foliage was fed untreated or dipped in a solution of 20% PEG

samples, ranging from 29.1 g DM.day^{-1} to 82.7 g DM.day^{-1}. Like the ringtails, brushtail possums also ate less as the sideroxylonal concentration of the foliage increased ($P < 0.0001$; Fig. 5a). However, this was partly offset by dipping the leaves in PEG, which allowed brushtails to eat more ($P = 0.0049$). Despite hypothesising that possums should increase DMI in response to PEG supplementation at low, but not at high, sideroxylonal concentrations and, although Fig. 5a suggests a trend in this direction, there was no interaction between sideroxylonal concentration and PEG treatment ($P = 0.15$). In other words, brushtail possums increased their intake of the sideroxylonal-rich *E. melliodora* foliage just as much as they did the sideroxylonal-poor foliage after tannins were inactivated with PEG.

By re-analysing the food intake data as two separate groups (untreated and PEG-treated foliage) we found that the amount of PEG-binding material in each sample influenced the amount of untreated foliage that brushtails ate ($P = 0.005$). Furthermore, coating the leaves

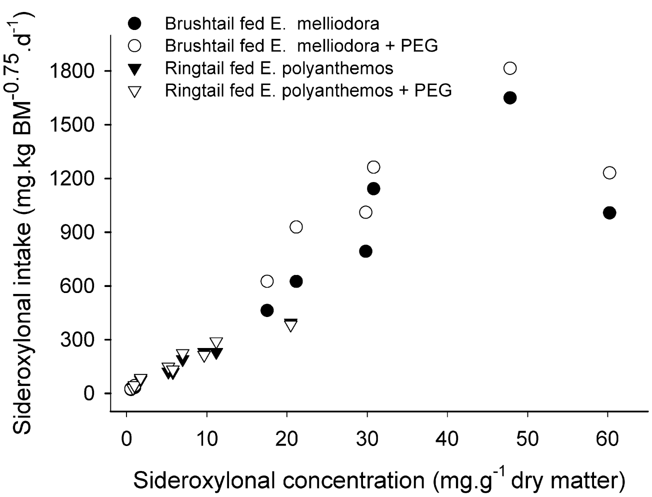

Fig. 6 Sideroxylonal intake by brushtail and ringtail possums offered foliage from nine *E. melliodora* or from nine *E. polyanthemos*, respectively, that varied in their concentration of sideroxylonals. The foliage was fed untreated or dipped in a solution of 20% PEG

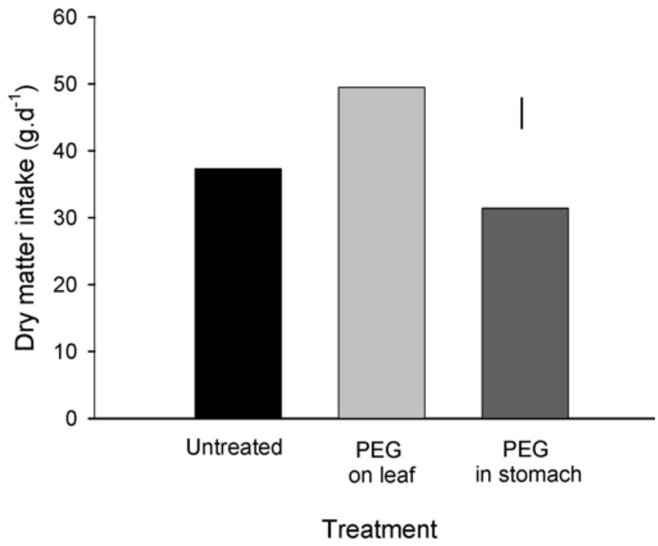

Fig. 7 The effect of PEG on the DMI by brushtail possums fed *Eucalyptus melliodora* foliage. The PEG was administered as a coating on the foliage or by gavage. The bar represents the 5% least significant difference. $P_{treatment} < 0.001$

with PEG inactivated this fraction and it then ceased being a determinant of feeding ($P = 0.16$). In both sets of data, sideroxylonal was the prime determinant of feeding ($P < 0.0001$), with no interaction between sideroxylonal concentration and PEG-binding.

Figure 6 shows sideroxylonal intake by ringtail and brushtail possums as a function of metabolic body mass, plotted against the sideroxylonal content of foliage. These data show three findings of interest: (1) brushtail possums ingest more sideroxylonal when PEG is added, (2) brushtail possums can ingest more sideroxylonal than can ringtail possums, and (3) the pair of points showing a sideroxylonal intake of approximately 1650 mg.kg$^{-0.75}$.day^{-1} before, and 1820 mg.kg$^{-0.75}$.day^{-1} after PEG-supplementation, are much higher than the other points.

Dry matter and nitrogen digestibility of *Eucalyptus* foliage for brushtail and ringtail possums in the presence and absence of PEG

Brushtail possums digested the DM in the four *E. melliodora* trees equally well (mean ± SD = 46.4 ± 3.3%) and did not digest any more when the leaves were coated with PEG (mean ± SD = 47.3 ± 1.7%; $P = 0.40$). In contrast, apparent nitrogen digestibility differed significantly among the four trees (range = 25.9–40.5%; mean ± SD = 32.7 ± 6.2; $P < 0.001$). Trees with the lowest nitrogen digestibility were those with the highest capacity to bind PEG in the in vitro analysis ($P = 0.024$). Presumably they also had a high capacity to bind protein (Silanikove et al. 2001). Thus, it was not surprising that brushtail possums digested more nitrogen following inactivation of the tannins by PEG (from 32.7% to 43.5%, SD = 5.2%; $P < 0.001$).

Ringtail possums digested 59.1% of the DM and 67.0% of the nitrogen in the foliage of *E. rossii*. Neither DM digestibility (56.0%; sed = 2.0%; $P = 0.69$) nor apparent nitrogen digestibility (66.5%; sed = 1.2%; $P = 0.59$) were affected by inactivating tannins with PEG.

The responses of common brushtail possums to PEG administered on the foliage or by gavage

The amount that brushtail possums ate on the day of gavaging did not differ from that on the other days, suggesting that they were little disturbed by the procedure ($P = 0.43$). Possums fed PEG-coated foliage (disturbed by gavage or undisturbed) ate more than did possums fed untreated foliage or possums that received untreated foliage and PEG by gavage ($P < 0.001$; Fig. 7).

Discussion

Interactions between PEG and FPCs

Our results showed conclusively that PEG did not reduce the antifeedant effects of the FPC, jensenone, when it was added to an artificial diet at concentrations typical of foliage. Since jensenone is a monomer of sideroxylonal and the two compounds act similarly to reduce feeding (Lawler et al. 2000), it appears that PEG does not bind FPCs in the gut and that any response to PEG is independent of FPCs. This is not surprising; FPCs are relatively non-polar compounds whose phenolic groups exhibit intra-molecular hydrogen bonding to aldehyde groups, while the phenolic groups of tannins are free and bind readily to molecules like PEG (Fig. 1).

This experiment showed also that brushtails do not eat more when PEG is added to a tannin-free diet. Therefore, if brushtails eat more of a diet that contains both tannins and PEG, the effect is probably due to PEG binding tannins rather than any other attribute of PEG.

The feeding responses of common ringtail and brushtail possums offered eucalypt foliage containing both tannins and FPCs

We do not know of a eucalypt species that contains FPCs and is a favoured food of both ringtail and brushtail possums—the best option for testing our hypothesis (Fig. 2). We thus studied the interaction between tannins and the FPC, sideroxylonal, by feeding *E. melliodora* to brushtail possums and *E. polyanthemos* to ringtail possums and accepted that we could not compare the possum species statistically.

Increasing foliar concentrations of sideroxylonal depressed DMI by both ringtail and brushtail possums, but the magnitude of the depression differed. The *E. melliodora* foliage contained up to three times more sideroxylonal than did the *E. polyanthemos* (60 $mg.g^{-1}$ vs. 20 $mg.g^{-1}$ DM). Nonetheless, it took an increase of sideroxylonal concentration from 0.5 $mg.g^{-1}$ to 60 $mg.g^{-1}$ DM to halve DMI by brushtail possums, while an increase of sideroxylonal from 0.5 $mg.g^{-1}$ to only 5 $mg.g^{-1}$ DM gave a similar reduction in DMI by ringtail possums. Furthermore, when sideroxylonal intake was scaled to metabolic body mass, brushtail possums tolerated far more sideroxylonal (up to 1800 $mg.kg^{-0.75}.day^{-1}$) than did ringtail possums (400 $mg.kg^{-0.75}.day^{-1}$), which supports a previous finding from this laboratory (I.R. Wallis and W.J. Foley, unpublished data). This contradicts the widespread assumption that, as specialist folivores, ringtail possums should be better equipped than brushtail possums to cope with the PSMs in *Eucalyptus* and begs discussion of what defines a specialist folivore.

Unfortunately, there are not enough data to describe the ingestion of sideroxylonals by ringtail and brushtail possums in relation to the concentration of sideroxylonals in the leaves. However, Fig. 6 suggests that sideroxylonal intake reaches a threshold in ringtail possums while in brushtails it rises to a maximum and then declines. This finding for brushtail possums contrasts with those of Lawler et al. (1998b) and Stapley et al. (2000) who showed, by feeding synthetic diets, that possums eat at a rate that keeps ingested FPCs below a threshold. Our data suggest that there may be a difference between synthetic and natural diets.

The two possum species differed in their responses to foliage coated with PEG. Brushtail possums consistently ate more *E. melliodora* foliage when it was coated with PEG. In contrast, despite up to 28% of *E. polyanthemos* DM binding to PEG in vitro (unpublished results), ringtail possums did not eat more when foliage was coated with PEG. Although agreeing with the results of McArthur and Sanson (1991) who showed that PEG does not influence the amount of *E. ovata* foliage eaten by ringtails, the result was unexpected. Treatment with PEG enabled ringtails to eat more foliage from two eucalypt species, *E. rossii* and *E. consideniana*, which lack FPCs (Marsh et al. 2003), and the same was expected when they were offered *E. polyanthemos* with low concentrations of sideroxylonal.

There are several possible explanations for why ringtail possums did not respond to PEG on *E. polyanthemos*, while brushtail possums always responded to PEG on *E. melliodora*. For example, *E. polyanthemos* may contain tannins that do not deter feeding, ringtails may have ingested insufficient PEG, or the nutritional plane provided by *E. polyanthemos* may have been too low to enable ringtails to tolerate sideroxylonal. To test whether possums were ingesting enough PEG to bind tannins we limited the amount of PEG in the in vitro PEG-binding assay to 6% of DM, thus mimicking the amount of PEG on foliage dipped in a 20% PEG solution. With this assay, 50% and 30% of the possible PEG-binding occurred for *E. melliodora* and *E. polyanthemos*, respectively. While this latter amount may seem paltry, a similar level of PEG-binding in *E. rossii* and *E. consideniana* enabled ringtail possums to eat about 10% more (Marsh et al. 2003).

Tannins comprise a large class of compounds that do not all affect feeding the same way and whose occurrence varies between plants. For example, neither sheep fed *Acacia cyanophylla* foliage (Ben Salem et al. 1999) nor brushtail possums fed black mangrove foliage (*Lumnitzera racemosa*; S. Dalla Pozza, unpublished data) ate more after the foods, known to be rich in tannins, were supplemented with PEG. Both species eat more when PEG is provided with other tannin-rich foods (Barry and Duncan 1984; Foley and Hume 1987).

While it is difficult to compare the individual tannins present in foliage samples, analyses by liquid chromatography–mass spectrometry (LC–MS) revealed substantial similarities in the qualitative profiles of low molecular weight phenolic compounds. Foliage from both *E. melliodora* and *E. polyanthemos* contained peaks corresponding to known flavanoids and hydrolysable tannins (e.g. engelitin, rutin and trigalloylglucoses) as well as unknown compounds (N. Davies, personal communication). Condensed tannins are too large to be reliably identified by LC–MS (N. Davies, personal communication).

Because brushtail possums ate more *E. melliodora* foliage when it was coated with PEG, sideroxylonal intake also increased. Stapley et al. (2000) and Lawler et al. (2000) argued that FPC intake is regulated at a level that avoids toxication, so why does PEG treatment allow brushtails to eat up to 300 mg more sideroxylonals per day? One explanation is that a better nutritional environment allows animals to ingest more PSMs. This was shown by Wang and Provenza (1996) who found that food-deprived lambs did not ingest as much of a diet containing lithium chloride as did lambs fed ad libitum. PEG improves nitrogen digestibility for brushtail possums fed *E. melliodora* and this additional supply of digestible nitrogen may allow brushtails to ingest more sideroxylonals. However, this remains to be tested. Interestingly, PEG did not improve nitrogen digestibility for ringtail possums fed *E. rossii* foliage and it is therefore unlikely that it would have any effect on the nitrogen digestibility of *E. polyanthemos*. In keeping with the conclusion of Lawler et al. (1998a) that another FPC, macrocarpal G, largely determined feeding by ringtails on *E. ovata*, we suggest that ringtails avoid eating foliage that contains even moderate amounts of FPCs, but are less concerned about tannins. In contrast, brushtail possums have a higher tolerance of FPCs but are poorly equipped to cope with tannins.

A likely explanation is that the specialist ringtail possum has anatomical and physiological means for coping with ingested tannins that the generalist brushtail possum does not possess. From a nutritional perspective the most obvious difference between the two possum species lies in their gastrointestinal morphology. Both are hindgut fermenters but the ringtail possum has a large and more complex caecum. This organ is matched with a separation mechanism for the selective retention of bacteria and other nitrogen-rich small particles and a dentition that reduces highly resilient leaf to fine particles (Hume 1999). In addition, the species practices caecotrophy and thus returns the nutritionally rich caecal contents to the proximal digestive tract (Chilcott 1984). Finally, as suggested by McArthur and Sanson (1991), tannin-protein complexes may dissociate in the caecum so a proportion of tanned protein can be retrieved through caecotrophy. In contrast, the dentition of the brushtail has not evolved to reduce leaf into fine particles, relative to body size its caecum is only half as big as the ringtail's and there is no evidence for a separation mechanism to selectively retain small particles.

As has been found previously (Lawler et al. 2000; Wallis et al. 2002), there was wide variation in how much possums ate at a given concentration of dietary sideroxylonals. For example, brushtail possums ate 26 g more from one tree than they did of another with an almost identical concentration of sideroxylonals. We proposed originally that tannins accounted for some of this variation in food intake. Since the ringtail possums did not respond to PEG, there was no scope for tannin inactivation to explain variation in feeding. However, two pieces of evidence support the proposal for brushtail possums. First, the concentration of PEG-binding material in the foliage influenced DMI when the foliage was fed untreated so that, at a given sideroxylonal concentration, brushtails ate less when the tree foliage had a higher PEG-binding capacity. Second, coating the foliage with PEG inactivated much of the PEG-binding fraction, leaving sideroxylonals as the only factor significantly affecting DMI.

The response of common brushtail possums administered with PEG coated on foliage or by gavage

The finding that possums responded to PEG-coated leaves by eating more invites questions about how PEG works. For example, is the effect related to taste or is it post-ingestive, perhaps regulated through the emetic system? Possums ate significantly more PEG-coated foliage than they did untreated foliage on both the pretreatment day and the treatment day, indicating firstly that feeding was not disrupted by the gavaging process and, secondly, that a PEG gavage did not alleviate the systemic effects of tannins. Because we know little about how PEG works we must make several assumptions in interpreting the results. The main assumption was that PEG had not passed beyond the site of any systemic effects by the time possums started to eat. This gains support from Silanikove et al. (1994) who found that providing PEG to goats in two doses during the day was no more effective than providing a single dose. They attributed this finding to the long mean retention times of digesta in the gut of the species (35–40 h). Although brushtail possums eating *E. melliodora* foliage also have long mean retention times (50 h; Foley and Hume 1987), unlike goats they are not foregut fermenters and would not have digesta in the foregut for as long as do goats.

We assumed also that the results of our short-term experiments equate with those from longer studies. This may not be true. Foley et al. (1999) suggested that herbivores might take many hours or even days to sense changes in protein digestibility. Our experiment was deliberately short because previous work showed immediate effects on feeding of applying PEG to the leaf. The gavage experiment may have been too short to assess the effects on protein digestibility of providing PEG directly into the stomach. However, the same possums had experienced PEG many times, albeit as a coating on the leaf, and would be expected to respond to a systemic signal.

Thus, increased feeding by brushtail possums when PEG is administered on the foliage shows that tannins elicit immediate oral effects. This agrees with many studies, which suggest that animals use the oral effects of tannins to regulate tannin intake. For example, Landau et al. (2000) noted that cattle modified their feeding behaviour within minutes of the tannin, quebracho, being added or removed from their diet. Prinz and Lucas (2000) hypothesised that astringency due to the interactions of tannins with salivary and mucosal proteins in the mouth is the signal used by animals to detect tannins. In this way, PEG may reduce astringency by releasing protein back into the saliva (Villalba and Provenza 2001). These researchers found no difference in the amount of PEG lambs voluntarily ingested when fed a control diet, or a control diet coupled with an intraruminal infusion of quebracho, even though lambs given quebracho in their food voluntarily ingested more PEG. Similarly, Kyriazakis et al. (1997) were unable to induce a conditioned aversion in sheep towards flavoured hay that was paired with a quebracho gavage. This last result confirms that oral effects can provide an immediate signal to the animal to stop eating but may not condition long-term aversions to the food (Clark 1996).

Conclusions

These experiments with *E. melliodora* and *E. polyanthemos* illustrate the complexity of working with animals fed natural diets. We have shown that two groups of PSMs, sideroxylonals and tannins, can simultaneously affect feeding by ringtail and brushtail possums but that the species have differing capacities to handle these compounds. It appears that ringtail possums can eat foliage rich in tannins while brushtail possums use oral detection to avoid ingesting large quantities of tannins. Thus, they avoid the high cost that tannins impose on the animals' nitrogen metabolism. In contrast, ringtail possums have a much lower tolerance of FPCs than do brushtail possums. Surprisingly, food intake was not influenced by an interaction between FPCs and tannins, thus rejecting one of our hypotheses. These findings agree with our experiences in keeping both species in captivity. Ringtails are easily maintained on eucalypts from the informal subgenus *Monocalyptus*, which do not contain FPCs. They will also eat from trees in the subgenus *Symphyomyrtus* provided the foliage contains low concentrations of FPCs. In contrast, it is harder to maintain brushtails on an exclusive eucalypt diet and the foliage from the individual trees they prefer tends to contain low concentrations of tannins and high concentrations of nitrogen and be from species within the sub-genus *Symphyomyrtus*. This leaves the outstanding question of why ringtails have not evolved a tolerance for FPCs and why brushtail possums have. Are both species specialists in their own ways?

Acknowledgements Dr Noel Davies kindly analysed eucalypt extracts for tannins and interpreted the results. Ms Cynthia Cheung helped with various aspects of the possum feeding. Ben Moore provided many useful comments on the manuscript. The work described in this paper was approved by the Animal Experimentation Ethics Committee of the Australian National University and conforms to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

References

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. Brit J Nutr 51:485–491

Ben Salem H, Nefzaoui A, Ben Salem L, Tisserand JL (1999) Intake, digestibility, urinary excretion of purine derivatives and growth by sheep given fresh, air-dried or polyethylene glycol-treated foliage of *Acacia cyanophylla* Lindl. Anim Feed Sci Tech 78:297–311

Burritt EA, Provenza FD (2000) Role of toxins in intake of varied diets by sheep. J Chem Ecol 26:1991–2005

Chilcott MJ (1984) Coprophagy in the common ringtail possum (*Pseudocheirus peregrinus*). Aust Mamm 7:107–110

Clark L (1996) Trigeminal repellents do not promote conditioned odor avoidance in European starlings. Wilson Bull 108:36–52

Eschler BM, Pass DM, Willis R, Foley WJ (2000) Distribution of foliar formylated phloroglucinol derivatives amongst Eucalyptus species. Biochem Syst Ecol 28:813–824

Foley WJ, Hume ID (1987) Digestion and metabolism of high-tannin *Eucalyptus* foliage by the brushtail possum (*Trichosurus vulpecula*) (Marsupialia: Phalangeridae). J Comp Physiol B 157:67–76

Foley WJ, Iason GR, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Jung H-JG, Fahey GCJ (ed) Nutritional ecology of herbivores. Proceedings of the Vth International Symposium on the Nutrition of Herbivores. American Society of Animal Science, Savoy, pp 130–209

Hume ID (1999) Marsupial nutrition. Cambridge University Press, Cambridge

Jones WT, Mangan JL (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis vicifolia* Scop.) with fraction 1 leaf protein and with submaxillay mucoprotein, and their reversal by polyethylene glycol and pH. J Sci Food Agr 28:126–136

Kerle JA (1984) Variation in the ecology of *Trichosurus*: its adaptive significance. In: Smith AP, Hume ID (ed) Possums and gliders. Surrey Beatty, Sydney, pp 115–128

Kyriazakis I, Papachristou TG, Duncan AJ, Gordon IJ (1997) Mild conditioned aversions developed by sheep towards flavours associated with plant secondary compounds. J Chem Ecol 23:727–746

Landau S, Silanikove N, Nitsan Z, Barkai D, Baram H, Provenza FD, Perevolotsky A (2000) Short-term changes in eating patterns explain the effects of condensed tannins on feed intake in heifers. Appl Anim Behav Sci 69:199–213

Lawler IR, Foley WJ, Eschler BM, Pass DM, Handasyde K (1998a) Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. Oecologia 116:160–169

Lawler IR, Foley WJ, Pass GJ, Eschler BM (1998b) Administration of a 5HT(3) receptor antagonist increases the intake of diets containing Eucalyptus secondary metabolites by marsupials. J Comp Physiol B 168:611–618

Lawler IR, Eschler BM, Schliebs DM, Foley WJ (1999) Relationship between chemical functional groups on *Eucalyptus* secondary metabolites and their effectiveness as marsupial antifeedants. J Chem Ecol 25:2561–2573

Lawler IR, Foley WJ, Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. Ecology 81:1327–1338

Marsh KJ, Wallis IR, Foley WJ (2003) The effect of inactivating tannins on the intake of *Eucalyptus* foliage by a specialist *Eucalyptus* folivore (*Psuedocheirus peregrinus*) and a generalist herbivore (*Trichosurus vulpecula*). Aust J Zool 51 (In press)

McArthur C, Sanson GD (1991) Effects of tannins on digestion in the common ringtail possum (*Pseudocheirus peregrinus*), a specialized marsupial folivore. J Zool 225:233–251

Pahl L (1984) Diet preference, diet composition and population density of the ringtail possum (*Pseudocheirus peregrinus*) in several plant communities in southern Victoria. In: Smith AP, Hume ID (ed) Possums and gliders. Surrey Beatty, Sydney, pp 252–260

Prinz JF, Lucas PW (2000) Saliva tannin interactions. J Oral Rehabil 27:991–994

Ratkowsky DA, Evans MA, Alldredge JR (1993) Cross-over experiments—design, analysis, and application. Marcel Dekker, New York

Reed JD (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. J Anim Sci 73:1516–1528

Silanikove N, Nitsan Z, Perevolotsky A (1994) Effect of polyethylene glycol supplementation on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by goats. J Agr Food Chem 44:199–205

Silanikove N, Gilboa N, Nir I, Perevolotsky A, Nitsan Z (1996a) Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Quercus calliprinos*, *Pistacia lentiscus*, and *Ceratonia siliqua*) by goats. J Agr Food Chem 44:199–205

Silanikove N, Shinder D, Gilboa N, Eyal M, Nitsan Z (1996b) Binding of poly(ethylene glycol) to samples of forage plants as an assay of tannins and their negative effects on ruminal degradation. J Agr Food Chem 44:3230–3234

Silanikove N, Perevolotsky A, Provenza FD (2001) Use of tannin-binding chemicals to assay for tannins and their negative post-ingestive effects in ruminants. Anim Feed Sci Tech 91:69–81

Stapley J, Foley WJ, Cunningham R, Eschler B (2000) How well can common brushtail possums regulate their intake of Eucalyptus toxins? J Comp Physiol B 170:211–218

Steinly BA, Berenbaum M (1985) Histopathological effects of tannins on the midgut epithelium of *Papilio polyxenes* and *Papilio glaucus*. Entomol Exp Appl 39:3–9

Villalba JJ, Provenza FD (2001) Preference for polyethylene glycol by sheep fed a quebracho tannin diet. J Anim Sci 79:2066–2074

Wallis IR, Watson ML, Foley WJ (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. Aust J Zool 50:1–13

Wallis IR, Herlt AJ, Eschler BM, Takasaki M, Foley WJ (2003) Quantification of sideroxylonals in *Eucalyptus* foliage by HPLC. Phytochem Anal (In press)

Wang J, Provenza FD (1996) Food deprivation affects preference of sheep for foods varying in nutrients and a toxin. J Chem Ecol 22:2011–2021