# ECOLOGICAL EXAMPLE OF CONDITIONED FLAVOR AVERSION IN PLANT–HERBIVORE INTERACTIONS: EFFECT OF TERPENES OF *Eucalyptus* LEAVES ON FEEDING BY COMMON RINGTAIL AND BRUSHTAIL POSSUMS

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**Abstract**—We investigated the hypothesis that feeding deterrence of common ringtail possums (*Pseudocheirus peregrinus*) and common brushtail possums (*Trichosurus vulpecula*) by *Eucalyptus* terpenes (in this case 1,8-cineole) is a result of a conditioned flavor aversion (CFA), due to the association of terpenes with postingestive effects of another group of *Eucalyptus* toxins, the diformylphloroglucinol compounds (DFPCs). Wild-caught common ringtail and common brushtail possums showed a dose-dependent reduction in food intake when 1,8-cineole was added to the diet. However, after continued exposure over 12 days to increasing amounts of cineole in the diet, both species substantially increased their intakes of cineole relative to control animals. This indicated that the aversion to cineole was a conditioned response rather than a physiological limitation in their ability to detoxify terpenes. Subsequent exposure to a diet including both cineole and jensenone (a simple DFPC also found in *Eucalyptus* and considered to cause postingestive emesis) in corresponding amounts was able to recondition the dose-dependent aversion. Consequently, animals that had been given jensenone showed an aversion to cineole-rich diets that matched the behavior of animals in the control group. This supported our hypothesis that the effect of terpenes on feeding of these marsupial folivores on *Eucalyptus* is due to a CFA. Possums can cope with levels of terpenes in the diet that far exceed those occurring naturally. The role of terpenes in marsupial folivore–*Eucalyptus* interactions

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appears to be to act as a cue to levels of toxic DFPCs in the leaves, rather than acting as toxins in their own right.

**Key Words**—Conditioned flavor aversion, plant toxin, *Eucalyptus*, *Pseudocheirus perigrinus*, *Trichosurus vulpecula*, terpene, cineole, jensenone, food intake, emesis.

## INTRODUCTION

Conditioned flavor aversions (CFAs) are considered to be an important mechanism modulating diet choice in free-ranging herbivores (Provenza, 1996). Animals are thought to regulate their intake of some plant secondary metabolites (PSMs) below toxic levels by learning to associate a distinctive taste of a diet with negative postingestive feedback resulting from the PSM. Studies of this phenomenon in browsing mammals mostly have been conducted with ruminants and have used toxins (principally LiCl) (Wang and Provenza, 1997) and/or artificial flavors such as orange or onion as the taste stimulus (Ralphs et al., 1995; Wang and Provenza, 1997), which do not occur naturally in the diets studied. Although these studies have shown how CFAs could develop in free-ranging animals, the stimuli used are not ecologically realistic. In contrast, Kyriazakis et al. (1997) used two naturally occurring plant secondary metabolites (PSMs) to condition aversions in sheep, but again they used flavor stimuli that the animals are unlikely to encounter in nature. In this study we examine the development of a conditioned flavor aversion in the common ringtail possum (*Pseudocheirus peregrinus*) and the common brushtail possum (*Trichosurus vulpecula*), both arboreal marsupial folivores of *Eucalyptus*. We used both a flavor stimulus (1,8-cineole, a monoterpene) and a toxin (jensenone, a diformylphloroglucinol compound; see below) which occur together in *Eucalyptus* leaves.

It has been suggested that terpenes deter feeding of a wide range of browsing mammals, including the marsupial folivores of *Eucalyptus* (Southwell, 1978; Hume and Esson, 1993) because small amounts can be toxic to some animals (McLean and Foley, 1997). However, correlations between food intake and the concentrations of foliar terpenes have explained little, if any, of the differences in foliage intake by these animals (Southwell, 1978; Hume and Esson, 1993). In contrast, we showed that intraspecific variation in food intake by common ringtail possums correlates strongly with the total terpene concentration of the leaves (Lawler et al., 1998a). However, in these experiments, food intake also correlated with the concentration of a newly discovered group of compounds, the diformylphloroglucinol compounds (DFPCs). A strong correlation between food intake and foliar DFPC content has now also been found with two other species of *Eucalyptus* (Lawler, Foley, Eschler, unpublished data).

We suggested that foliar terpenes could not cause the variation in intakes

at the concentrations observed between individual trees (Lawler et al., 1998a). When added to an artificial diet, the terpene concentration required to reduce food intake was far greater than that found in leaves on which food intakes were reduced to similar levels. In contrast, addition of DFPCs to the diet reduced intakes at levels in close agreement with the trend seen in leaves. This finding was supported also by more recent studies of two other *Eucalyptus* species (Lawler, Foley, Eschler, Schliebs, unpublished data). Consequently, we suggested that foliar terpenes may cue animals to the concentration of the true toxins in the leaves. Terpenes have strong and distinctive odors and tastes and are responsible for the characteristic smell of *Eucalyptus* leaves, while DFPCs are nonvolatile and give off very little noticeable odor (Lawler, personal observation). All DFPCs contain a side chain derived from common terpenes (such as $\beta$-phellandrene and bicyclogermacrene) (Ghisalberti, 1996), and so we suspected that concentrations of the two compounds would be correlated if production of DFPCs is substrate-limited (Lawler et al., 1998a). Such correlations have now been found in three *Eucalyptus* species (Lawler and Foley, unpublished). Upon initial inspection and tasting of a leaf, a folivore would first encounter information about the terpene components of the leaf and might use this to estimate the DFPC content of the leaves and its likely postingestive effects, based on previous experience. Indeed, we have found that even with our relatively limited olfactory capacity, we could predict the relative amounts of DFPCs between foliage samples of different individual trees within *Eucalyptus* species, simply by crushing and smelling the leaves upon collection (Lawler and Foley, unpublished).

In this study we aimed to test the following three hypotheses: (1) Wild-caught folivorous possums held in captivity will show a strong aversion to *Eucalyptus* terpenes, due to their previous experience. (2) This aversion can be reduced or removed by feeding the animals an artificial diet to which increasing amounts of terpene are added. (3) Once removed, the aversion can be reconditioned by giving the animals a diet consisting of both the terpene and another *Eucalyptus* toxin known to produce negative postingestive effects in corresponding concentrations.

## METHODS AND MATERIALS

This research was approved by the Animal Experimentation Ethics Committee of the Australian National University and conforms with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Twelve common ringtail (*Pseudocheirus peregrinus*) and 12 common brushtail (*Trichosurus vulpecula*) possums were collected from *Eucalyptus* woodlands, kept individually in metabolism cages (described by Lawler et al., 1998a), and maintained on a basal diet made fresh each day. The diet fed to ringtail possums consisted of (percent

wet matter) 55.5% grated apple, 28.3% banana pulp, 4.7% ground lucerne hay (to pass a 2-mm sieve), 5.5% ground rice hulls, 4.7% ground Weetbix (a wheat-based breakfast cereal), and 1.6% acid casein. The diet for common brushtails was modified for the purposes of other experiments and consisted of 55% apple, 15% carrot, 15% banana, 5.8% sugar, 1% lucerne hay, 3% Weetbix, 5% rice hulls, and 0.2% casein. All animals maintained body mass on this basal diet.

For each experiment, a *Eucalyptus* terpene (1,8-cineole) and a simple DFPC (jensenone) (Boland et al., 1992) were added to the diet in varying concentrations and aversion/deterrence measured as a reduction in food intake relative to control values. No alternative food was offered during experimental periods. For ringtail possums, all experimental periods were separated by a day on which only the basal diet was offered, in order to reduce carryover effects and ensure the welfare of the animals. This was not considered necessary for brushtail possums as they readily feel during the day and so were offered an untreated diet between 08:00 hr and 12:00 hr on each experimental day, which similarly reduced carry over effects and ensured the health of the animals.

Cineole was chosen as a representative terpene as it is one of the most common terpenes found in *Eucalyptus*, often dominating the total volatile terpene extract (Boland et al., 1991), and has specifically been suggested as a feeding deterrent in studies of both mammals (Southwell, 1978; Reichardt et al., 1990; Zoidis and Markowitz, 1992) and insects (Edwards et al., 1993). Cineole was purchased from Sigma Chemical Company, Australia.

Jensenone was used as a representative DFPC as it is structurally similar to other active DFPCs, is active in similar molar quantities (Lawler et al., 1998a), and is the only DFPC that we have thus far been able to isolate in sufficient quantity for these kinds of experiments (Lawler et al., 1998b). We also have evidence that jensenone acts by stimulation of the emetic system, as much of its effect on intake can be attenuated by administration of ondansetron (Lawler et al., 1998b), an antiemetic drug that is a 5HT$_3$ receptor antagonist (Butler et al., 1988). In this way it is similar to LiCl, the emetic stimulant so frequently used in other studies (Launchbaugh and Provenza, 1993; Provenza et al., 1994b; Wang and Provenza, 1997). Jensenone was extracted from air-dried foliage of *Eucalyptus jensenii*, ground to pass a 2-mm sieve, and extracted in 20% acetone–light petroleum in a Soxhlet apparatus. The extracts were concentrated and combined in diethyl ether and washed with 0.3 M NaOH. These washes were then acidified with HCl, and the precipitate was washed with ethanol and recrystallized from acetone to give jensenone (98% by $^1$H NMR) (Eschler and Foley, unpublished data).

As cineole is volatile, it was added to the diet by adsorbing it onto the dry components immediately before adding the fruit. Jensenone is nonvolatile and insoluble in water, so it was dissolved in acetone and then added to the dry component of the diet, after which the acetone was evaporated before mixing with the fruit component of the diet. Control diets were treated similarly with acetone.

*Independence of Effects of Cineole and Jensenone in Common Ringtail*
*Possums*

As this experiment relied on our ability to predict the amount of reduction in food intake associated with jensenone and cineole together, it was necessary to investigate firstly whether there was any interaction between the effects of the two compounds before attempting to remove any aversion to cineole. In this experiment, carried out on ringtail possums, there was no significant interaction, so we considered it unnecessary to also conduct this experiment with brushtail possums as their reactions to both compounds are similar (Lawler et al., 1998b; Stapley, unpublished).

Three concentrations of each compound were used. Cineole was added to the diet at concentrations of 0, 6.3, and 9.8% of total dry matter (DM) and jensenone was added at 0, 0.84, and 1.26% of the total DM of the diet. In an orthogonal design this resulted in nine possible treatment combinations. A truncated Latin-square design was used with nine possums over five treatment periods, so that over the experiment each possum received five different randomly allocated treatments.

*Removal and Reconditioning of Aversion to Cineole*

The experiments with the ringtail possums and brushtail possums were carried out separately, with those on ringtail possums conducted earlier. Following the ringtail possum experiments, some adjustments to experimental design were made for the brushtail possums and differences in the protocols (principally for stages 1 and 3) are listed below. For both species the animals were divided randomly into two groups of six (a control and a treatment group) and the experiments were carried out in five sequential stages. The complete series of experiments took 35 days for ringtail possums and 27 days for brushtail possums.

For clarity, the five stages of the basic design and the expectations and hypotheses are summarized here, with full details of each experiment given below. In stage 1—initial/preacclimation aversion to cineole—the ability of both groups to ingest cineole in no-choice experiments was tested. The hypothesis to be tested is that both groups should show a similar strong reduction in food intake when cineole is added to the diet. In stage 2—acclimation to cineole—the treatment group was acclimated to increasing levels of dietary cineole over 12 days while the control group was fed only the basal diet. The expectation is that animals in the treatment group will increase their intake to cineole to levels well beyond the tolerance shown in stage 1. In stage 3—postacclimation to cineole—the tolerance of each group to cineole was again tested, and animals in the treatment group were expected to show significantly less reduction in food intake than the animals in the control group in response to dietary cineole. In stage 4—reconditioning aversion to cineole—the aversion was reconditioned by giving

the treatment group a diet including both jensenone and cineole in corresponding concentrations. The hypothesis is that jensenone should reduce food intake to levels similar to those expected on the basis of the initial aversion to cineole. In stage 5—postreconditioning aversion to cineole—the tolerance of both groups to added cineole (without jensenone) was again tested, and we expected that the reduction in food intake due to dietary cineole (in the absence of jensenone) should be similar to that seen in the control group and be dose-dependent.

*Stages 1 and 3.* In ringtail possums tests of the ability of the two groups of animals to tolerate cineole were carried out as crossover designs involving two treatments and two periods. On the first day, three animals in each group were given diets of 9.8% cineole on a dry matter (DM) basis, while the remaining three were given the basal diet alone. The following day all animals were fed the basal diet and the next day the treatments were reversed.

In brushtail possums similar tests were made with three concentrations of cineole (0, 4.9, and 9.8% DM). Animals in both the treatment and control groups were divided into two subgroups and the diets offered in a 3 × 3 Latin-square design to each group of three animals over three consecutive nights. A more extensive design with more concentrations was avoided as it was thought that there may be some loss of the aversion in a longer experiment.

*Stage 2.* Six animals were acclimated to increasing levels of cineole in their diets while the remaining six were fed the basal diet only. The initial concentration of cineole was 1.75% DM; this was increased by approximately 0.7% DM per day to a maximum of 10.5% DM for ringtail possums and by approximately 1.2% DM per day to a maximum of 16.5% DM for brushtail possums. Stages 2 and 1 were necessary as the animals were caught from *Eucalyptus* woodland and their intakes of foliage diets had been shown to correlate strongly with leaf terpene concentrations (Lawler, unpublished); hence, they were likely to already have a CFA towards cineole. That is, it is possible that the animals had already made associations between cineole or some other foliar terpene and diet choice. This possibility had to be evaluated and its influence removed in the later stages of the experiment. It was not possible to find possums that we could confidently say had not been exposed to terpenes in their natural diets.

*Stage 4.* The treatment group was given the basal diet to which cineole and jensenone was added in corresponding concentrations, that is, concentrations of each compound, which, on the basis of the first experiment described above (for ringtails or Stapley's unpublished data (for brushtails) would produce the same level of reduction in food intake. The amount of jensenone added to the cineole-rich diet was that required to reduce food intake to the same level as seen when cineole alone was incorporated before removal of the aversion. The purpose of this was to allow the animals to learn to associate the concentration of cineole with a given level of postingestive discomfort. Three concentrations of each compound were used. These were (jensenone/cineole as %

DM): 0.42/4.39, 0.84/8.78, and 1.26/13.17 for ringtail possums and 0.176/4.5, 0.29/7.5, and 0.43/9.8 for brushtail possums.

A 6 × 6 Latin-square design was used to allocate treatments among the six animals, so that each animal was given each concentration twice over the course of the experiment.

*Stage 5.* For both species, three treatment levels were used as described above for brushtail possums in stages 1 and 3. This procedure of using stages 1 and 3 for ringtails (and all stages for brushtails) was done in order to show that any reconditioned aversion was also dose-dependent, rather than a simple avoidance. It was not initially considered necessary to examine dose-dependence for the previous cineole aversion of ringtail possums as this was shown in the experiment that tested for interactive effects of jensenone and cineole.

## Statistical Analysis

All experiments were treated as mixed model, row–column designs with possums and days as random effects and cineole concentration, group (treatment or control), and group × cineole interaction as fixed effects. Variables of the models were estimated by restricted maximum likelihood (REML) theory (R. Cunningham, personal communication). For the key experiments, data presented are for the effect of cineole on DM intake and the cineole × group interaction effect on DM intake. We expected that cineole would reduce DM intake and, where the aversion to cineole was similar between control and treatment groups (stages 1 and 5), the interaction would be nonsignificant, while where the treatment group had a reduced aversion, relative to the controls, the interaction would be highly significant (stage 3).

## Data Presentation

All data are presented graphically. Where formal statistical tests have been performed (stages 1, 3, and 5) means are presented with a bar showing least significant difference (LSD) derived from the analysis. Where we have not performed formal tests of differences (stages 2 and 4), data are shown as means ± standard errors.

## RESULTS

### Independence of Effects of Cineole and Jensenone in Common Ringtail Possums

There was a significant interaction between dietary cineole and jensenone on food intake of common ringtail possums (ANOVA: $P < 0.001$). However, this was mainly attributable to the overriding influence of jensenone on food intake,
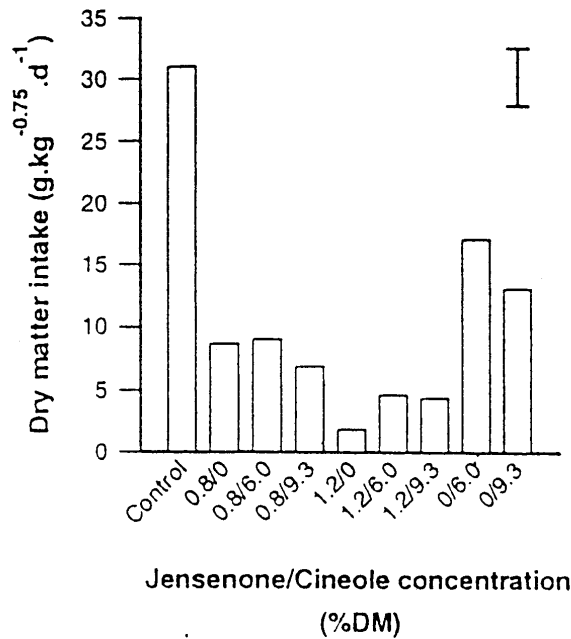
Jensenone/Cineole concentration
(%DM)

FIG 1. Effects of combinations of 1,8-cineole and jensenone on food intake of common ringtail possums. Data are means with least significant difference in the analysis represented by the bar in the upper right part of the figure. Food consumption is based on dry matter (DM) intake.

rather than any strong additive or synergistic effect of the two compounds combined (Figure 1). Thus, the combination of the two in the reconditioning of any CFA was considered appropriate.

*Removal and Reconditioning an Aversion to Cineole*

The salient data are given in Figure 2, with data for ringtail possums listed on the left (Figure 2a) and those for brushtail possums on the right (Figure 2b). Statistical tests are summarised in Table 1. In summary, both species showed an

FIG. 2. Effects of 1,8-cineole on food intake of (a) common ringtail possums and (b) common brushtail possums at different stages in the experimental sequence. [i] Initial/preacclimation aversion after capture from *Eucalyptus* woodland; [ii] postacclimation of animals in the treatment group to increasing dietary cineole; [iii] postreconditioning of the aversion in animals in the treatment group by combining added dietary cineole and jensenone in corresponding amounts. Shaded bars represent the control group; unshaded bars represent the treatment group. Data are means with least significant difference in the analysis represented by the bar in the upper right part of the figure.
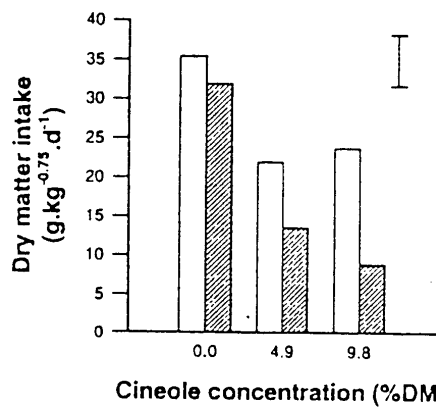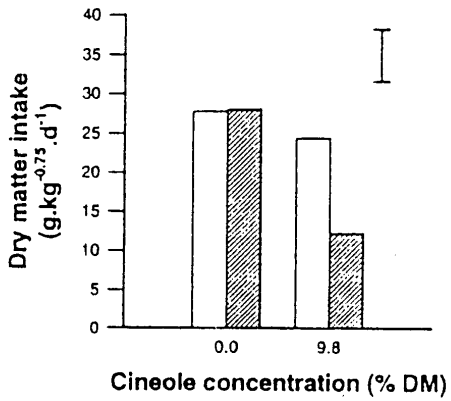
## a. Common ringtail possums    b. Common brushtail possums

### [i] Stage 1. pre-acclimation
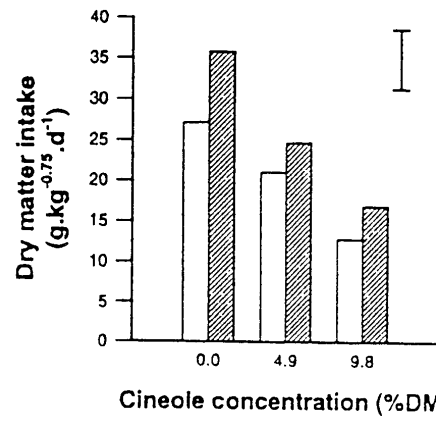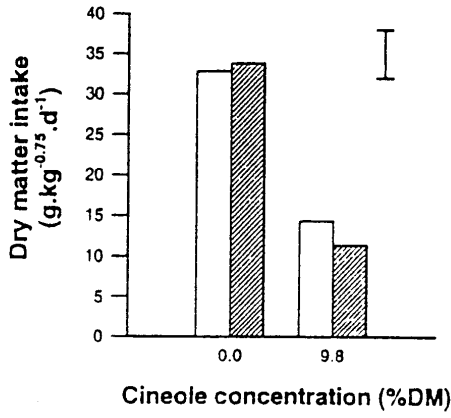


### [ii] Stage 3. post-acclimation



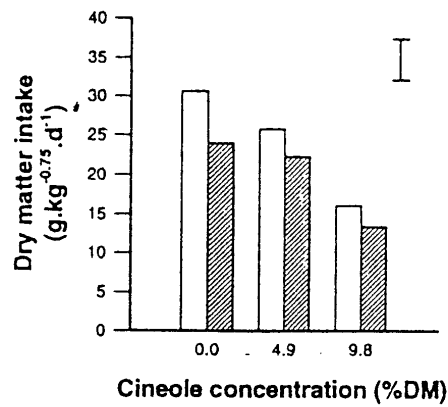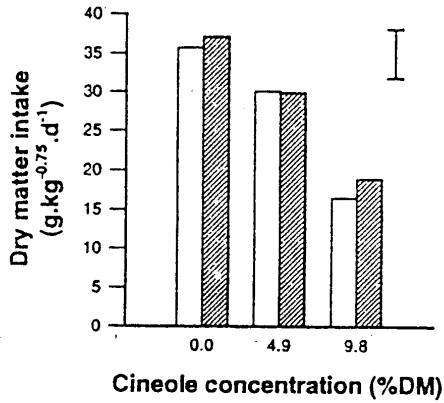### [iii] Stage 5. post-reconditioning

TABLE 1. SUMMARY OF PROBABILITY VALUES FROM STATISTICAL ANALYSES OF COMPARISON OF AVERSION TO 1,8-CINEOLE BETWEEN ANIMALS IN CONTROL AND TREATMENT GROUPS

| Experiment stage | Ringtail possums | | Brushtail possums | |
|---|---|---|---|---|
| | Cineole main effect | Cineole × treatment interaction | Cineole main effect | Cineole × treatment interaction |
| Preacclimation | <0.001 | 0.403 | <0.001 | 0.472 |
| Postacclimation | <0.001 | 0.001 | <0.001 | 0.02 |
| Postreconditioning | <0.001 | 0.778 | <0.001 | 0.861 |

initial aversion to cineole (stage 1: Figure 2[i]), which was substantially reduced after continued exposure (acclimation) to cineole (stage 3: Figure 2[ii]) and was reconditioned after exposure to dietary cineole with corresponding amounts of dietary jensenone (stage 5: Figure 2[iii]). Results for each stage [including intermediate acclimation (stage 2) and reconditioning (stage 4)] are described fully below.

*Stage 1. Initial/Preacclimation Aversion to Cineole.* Common ringtail possums in both treatment and control groups showed a clear initial aversion to dietary cineole, which was consistent between groups (Table 1, Figure 2a[i]). Common brushtail possums showed a similar response to both cineole as a main effect and the cineole by group interaction (Table 1, Figure 2b[i]).

*Stage 2. Acclimation to Cineole.* Both common ringtail and common brushtail possums substantially increased their intakes of cineole over the acclimation period (Figure 3a and b).

*Stage 3. Postacclimation Aversion to Cineole.* After acclimation to dietary cineole, both species showed substantially reduced aversions relative to animals that were not acclimated. There was still a significant main effect of cineole, due principally to the remaining strong aversion in the control group (Table 1, Figure 2a and b[ii]).

*Stage 4. Reconditioning of Aversion to Cineole.* During the reconditioning phase of the experiment, animals of both species clearly regulated food intakes in relation to the jensenone (and hence cineole) concentration of the diet (Figure 4a and b).

*Stage 5. Postreconditioning Aversion to Cineole.* After reconditioning, the aversions of animals to cineole in the treatment groups for both species matched those of the animals in the control groups that had never had an opportunity to learn that cineole is not always associated with negative postingestive feedbacks (Figure 2c[i] and [ii]).
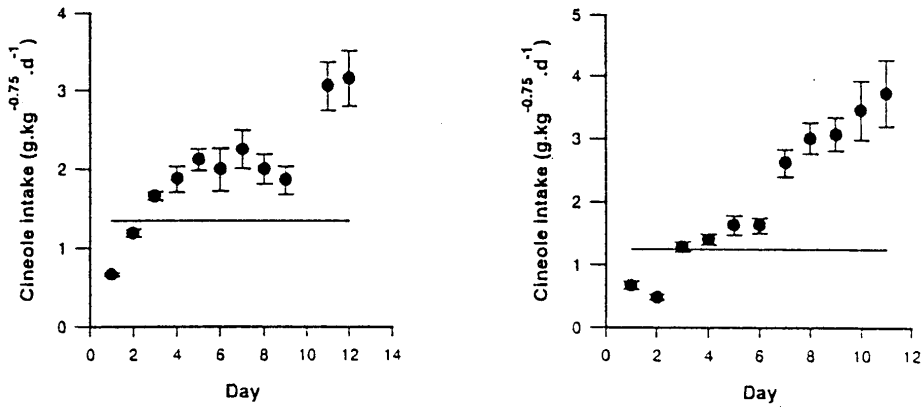
FIG. 3. Intake of 1,8-cineole by common ringtail possums (left) and common brushtail possums (right) during acclimation to increasing dietary cineole. Solid lines represent previous mean maximum intake of cineole by the same animals. Data are means ± standard errors.

## DISCUSSION

The data presented here show that the feeding deterrence of terpenes in *Eucalyptus* is mediated largely through a conditioned flavor aversion. This aversion can be removed and then reconditioned by dissociating the taste and toxic stimuli and then reinstating the association. Several studies have examined the
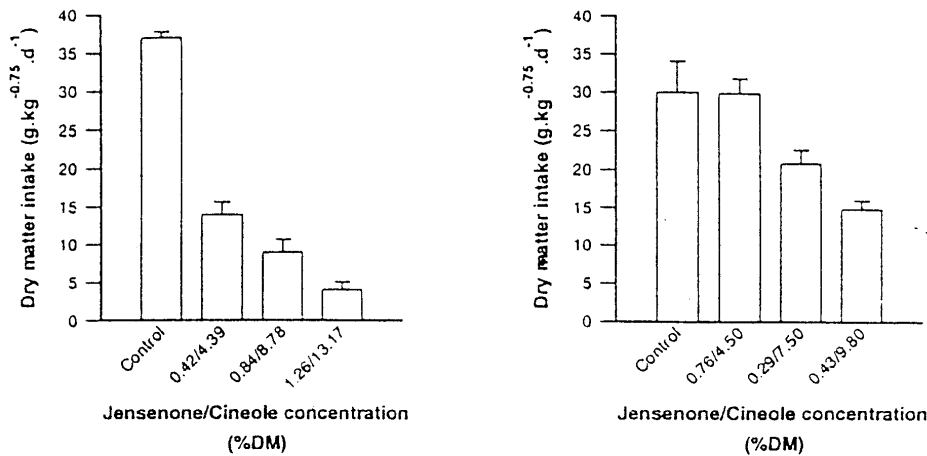


FIG. 4. Effect of 1,8-cineole and jensenone in combination on food intake of treatment animals only for common ringtail possums (left) and common brushtail possums (right). First column shows intakes of animals in the control group concurrent with measured intakes of animals in the treatment group. Data are means ± standard errors.

conditions required to produce a flavor aversion in mammals, but we believe that this is the first study to identify both a salient taste and a toxin that influence a natural plant–herbivore interaction. In studies of browsing ruminants, there have been examples of identified aversive compounds (Kronberg et al., 1995; Provenza et al., 1994a), and it has been suggested that the degree of aversion may relate to the concentration of a salient flavor; however, the compound responsible for the flavor was not identified (Provenza et al., 1994a). It has also been shown that, when flavor and toxins are highly correlated, herbivores learn to regulate food intake based on interactions between postingestive feedback and flavor and then adjust intake on the basis of changes in flavor alone (Launchbaugh et al., 1993).

In *Eucalyptus*, the underlying negative feedback is caused by the DFPCs and this causes an aversion to the taste of terpenes, which occur in concentrations correlating with the DFPCs in the three eucalypt species studied to date (Lawler, Foley, Eschler, unpublished data). The aversion to the taste of cineole is dose-dependent: animals eat less of diets with high concentrations of cineole, apparently conditioned to a potentially greater "internal malaise" (sensu Provenza, 1995). It has been shown that one cause of CFAs is stimulation of the emetic system of the body (Provenza et al., 1994b). This appears to be at least part of the mechanism for conditioning an aversion due to jensenone (and, we suspect, other DFPCs). Administration of an antiemetic drug (ondansetron, an antagonist of the $5HT_3$ serotonin receptors) allowed both common ringtail and common brushtail possums to significantly increase their intake of a jensenone-treated diet relative to animals given only the treated diet (Lawler et al., 1998b).

The role of terpenes in deterrence of mammalian folivores of *Eucalyptus* appears to have been overstated (Lawler and Foley, 1998). They appear not to be the primary cause of the deterrence, but rather their strong, overriding smell and taste serve as a cue to levels of the true deterrent compounds. Whereas it has been shown that there are physiolgoical costs associated with the detoxification of terpenes by these animals (Foley, 1987, 1992), no study to date has examined whether these costs are indeed limiting to food intake of the animals (Foley and MacArthur, 1994). The limiting cost would probably not be reached in the natural diet, in which concentrations of terpenes rarely exceed 5% (Hume and Esson, 1993; Lawler et al., 1998a), since this was clearly not deterrent to either the common ringtail possums or the brushtail possums in this study. In contrast, previous work (Lawler et al., 1998a) and ongoing work (Lawler, unpublished) show that the DFPCs can deter marsupials from eating when applied to food in ecologically realistic concentrations.

Assays of the terpene content of leaves may still be useful if we use them as do the animals, as a de-facto indicator of leaf toxicity. The DFPCs have been detected in 38 of 40 *Eucalyptus* species examined (Eschler, Pass, Foley, unpublished). In the three eucalypt species studied in detail to date, the concentrations

of the two groups of compounds are closely correlated (Lawler, Foley, Eschler, unpublished data). However, the threshold DFPC (and terpene) concentration that results in a reduction in food intake may vary between different species of *Eucalyptus*. Whereas the terpene and DFPC concentration of the foliage within a single species correlate strongly, the slope of this relationship appears to vary significantly between species (Lawler, unpublished data). Furthermore structural diversity of DFPCs is such that the biological activities of the different DFPCs may vary as well. The biosynthesis of the DFPCs and the biochemical relationships between the DFPCs and terpenes is an area needing further study.

This begs the question of whether high concentrations of terpenes can confer resistance to herbivores in the absence of DFPCs. Can susceptible plants of the same or different species as resistant ones use mimicry to escape herbivory? Launchbaugh and Provenza (1993) concluded that flavor mimicry could reduce the amount of damage that herbivores inflict on plants if those plants had a similar taste and odor to genuinely resistant plants. It is conceivable that plants rich in terpenes but poor in DFPCs could gain some measure of protection from marsupial folivores, especially if the different types (low or high DFPC) are individuals of the same species, with very similar terpene profiles. However, CFAs are rapidly extinguished under a range of conditions, and there are restrictions also on the effectivenes of mimicry, which are considered below.

While the long-lived nature of CFAs has been emphasized in the past, recent work emphasizes the dynamic nature of foraging in environments where toxins and nutrients are spatially heterogeneous, requiring constant reassessment of the consequences of ingestion of particular foods (Wang and Provenza, 1997). Repeated exposure to the flavor stimulus alone leads to rapid reduction in the strength of the aversion (O'Connor and Matthews, 1997; this study), and several situations may arise that encourage animals to resample a diet possessing a flavor that previously led to an aversion. Where the amount of nonaversive food is limited, hunger will force the animals to sample aversive foods (Provenza, 1996), and, in fact, common ringtail possums continued to sample a diet containing toxins even when a toxin-free control was available *ad libitum* (D. Pass, personal communication). In free-ranging aninrals the social context is also important. For example, when associated with animals not possessing the aversion, averted cattle lose an aversion more rapidly (Ralphs, 1997).

Mimicry frequently requires a high proportion of the toxic model before the mimic gains significant advantage (e.g., Lindstrom et al., 1997). It seems likely that flavor mimicry in plants would be an effective strategy to avoid herbivory only when the proportion of mimics in the habitat is very low relative to truly toxic plants of the same flavor. This would be especially true where the herbivore can also incorporate spatial information about the models and the mimics. It is likely also to be necessary to have an abundance of alternative palatable food available to prevent the animals from needing to sample unpalatable items. Ter-

penes themselves are therefore unlikely to constitute an effective defense against folivorous possums.

The concepts discussed above may prove useful in the management of folivorous possums where they occur as pests of commercial *Eucalyptus* plantations. Commercial operations have suffered significant losses due to predation of trees by marsupials (Montague, 1994), as well as from chewing insects. The use of genotypes that are naturally resistant to herbivores may be one means of managing these losses, and entomologists have suggested selecting for cineole-rich genotypes that may deter some insect herbivores (Edwards et al., 1993; Farrow, 1993). This may be successful in providing cross-resistance against marsupial browsers in the short-term but, given our current lack of knowledge of the biosynthesis of the DFPCs (Ghisalberti, 1996), we cannot safely assume that selection for high terpene plants will always result in high DFPC plants. Considerable effort may be expended on producing genotypes that have substantial leaf terpenes but insufficient DFPCs to deter marsupial folivores in the longer term. Folivorous marsupials living within or adjacent to these kinds of trees may quickly lose any aversion based on the taste of terpenes.

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