Australian Forestry Vol 62, No. 1 pp. 17–20

Swamp wallabies and Tasmanian pademelons show intraspecific preferences for foliage

Ivan R. Lawler and William J. Foley

Division of Botany and Zoology, Australian National University, 0200, Canberra, ACT

Revised manuscript received 9 September 1998

Summary

A preliminary experiment is reported in which differences in the palatability of the foliage of individual *E. ovata* trees were tested, using cafeteria-style experiments with captive swamp wallabies (*Wallabia bicolor*) and Tasmanian pademelons (*Thylogale billardierii*). Strong and consistent preferences were shown by both macropod species. These preferences were not correlated with any of the conventional measures of nutritional quality or putative defence compounds often made on *Eucalyptus* foliage. However, the preferences shown by these macropods correlate strongly with preferences shown by common ringtail possums (*Pseudocheirus peregrinus*), an interaction which is far better understood. Thus the same chemical factors may influence the palatability of the foliage for all three marsupial species. Continuing research on the relationship between chemical compounds in *Eucalyptus* foliage and the palatability of the foliage for browsing animals could potentially yield significant advantages in plantation pest management. The utility of near infrared (NIR) spectroscopy as a tool for screening plants for resistance to browsing is also discussed.

Introduction

Browsing of seedlings by marsupials is a major obstacle to the establishment of viable *Eucalyptus* plantations (Wilkinson & Neilsen 1995). Even non-fatal browsing by an animal is sufficient to reduce the future timber value of a seedling through its effect on the form of the tree (Montague 1996). This damage, inflicted in many cases principally by macropods, causes losses estimated at up to $400/ha in Victorian plantations (Montague 1996) and the failure of up to 63% of unprotected areas in Tasmania (Neilsen and Wilkinson 1995). Consequently, methods to reduce or eliminate marsupial browsing have been a primary focus of research. Methods such as fencing or poisoning are considered less than ideal due to their cost and negative public perceptions respectively (Montague 1993, 1996; Wilkinson & Neilsen 1995).

One approach often suggested for dealing with this problem, for both vertebrate and insect herbivores, is to exploit natural resistance to herbivory (Floyd & Farrow 1994; Montague 1994). Field studies have shown that some *Eucalyptus* species display very strong intraspecific (i.e. between individuals) variation in their susceptibility to browsing by marsupials (e.g. Hindell & Lee 1987; Pahl 1987). If the factors causing natural resistance can be identified, then breeding programmes may select for these traits, conferring a resistance which costs little in dollar terms and minimises the impact on the environment (Floyd and Farrow 1994). However, the allocation of resources to defensive chemistry by the plants may incur a trade-off in growth (Gulmon & Mooney 1986). The relative costs of these trade-offs must be assessed in terms of plantation viability and growth rates of susceptible genotypes under field conditions.

A significant constraint to this approach is our poor understanding of what chemical factors in *Eucalyptus* foliage confer resistance to marsupial herbivory. The foliage of eucalypts has long been recognised to be rich in terpenes and phenolics and research into food preferences of marsupial herbivores has focussed on these groups of compounds. However, few data have accumulated to support a significant role for these compounds (e.g. Cork & Sanson 1990; Hume & Esson 1993). Recently, it has been shown that the there is a well-defined group of chemical compounds which can determine the rate at which arboreal marsupials can ingest foliage, and which, in some individual trees, occur in concentrations sufficient to completely deter feeding (Lawler 1999; Lawler *et al.* 1998a; Pass *et al.* 1998).

In this paper, we report on the high degree of variation in palatability of individual trees of *E. ovata* to swamp wallabies (*Wallabia bicolor*) and Tasmanian pademelons (*Thylogale billardierii*). We show that the ranking of palatabilities of individual trees is highly consistent between macropod species, and also between the macropods and common ringtail possums (*Pseudocheirus peregrinus*). We discuss factors likely influencing palatability of *Eucalyptus* foliage to macropods, in relation to the more detailed available knowledge of such factors affecting possums and koalas.

Methods

Animals

This experiment was approved by the Animal Experimentation Ethics Committee of Monash University and conforms with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Four adult male swamp wallabies and six Tasmanian pademelons (4 female, 2 male) were housed in separate outdoor enclosures at Monash University in Melbourne. Enclosures measured approximately 15 m x 20 m and had a thick covering of grass. Pelleted foods and water were supplied *ad libitum*. To reduce disturbance to the animals, body mass was not directly measured but average weight was approximately 17 kg for swamp wallabies, 7 kg for male Tasmanian pademelons and 3.9 kg for female Tasmanian pademelons (Strachan 1983).

Eucalyptus ovata was chosen as the study species based on prior knowledge of the way food intake of ringtail possums varied from tree to tree within this species (Lawler *et al.* 1998a). Although *E. ovata* is not a commercial species, we considered that it was more useful to begin these investigations with a species that we knew to exhibit significant variation in deterrence, to identify a chemical principle, and then to look for that chemical in other eucalypts of commercial value. Foliage of six individual trees of *E. ovata* were collected from areas within 20 km of Monash University. Trees were chosen on the basis of an abundance of young, fully expanded foliage and on data from feeding experiments with common ringtail possums (reported in Lawler *et al.* 1998a). All were healthy adult trees (6-12 m) growing in natural conditions.

A cafeteria-style experimental design was used in which six feeding stations were established at even intervals around the perimeter of the pens. Each station consisted of a bucket of water into which branches approximately one metre long were placed and fastened to the fence to prevent their ends coming out of the water. A sample of foliage from one tree was placed at each feeding station, such that foliage from all trees was offered each night. The experiment was repeated over 6 nights, with positions of foliage re-randomised each night, ensuring that all trees were positioned at all stations by the end of the experiment. Foliage was put in the pens at 1700h and removed the following morning at 0800h. The amount of foliage offered was determined by preliminary feeding in which an abundance of highly palatable foliage was offered and the amount eaten recorded. Branches were weighed before placing in cages and after removal. Leaves dropped on the ground around each station were collected and dried at 80°C for 24 hours. Separate buckets in unoccupied pens were used with foliage samples to correct for evaporative loss. A sample of this foliage was collected and frozen for later chemical analysis and determination of appropriate dry matter conversion by drying a subsample of foliage. Differences in food intake between trees were compared by analysis of variance, with data transformed by log(x+1).

Chemical analysis

All samples were freeze-dried and ground to pass a 1 mm sieve. Samples were analysed for nitrogen, cyanogenic glycosides, total phenolics, condensed tannins, neutral detergent fibre, cellulose, hemicellulose and lignin as described in Lawler *et al.* (1998a). Relationships between food intake and leaf chemistry were compared by Pearson's correlations.

Comparison of intakes of *Eucalyptus ovata* foliage by macropods and ringtail possums

These experiments were run in conjunction with similar, but more comprehensive experiments using common ringtail possums, as described in Lawler *et al.* (1998a). Hence we were interested to know if those trees which showed resistance to browsing by the macropods were also resistant to browsing by ringtail possums. The experiments with ringtail possums employed a slightly different protocol, in which foliage from only a single tree was offered to the animals each night (Lawler *et al.* 1998a). The small size and low energy reserves of ringtail possums meant that we were unable to offer them the three least palatable trees offered to macropods because they would not eat enough to maintain themselves. A direct comparison is hence not possible. However, we have recently developed a technique for predicting food intake of common ringtail possums using near infrared reflectance (NIR) spectroscopy, calibrating food intakes against leaf chemistry as reflected by differences in sample spectra (McIlwee *et al.* 1999). This model is highly accurate, producing an r^2 value of the relationship between observed and predicted values of 0.94. We have used this equation to estimate the ringtail possum intakes of samples of this foliage in order to compare preferences across taxonomic groups.

Results

Both swamp wallabies ($F_{5,25}$ = 19.00, p<0.001; Fig. 1a) and Tasmanian pademelons ($F_{5,25}$ = 5.92, p=0.001; Fig. 1b) showed strong preferences between trees which were consistent between the two macropod species (r=0.93). Similarly, the preferences of both macropods were highly consistent with the preferences predicted to be shown by common ringtail possums (swamp wallabies r=0.97; Tasmanian pademelons r=0.91). No relationships were found between food intake by macropods and any of the leaf chemical measures made.

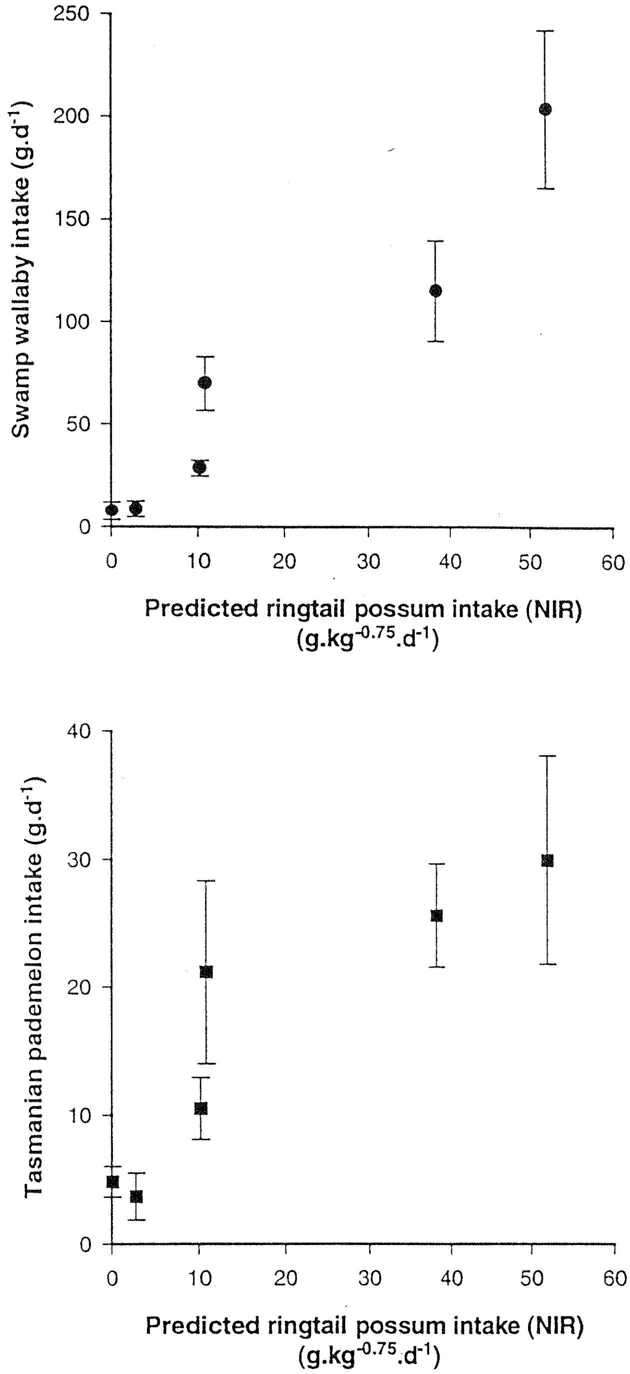

Figure 1. Intake of foliage of six individual *E. ovata* trees by a) swamp wallabies and b) Tasmanian pademelons in cafeteria-style experiments. Data are plotted using intakes by ringtail possums (as predicted by NIR spectroscopy) as the X axis to show the correlation between these measures. Data are means ± standard errors.

Discussion

Variation in the palatability of different species of *Eucalyptus* to swamp wallabies, one of the major pests of eucalypt plantations, has previously been investigated and found to be significant (Montague 1994). We have now shown that there are significant differences in palatability between individual *E. ovata* trees which appear stronger than the interspecific differences reported by Montague (1994); these differences are likely to be even greater in a field situation (Montague 1994). If such variation can be found within commercial species (e.g. *E. regnans, E. nitens, E. globulus*) then there could be the potential to exploit this in breeding programmes to confer natural resistance to macropod browsing of eucalypt seedlings in plantations. Further, it would appear that selection of trees resistant to macropods will also have the added benefit of conferring resistance to browsing by arboreal marsupials. However, to do this effectively requires that such variation be found, and ideally, that the chemical attributes causing it be identified.

In this study, none of the chemical characteristics measured showed any relationship with palatability. However, the trend in palatability was strongly consistent with levels of resistance to herbivory by common ringtail possums, an interaction which is now far better understood (Lawler 1999; Lawler *et al.* 1998a,b, 1999; Pass *et al.* 1998). Given such consistency, it is reasonable to surmise that the same chemical features are responsible for a reduction in palatability of *E. ovata* foliage to macropods. In experiments in which *E. ovata* foliage was fed to ringtail possums, two groups of chemical compounds correlated negatively with possum herbivory (Lawler *et al.* 1998a). These were the terpenes and a recently discovered group of compounds known as diformylphloroglucinol compounds (DFPCs). It was further shown that only the DFPCs could reduce food intake similarly in bioassay experiments. Two other species of marsupial browsers, the koala (*Phascolarctos cinereus*) and the brushtail possum (*Trichosurus vulpecula*) also reduce food intakes in response to dietary DFPCs (Lawler *et. al.* 1998a,b, 1999). In addition, concentrations of the two groups of compounds are correlated (Lawler 1999) and ringtail possums appear to use the smell and taste cues of the foliar terpene concentration as a proximal cue to the concentration of the real deterrent, the DFPCs (Lawler *et al.* 1999). We were unable to measure the specific DFPC (macrocarpal G: Lawler *et al.* 1998a) likely to cause the effect seen in this experiment due to insufficient sample for gravimetric isolation (Pass *et al.* 1998), and because a suitable quantitative method has not yet been developed. We consider that variation in the concentration of DFPCs, and macrocarpal G in particular, in foliage of the individual *E. ovata* trees used here is the most likely explanation for the variation in feeding rates of macropod browsers.

Further investigation of the effect of DFPCs on macropod feeding, and variation in their concentrations in commercial eucalypt species, would potentially yield significant advantages in plantation pest management. Other commercial species, *E. globulus, E. delegatensis* and *E. nitens* contain DFPC compounds, the latter two having predominantly sideroxylonals (Pass, Eschler, Foley unpublished data), a group of DFPCs which is highly effective in deterring common ringtail possums from feeding (Lawler 1999). Further, in all DFPC-containing species which we have investigated, we have found significant intraspecific variation in concentrations, covering a range that we consider to confer resistance. There are also strong indications of a genetic basis determining the concentrations of DFPCs in foliage, which would make the system amenable to breeding programmes. We have often noted both susceptible and resistant individual trees of the same species growing side by side in nature, where microclimatic conditions could not vary significantly. A survey of 87 *E. polyanthemos* trees in one 100 x 50 m site observed sideroxylonal concentrations that varied from nil to over 12 mg/g dry matter (a concentration at which ringtail possums would be unable to feed) (Lawler 1999). The frequency distribution of foliar sideroxylonal concentrations in this population was approximately normal, indicating a multi-locus genetic basis (Lawler 1999).

One of the greater obstacles to making progress in the development of browser-resistant eucalypt plantations is the identification of resistant individuals within each eucalypt species. However, there are two alternatives which may prove practical. The first is the selection of high-terpene genotypes. As described above, browsing by ringtail possums correlates negatively with terpene concentrations while DFPC concentrations are positively correlated with terpenes. As a defacto measure of marsupial resistance, the foliar terpene concentration is useful (Lawler *personal observation*). However, the biochemical relationship between the two groups of compounds is unknown (Ghisalberti 1996). Thus there may be the potential for selection of high-terpene genotypes to cause some uncoupling of the relationship, perhaps resulting in selection for high-terpene, low-DFPC genotypes which ultimately are not resistant to browsing (Lawler and Foley *in press*).

A more rigorous technique for identifying and quantifying resistance is NIR spectroscopy. The technique is fully described in Foley *et al.* (1998), but in essence provides a means for the rapid and accurate analysis of large numbers of samples for multiple chemical components. Samples are irradiated with NIR light and light is absorbed by the material at wavelengths that correspond to particular types of chemical bonds (Foley *et al.* 1998). The resulting spectrum reflected from the sample thus gives a composite measure of leaf chemistry, which can be calibrated against laboratory reference values to develop statistical models for the estimation of those features in new samples. There is minimal sample preparation and no use of laboratory reagents, thus conferring significant cost and time savings in analysis. Conventional measures of leaf chemistry can be made in this manner and so to can DFPC compounds be rapidly and precisely quantified (Lawler 1998). The other substantial advantage of using NIRS is that functional attributes, such as potential animal intakes (as we have done here; McIlwee *et al.* 1999; Lawler 1999) can be calibrated against NIR spectra, even when the specific chemical basis is unknown (Foley *et al.* 1998 and references therein). Therefore, while it is clearly desirable to develop a strong mechanistic understanding of the characteristics governing susceptibility of *Eucalyptus* to macropod browsing, it is not essential before NIR spectroscopy can be used as a functional tool for screening genotypes for inclusion in breeding programmes. For this purpose, we need only identify and quantitatively measure, via bioassay experiments, varying levels of resistance within commercial species and calibrate these against their NIR spectra.

In conclusion, there exists significant variation in palatability of individual trees of *E. ovata* to macropod browsers, and this is likely to occur in other, commercial *Eucalyptus* species. Further work is required to identify this variation, and to define its chemical basis, however the concentration of DFPCs at this stage offers the most likely explanation. NIR spectroscopy offers significant potential for developing the means to assess resistance to browsing, both on a mechanistic level and as a purely functional tool.

Acknowledgments

This work was funded by a grant from the Australian Research Council to WJF. We wish to thank Gordon Sanson and Graeme Farrington, of the Department of Biology, Monash University for allowing us the use of their animal-holding facilities. David Pass (James Cook University) and David de Little (North Forest Products) contributed useful discussion on these topics.

References

Cork, S.J. and Sanson, G.D. (1990). Digestion and nutrition in the koala: a review. In (A.K. Lee, K.A. Handasyde and G.D. Sanson eds.) *Biology of the Koala*. pp. 129-144, Surrey Beatty and Sons, Chipping Norton, NSW.

Floyd, R.B. and Farrow, R.A. (1994). The potential role of natural insect resistance in the integrated pest management of eucalypt plantations in Australia. In (S.C. Halos, R.R. Natividad, L.J. Escote-Carlson, G.L. Enriquezand I. Umboh eds.) *Proceedings of the symposium on biotechnological and environmental approaches to forest pest and disease management.* pp. 55-76, Quezon City, Philippines.

Foley, W.J., McIlwee, A.P., Lawler, I.R., Aragones, L., Woolnough, A.P. and Berding, N. (1998). Ecological applications of near infrared spectroscopy - a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia* **116**:293-305.

Ghisalberti, E.L. (1996). Bioactive acylphloroglucinol derivatives from *Eucalyptus* species. *Phytochemistry* **41**:7-22.

Gulmon, S.L. and Mooney, H.A. (1986). Costs of defense and their effects on plant productivity. In (T.J. Givnish ed.) *On the Economy of Plant Form and Function*. pp. 681-698, Cambridge University Press, Sydney.

Hindell, M.A. and Lee, A.K. (1987). Habitat use and tree preferences of koalas in a mixed eucalypt forest. *Australian Wildlife Research* **14**:349-360.

Hume, I.D. and Esson, C. (1993). Nutrients, antinutrients and leaf selection by captive koalas (*Phascolarctos cinereus*). *Australian Journal of Zoology* **41**:379-392.

Lawler, I.R., Foley, W.J., Eschler, B.M., Pass, D.M. and Handasyde, K. (1998a). Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116**:160-169.

Lawler, I.R., Foley, W.J., Pass, G.J. and Eschler, B.M. (1998b). Administration of a $5HT_3$ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials. *Journal of Comparative Physiology B* **168**: 611–618.

Lawler, I.R. (1999). Variation in Marsupial Folivory Between and Within *Eucalyptus* Species: The Roles and Actions of Plant Secondary Metabolites. PhD Thesis. The Australian National University, Canberra, Australia.

Lawler, I.R., Stapley, J., Foley, W.J. and Eschler, B.M. (1999). Ecological example of conditioned flavor aversion in plant-herbivore interactions: effect of terpenes of *Eucalyptus* on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology* **25**: 401–405.

Lawler, I.R., Foley, W.J. (*in press*). Chemical ecology of *Eucalyptus* essential oils in plant-herbivore interactions. In (J. Coppen ed.) *Medicinal and Aromatic Plants - Industrial Profiles:* Eucalyptus. Hardwood Academic Press, UK.

McIlwee, A.P., Lawler, I.R., Cork, S.J. and Foley, W.J. (1999). Coping with chemical complexity in mammal-plant interactions: Near infrared spectroscopy as a predictor of foliar nutrients and of the feeding rates of folivorous marsupials. *Oecologia* submitted.

Montague, T.L. (1993). An assessment of the ability of tree guards to prevent browsing damage using captive swamp wallabies (*Wallabia bicolor*). *Australian Forestry* **56**:145-147.

Montague, T.L. (1994). Wallaby browsing and seedling palatability. *Australian Forestry* **57**:171-175.

Montague, T.L. (1996). The extent, timing and economics of browsing damage in eucalypt and pine plantations of Gippsland, Victoria. *Australian Forestry* **59**:120-129.

Neilsen, W.A. and Wilkinson, G.R. (1995). Browsing damage, site quality and species selection — a case study of their effects on the economic viability of eucalypt plantations in north eastern Tasmania. In *Proceedings of the 10th Australian vertebrate pest control conference*, pp. 161–170 Department of Primary Industries and Fisheries, Tasmania

Pahl, L.I. (1987). Feeding behaviour and diet of the common ringtail possum, *Pseudocheirus peregrinus,* in *Eucalyptus* woodlands and *Leptospermum* thickets in Southern Victoria. *Australian Journal of Zoology* **35**:487-506.

Pass, D.M., Foley, W.J. and Bowden, B. (1998). Vertebrate herbivory on *Eucalyptus* - identification of specific feeding deterrents for common ringtail possums (*Pseudocheirus peregrinus*) by bioassay-guided fractionation of *Eucalyptus ovata* foliage. *Journal of Chemical Ecology* **24**:1513-1527.

Strachan, R. (1983). *Complete Book of Australian Mammals.* Angus and Robertson Publishers. Sydney.

Wilkinson, G.R. and Neilsen, W.A. (1995). Implications of early browsing damage on the long term productivity of eucalypt forests. *Forest Ecology and Management* **74**:117-124.