



Identifying Pest Resistant Eucalypts

using near-infrared spectroscopy

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and Development Corporation**

by Robert B. Floyd and William J. Foley

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Foreword

In agroforestry and farm forestry, the traditional method of managing insect and vertebrate pests is the application of chemical pesticides. However, spraying chemicals is costly to plantation owners and also incurs hidden costs in the form of health hazards and loss of biodiversity.

Incorporating naturally occurring resistant *Eucalyptus* plants in plantations has been proposed as an alternative to spraying. The Joint Venture Agroforestry Program (RIRDC/LWRRDC/FWPRDC) commissioned this project to develop a method of rapid identification of plant resistance using a newly available technology, near-infrared reflectance spectroscopy (NIRS).

This report is a summary of two and half years of research, examining natural variation in resistance in selected *Eucalyptus* species to insect and vertebrate pests, mechanisms of resistance, and development of a method of rapid identification of resistant trees using NIRS. As a result, more efficient and rapid methods of NIRS analysis of eucalypt foliage have been developed, a better understanding of the variation in chemistry of *Eucalyptus* leaves in plants of different ages and between individual trees has been gained and, the chemical basis of resistance to a number of insect and marsupial herbivores has been elucidated. These advances are critical to the future incorporation of pest resistance into plantation eucalypts and assists with the overall aim of reducing reliance on chemical pesticides.

In addition to the technological and knowledge advances, this report also briefly addresses some aspects of the appropriate deployment of resistant trees, taking account of the ecological and evolutionary implication.

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This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our Joint Venture Agroforestry R&D Program, which aims to integrate sustainable and productive agroforestry within Australian farming systems.

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Peter Core
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Executive Summary

Application of chemical pesticides and poisons to reduce damage by insect and vertebrate pests in plantations is costly to plantation owners and also incurs hidden costs of health hazards and loss of biodiversity. It has been proposed that incorporating naturally resistant trees in plantation is an alternative to applying chemical pesticides. Many *Eucalyptus* species have shown considerable natural differences among individual trees in resistance to insect and vertebrate pests. For example, an eucalypt tree may be completely defoliated by Christmas beetles, while another tree of the same species, and less than five meters away, is hardly attacked. It is this type of natural resistance that we have studied in this project. In southern blue gum (*Eucalyptus globulus*) we observed substantial differences in growth and resistance to insect pests. These differences were found among 18 provenances and individual trees within each provenance.

The objective of this study was to develop our understanding of the mechanisms that confer resistance of eucalypts to both vertebrate and invertebrate herbivores and to assess the potential of near-infrared reflectance spectroscopy as a method of rapid identification of resistant trees for use in agroforestry. We determined the chemical basis for resistance to insect and marsupial folivores in farm forestry. Very little was known about either of these two subjects prior to the commencement of this study and hence this project was very much the pioneering study in this area. An excellent understanding of some important resistance mechanisms and of the application of near-infrared spectroscopy to identifying plants that express key resistance traits has been gained.

Near-infrared spectroscopy proved to be a highly suitable tool for rapidly assessing components of natural herbivore resistance in eucalypts. However, given the poor understanding of the basis of resistance to herbivores in eucalypts and the large number of inconclusive studies that preceded the current work, we felt that it was important to show that the wavelengths that contributed most to the resistance models that we developed were in fact based on known traits. One of the strengths of this work is that the resistance traits that we have demonstrated (e.g. sideroxylonal and cineole) are strongly represented in the NIRS models that we have developed. We are now able to use NIRS to predict resistance rapidly and with great confidence since the resistance traits have been correctly identified.

Different insect and vertebrate pests attack *Eucalyptus* plantings in farm forestry. We have demonstrated substantial cross-resistance between marsupial herbivores and one major group of insect defoliators, the Christmas beetles (*Anoplognathus* spp). This is the first time that cross-resistance has been demonstrated between invertebrate and vertebrate herbivores of *Eucalyptus* and the first time that the chemical basis of cross-resistance between vertebrate and invertebrate herbivores has been identified. Although all the marsupial species examined are susceptible to the effects of sideroxylonal (albeit at different concentrations), we found that a range of different insect defoliators did not respond the same way as Christmas beetles. Given the wide variety of Orders represented and the different evolutionary histories of these species, the lack of full cross-resistance is perhaps not surprising. Nonetheless it is evident from other studies that there are other uncharacterised resistant traits against some of the species that we studied (e.g. *Uraba lugens*).

Major differences between the chemical composition of seedlings and adult leaves of some eucalypt species have been shown (Doran and Matheson 1994). This important trait of eucalypts has only been studied to a limited extent in the past yet is of major ecological importance. It is likely, based on the little available data, that eucalypts undergo major chemical changes at about the time that they finally acquire their adult-type foliage. In this study we have followed a group of *E. sideroxylon* and *E. globulus* seedlings from parents with distinct chemical profiles for 24 months but have yet to detect any significant change in the concentrations of defensive chemistry and many of them are not yet showing the profile of their parents. Nonetheless we believe that it is worthwhile to continue to chart the progress of this material since all available evidence suggests that these resistance traits are

determined predominantly by genetic makeup rather than environmental conditions (Lawler et al. 2000; Adamson, Foley and Woodrow unpublished).

NIRS based models of resistance and resistance traits have been developed. We have improved the methods of acquiring spectra and of building models once those spectra had been acquired, however the measurement of resistance itself through feeding experiments still remains as very time consuming. However, in many cases where we did these experiments, their value was proved since we were able to show that traits that others had claimed as resistant (e.g. cineole for Christmas beetles; Edwards *et al.* 1993) were in fact not causally associated with resistance.

In the case of vertebrates, we believe that there is now sufficient understanding of resistance to be able to recommend that trees expressing high concentrations of sideroxylonal will confer partial resistance to herbivorous marsupials. However, the presence of sideroxylonal (unless in very high concentrations) is not in itself an absolute deterrent. As would be expected, animals can eat some of the compound but the nausea that results if a threshold dose is exceeded is a very strong promoter of future avoidance.

Not all eucalypt species contain sideroxylonal and it is absent from several commercially important species such as *E. globulus*, *E. camaldulensis* and *E. dunnii*. In these species, there is a related group of compounds called macrocarpals that are also believed to confer resistance against marsupial herbivores. Although these are closely related to sideroxylonals they are a more complex group of compounds that are poorly resolved chromatographically. Consequently there is as yet no reliable way of assaying the concentration of the compounds but we do know that NIRS can detect and rank plants containing these compounds.

Finally, whilst we believe that incorporating resistant trees in plantations is an excellent alternative to the use of pesticides, we do caution against uniform and uncontrolled deployment of strongly resistant trees in plantations, for two main reasons. Firstly, since resistance in eucalypts is based on leaf chemistry, trees are not likely to be fast growing and strongly resistant at the same time. This is because growth and resistance require the same resources, and trees normally can not allocate unlimited amounts of resources to these two functions. Secondly, if strongly resistant trees were the only trees planted in plantations, then we might witness rapid counter-adaptation by insect pests. Both these issues can be easily managed in farm forestry by avoiding deploying large areas of uniformly resistant trees expressing the same resistance trait and considering the relative benefits of moderate levels of resistance rather than strong resistance.

1. Introduction

In agroforestry and farm forestry, damage by insects and marsupial pests may result in economic loss. Damage by pests has been shown to reduce growth of trees and sometimes results in death, particularly when young. The most commonly used method of managing pests in agroforestry and farm forestry is application of chemical pesticides. However, use of pesticides can be costly in the absence of effective damage prediction. Thus, to make spraying effective, trees must be sprayed frequently as a preventative measure, or sprayed infrequently but surveyed frequently to detect pests. Moreover, use of pesticides may incur additional costs in the form of health hazard and loss of biodiversity.

An alternative to the use of pesticides is to incorporate naturally resistant trees into plantations. Plant resistance to their pests can be based on physical, chemical or ecological traits. Physical traits such as tough leaves and hairs can make feeding difficult. Chemical traits are based on compounds in plant tissues. These compounds may act as toxins, feeding deterrents, and digestibility reducers that reduce growth of pests. At least some insects use plant chemistry to identify their host plants. Therefore, lack of such attractants may result in 'resistance'. Two examples of ecological traits of resistance are tolerance and rapid growth. Resistant plants may be able to tolerate damage or be able to quickly grow out of susceptible stages such as seedling and sapling stages.

In agroforestry and farm forestry, resistant trees have been selected by screening in provenance trials. Plants from many different provenances are planted in a plantation, and the growth of plants is measured under natural conditions of pest attack. Provenances and individuals showing fast growth have a combination of fast growing traits and pest resistant traits. This process, however, is time consuming as it takes years for trees to grow and reproduce.

In this study an alternative to provenance trials for selecting resistant trees has been explored. Variation in resistance to herbivores between individuals of the same species has been reported in a number of *Eucalyptus* species (*E. melliodora*, *E. conica*, *E. sideroxylon*, *E. blakelyi*, and *E. camaldulensis* (Edwards *et al.*, 1990, 1993); *E. regnans* and *E. nitens*, (Raymond, 1995); *E. blakelyi* and *E. camaldulensis* (Floyd *et al.*, 1994); *E. camaldulensis* (Stone and Bacon, 1994); and *E. globulus* (Farrow *et al.*, 1994); *E. ovata*, *E. viminalis*, *E. sideroxylon* and *E. polyanthemos* (Lawler *et al.* 1998, 2000)). At least in some *Eucalyptus* species, resistance to Christmas beetles has been shown to be related to leaf chemistry (Edwards *et al.*, 1991, 1993). If we were able to identify mechanisms of pest resistance such as some traits of leaf chemistry, and if we were able to detect differences in such traits in seedlings, then we may be able to effectively and rapidly select for resistant plants.

Near-infrared reflectance spectroscopy (NIRS) has been used to characterise chemical composition of plant materials in other industries (e.g., Burns and Ciureczak, 1992; Davies and Williams 1996; Foley *et al.*, 1998). NIRS requires only small samples of a material, and the chemical composition of the material can be determined quickly. Therefore, in theory, we should be able to determine the chemical composition of a leaf from a seedling, identify its resistance, and utilise that knowledge to plant only resistant trees in plantations.

The objective of this study was to develop a method of rapid identification of resistant trees, for agroforestry and farm forestry, using near-infrared reflectance spectroscopy. To achieve this, we took the following three steps:

1. Quantify natural between-species variation in resistance to insect herbivores and marsupial folivores;
2. Determine mechanisms of resistance; and
3. Develop NIRS methods for predicting resistance of untested plants.

A detailed overview of the methodology and results in each of these steps will be described in this report. More extensive details of the studies that constitute this project are presented in Chapters 2 to 7. The final chapter discusses some general issues arising from these studies, presents the overall conclusions and implications and identifies aspects requiring further research.

2. Within species variation in insect damage and growth in *Eucalyptus globulus*¹

Introduction

Plants show considerable variation in growth and susceptibility to herbivores within and between genotypes (Bryant *et al.* 1983, Denno and McClure 1983, Coley *et al.* 1985, Herms and Mattson 1992). It has been hypothesised that variation in susceptibility to herbivores is largely determined by variation in amounts and types of plant secondary metabolites (Fraenkel 1959). Moreover, it is widely accepted that there is a trade-off between plant growth and chemical defence because these functions compete for limiting resources (Bazzaz *et al.* 1987, Chapin *et al.* 1990). Thus, fast growing plants (species, populations, or individuals) are hypothesised to be more susceptible to herbivores than slow growing plants (species, populations, and individuals).

Trade-off may pose an important practical problem in agriculture and forestry, where fast growing genotypes with high yield have been artificially selected and where growth and yield are enhanced by irrigation and fertiliser applications. Moreover, the level of spatial variation in plant chemical defence in crop fields and plantations is typically much less than that in natural environments (e.g., Denno and McClure 1983), and once pest species establish themselves, they can spread quickly and cause considerable damage to crops or plantation trees. Insecticides are used to control pests in fields and plantations. However, periodic large-scale preventative spraying is costly to farmers and plantation owners, as well as to the general public through the hidden cost of environmental damage. To optimise effects of spraying, regular monitoring of population levels of pests has been suggested for certain pest species (Candy *et al.* 1992, Elliott *et al.* 1992). However, it may be costly to conduct frequent pest surveys to detect establishment of pests before they incur substantial damage. One possible approach to reducing dependence on the use of insecticides is to incorporate genetically resistant plants to agriculture and forestry (Floyd and Farrow 1994). Moderate increases in resistance to insect pests might result in equivalent or enhanced growth compared with susceptible genotypes if reduced growth due to resistance in resistant genotypes were less than reduced growth due to insect damage in susceptible genotypes. Also, if a total loss of crops or plantation trees due to pest damage can be avoided by the use of resistant (but slightly slower growing) genotypes, then the net benefit of planting resistant genotypes may be greater than planting susceptible (but fast growing) genotypes.

The objective of this study is to examine a relationship between growth and susceptibility to insect pests in *Eucalyptus globulus* Labill., an important species for plantation in southern Australia, at within and between provenance levels. Considerable variation in susceptibility to various insect pests in *Eucalyptus* spp. has been documented: Between and within species variation in *E. melliodora*, *E. conica*, *E. sideroxylon*, *E. viminalis*, *E. blakelyi*, *E. caliginosa*, and *E. camaldulensis* (Edwards *et al.* 1990, 1993; Lowman and Heatwole 1987); within species variation in *E. regnans*, *E. nitens*, *E. blakelyi* and *E. camaldulensis* (Floyd *et al.* 1994, Stone and Bacon 1994, Raymond 1995); and within and between provenance variation in *E. globulus* (Farrow *et al.* 1994). These studies have shown that within a species, susceptible individuals or provenances suffer at least twice the amount of damage by insect pests as resistant individuals or provenances.

This study directly follows from a previous study of variation in resistance to insect herbivores in *E. globulus* (Farrow *et al.* 1994). The main results of the previous study were:

- (1) Provenances from Tasmania and the Bass Strait islands were more resistant to herbivores than provenances from the mainland Australia (Victoria); and

¹ Based on a manuscript developed by R. B. Floyd, R. A. Farrow, M. Matsuki.

- (2) The resistant provenances showed cross-resistance to two insect species, *Mnesampela privata* Guenée (Lepidoptera: Geometridae) and *Phylacteophaga froggatti* Riek (Hymenoptera: Pergidae). However, tree growth was not included in the previous study.

The main contributions of the present study are:

- (1) Comparison of 18 provenances (all four subspecies) from Tasmania, the Bass Strait islands, and the mainland (Victoria and NSW);
- (2) Examination of the relationships between growth and resistance to insect pests;
- (3) Comparison of within and between provenance variation in growth and resistance to insect pests; and
- (4) Investigation of cross-resistance to another pest species, *Anoplognathus* spp. (Coleoptera, Scarabaeidae).

Materials and methods

Study organisms

Eucalyptus globulus occurs naturally in southeast Australia. Four subspecies have been recognised (Kirkpatrick 1974), and we included all four subspecies (18 provenances) in this study (Figure 2.1): eight provenances of *E. globulus globulus* Labill., four provenances of *E. globulus bicostata* (Maiden *et al.*) Kirkpatr., four provenances of *E. globulus maidenii* (F. Muell.) and two provenances of *E. globulus pseudoglobulus* (Naudin ex Maiden) Kirkpatr.. Except for *E. g. bicostata* which occurs predominantly on plateaus, *E. globulus* tends to occur on mid to lower slopes in sheltered valleys. All subspecies occur in areas with 600 - 1400 mm rainfall and some frosts each winter.

Eucalyptus globulus is considered to be a fast growing species among eucalypts and is an important species for the plantation industry in southern Australia because of its high fibre quality (Cotterill and MacRae 1997). All subspecies typically grow to 30 - 45 m in height, and up to 75 m under favourable conditions. Severe herbivory by *M. privata* and *P. froggatti* on *E. globulus* in plantations have previously been reported (Elliott and Bashford 1978, Farrow *et al.* 1994, Neumann 1993).

Anoplognathus species are common herbivores on several *Eucalyptus* species on farmland and totally defoliate some trees in some years (Carne *et al.* 1974, Edwards *et al.* 1990, 1993). At least seven species of *Anoplognathus* have been observed at and around the study site in Canberra (*A. pallidicollis*, *A. velutinus*, *A. chloropyrus*, *A. hirsutus*, *A. montanus*, *A. suturalis*, and *A. viriditarsis*). Drought in late spring/early summer is correlated with low abundance of *Anoplognathus* spp. (Carne *et al.* 1981). Adults feed on *Eucalyptus* leaves, and larvae feed on roots of grasses and crop plants. Severe defoliation of farmland *Eucalyptus* trees has been considered as a result of changes in land use practice. Increased areas of farmland provide favourable conditions for larval growth, and reduced areas of woodland resulted in high concentration of adults on a small number of farmland trees (Ohmart and Edwards 1991).

Plantation experiment

Seeds were obtained from the Australian Tree Seed Centre, CSIRO Forestry and Forest Products, Canberra, Australian Capital Territory, Australia. Seedlings were planted at Lyneham Ridge, Canberra, Australian Capital Territory (35°14'S, 149°07'E; 620 masl) in May 1994. The study site is located on a small ridge oriented north-south, and the seedlings were planted on an east-facing slope from the top of the ridge downwards. There are six blocks, located along the ridgeline. Within each block, there are 18 plots, one plot for each provenance: six rows down the slope × three plots along the ridgeline. Four seedlings were planted in each plot. Provenances were assigned randomly to the plots. Seedlings were planted 2.5 m apart in each row, and rows were 4 m apart. Two rows of seedlings were planted as a buffer around the experimental blocks. Three blocks were sprayed with insecticide, and

three others were left unsprayed. Spraying treatment was randomly assigned to each block. At the time of planting, seedling height ranged from 14 to 51 cm (mean = 34 cm, SD = 7 cm, $n = 432$).

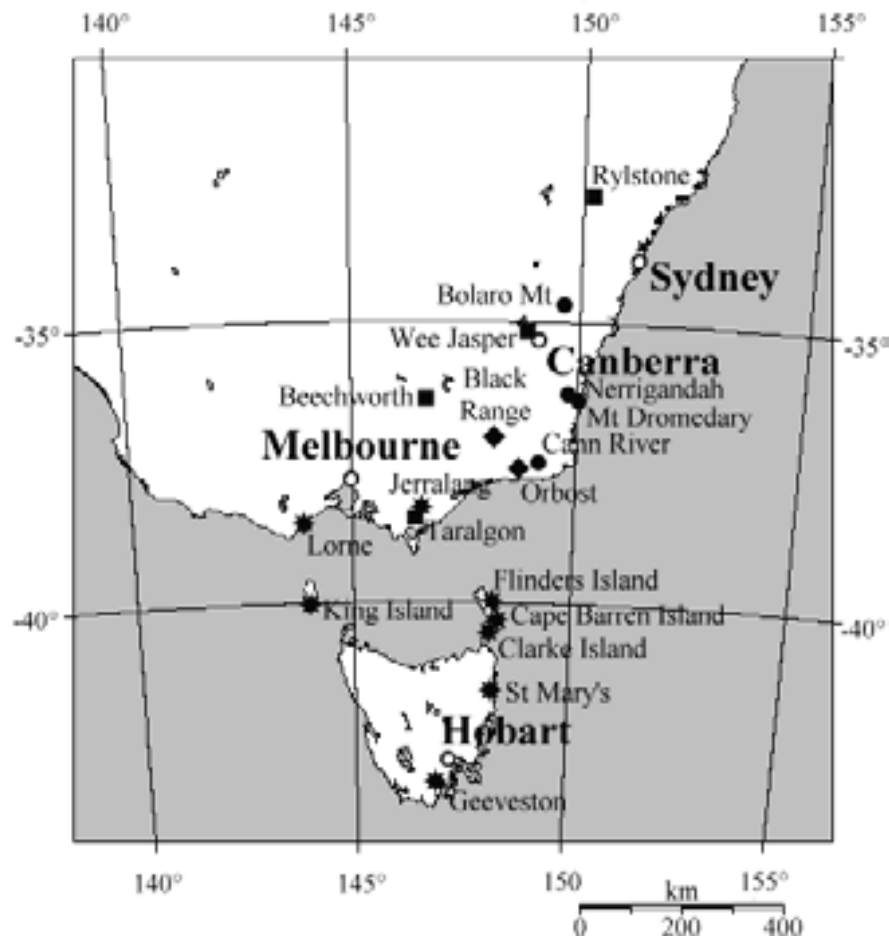


Figure 2.1. Map showing locations of 18 provenances of *E. globulus* used in this study. Key to *Eucalyptus* subspecies: *E. globulus bicostata* ■, *E. globulus globulus* ★, *E. globulus maidenii* ● and *E. globulus pseudoglobulus* ◆.

Height and stem diameter of each plant were measured at the time of planting (May 1994), June 1995, February 1996, August 1996, September 1997, September 1998, and September 1999. Volume was calculated as: $\text{volume} = ((\text{stem radius})^2 \times \pi \times \text{height}) / 3$, and was square root transformed. We used plant growth rate corrected for the initial plant size: $365 \times (\text{vol}(t+1) - \text{vol}(t)) / (\text{vol}(t) \times ((t+1) - (t)))$, where t = date of initial measurement and $(t+1)$ = date of final measurement. Plant volume was square root transformed.

The extent of insect damage was visually assessed at least every six months starting June 1995. Damage assessments were scheduled so as to quantify damage before, during, and after peak periods of herbivory by *Anoplognathus* spp. and *M. privata*. Herbivory on juvenile and adult leaves was assessed separately, and herbivory by each insect species was recorded separately. All damage assessments were conducted by the same observers for consistency. Damage by each species was estimated to the nearest 5%. Separately assessing damage by different insect species was possible because each insect species produced characteristic damage.

Statistical analyses

We examined the effects of spraying insecticide and differences among provenances in growth and resistance to insects. As the study progressed, it became apparent that plants on the top of the ridge were growing slower than those down the slope. Therefore, we first examined effects of plant location using restricted maximum likelihood analysis (REML: Corbeil and Searle 1976). Location of plants, specified by block, row, plot, and tree position, was used as the random effect, and insecticide treatment and population were treated as the fixed effects. Plant growth was significantly affected by location, especially that specified by row and row $\times$ block. Therefore, we estimated mean growth rates of plants for each population using REML. Insect damage was not, however, affected by the location of plants, and therefore, we used analysis of variance (ANOVA) to estimate mean damage for each population.

For the analysis at the level of individuals within each population, we used residuals from trend surface regressions (Legendre 1990, 1993) with the plant growth as the response variable and row, block, and row $\times$ block as explanatory variables. The residuals from the trend surface regressions represent variation in plant growth rates associated with factors other than the physical location of plants within the experimental site. All statistical analyses were performed using Genstat (Genstat 5 Committee 1993) and SAS (SAS Institute 1989).

Results

Insect damage

There was approximately three-fold difference in cumulative damage by insects among 18 provenances of *E. globulus* during the five years of study (Figure 2.2a). There was no clear trend in insect damage with respect to geographic locations or subspecies. The top five resistant provenances are: Geeveston (Tasmania, *E. g. globulus*), Rylstone (Victoria, *E. g. bicostata*), Beechworth (Victoria, *E. g. bicostata*), King Island (Bass Strait, *E. g. globulus*), and Bolaro Mt. (Victoria, *E. g. maidenii*). The five least resistant provenances are: Lorne (Victoria, *E. g. globulus*), Jeeralang (Victoria, *E. g. globulus*), Mt. Dromedary (Victoria, *E. g. maidenii*), Cape Barren Island (Bass Strait, *E. g. globulus*), St. Mary's (Tasmania, *E. g. globulus*).

The largest damage was caused by *Anoplognathus* spp. between December 1995 and January 1996. All except one out of 186 plants in the unsprayed treatment were affected, and some of the plants suffered 85% defoliation (mean = 54%, SD = 20%, $n = 186$). Zero to 88% of the total cumulative damage was caused by this event (mean = 32%, SD = 16%, $n = 186$). Provenances from Tasmania and Bass Strait Islands tended to receive less damage than the mainland provenances (Figure 2.2b). However, provenances from Cape Barren Island and Flinders Island received comparatively more damage in subsequent years. When individual trees were examined separately, very short trees tended to escape from damage by *Anoplognathus* spp., and taller trees tended to receive more damage (Figure 2.3). However, as the trees became taller, more severe damage was found on trees with intermediate height.

Scale insects (*Eriococcus* spp.) also caused extensive damage in 1996 and 97 (Figure 2.2c). Provenances of *E. g. globulus*, especially Jeeralang, Lorne, Clarke Island, and St. Mary's, were affected comparatively more than provenances of other subspecies. The provenance from Mt. Dromedary was heavily damaged by *M. privata*, *P. froggatti*, *Paropsis atomaria* (Coleoptera: Chrysomelidae), and *Gonipterus scutellatus* (Coleoptera: Curculionidae), between 1997 and 99 (Figure 2.2d).

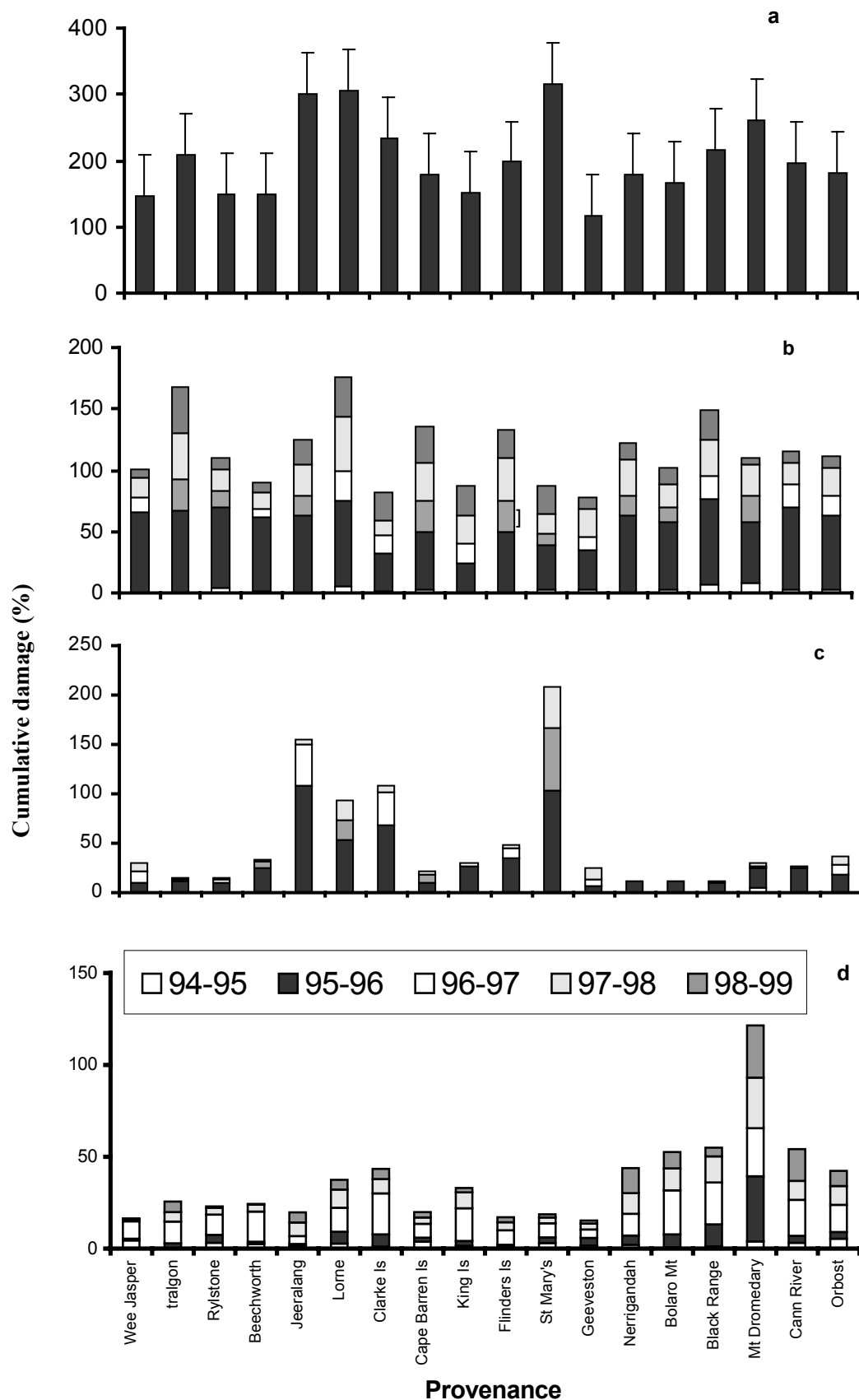


Figure 2.2. Insect damage on 18 provenances of *E. globulus*. (a) Cumulative total damage (Mean + SE) between 1994 and 99. (b) Damage by *Anoplognathus* spp. (c) Damage by *Eriococcus* spp. No damage in 1998-99. (d) Damage by herbivores other than *Anoplognathus* spp.

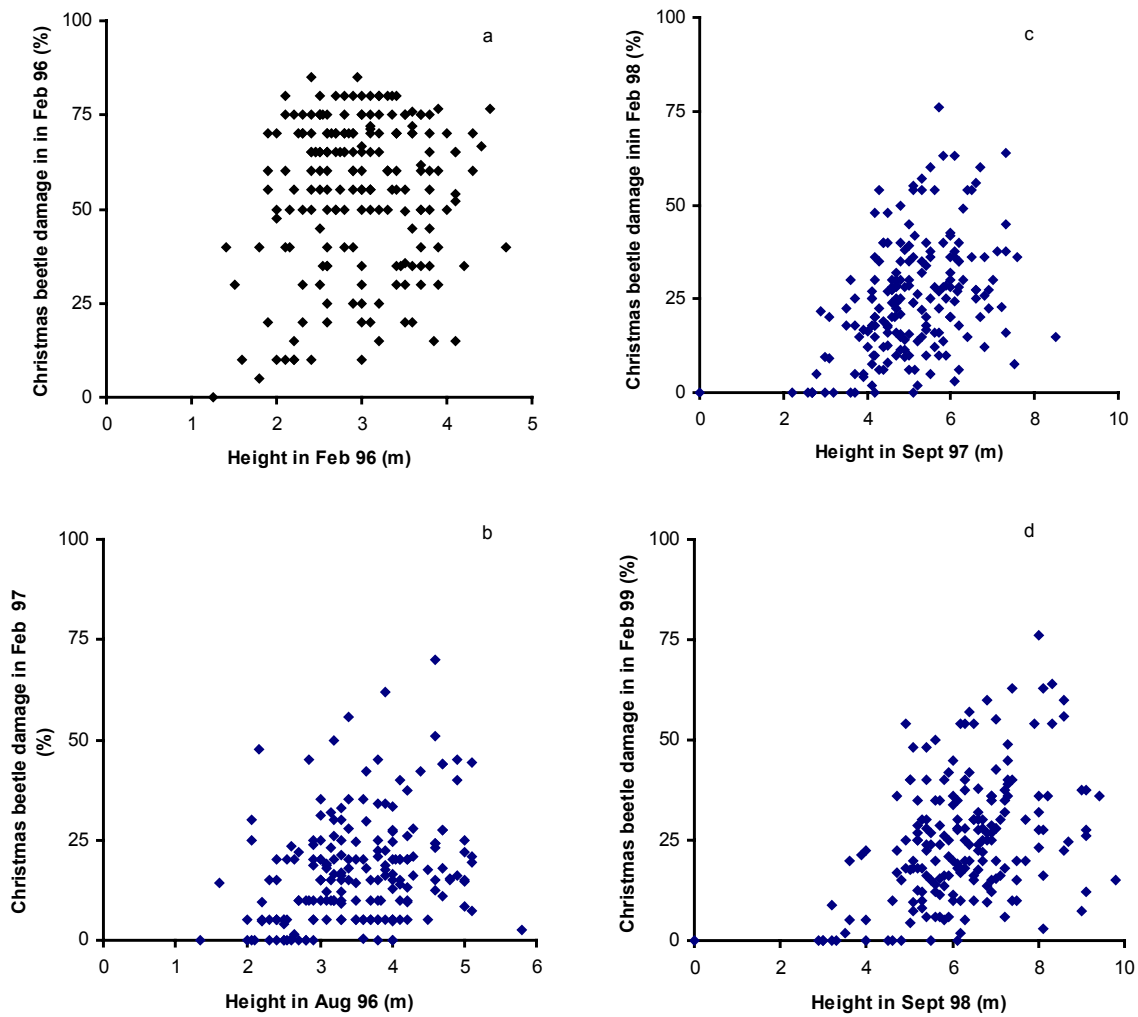


Figure 2.3. Relationship between tree height and damage by *Anoplognathus* spp.

Plant growth

Volume of sprayed plants was significantly different among the 18 provenances (Figure 2.4a) (REML testing for the provenance effect: $\Delta dev = 49$, $df. = 17$, $P < 0.001$). There was a four-fold difference in mean plant volume. Provenances of *E. g. bicostata* and *E. g. globulus* showed variable mean plant volume, while those of *E. g. maidenii* and *E. g. pseudoglobulus* tended to be uniformly small. The top five provenances with large volume are: Geeveston (Tasmania, *E. g. globulus*), Taralgon (Victoria, *E. g. bicostata*), Flinders Island (Bass Strait, *E. g. globulus*), St. Mary's (Tasmania, *E. g. globulus*), and Wee Jasper (N.S.W., *E. g. bicostata*).

Insect damage did reduce plant volume in 12 out of 18 provenances (Figure 2.4a) (REML testing for the overall effect of spraying: $\Delta dev = 4.5$, $df. = 1$, $P = 0.03$). Mean plant growth rates tended to be greater in unsprayed treatment than in sprayed treatment in the first one and half years during which insect damage was minimal (Figure 2.4b) due to damage by insecticides in the sprayed treatment. Sixteen out of 18 provenances show greater mean plant growth rates in sprayed than unsprayed treatment after the major damage by *Anoplognathus* spp. in December 1995 – January 1996 (Figure 2.4c).

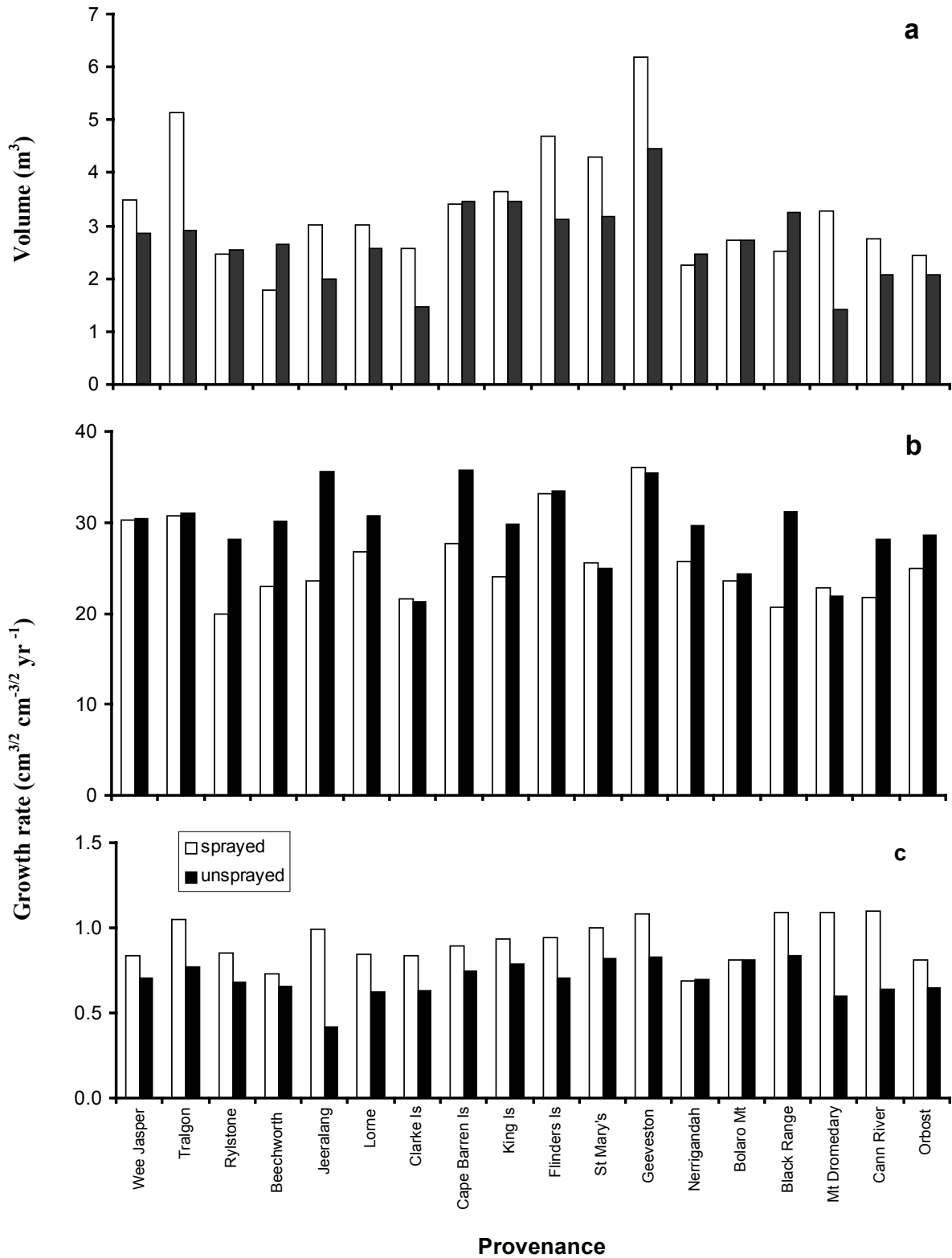


Figure 2.4. (a) Volume of 18 provenances of *E. globulus* after 5 yr and 4 months (b) Relative growth rate between planting and the major damage by *Anoplognathus* spp. (May 1994 – February 1996). (c) Relative growth rate after the major damage by *Anoplognathus* spp. (February 1996 – September 1999).

Based on the mean plant volume of sprayed and unsprayed treatments, we calculated predicted ranking of plant volume under different probability of occurrence of insect damage (Table 2.1). Predicted mean plant volume of a provenance when probability of occurrence of insect damage is p is:

$$\text{Volume}(p) = [\text{mean volume of unsprayed plants} \times p + \text{mean volume of sprayed trees} \times (1 - p)] / 2.$$

Table 2.1. Predicted rank order of 18 provenances of *E. globulus* at different probability of insect attack.

Provenance	Probability of insect attack										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Wee Jasper	6	7	7	7	7	7	7	7	8	8	8
Taralgon	2	2	2	2	2	2	2	3	6	7	7
Rylstone	15	14	14	14	12	11	11	11	11	12	12
Beechworth	18	18	18	18	17	17	15	13	12	11	10
Jeeralang	10	10	10	11	11	12	13	14	15	16	16
Lorne	9	9	8	8	8	9	9	10	10	10	11
Clarke Is	13	15	16	17	18	18	18	18	18	18	17
Cape Barren Is	7	6	6	6	6	6	6	6	3	3	2
King Is	5	5	5	5	5	5	5	4	2	2	3
Flinders Is	3	3	3	3	3	3	3	2	4	5	6
St Mary's	4	4	4	4	4	4	4	5	5	4	5
Geeveston	1	1	1	1	1	1	1	1	1	1	1
Nerrigundah	17	17	17	16	15	14	12	12	13	13	13
Bolaro Mt	12	11	11	9	10	10	10	9	9	9	9
Black Range	14	13	12	10	9	8	8	8	7	6	4
Mt Dromedary	8	8	9	12	14	16	17	17	17	17	18
Cann River	11	12	13	13	13	13	14	15	14	14	15
Orbost	16	16	15	15	16	15	16	16	16	15	14

Regardless of probability of occurrence of insect damage, the provenance from Geeveston is predicted to be the best provenance in terms of plant volume after five years. Provenances from Taralgon, Flinders Island, and St. Mary's are predicted to be good provenances when the probability of occurrence of insect damage is low. In contrast, provenances from King Island, Cape Barren Island, and Black Range are predicted to be good provenances when the probability of occurrence of insect damage is high.

Discussion

Previous plantation experiments had shown that provenances of *E. globulus* from Tasmania and the Bass Strait islands were less susceptible to two other herbivore species, *M. privata* and *P. frogatti*, compared with provenances from mainland Australia (Farrow *et al.* 1994). Results of this study showed that some provenances from Tasmania and Bass Strait islands were also less susceptible to *Anoplognathus* spp. However, there was no clear relationship between damage by *Anoplognathus* spp. and that by other herbivores (e.g., *M. privata*) in this study. It is possible that differential resistance to insects can be observed only when damage levels are above a certain threshold, as evident from damage by *Anoplognathus* spp. in the 1996 – 97 season. Damage by herbivores other than *Anoplognathus* spp. at any one time was much smaller than that by *Anoplognathus* spp. in the summer of 1995 - 96 or damage by *M. privata* and *P. frogatti* described in Farrow *et al.* (1994).

In addition to between-provenance variation, we observed between-individual variation in plant growth and resistance to *Anoplognathus* spp. There has been a suggestion that *Anoplognathus* spp. tend to be attracted to tall trees in a plantation (M. J. Steinbauer, personal communication). However, at least in this study, trees that received most damage tended to be intermediate in their height.

Alternatively, differences among individual trees within each provenance may be due to genetic differences. Although we did not examine the heritability of plant growth and resistance to insect herbivores, a study using *E. regnans* and *E. nitens* showed that the resistance of these two species to *Chrysophtharta bimaculata* Olivier (Coleoptera: Chrysomelidae) was heritable (Raymond 1995). The next step in selection of herbivore resistant *E. globulus* will be to examine the heritability of plant growth and resistance to herbivores.

Although the provenance from Geeveston showed fast mean plant growth and resistance to insects, fast growing individuals and provenances, in general, tended to be more susceptible to insects. This result, combined with reduced growth due to insect damage, may pose a practical problem for plantation owners because fast growing trees are likely to receive more insect damage than slow growing trees. Therefore, selecting the fastest growing trees may not maximise the return if trees are attacked by insects. Unfortunately, insect outbreaks are highly variable in space and time. Perhaps, a safe option for plantation owners is to mix provenances which are less resistant but fast growing such as those from St. Mary's, Taralgon, and Flinders Island and provenances which are more resistant but slower growing such as those from King Island, Black Range, and Cape Barren Island.

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3. Effects of acute damage and chronic damage by insects on growth in *Eucalyptus globulus*²

Introduction

Phytophagous and sap-sucking insects reduce plant growth. However, not all insect damage has the same effect on plant growth. For example, evenly distributed defoliation results in a smaller reduction in the subsequent plant growth and reproduction than the same amount of defoliation concentrated on only one part of a plant (Marquis 1991, 1993; Price and Hutchings 1992; Mauricio *et al.* 1993). In nature, intra-crown variation in defoliation can be caused by spatial variation in insect population densities possibly due to spatial variation in plant leaf chemistry.

Insect populations vary not only in space but also in time. A number of phytophagous insects attack young leaves (Ayres and MacLean 1987; Coley 1980, 1983; Coley and Aide 1991; Landsberg 1988; Lowman 1985; Raupp and Denno 1983). Damage caused during leaf development is hypothesised to have stronger effects on plant growth and reproduction than that caused in other times (Harper 1989; Jurik and Cabot 1986; Krischik and Denno 1983; Mendoza *et al.* 1987; but see Haukioja *et al.* 1990). In certain parts of the world, plants also suffer from periodic or irregular insect outbreaks during which the entire plant or the entire landscape may be defoliated by a single species of insect (Edwards *et al.* 1990, 1993; Tenow 1972). Also, damage can be intense (i.e., occurring in a relatively short period of time) or spread out over a long time (Landsberg 1990; Lowman and Heatwole 1992).

The purpose of this paper is to report results of exploratory data analyses on relationships between growth of a long-lived plant species and acute and chronic insect damage. During a plantation trial of Tasmanian blue gum (*Eucalyptus globulus*) in southeast Australia, to select for populations which are insect resistant, the trial trees suffered small amounts of damage throughout the trial and intense annual defoliation events by Christmas beetles (*Anoplognathus* spp.: Scarabaeidae, Coleoptera) (Floyd *et al.* in prep). In this study, we define acute damage to be defoliation by *Anoplognathus* spp. that occurred annually in mid December to mid January and chronic damage to be damage by other species of insects. *Anoplognathus* spp. typically feed in large groups, and a group of beetles spend time feeding in a patch of trees (e.g., plantations) from a few days to a few weeks. In contrast, chronic damage by other insects such as scale insects (*Eriococcus* spp.: Eriococcidae, Hemiptera) and autumn gum moth (*Mnesampela privata*: Geometridae, Lepidoptera) occurred over much longer time scale (3 months or longer) each year.

Study organisms

Eucalyptus globulus occurs naturally in southeast Australia. Four subspecies have been recognised (Kirkpatrick 1974), and we included all four subspecies (18 populations) in this study (Figure 3.1): eight populations of *E. globulus globulus*, four populations of *E. globulus bicostata*, four populations of *E. globulus maidenii*, and two populations of *E. globulus pseudoglobulus*. Except for *E. g. bicostata* which occurs predominantly on plateaus, *E. globulus* tends to occur on mid to lower slopes in sheltered valleys. New leaves are typically produced from early summer to mid autumn. Populations of *E. g. globulus* from Tasmania and Bass Strait Islands have been shown to be cross resistant to at least three species of major insect pests in southeast Australia: Autumn gum moth (*Mnesampela privata*: Lepidoptera, Geometridae) (Floyd *et al.* 1994), Leaf blister sawfly (*Phylacteophaga froggatti*: Hymenoptera, Pergidae) (Farrow *et al.* 1994), and Christmas beetles (*Anoplognathus* spp.) (Floyd *et al.* in prep).

² Based on a manuscript developed by M. Matsuki, R. A. Farrow and R. B. Floyd

Anoplognathus species are common herbivores on several *Eucalyptus* species on farmland and totally defoliate some trees in some years (Carne *et al.* 1974, Edwards *et al.* 1992, 1993). At least seven species of *Anoplognathus* have been observed in and around the study site (*A. pallidicollis*, *A. velutinus*, *A. chloropyrus*, *A. hirsutus*, *A. montanus*, *A. suturalis*, and *A. viriditarsis*). Adult beetles feed on *Eucalyptus* leaves, and larvae feed on roots of grasses and crop plants.

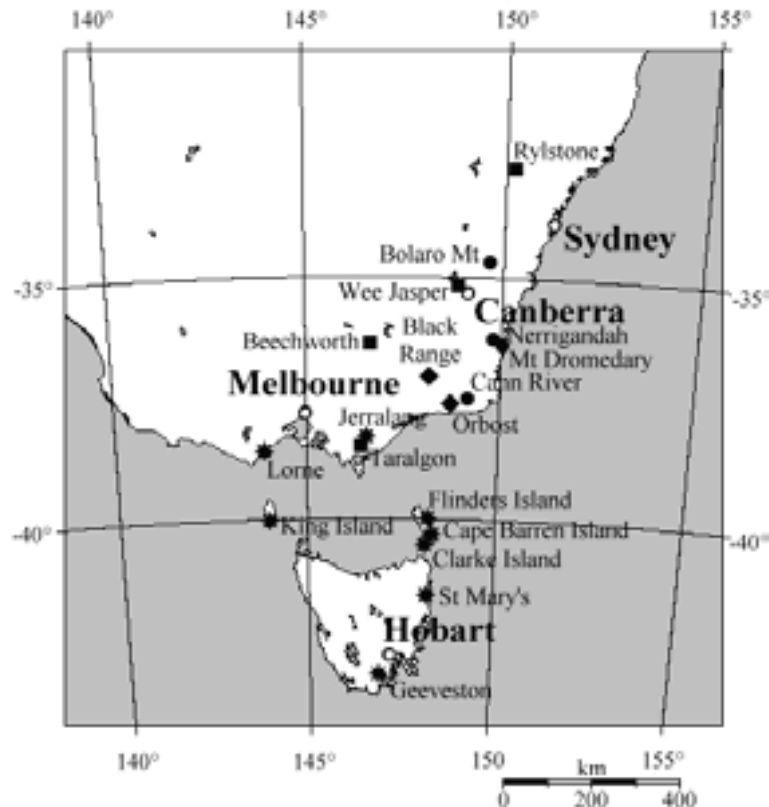


Figure 3.1. Map showing locations of 18 provenances of *E. globulus* used in this study. Key to *Eucalyptus* subspecies: *E. globulus bicostata* ■, *E. globulus globulus* ★, *E. globulus maidenii* ● and *E. globulus pseudoglobulus* ◆.

Methods

Plantation experiment

Seeds were obtained from the Australian Tree Seed Centre, CSIRO Forestry and Forest Products, Canberra, Australian Capital Territory, Australia. Seedlings were planted at Lyneham Ridge, Canberra, Australian Capital Territory (35°14'S, 149°07'E, 620 masl) in May 1994. The study site is located on a small ridge oriented north-south, and the seedlings were planted on an east facing slope from the top of the ridge downwards. There are six blocks, located along the ridgeline. Within each block, there are 18 plots, one plot for each provenance: six rows down the slope × three plots along the ridgeline. Four seedlings were planted in each plot. Provenances were assigned randomly to the plots within each block. Seedlings were planted 2.5 m apart in each row, and rows were 4 m apart. Two rows of seedlings were planted as a buffer around the experimental blocks. Three blocks were sprayed with insecticide, and three others were left unsprayed. Spraying allowed estimation of potential growth rate in the absence of damage. Treatments (spraying / no spraying) were randomly assigned to each block. At the time of planting, height of seedlings ranged from 14 to 51 cm (mean = 34 cm, SD = 7 cm, $n = 432$).

Height and stem diameter of each plant were measured at the time of planting (May 1994), June 1995, February 1996, August 1996, September 1997, September 1998, and September 1999. Volume was calculated as: $\text{volume} = ((\text{stem radius})^2 \times \pi \times \text{height}) / 3$, and was square root transformed. We used plant growth rate corrected for the initial plant size: $365 \times (\text{vol}(t+1) - \text{vol}(t)) / (\text{vol}(t) \times ((t+1) - (t)))$, where t = date of initial measurement and $(t+1)$ = date of final measurement. Plant volume was square root transformed.

The extent of insect damage was visually assessed at least every six months starting June 1995. Damage assessments were scheduled so as to quantify herbivory before, during, and after peak periods of herbivory by *Anoplognathus* spp. (December – January) and *M. privata* (March – August). Damage by each insect species was recorded separately. All damage assessments were conducted by the same observers for consistency. Damage by each species was estimated to the nearest 5%. Separately assessing damage by different insect species was possible because each insect species produced characteristic damage.

Statistical analyses

Between population analyses

Plants on the top of the ridge grew slower than those down the slope. Therefore, we first examined effects of plant location using restricted maximum likelihood analysis (REML: Corbeil and Searle 1976). Location of plants, specified by block, row, plot, and tree position, was used as the random effect, and insecticide treatment and population were treated as the fixed effects. Plant growth was significantly affected by location, especially that specified by row and row $\times$ block. Therefore, we estimated mean growth rates of plants for each population using REML. We used initial volume as the covariate to adjust for difference in plant size. Insect damage was not, however, affected by the location of plants, and therefore, we used analysis of variance (ANOVA) to estimate mean damage for each population.

Within population analyses

For the analysis at the level of individuals within each population, we used residuals from trend surface regressions (Legendre 1990, 1993) with the plant growth as the response variable and row, block, and row $\times$ block as explanatory variables. The residuals from the trend surface regressions represent variation in plant growth rates associated with factors other than the physical location of plants within the experimental site.

We examined relative importance of acute damage and chronic damage by estimating components of variation in plant growth due to these two types of damage. Components of variation in plant growth were estimated using a method described in Legendre (1993). The R^2 term of a multiple regression with plant growth as the response variable and both acute damage and chronic damage as explanatory variables (R^2_a) indicates combined effects of acute damage (xb), chronic damage (c), and the interaction between the acute damage and chronic damage (xb $\times$ c). Similarly, the R^2 term of a regression with just acute damage as the explanatory variable (R^2_{xb}) indicates combined effects of xb and xb $\times$ c, and the R^2 term of a regression with just chronic damage as the explanatory variable (R^2_c) indicates combined effects of c and xb $\times$ c. We estimated the component of variation in tree growth due to acute damage as ($R^2_a - R^2_c$), that due to chronic damage as ($R^2_a - R^2_{xb}$), that of xb $\times$ c as ($R^2_c + R^2_{xb} - R^2_a$), and unexplained variation as ($1 - R^2_a$).

For detecting relationships between growth and damage, we examined (1) growth rates and damage in the same year, (2) damage in year 1 and growth rates in year 2, and (3) damage in years 1 and 2 and growth rates in year 2. All three methods resulted in a similar pattern, and we report results from the relationship between growth and damage in the same year. All statistical analyses were performed using Genstat (Genstat 5 Committee 1993) and SAS (SAS Institute 1989).

Results

All except one out of 185 plants in the unsprayed treatment were affected by *Anoplognathus* spp., with up to 90% defoliation (Table 3.1). Zero to 88% of the total cumulative damage and 0 to 100% of total annual damage was caused by *Anoplognathus* spp. (Table 3.1). There was a slight tendency for trees that suffered acute damage to suffer less chronic damage in the same year (Table 3.2). Also, plants that suffered severe chronic damage in one year tended to suffer less acute damage the following year. However, plants that suffered acute damage in one year did not necessarily suffer less chronic damage in the following year. Plants that tended to suffer severe chronic damage or acute damage in one year also suffered severe chronic damage or acute damage, respectively, the following year.

Table 3.1. Summary of annual damage in 185 plants of *E. globulus*. Mean $\pm$ SD (range)

Year	Acute damage (%)	Proportion (%) of acute damage in total	
		Cumulative damage	Annual Damage
1995	2 $\pm$ 5 (0 - 30)	1 $\pm$ 3 (0 - 21)	37 $\pm$ 45 (0 - 100)
1996	55 $\pm$ 20 (0 - 85)	33 $\pm$ 16 (0 - 88)	70 $\pm$ 26 (0 - 100)
1997	17 $\pm$ 13 (0 - 70)	9 $\pm$ 7 (0 - 28)	64 $\pm$ 33 (0 - 100)
1998	24 $\pm$ 16 (0 - 76)	14 $\pm$ 9 (0 - 54)	69 $\pm$ 28 (0 - 100)
1999	18 $\pm$ 17 (0 - 90)	9 $\pm$ 8 (0 - 48)	72 $\pm$ 36 (0 - 100)

Table 3.2. Correlations between annual acute damage and annual chronic damage in 185 plants of *E. globulus*. The letter in front of each year stands for the type of damage (a = acute and c = chronic).

	c95	c96	c97	c98	c99	a95	a96	a97	a98
c96	-0.07								
c97	-0.07	0.41							
c98	0.09	0.25	0.66						
c99	0.34	-0.03	0.01	0.21					
a95	0.24	-0.05	-0.14	-0.04	0.13				
a96	0.13	-0.05	-0.16	0.01	0.12	0.18			
a97	0.10	-0.13	-0.22	-0.12	0.02	0.07	0.24		
a98	0.13	-0.09	-0.26	-0.12	0.08	0.14	0.28	0.56	
a99	-0.02	0.13	-0.12	-0.11	-0.28	0.07	0.11	0.49	0.52

Different populations of *E. globulus* showed more than two-fold differences in acute damage and more than four-fold differences in chronic damage (Figure 3.2). Some populations such as Geeveston and King Island received small amounts of mean chronic damage and mean acute damage, while other populations such as Lorne received large amounts of mean chronic damage and mean acute damage. However, the correlation between mean chronic damage and mean acute damage was not strong due to populations with intermediate amounts of mean damage (Table 3.3). Severity of chronic damage in 1996 - 97 was positively correlated with that in 1997 - 98. Also, severity of acute damage in 1997 - 98 was positively correlated with that in 1998 - 99.

Growth of *E. globulus* trees varied among populations (Figure 3.3). There were inherent differences in growth rates among populations, indicated by differences among mean plant growth rates in sprayed plants. However, the ranking of the populations based on potential growth rates changed from year to year.

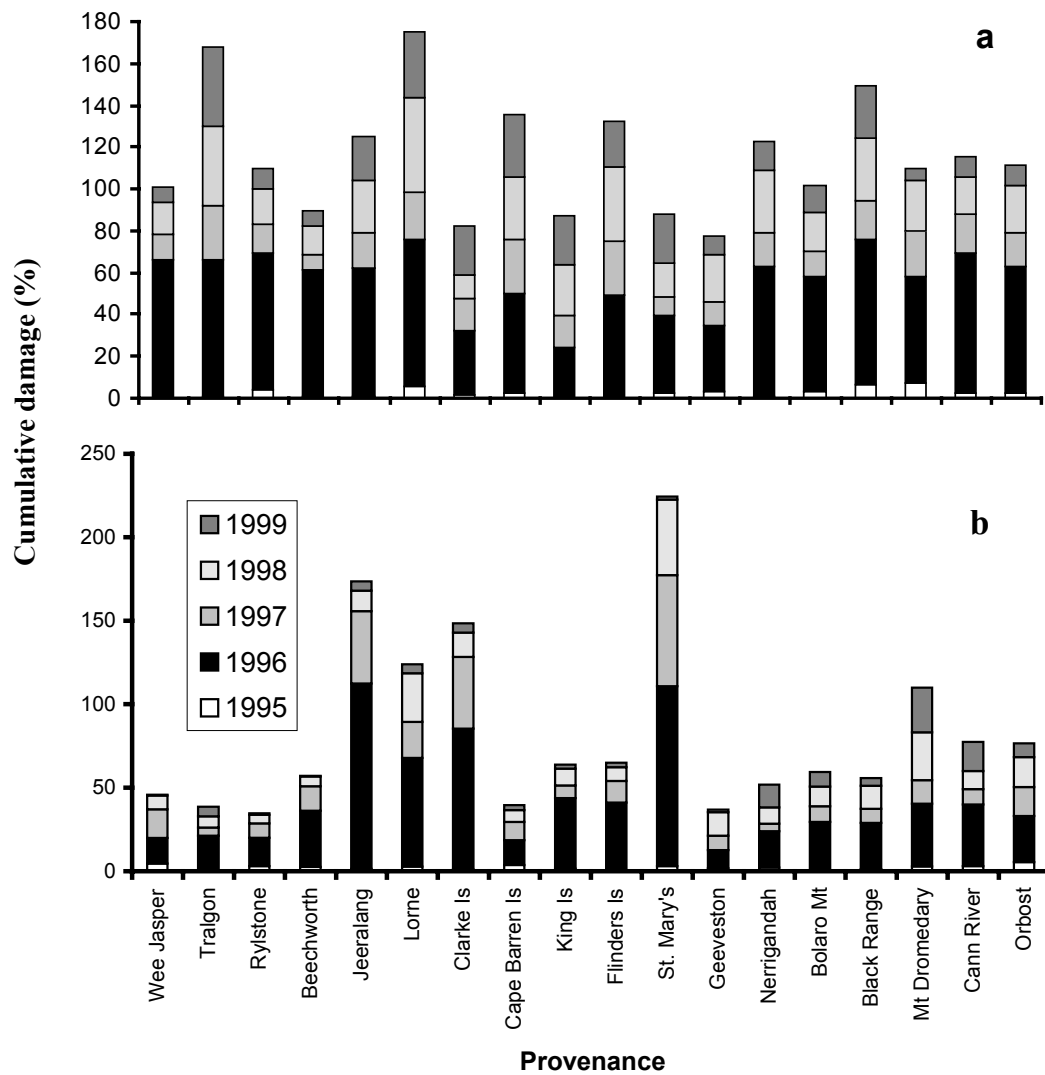


Figure 3.2. Annual acute (a) and chronic (b) damage in the 18 populations of *E. globulus*. Annual chronic damage in some populations in 1996 exceeds 100% because damage on leaves and stems were scored separately.

Table 3.3. Correlations between annual mean acute damage and annual mean chronic damage in 18 populations of *E. globulus*. The letter in front of each year stands for the type of damage (a = acute and c = chronic).

	c95	c96	c97	c98	c99	a95	a96	a97	a98
c96	-0.17								
c97	0.07	0.90							
c98	0.19	0.58	0.67						
c99	0.06	-0.04	-0.18	0.23					
a95	0.09	-0.07	-0.07	0.48	0.44				
a96	0.20	-0.24	-0.31	-0.22	0.17	0.19			
a97	-0.19	-0.12	-0.30	-0.10	0.31	0.14	0.22		
a98	-0.26	-0.11	-0.31	0.02	0.06	0.14	0.30	0.79	
a99	-0.45	0.29	0.17	0.10	-0.29	-0.11	-0.07	0.58	0.64

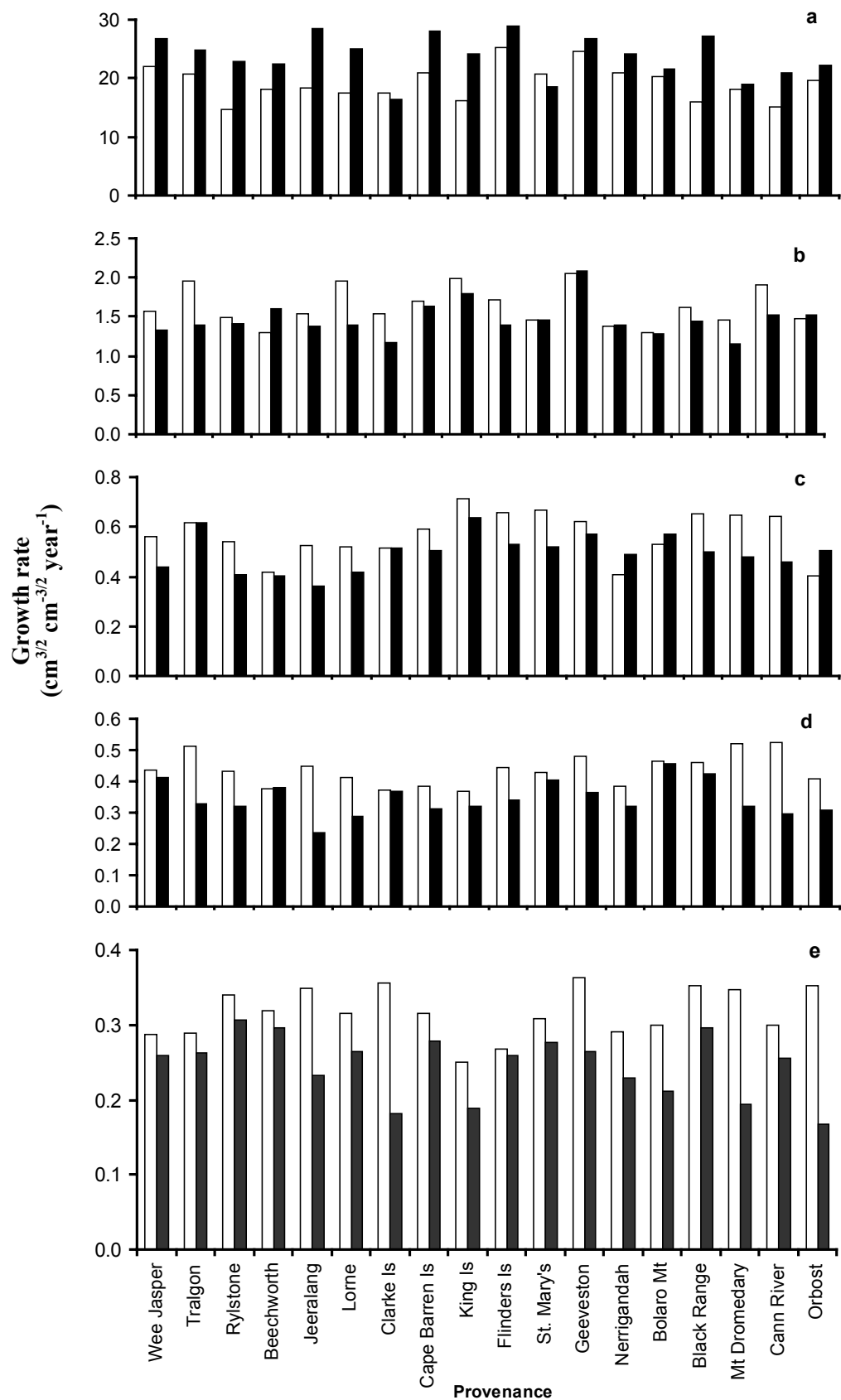


Figure 3.3. Annual growth rates of the 18 populations of *E. globulus*. a) 1994-95, b) 1995-96, c) 1996-97, d) 1997-98, e) 1998-99. Solid bars = growth rate of damaged plants. Open bars = growth rate of sprayed plants (potential growth rate).

For each population, the proportion of between-individual variation in annual growth associated with the variation in acute or chronic damage varied between years (Figure 3.4). For example, in the Cape Barren Island population, variation in individual growth rates is strongly associated with variation in chronic damage in 1995 and 1998, but not in 1996 - 97, and 1999. The observed differences in the strength of associations between variation in damage and variation in growth within populations is not due to differences in variability in damage or growth because there is no large differences in coefficient of variation for damage or growth among populations and years (data not shown).

In four of the five years during this study, strong associations between variation in annual growth rates and variation in acute damage were found in populations with intermediate potential growth rates (Figure 3.5). Populations with potentially fast or slow growth rates in a given year showed hardly any association between variation in annual growth rates and variation in acute or chronic damage. A higher proportion of variation in individual growth rates tended to be associated with variation in acute damage in populations with higher mean acute damage in three of the five years (Figure 3.6). However, a higher proportion of variation in individual growth rates tended to be associated with variation in chronic damage in populations with higher mean chronic damage only in 1996 (Figure 3.7).

Discussion

We observed variation in amounts of acute and chronic damage suffered by individuals within populations and among 18 populations of a long lived species, *Eucalyptus globulus*. Similarly, there was variation in annual growth rates among individuals within populations and among the populations. Insect damage did reduce growth of *E. globulus* in this study, and the level of reduction in growth in this study was comparable to those found in studies involving other species of *Eucalyptus* and insects (Mazanec 1968; Morrow and LaMarche 1978; Readshaw and Mazanec 1969; Stone and Bacon 1994a, 1994b; Vranjic and Gullan 1990).

Annual growth of *E. globulus* was associated with acute damage caused by *Anoplognathus* spp. in some populations with high mean acute damage. In a given population, however, there was no consistent pattern of relationship between variation in growth and variation in damage between years. This suggests that, for the levels and types of damage observed during this study, variation in growth in response to insect damage is more likely to be influenced by the environment rather than the genetic make-up of a population.

Variation in plant growth tended to be rarely associated with variation in neither acute nor chronic damage in populations when mean potential plant growth rates of those populations are high. Moreover, in populations when the potential growth rates of those populations are low, variation in plant growth tended to be hardly associated with variation in acute damage. These results suggest that insect damage affects plant growth more strongly in populations when the potential growth rates are at intermediate levels than those when the potential growth rates are very high or very low. Therefore, insect herbivores are more likely to exert strong selection pressure in populations when the potential growth rate is at intermediate levels than when the potential growth rate is very high or very low.

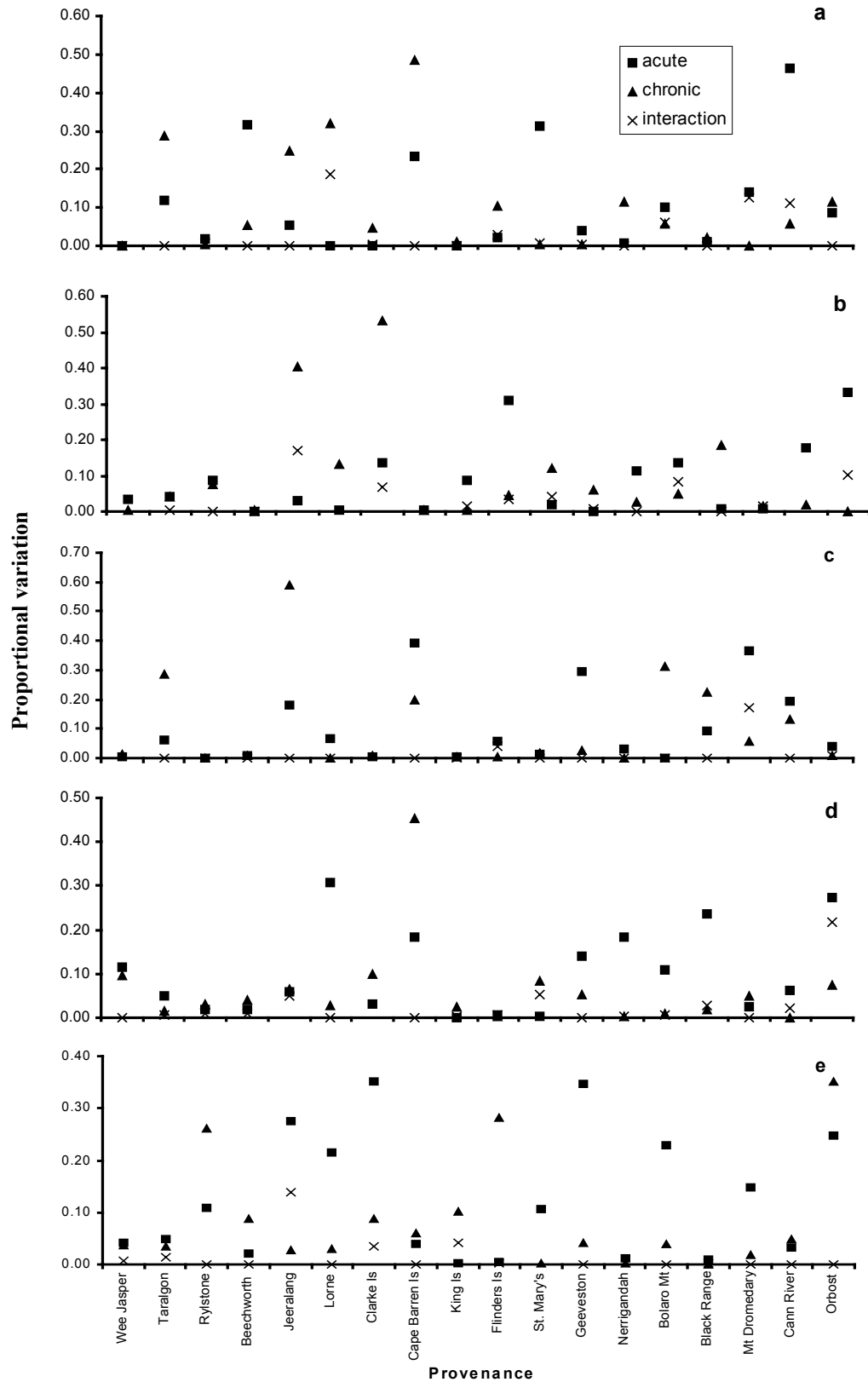


Figure 3.4. Annual change in proportion of variation in individual growth rate associated with variation in acute or chronic damage in the 18 populations of *E. globulus*. a) 1995, b) 1996, c) 1997, d) 1998, e) 1999. Solid square = association with acute damage. Open triangle = association with chronic damage. Cross = association with the interaction between acute and chronic damage.

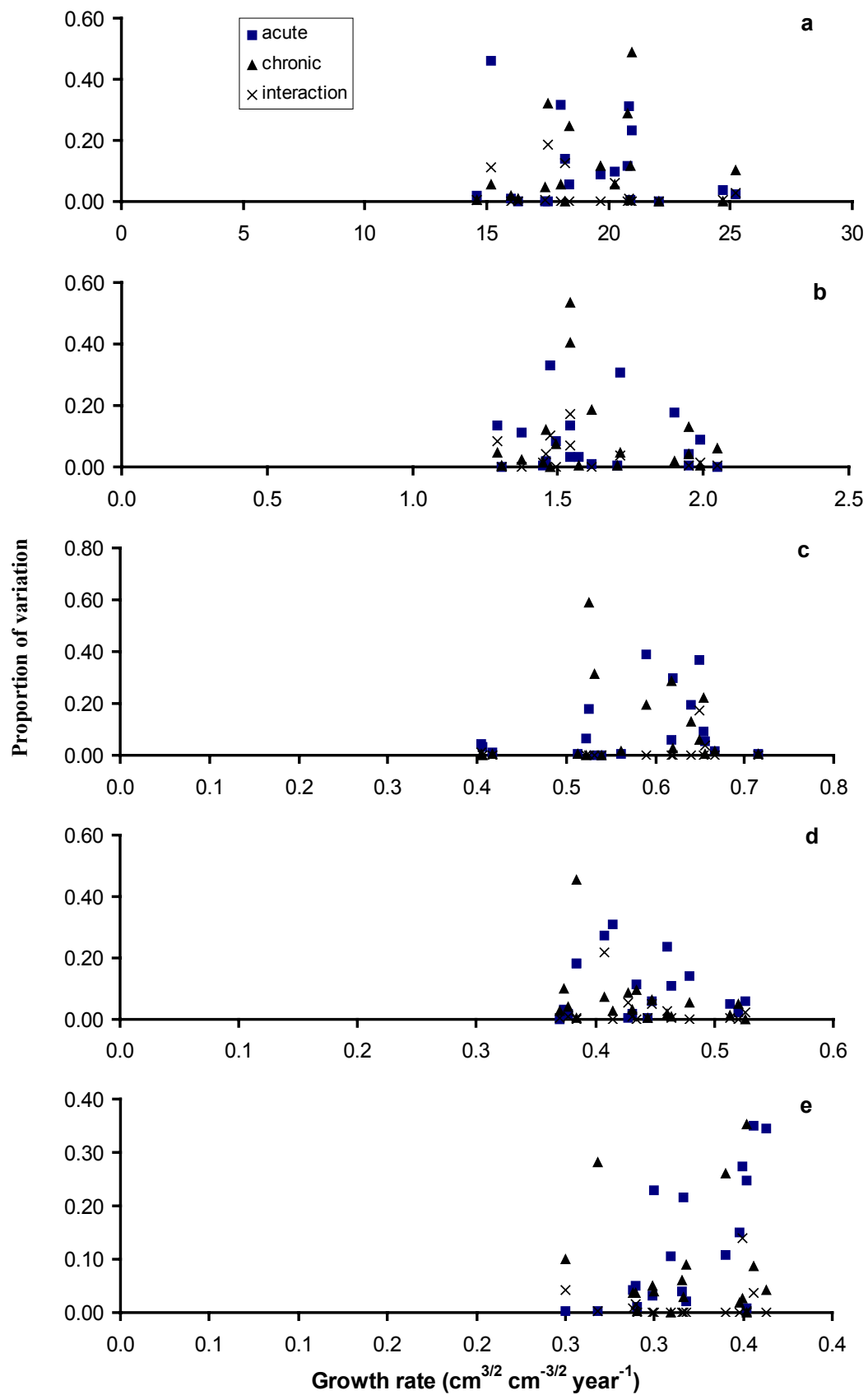


Figure 3.5. Relationships between population mean potential growth rates and damage – growth association among individuals within populations. Symbols are as in Figure 3.4.

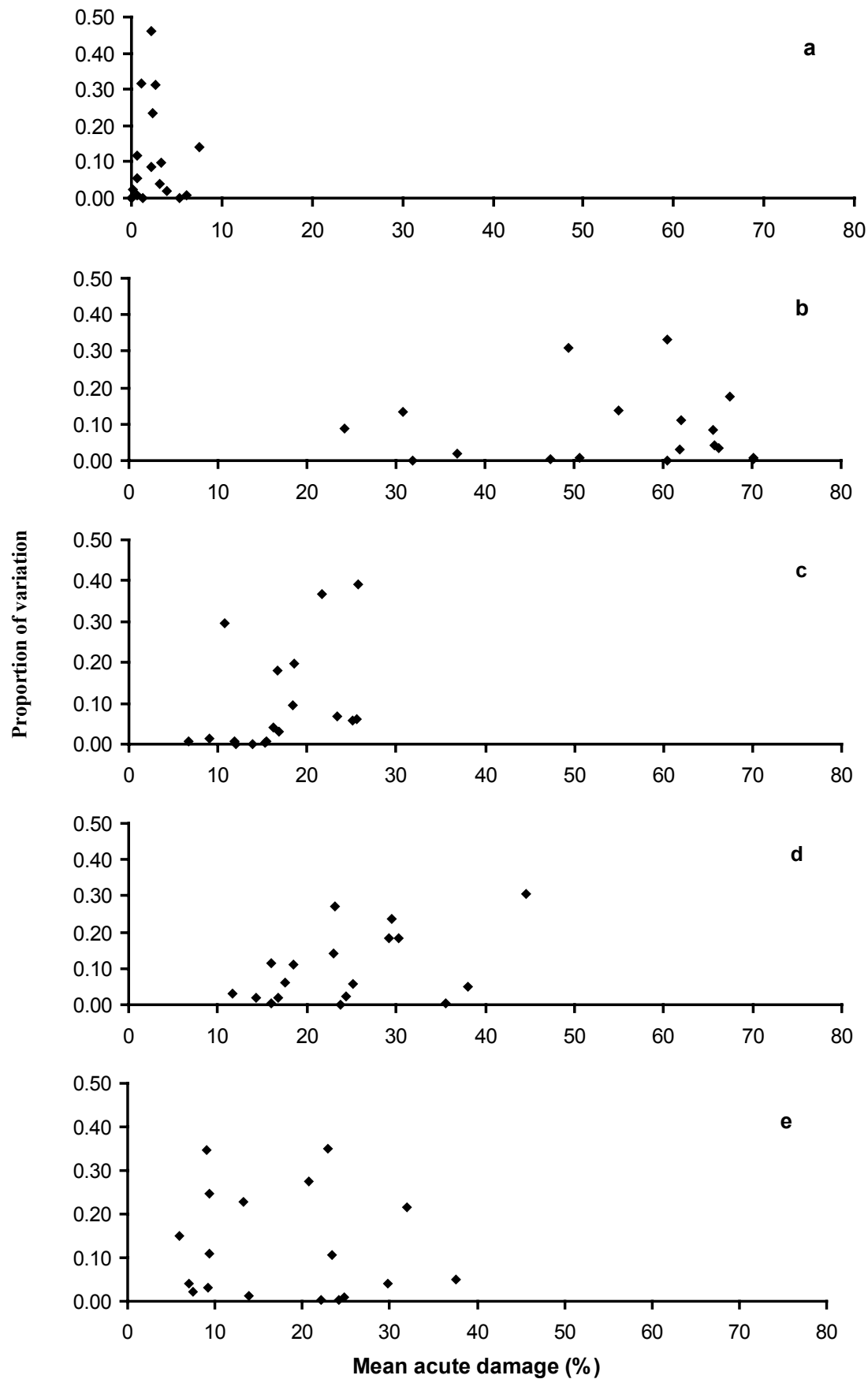


Figure 3.6. Relationships between population mean acute damage and damage - growth association among individuals within populations.

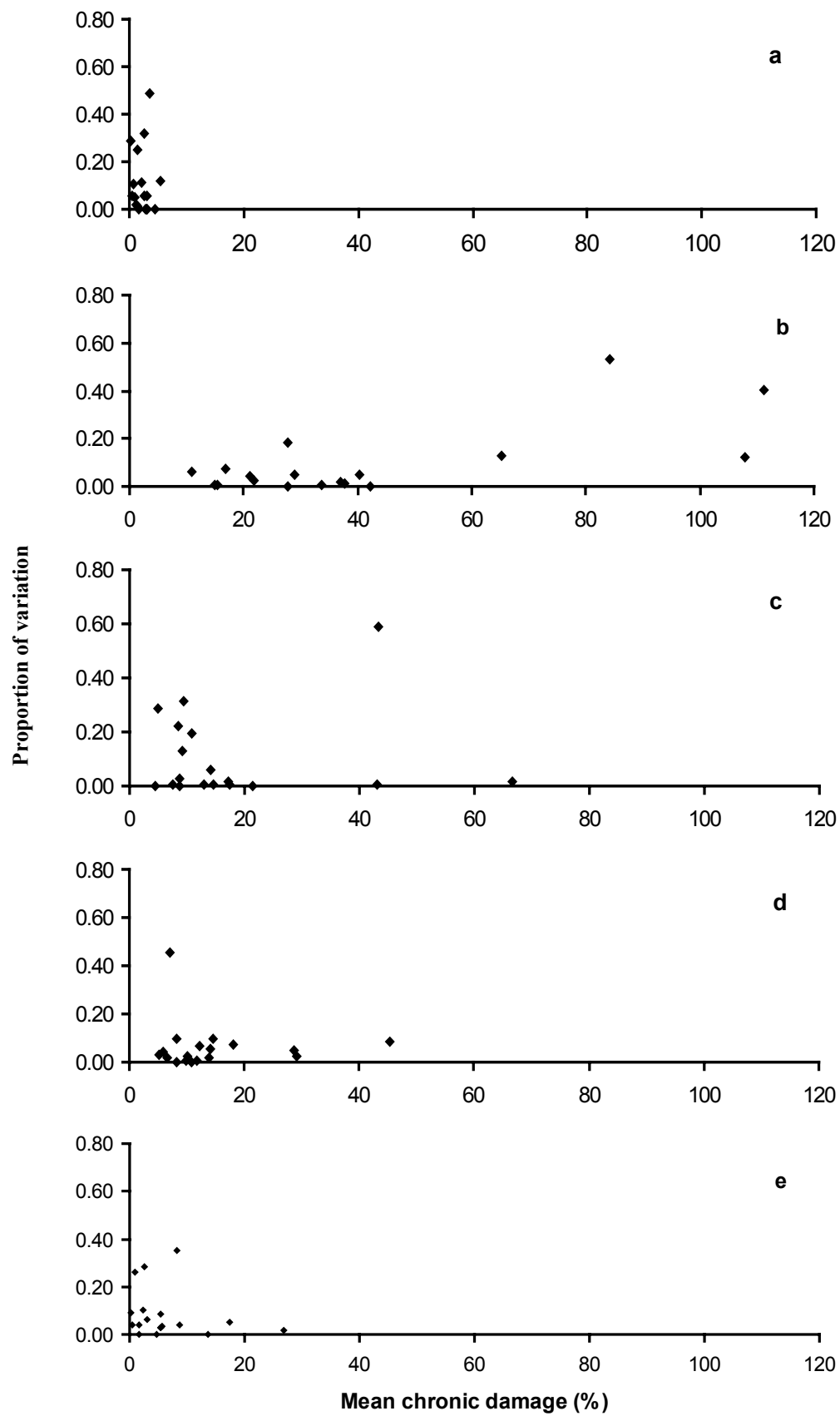


Figure 3.7. Relationships between population mean chronic damage and damage - growth association among individuals within populations.

We observed only few strong negative correlations between insect damage and plant growth at the level of individuals within populations. For example, many populations showed more than ten-fold difference (e.g., 5 – 80%) in the acute damage caused by *Anoplognathus* spp. in the summer of 1995-96, but less than 25% of variation in growth rates was associated with variation in acute damage in 16 of the 18 populations. In contrast, Marquis (1984) found reduced growth due to 30 to 100% removal of leaf area in small and medium sized plants of a tropical shrub (*Piper arieianum*) compared with plants receiving 0 to 10% removal of leaf area. The range of damage observed in this study is comparable to that imposed by Marquis.

A possible explanation for the rarity of direct relationships between damage and growth in this study is the contribution of genetic variation to both potential growth rates and responses of plants to damage. We used seeds from a mixture of parent trees in each population. Therefore, variation in growth rates of unsprayed plants is probably a result of at least (1) variation due to potential growth rates and (2) variation in damage.

Another possible explanation is that at least some populations of *E. globulus* have evolved to tolerate insect damage and other causes of stress. The highest insect damage observed in *P. arieianum* was 50% (mean = 16.7%, SD = 8.8%) (Marquis 1984). Although there is no specific examples involving natural stands of *E. globulus*, there are many records of complete defoliation of *Eucalyptus* stands by various insects (review by Ohmart and Edwards 1991). Therefore, even 30% damage on a *P. arieianum* plant may have more substantial impact than 50% damage on an *E. globulus* plant because of the possible difference in amounts of damage frequently encountered during their evolutionary history. Moreover, many *Eucalyptus* species is thought to have evolved to withstand severe environmental stress such as fire and severe drought.

The timing of defoliation and kinds of leaves lost to herbivores may also play important roles in determining effects of defoliation on growth and sometimes survivorship of plants. *Anoplognathus* spp. typically avoids feeding on expanding leaves, and *E. globulus* produce new leaves in summer to autumn. Thus, even after defoliation by *Anoplognathus* spp. in mid summer, *E. globulus* trees are able to produce new leaves. If *Anoplognathus* spp. feed also on expanding leaves or if defoliation by *Anoplognathus* spp. occurred in late autumn to early winter, then effects of defoliation may be greater than what is observed in this study.

Although there was relatively minor damage by *Mnesampela privata* in this study, this species sometimes causes major damage to *E. globulus*. Unlike *Anoplognathus* spp., *M. privata* feeds on leaves of young plants that are morphologically and chemically different from leaves of older plants. Moreover, *M. privata* larvae cause damage for a much longer time (from late summer to mid winter) than *Anoplognathus* spp. Combination of these traits sometimes results in death of young *E. globulus* plants in plantations during outbreaks (R. A. Farrow, *unpublished data*).

Long-lived plants perhaps deserve more attention. In short lived plants, one may be able to detect direct effects of damage by insects and other herbivores on growth and reproduction of plants and consequent evolutionary changes in plant traits such as inherent growth rate, chemical defence, and tolerance. In contrast, it is more time consuming, and perhaps more difficult, to detect direct effects of the damage on growth and reproduction of long-lived plants and evolutionary changes in the plant traits. We need to study both short lived and long-lived plants before making generalisations about effects of damage on plant growth. Results of this study suggests that, if insect damage was to have effects on plant growth, reproduction and consequent evolutionary change, a very severe or repeated very severe acute damage might have stronger effects than mild chronic damage repeated over the life time of plants. However, we did not have a chance to examine all different types of insect damage (e.g., severe chronic damage), and further studies are needed.

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4. Herbivory by Christmas beetles in Southeast Australia in relation to intra-specific variation in *Eucalyptus* leaf chemistry³

Introduction

The importance of intra-specific variation in plant secondary metabolites to plant - herbivore interactions is widely accepted (Denno and McClure 1983, Fritz and Simms 1992, Hunter 1997, Lowman and Heatwole 1992). Intra-specific variation in plant chemistry is essential for evolution of plant chemical defences (Kennedy and Barbour 1992, Simms and Rausher 1992, Tuomi and Augner 1994, Leimar and Tuomi 1998). Moreover, both spatial and temporal variation in plant chemistry is considered to affect population dynamics, and consequently community dynamics, of herbivores and their predators and parasitoids (Fritz 1992, Hare 1992, Karban 1992, Pollard 1992).

Some *Eucalyptus* (Myrtaceae) species show a striking intra-specific variation in susceptibility to Christmas beetles (*Anoplognathus* spp: Scarabaeidae) in southeast Australia. Trees growing in a close proximity may show marked variation in susceptibility to the beetles (Edwards *et al.* 1993), and occasionally, there are trees showing within tree variation in the susceptibility. For example, one branch of a particular study tree of *E. melliodora* suffered only 5% defoliation, while the rest of the tree suffered 85% defoliation (Edwards *et al.* 1990). Earlier studies showed negative correlations between herbivory by Christmas beetles and the proportion of 1,8-cineole (a monoterpene) in *Eucalyptus* oils in six species of *Eucalyptus* (Edwards *et al.* 1993, Stone and Bacon 1994).

While research on *Eucalyptus* - insect interactions have been focussed on the importance of 1,8-cineole and other components of *Eucalyptus* oils on insect herbivory (e.g., Morrow *et al.* 1976, Morrow and LaMarche 1978, Morrow and Fox 1980), recent studies on arboreal marsupial folivores on *Eucalyptus* (i.e., koala, common ringtail possum, and common brushtail possum) showed that consumption of the marsupial folivores are negatively affected by a group of compounds called acylphloroglucinol derivatives (Lawler *et al.* 1998a, 1998b, 1999, in press). Acylphloroglucinol derivatives are hybrids between phenolics and terpenes/isoprenes (Boland *et al.* 1992, Ghisalberti 1996). Leaves of many *Eucalyptus* species contain one to several different acylphloroglucinol derivatives (Ghisalberti 1996).

In this study, we combined the knowledge gained from the studies in *Eucalyptus* - insect herbivore interactions and *Eucalyptus* - mammalian folivore interactions and re-examined the relationship between variation in Christmas beetle herbivory and intra-specific variation in leaf chemistry. Objectives of this study are to examine (1) intra-specific variation in leaf chemistry of some *Eucalyptus* species and (2) relationships between leaf consumption by Christmas beetles and concentrations of 1,8-cineole and sideroxylonal (an acylphloroglucinol derivative).

Study organisms

We examined leaf consumption by six species of Christmas beetles commonly found in southeast Australia (*Anoplognathus montanus*, *A. viriditarsis*, *A. suturalis*, *A. chloropyrus*, *A. hirsutus*, and *A. pallidicollis*). Larvae of Christmas beetles feed on roots of grasses and crops in pastures. Adults of *A. montanus* and *A. suturalis* emerge late November to mid December, while those of *A. viriditarsis*, *A.*

³ Based on a manuscript developed by M. Matsuki, B. Clarke, M. Ebberts, B. Eschler, W. Foley, I. Lawler, B. Moore, M. Watson and R. B. Floyd.

chloropyrus, *A. hirsutus*, and *A. pallidicollis* emerge mid December to mid January. Population size of adult Christmas beetles shows marked spatial and between year variation. Low abundance of Christmas beetles has been shown to be associated with drought in late spring and early summer (Carne *et al.* 1981). Adult Christmas beetles preferentially feed on young leaves which are nearly or completely expanded (Carne *et al.* 1974). Leaves of less than three years of age are also eaten, but very old leaves are typically avoided. In southeast Australia, Christmas beetles feed on *E. melliodora*, *E. sideroxylon*, *E. globulus*, *E. camaldulensis*, *E. blakelyi*, *E. conica*, and *E. rubida*. However, when leaves of the host species are exhausted, Christmas beetles feed on leaves of some other *Eucalyptus* spp., other native trees such as *Acacia* spp., and exotic ornamental trees such as *Betula* spp. and *Schinus mole* (Steinbauer and Wanjura manuscript).

We used *E. melliodora*, *E. polyanthemos*, and *E. sideroxylon* in this study. *Eucalyptus melliodora*, *E. polyanthemos*, and *E. sideroxylon* are closely related to each other (i.e., within the same section: Boland *et al.* 1984), and sideroxylonal is the main acylphloroglucinol derivative found in these species. *Eucalyptus melliodora* and *E. sideroxylon* are typical hosts of Christmas beetles; however, our third study species, *E. polyanthemos* is avoided by Christmas beetles.

The three *Eucalyptus* species are similar in many aspects of their natural history. They are medium-sized trees (typically to 20 m in height) and are found in pastures and woodlands throughout southeast Australia (Boland *et al.* 1984). *Eucalyptus melliodora* and *E. polyanthemos* are found in large continuous populations at low to moderate density, while *E. sideroxylon* is found in small isolated populations at high density. Flowering follows production of new shoots in all three species and occurs in the Australian spring (October to December) in *E. polyanthemos*, and *E. sideroxylon* and in late spring to summer (December to February) in *E. melliodora*. However, reproduction and new shoot production in a given tree do not occur every year. Moreover, there is between individual variation in flowering and new shoot production at any site in a given year.

Methods

Choice of trees

Trees used in this study were selected based on records of Christmas beetle herbivory and leaf consumption by marsupial herbivores. We chose some susceptible (trees m1, m19, s12, & s13) and resistant (trees m2, m6, s15, & s21) trees (Table 4.1 and Figure 4.1), based on records of herbivory by Christmas beetles (mostly *A. montanus*) in the field (P. B. Edwards and W. J. Wanjura, *unpublished data*). We defined susceptible, resistant, and intermediate trees of the three *Eucalyptus* species based on leaf consumption by marsupial herbivores. Among eight *E. melliodora* trees, bm44 supported the highest leaf consumption, and bm20 supported the lowest leaf consumption by koala (*Phascolarctos cinereus*: B. Moore, *unpublished results*) and common brushtail possum (*Tricosurus vulpecula*: Watson 1998). Among 12 *E. sideroxylon* trees, s45 supported the highest leaf consumption, and s47 supported the lowest leaf consumption by common brushtail possum (Watson 1998) and common ringtail possum (*Pseudocheirus peregrinus*: Lawler 1999). Among 36 *E. polyanthemos* trees, p4 supported the highest leaf consumption, and p30 supported the lowest leaf consumption by common ringtail possum (Lawler 1999). Trees mm3 - mm9 were not used in the studies with the vertebrates.

Physical characteristics of leaves

We measured leaf water content and specific mass. Because *Eucalyptus* leaves contain volatile terpenes, leaves were dried at 40°C for 2 days. Specific mass of a leaf was measured by weighing a small disk of a unit area cut out from the leaf blade.

Chemical assays

We used gas chromatography (GC) to quantify concentrations of terpenes, high performance liquid chromatography (HPLC) to quantify concentrations of sideroxylonal in 1997 - 98, and near-infrared reflectance spectroscopy (NIRS) to estimate leaf nitrogen content in 1997 - 98 and sideroxylonal in

1998 - 99. Leaf materials used in chemical assays were kept in sealed plastic bags and stored in freezers until the analyses.

For the analysis of terpenes, thawed fresh leaves were cut into thin strips (< 1 mm wide), and 100 mg of sample was placed in a glass tube with the solvent (hexane). Each tube was sealed and placed in a 100°C oven for 1 hr. Extracts were elutriated with hexane and injected into a GC. Some common monoterpenes were identified using purified compounds. We used tridecane as the internal standard for calculating concentrations of terpenes.

Table 4.1. Summary of information about trees used in this study. Susceptibility of trees mm3-mm9 was not known prior to this study.

Tree #	Species	Population	Susceptibility	Herbivore species	% defoliation or leaf consumption
m1	<i>E. melliodora</i>	Yeoval	Susceptible	Christmas beetle	98%
m2	<i>E. melliodora</i>	Yeoval	Resistant	Christmas beetle	15%
m6	<i>E. melliodora</i>	Yeoval	Resistant	Christmas beetle	10%
m19	<i>E. melliodora</i>	Yeoval	Susceptible	Christmas beetle	100%
mb9	<i>E. melliodora</i>	Canberra	Intermediate	koala	23 g kg ⁻¹ day ⁻¹
				brushtail possum	--
bm20	<i>E. melliodora</i>	Canberra	Resistant	koala	9 g kg ⁻¹ day ⁻¹
				brushtail possum	16 g kg ⁻¹ day ⁻¹
bm44	<i>E. melliodora</i>	Canberra	Susceptible	koala	35 g kg ⁻¹ day ⁻¹
				brushtail possum	34 g kg ⁻¹ day ⁻¹
s12	<i>E. sideroxylon</i>	Cumnock	Susceptible	Christmas beetle	95%
s13	<i>E. sideroxylon</i>	Cumnock	susceptible	Christmas beetle	95%
s15	<i>E. sideroxylon</i>	Cumnock	resistant	Christmas beetle	15%
s21	<i>E. sideroxylon</i>	Cumnock	resistant	Christmas beetle	20%
mm3	<i>E. sideroxylon</i>	Canberra	--	--	--
mm4	<i>E. sideroxylon</i>	Canberra	--	--	--
mm6	<i>E. sideroxylon</i>	Canberra	--	--	--
mm9	<i>E. sideroxylon</i>	Canberra	--	--	--
s42	<i>E. sideroxylon</i>	Canberra	intermediate	brushtail possum	21 g kg ⁻¹ day ⁻¹
			intermediate	ringtail possum	32 g kg ⁻¹ day ⁻¹
s45	<i>E. sideroxylon</i>	Canberra	susceptible	brushtail possum	27 g kg ⁻¹ day ⁻¹
			susceptible	ringtail possum	68 g kg ⁻¹ day ⁻¹
s47	<i>E. sideroxylon</i>	Canberra	resistant	brushtail possum	10 g kg ⁻¹ day ⁻¹
			resistant	ringtail possum	2 g kg ⁻¹ day ⁻¹
p4	<i>E. polyanthemos</i>	Canberra	susceptible	ringtail possum	41 g kg ⁻¹ day ⁻¹
p30	<i>E. polyanthemos</i>	Canberra	resistant	ringtail possum	5 g kg ⁻¹ day ⁻¹
p33	<i>E. polyanthemos</i>	Canberra	Intermediate	ringtail possum	11 g kg ⁻¹ day ⁻¹

For the quantification of sideroxylonal, frozen leaves were freeze dried for 48 hrs. Freeze dried leaves were ground using Wiley mill with 1 mm mesh. Ground samples were placed in plastic twist top vials and stored in a cold room (4°C) until extraction and were oven dried at 40°C for 12 hrs immediately before extraction. Approximately 3 g of the ground sample was used for extraction with the relevant Soxhlet apparatus. Samples were extracted in 20% acetone: 80% light petroleum (125 ml) for 12 hrs, and the solvent was rotovapped off. Approximately 10 mg of crude extract was elutriated with 95% methanol: 4.9% water: 0.1% trifluoroacetic acid for HPLC injection. We carried out duplicate extractions from each sample and duplicate HPLC injections from each extract.

For extraction and purification of sideroxylonal, ground and air dried *E. melliodora* leaf material (approximately 1 kg) was extracted with 10% ethanol: 90% hexane (6.6 l) for up to 48 hrs using the relevant Soxhlet apparatus. The solvent was removed to give approximately 100-130 g of green residue. The residue was dissolved in dichloromethane (300 ml). Then celite (150 g) was added, and the solvent was removed. This was placed on top of fresh celite (150 g) in a vacuum column and washed successively with hexane (1000 ml), ethyl acetate (1000 ml), 10% methanol in dichloromethane (1000 ml), and methanol (500 ml). The sideroxylonal-enriched fractions were combined, and the solvent was removed. This material was chromatographed on silica gel and elutriated with solvents in the following sequence: 50% dichloromethane: 50% light petroleum (500 ml), dichloromethane (1000 ml), 5% methanol: 95% dichloromethane (1000 ml), 25% methanol: 75% dichloromethane (500 ml), and methanol (500 ml). The sideroxylonal-enriched fractions were combined, and the solvent was removed. This material was dissolved in acetone (100 ml) and chromatographed through sephadex (LH-20, ~50 g) with acetone. Four fractions were collected. The solvent was removed from fraction 2 and 3, and the residue was dissolved in diethyl ether (200 ml). The ether was allowed to slowly evaporate overnight. The residue was filtered, and the solid was washed with ether to give sideroxylonal.

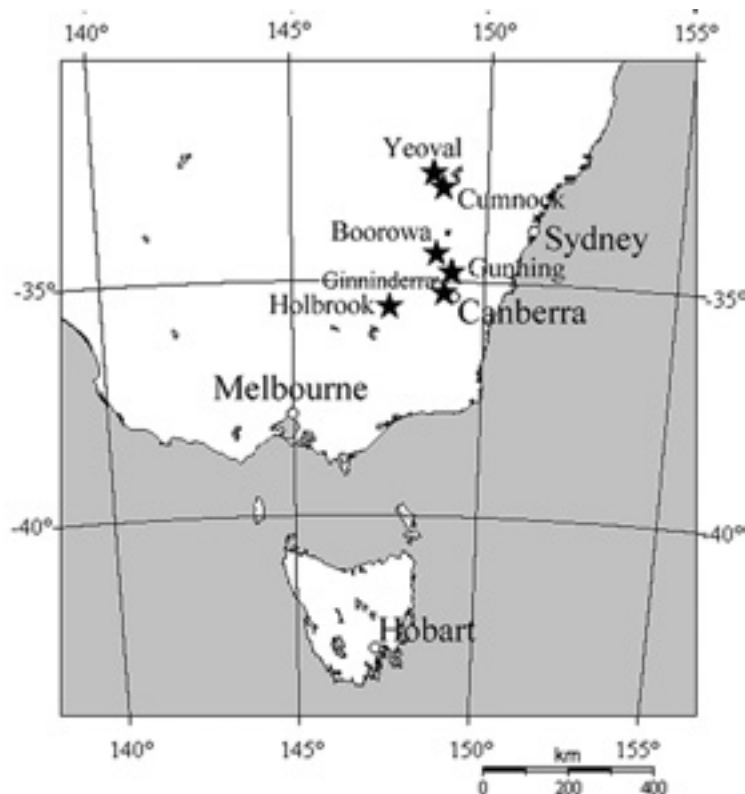


Figure 4.1. Locations of *Eucalyptus* and *Anoplognathus* species/populations used in this study. Table 4.1 summarises information on *Eucalyptus* species/populations. Table 4.2 summarises *Anoplognathus* species/populations used in different seasons.

Leaf nitrogen content in 1997-98 and sideroxylonal concentration in 1998-99 were estimated using near-infrared reflectance spectroscopy (NIRS). Freeze dried and ground samples were oven dried at 40°C for 12 hrs immediately prior to scanning using a NIRS device. Chemical composition of the leaf samples was estimated using NIRS in the following manner. The NIRS device emits a light beam of known quality (visible to near-infrared region) to a sample and measures the spectra of reflected light from the sample. The amount of light absorbed by the sample at different wavelength is a function of amounts and type of chemical bonding present in the sample. Consequently, by measuring the light

spectra reflected from the sample, we are able to estimate amounts and type of chemical bonding present in the sample.

To predict the amount of a chemical compound in a sample, a calibration equation for each chemical compound is used. A calibration equation is a regression of the amounts of chemical compound on the NIRS spectra. Therefore, to build a calibration equation, we need both NIRS spectra and the amount of a chemical compound measured using chemical analysis from a set of samples. Typically, the information contained in the NIRS spectra is summarised using principal component analysis (PCA regression) or canonical covariance analysis (partial least square regression) (Brown 1993, Infrasoftware International 1996). Amounts of a chemical compound in new samples can be predicted using the calibration equation and the NIRS spectra of the new samples.

The calibration equation for nitrogen content was developed based on a set of NIRS spectra and measured nitrogen content of 226 pre-existing samples using a partial least square regression. In order to examine the validity of the nitrogen content predicted by NIRS, we measured nitrogen content of nine samples using the standard micro Kjeldahl method. The difference between the measured and the predicted values of nitrogen content were small (mean difference = 0.058, SE = 0.215; $P = 0.78$ for a two-tailed t - test for the mean difference = 0). The calibration equations for sideroxylonal and 1,8-cineole were developed based on a set of NIRS spectra and measured sideroxylonal and 1,8-cineole concentrations of 27 pre-existing samples using a partial least square regression. Detailed description of NIRS methods and application of NIRS in ecological studies can be found in Foley *et al.* (1998).

Consumption trials

Adult beetles were collected in the field around Canberra, Australian Capital Territory, whenever they were found in large numbers (200+ individuals) (Figure 4.1 and Table 4.2). Beetles were kept in cages (37 × 28 × 55 cm each), and freshly cut branches of *E. melliodora*, *E. sideroxylon*, *E. globulus*, *E. bridgesiana*, and *E. leucoxylon* in small vases were placed in the cages each day. Christmas beetles readily feed on leaves of certain trees of these *Eucalyptus* species.

Table 4.2. Origin of *Anoplognathus* spp. used in this study.

Species	97 - 98	98 - 99	99 - 00
<i>A. montanus</i>	Boorowa	Holbrook	-
<i>A. viriditursus</i>	-	Boorowa	Boorowa
<i>A. chloropyrus</i>	Boorowa, Canberra	Gunning	Ginninderra, Gunning Boorowa
<i>A. pallidicollis</i>	Gunning	Gunning	-
<i>A. suturalis</i>	Gunning	Gunning	-
<i>A. hirsutus</i>	-	-	Ginninderra

We conducted five types of experiments from late December 1997 to late January 1998, early December 1998 to early February 1999, and late December 1999 to late January 2000: (1) no-choice experiments; (2) choice/no-choice experiments; (3) sideroxylonal paint experiments; (4) a steamed leaf experiment; and (5) cineole addition experiments. For all types of experiments, adult beetles were individually sexed, weighed, and placed in clear plastic screw-top vials (250 ml) with moist plaster of paris to maintain leaf turgor pressure. Experiments were terminated after 5 to 14 hrs when some leaves were nearly completely consumed. Each beetle was weighed again at the end of each experiment. All experiments were started in the morning and were terminated in the evening of the same day.

For each experiment, several small branches were taken from each tree so that leaves used in our experiments were representatives of leaves on the experimental trees. Size and age of leaves used in the experiments in a given season were carefully standardised in order to minimise effects of age and size on leaf consumption. In the summer of 1997 - 1998, we used leaves of one to two years old because leaves less than one year old were rare. However, the study trees produced new leaves in the autumn - spring of 1998, and by late December 1998, almost all old leaves had dropped and there were most new leaves in the canopy of the study trees. Therefore, we used leaves of two years old (mid December) and six to eight months old (late December to early February) in the summer of 1998 - 1999. We used leaves of approximately one and half years old in the summer of 1999 - 2000. Christmas beetles typically avoid feeding on old leaves (3+ yr old).

In no-choice experiments, one fresh leaf was placed in each vial. In choice/no-choice experiments, two fresh leaves were placed in each vial. Three trees were used in each choice/no-choice experiment: the susceptible, the resistant, and an intermediate tree based on consumption trials using marsupial herbivores. We used all six pair-wise combinations of trees, i.e., susceptible/susceptible, susceptible/intermediate, susceptible/resistant, intermediate/intermediate, intermediate/resistant, and resistant/resistant.

Dry matter of leaves consumed during the experiments was calculated as:

$$consumption = L_{tw} \times \frac{L_{cd}}{L_{cw}} - L_{td},$$

where

L_{tw} = initial fresh mass of consumed leaf,
 L_{cw} = initial fresh mass of control leaf,
 L_{cd} = final dry mass of control leaf, and
 L_{td} = final dry mass of consumed leaf.

Control leaves were individually placed in a plastic vial with moist plaster of paris. Within each block, location of vials with control leaves and those with experimental leaves and beetles was randomised.

Relative consumption rate (*RCR*) was calculated as:

$$RCR = \frac{consumption}{(T_f - T_i)(M_i + M_f) / 2},$$

where

M_i = initial dry mass of beetle measured at time T_i , and
 M_f = final dry mass of beetle measured at time T_f .

Since adults beetles did not grow exponentially, we used the mean dry mass of beetles to calculate *RCR* as suggested by Waldbauer (1968) instead of the ratio of the mean dry mass to the mean of natural log dry mass as suggested by Gordon (1968). Using a subset of beetles, dry mass of beetles was estimated from a wet mass:dry mass ratio for each beetle species/sex combination.

To examine preference of a tree i , we used the index D , as suggested by Jacobs (1974):

$$D_i = \frac{DR_i - RA_i}{RA_i + DR_i + 2RA_iDR_i},$$

where

DR_i is the dietary ratio, defined as the ratio between dry matter consumption of a leaf of tree i (DMC_i) and the total dry matter consumption of leaves from tree i and tree j (DMC_j):

$$DR_i = \frac{DMC_i}{DMC_t}.$$

RA_i is the relative amounts of leaf matter of tree i offered to a beetle during an experiment. RA_i is defined as the ratio between dry matter of a leaf of tree i offered (DMO_i) and the total dry matter of leaves of tree i and tree j (DMO_t):

$$RA_i = \frac{DMO_i}{DMO_t}.$$

Negative values of the index D indicate avoidance, and positive values indicates preference. We used the index D because it meets all three criteria for a good index of diet preference proposed by Cock (1978) and is sensitive to small changes in preference (Kam *et al.* 1997).

We also examined changes in leaf consumption due to variation in leaf quality of the alternative leaf. As a measure of leaf consumption adjusted for the leaf mass offered, we used the index of exploitation (IE_i), where IE_i is defined as (Kam *et al.* 1997):

$$IE_i = \frac{DMI_i}{DMO_i}.$$

The change in consumption of the target leaf (t) due to leaf quality of the alternative leaf (a) was estimated as:

$$\Delta consumption = \left(\frac{\overline{IE}_{t/a} \bullet - \overline{IE}_{t/t} \bullet}{\overline{IE}_{t/t} \bullet} \right) \times 100,$$

where the subscript t/t indicates that two leaves of the target tree were offered to each beetle, and t/a indicates that one leaf each of the target tree and the alternative tree was offered. $\overline{IE}_{t/t} \bullet$ and $\overline{IE}_{t/a} \bullet$ are the means of $IE_{t/t}$ and $IE_{t/a}$, respectively, calculated over the four Christmas beetle species. $IE_{t/t}$ is the mean of $IE_{t/t}$ in each beetle / *Eucalyptus* species combination. Similarly, $IE_{t/a}$ is the mean of $IE_{t/a}$ in each beetle / *Eucalyptus* species combination. For example, the change in consumption of a leaf of the susceptible tree due to having a leaf of the resistant tree is estimated as:

$$\Delta consumption = \left(\frac{\overline{IE}_{susceptible / resistant} \bullet - \overline{IE}_{susceptible / susceptible} \bullet}{\overline{IE}_{susceptible / susceptible} \bullet} \right) \times 100.$$

We manipulated amounts of sideroxylonal by painting solutions to leaves. In the sideroxylonal paint experiments, we used leaves of an *E. melliodora* tree with very low concentrations of sideroxylonal and 1,8-cineole (bm44) and another *E. melliodora* tree with high concentrations of sideroxylonal and 1,8-cineole (bm20)(see results). Purified sideroxylonal was dissolved into a solvent of 10% methanol: 90% dichloromethane. We painted sideroxylonal solutions to leaves of tree 6 so that treatment leaves would have approximately 20, 40, and 80 mg / g leaf dry mass. The maximum concentration of sideroxylonal found in *E. sideroxylon* and *E. melliodora* in the field is approximately 40 mg / g leaf dry mass. We also painted solvent only to leaves of both tree 6 and tree 7 as the solvent control. We prepared sideroxylonal solutions in three different concentrations so that approximately the same amount of the solution was applied to each leaf.

We manipulated amounts of 1,8-cineole by steaming leaves. In this experiment, we used leaves of the two *E. melliodora* trees used in the sideroxylonal paint experiments (i.e., bm20 and bm44) and an *E. sideroxylon* tree with a moderate concentration of sideroxylonal and a high concentration of 1,8-cineole (mm4). Leaves were steamed for 1 min. Fresh (i.e., unsteamed) leaves of the three trees were used as controls. We also artificially increased concentration of 1,8-cineole. Approximately 0.1 ml of purified 1,8-cineole was applied to leaves of bm44. In the cineole addition experiments, we used meshed screw-caps in order to avoid building up of pressure inside the vials due to evaporation of 1,8-cineole.

Statistical analyses

Designs of experiments are as follows: In no-choice experiments, we used 5 blocks $\times$ 3 to 6 treatments (trees) $\times$ 3 replicates. In choice/no-choice experiments, we used 5 blocks $\times$ 6 treatments (tree combinations) $\times$ 3 replicates. The experimental design of the sideroxylonal paint experiments was 5 blocks $\times$ 7 treatments (2 trees $\times$ no solvent or solvent control + 3 concentrations of sideroxylonal) $\times$ 3 replicates, and that of the leaf steaming experiment was 5 blocks $\times$ 6 treatments (3 trees $\times$ steaming or control) $\times$ 3 replicates. The design of cineole addition experiments was 1 block $\times$ 2 treatments (1 tree $\times$ cineole addition or control) $\times$ 10 replicates. Blocks in the experiments represented rooms in three different buildings; however, the same five rooms were used in all experiments.

We analysed data on leaf physical and chemical characteristics, the consumption, and feeding preference using ANOVAs (when the data set was balanced) and restricted maximum likelihood analyses (when the data set was unbalanced). When differences among species, populations, and individual trees in leaf physical and chemical traits were examined, we treated species, populations, and trees as fixed factors. Since we used only three individuals of *E. polyanthemus* from one population in this study, we excluded *E. polyanthemus* from the analysis of differences between species. Two monoterpenes, i.e., α -phellandrene and *p*-cymene, were found in only 13 out of 21 trees, and therefore, results of statistical analyses on these two compounds (cf, Table 4.3) may need to be examined with caution. We used GENSTAT (Genstat 5 Committee 1993) and SAS (SAS Institute 1989) for statistical analyses.

In this study, we did not statistically examine effects of host plant population/beetle species on leaf consumption by beetles. Reasons for this are: (1) Separate experiments are conducted for each beetle/host population combination; and (2) Leaf consumption by beetles gradually decreased as the season progressed. Therefore, if a particular species of Christmas beetle shows lower consumption in an experiment conducted late in the season with a host population than consumption in an experiment conducted early in the season with another host population, the difference in consumption is likely due to combined effects of season and differences in host plant leaf chemistry.

Results

Eucalyptus melliodora and *E. sideroxylon*

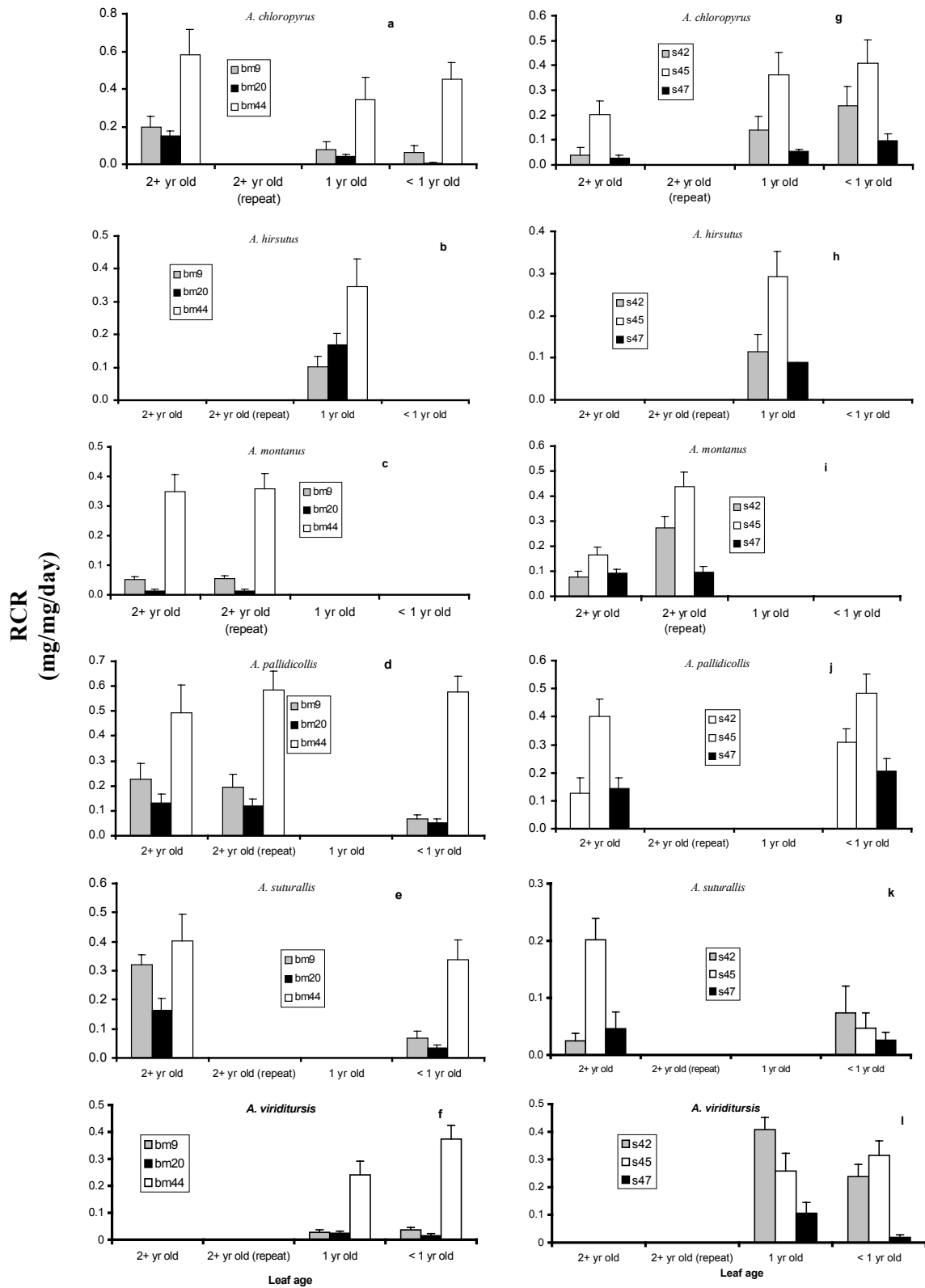
Under no-choice situations, leaf consumption by all six species of Christmas beetles showed marked variation among the six trees from Canberra, and all six species of beetles showed qualitatively consistent pattern of leaf consumption (Figure 4.2). All six species of Christmas beetles consumed more leaf material of trees with markedly lower concentrations of sideroxylonal and 1,8-cineole than trees with high concentrations of those compounds. Leaves of the trees with high concentrations of sideroxylonal and 1,8-cineole also tended to contain higher concentrations of some other monoterpenes such as α -pinene, limonene, and terpineol but lower concentrations of *p*-cymene and α -phellandrene, than leaves of trees with low concentrations of sideroxylonal and 1,8-cineole (Table 4.3). However, 1,8-cineole was the dominant component of the *Eucalyptus* oils. The concentration of 1,8-cineole ranged from 0 to 27 mg g⁻¹ leaf dry mass, while the maximum concentration of other terpenes was 3.5 mg g⁻¹ leaf dry mass. The difference between susceptible and resistant trees was not correlated with leaf water content or leaf specific mass (Table 4.3).

The same qualitative pattern of difference in leaf consumption of the same six trees was observed between years, between young (< 1 yr old) and old leaves (> 2 yrs old), and with at least one species (*A. chloropyrus*), three different populations in the same year (Figure 4.3). Moreover, for at least two species of Christmas beetles (*A. montanus* and *A. chloropyrus*) the same qualitative pattern of tree discrimination and the same pattern of leaf chemistry were observed in trees from other populations

(Figure 4.4). For these trees, leaf consumption in the laboratory was directly correlated with the historical record of defoliation by Christmas beetles (mostly *A. montanus*).

Table 4.3. Feeding preference by Christmas beetles. Mean values of the index *D* (see text). In the “Trees compared” column, susceptible, intermediate, and resistant trees are determined based on Christmas beetle no-choice experiments. Positive values indicate preference of trees shown in the “Trees compared” column. Negative values of the index *D* indicate reversal of preference from that shown in the “Trees compared” column. -- = no data. Values in parentheses after each index *D* indicate sample size. Bold-faced values are significantly different from zero at $\alpha = 0.05$ level. Index values with the same letters within each beetle / *Eucalyptus* species combination did not differ from each other at $\alpha = 0.1$ level (LSD).

Trees compared	<i>A. montanus</i>	<i>A. chloropyrus</i>	<i>A. pallidicollis</i>	<i>A. sutularis</i>
<i>E. melliodora</i>				
Susceptible over intermediate	0.81 (15) ^{ab}	0.43 (15) ^{ab}	0.96 (14) ^a	0.43 (10) ^a
Susceptible over resistant	0.94 (14) ^a	0.80 (14) ^a	0.93 (13) ^a	0.41 (14) ^a
Intermediate over resistant	0.60 (14) ^b	0.42 (15) ^b	0.63 (13) ^b	0.48 (15) ^a
<i>E. sideroxylon</i>				
Susceptible over intermediate	0.43 (13) ^a	0.46 (13) ^a	0.65 (12) ^a	0.77 (13) ^a
Susceptible over resistant	0.48 (13) ^a	0.26 (15) ^a	0.46 (12) ^a	0.49 (12) ^a
Intermediate over resistant	0.14 (10) ^a	-0.81 (3) ^a	0.61 (10) ^a	0.96 (5) ^a
<i>E. polyanthemos</i>				
Susceptible over resistant	0.34 (10) ^a	0.56 (5) ^a	--	--
Susceptible over intermediate	-0.05 (10) ^a	1.04 (5) ^a	--	--
Intermediate over resistant	0.52 (10) ^a	0.99 (6) ^a	--	--



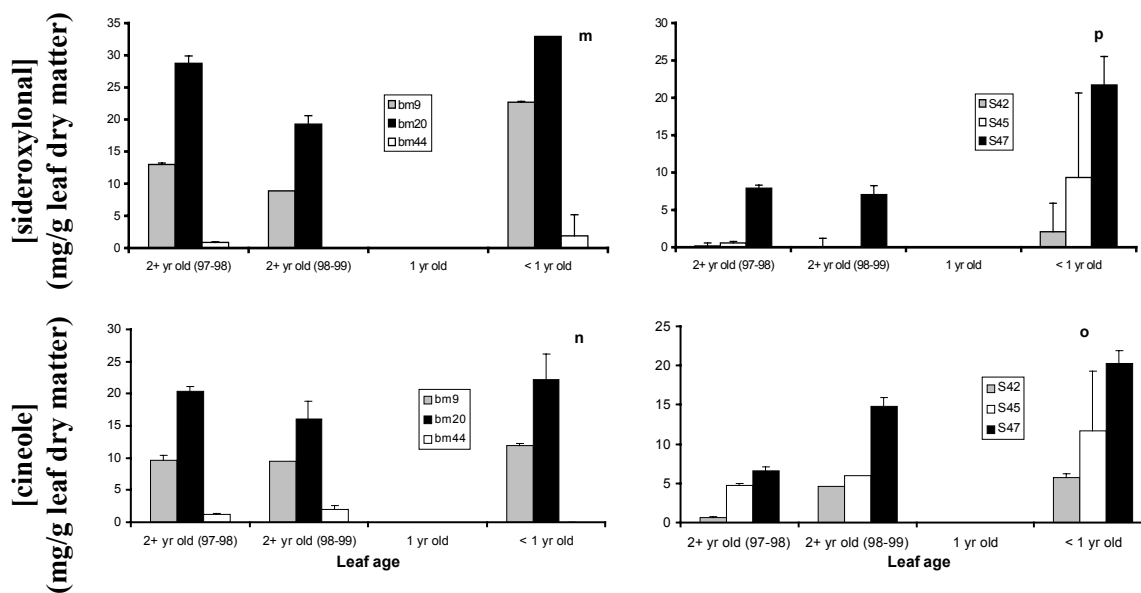


Figure 4.2. Relative consumption rates (RCR) of Christmas beetles (a-l) and concentrations of sideroxylonal and 1,8-cineole of *E. melliodora* (m & n) and *E. sideroxylon* trees (o & p). Experiments with 2+ yr old leaves were conducted in the 1997 - 98 season, with 1 yr old leaves in the 1999 - 2000 season, and with < 1 yr old leaves in the 1998 - 99 season. The repeat experiments with 2+ yr old leaves were conducted in the 1997 - 98 season for *A. pallidicollis* and in the 1998 - 99 season for *A. montanus*. Mean + SE, $n = 9-15$ beetles / tree.

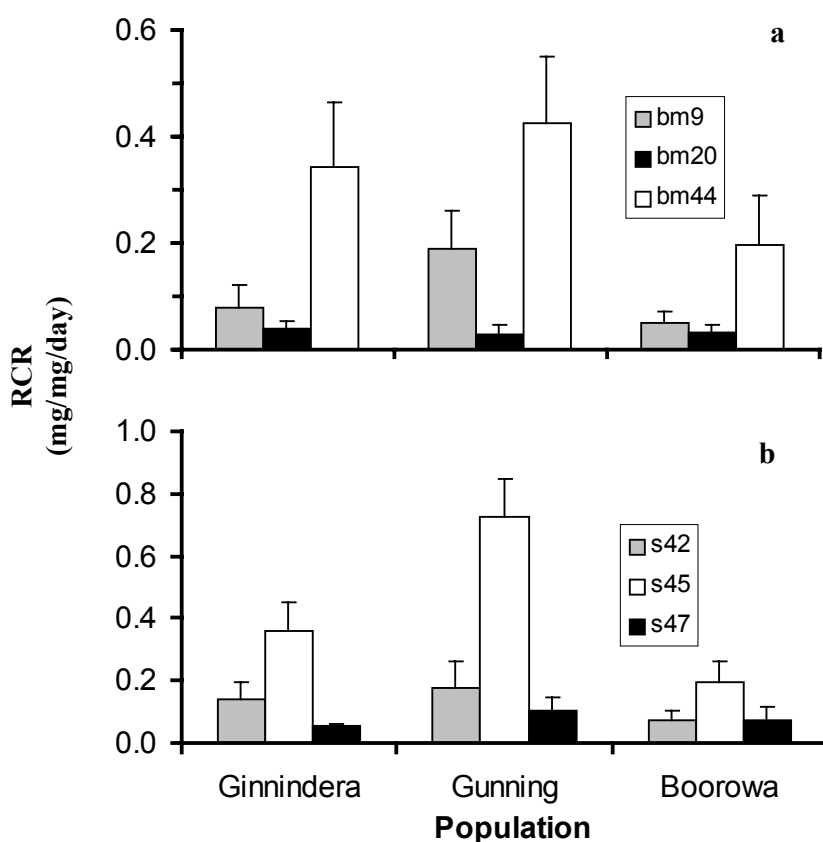


Figure 4.3. Relative consumption rates (RCR) of three different populations of *A. chloropyrus* on a) *E. melliodora* and b) *E. sideroxylon* trees. All experiments were conducted in the 1999 - 2000 season. Mean + SE, $n = 9 - 15$ beetles per tree.

Of the six species of Christmas beetles, *A. montanus* and *A. viriditarsis* appeared to be most sensitive to leaf chemistry. Large variation in leaf consumption was due to greatly reduced consumption of leaves of trees with high concentrations of sideroxylonal and 1,8-cineole in *A. montanus* and *A. viriditarsis* (Figures 4.2 – 4.4).

There was a striking difference in behaviour of Christmas beetles depending on leaves offered. The entire leaf, except mid vein and leaf margins, was consumed when leaves of trees with low concentrations of sideroxylonal and 1,8-cineole. In contrast, when leaves of trees with high concentrations of sideroxylonal and 1,8-cineole were offered, Christmas beetles took one to several bites at a number of locations along leaf margins but never continued feeding. Leaves of some trees (e.g., mm4) were either consumed to a similar extent leaves of susceptible trees or not touched at all. This observation lead to a hypothesis that some trees with low concentration of sideroxylonal might be avoided by Christmas beetles due to presence of strong negative cues (e.g., high concentration of 1,8-cineole). This hypothesis was tested by artificially manipulating concentrations of sideroxylonal and 1,8-cineole (see below).

When beetles were given a choice between leaves from two different trees of *E. melliodora*, the beetles always preferred the susceptible tree over the resistant tree or the intermediate tree (Table 4.4). The intermediate tree was always preferred over the resistant tree. In *A. montanus* and *A. pallidicollis*, the strongest preference tended to be exhibited when the susceptible tree and the resistant tree were offered together to beetles. Consumption of leaves of the susceptible and the intermediate trees tended to be increased when paired with leaves of the resistant tree (Table 4.5). Also, consumption of leaves of the resistant tree tended to be increased when paired with leaves of the intermediate tree.

Table 4.4. Mean changes in consumption of the target leaf due to quality of the alternative leaf. The mean represent four Christmas beetle species for each *Eucalyptus* species. Mean $\pm$ SE. Mean changes in leaf consumption of *E. polyanthemos* were not examined because only two beetle species were used in experiments with *E. polyanthemos*.

Effects of	<i>E. melliodora</i>	<i>E. sideroxylon</i>
resistant tree on susceptible tree	45.6 $\pm$ 5.8	-6.3 $\pm$ 3.8
resistant tree on intermediate tree	70.5 $\pm$ 34.6	91.3 $\pm$ 79.9
susceptible tree on resistant tree	1.0	36.6 $\pm$ 26.0
susceptible tree on intermediate tree	13.2	13.4 $\pm$ 23.4
intermediate tree on susceptible tree	8.3 $\pm$ 19.7	21.4 $\pm$ 28.0
intermediate tree on resistant tree	-44.2	13.0 $\pm$ 52.5

When leaves from two trees of *E. sideroxylon* were offered together, beetles again always preferred the susceptible tree over the resistant tree or the intermediate tree (Table 4.4). The intermediate tree was preferred over the resistant tree by three species of Christmas beetles, but not by *A. chloropyrus*. Differences in preference among the three *E. sideroxylon* trees were not as large as those among the three *E. melliodora* trees. Amounts of leaves consumed were not significantly altered by the quality of the alternative leaf (Table 4.5).

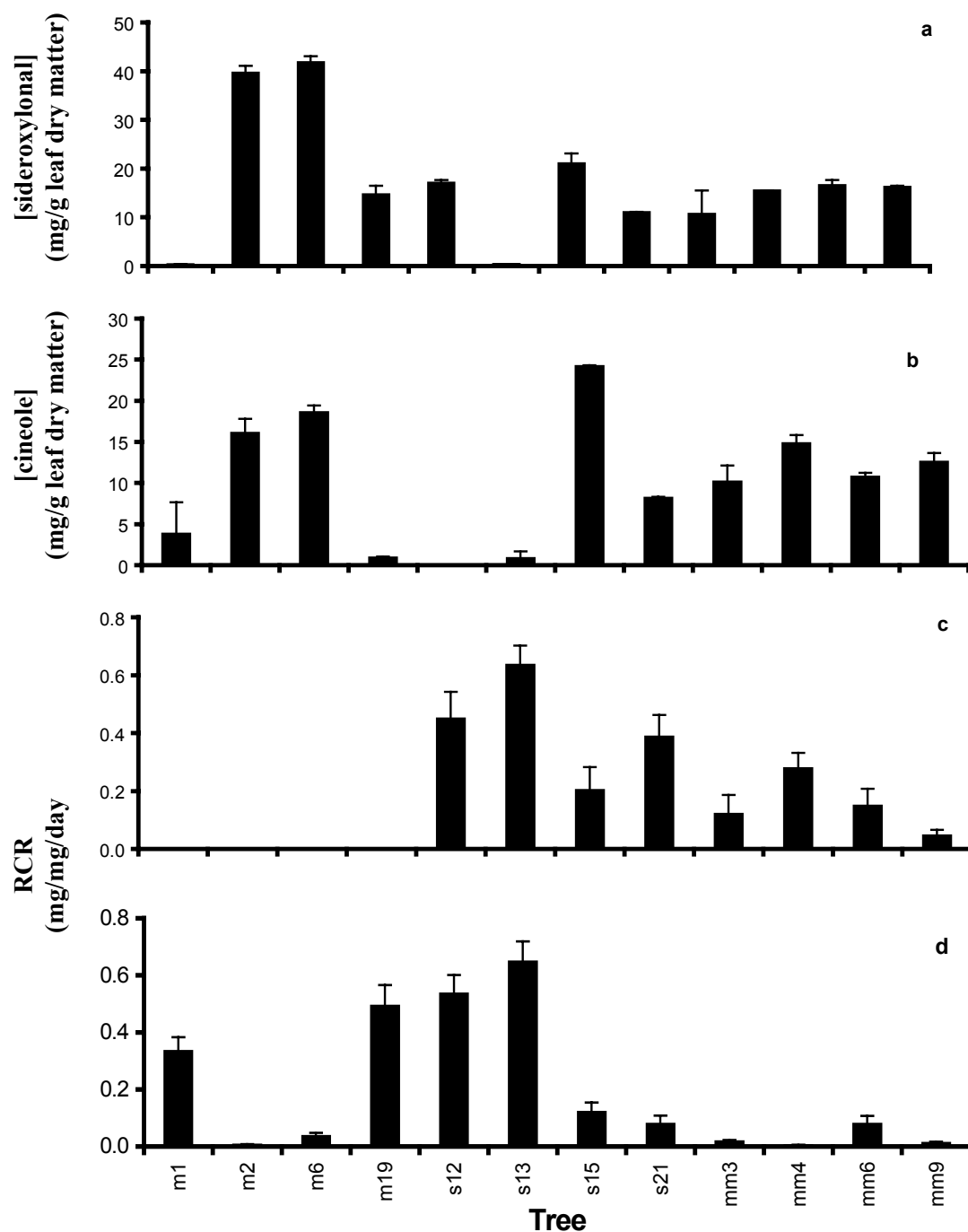


Figure 4.4 Concentrations of a) sideroxylonal and b) 1,8-cineole from *E. melliodora* trees from Yeoval, NSW (m1 - m19) and *E. sideroxylon* from Cumnock, NSW (s12 - s21) and Canberra, ACT (mm3 - mm9) and relative consumption rates (RCR) of c) *A. montanus* and d) *A. chloropyrus* on those trees. All experiments were conducted in the 1997 - 98 season. Mean + SE, $n = 9 - 15$ beetles per tree.

Table 4.5. Summary of statistical tests (nested ANOVAs and contrasts) examining differences in physical and chemical characteristics among *Eucalyptus* species, populations, individual trees, and the difference between resistant and susceptible trees to Christmas beetles. Bold faced values indicate significant differences at $\alpha = 0.05$ level. All measurements were taken on 2+ yr old leaves in the 1997 - 98 season.

Variable	Differences among			Resistant vs susceptible		
	Species <i>P</i> - value	Populations <i>P</i> - value	Trees <i>P</i> - value	<i>P</i> - value	Means	Units
water content	0.20	0.41	0.0001	0.400	44.4 vs 46.0	(%)
Specific mass	0.23	0.87	0.0001	0.080	20.3 vs 18.5	(mg mm ⁻²)
[nitrogen]	0.55	0.02	--	0.200	13.5 vs 14.7	(mg g ⁻¹)
[sideroxylonal]	0.25	0.25	0.0001	0.070	16.1 vs 5.7	(mg g ⁻¹)
[α -pinene]	0.07	0.04	0.0001	0.240	1.5 vs 0.6	(mg g ⁻¹)
[α -phellandrene]	0.92	0.49	0.0001	0.020	0.2 vs 1.1	(mg g ⁻¹)
[limonene]	0.14	0.96	0.0001	0.007	0.9 vs 0.1	(mg g ⁻¹)
[1,8-cineole]	0.73	0.72	0.0001	0.003	15.2 vs 2.1	(mg g ⁻¹)
[<i>p</i> -cymene]	0.44	0.96	0.0001	0.007	0.1 vs 0.8	(mg g ⁻¹)
[terpineol]	0.15	0.69	0.0001	0.060	0.9 vs 0.1	(mg g ⁻¹)

Eucalyptus polyanthemos

Eucalyptus polyanthemos, which was not a normal host of Christmas beetles, did not differ from *E. melliodora* and *E. sideroxylon* in terms of leaf water content and leaf specific mass. Leaf chemistry of the three *E. polyanthemos* trees used in this study also differed markedly (Figure 4.5). Leaf consumption by Christmas beetles, however, did not show corresponding differences (Figure 4.5). *Anoplognathus montanus*, *A. viriditarsis*, *A. chloropyrus*, and *A. pallidicollis* consumed much less leaf material of *E. polyanthemos* compared with *E. melliodora* and *E. sideroxylon*, while *A. suturalis* consumed as much *E. polyanthemos* leaves as leaves of other *Eucalyptus* species.

Among leaves of *E. polyanthemos*, the strongest preference was again observed between the susceptible tree and the resistant tree. *Anoplognathus montanus* consumed small amounts of leaves of *E. polyanthemos* regardless of leaf sources, while *A. chloropyrus* showed stronger preference.

Exotic species

At least one species of Christmas beetles, *A. chloropyrus*, was able to feed on leaves of two of three species of exotic tree species commonly found in southeast Australia (Figure 4.6). *Anoplognathus chloropyrus* avoided feeding on leaves of *Populus* sp. The beetles either completely avoided *Populus* leaves or took only a small number of bites. In contrast, leaves of *Quercus* sp. and *Betula pendula* were eaten almost as much as leaves of the susceptible *E. melliodora* tree (bm44).

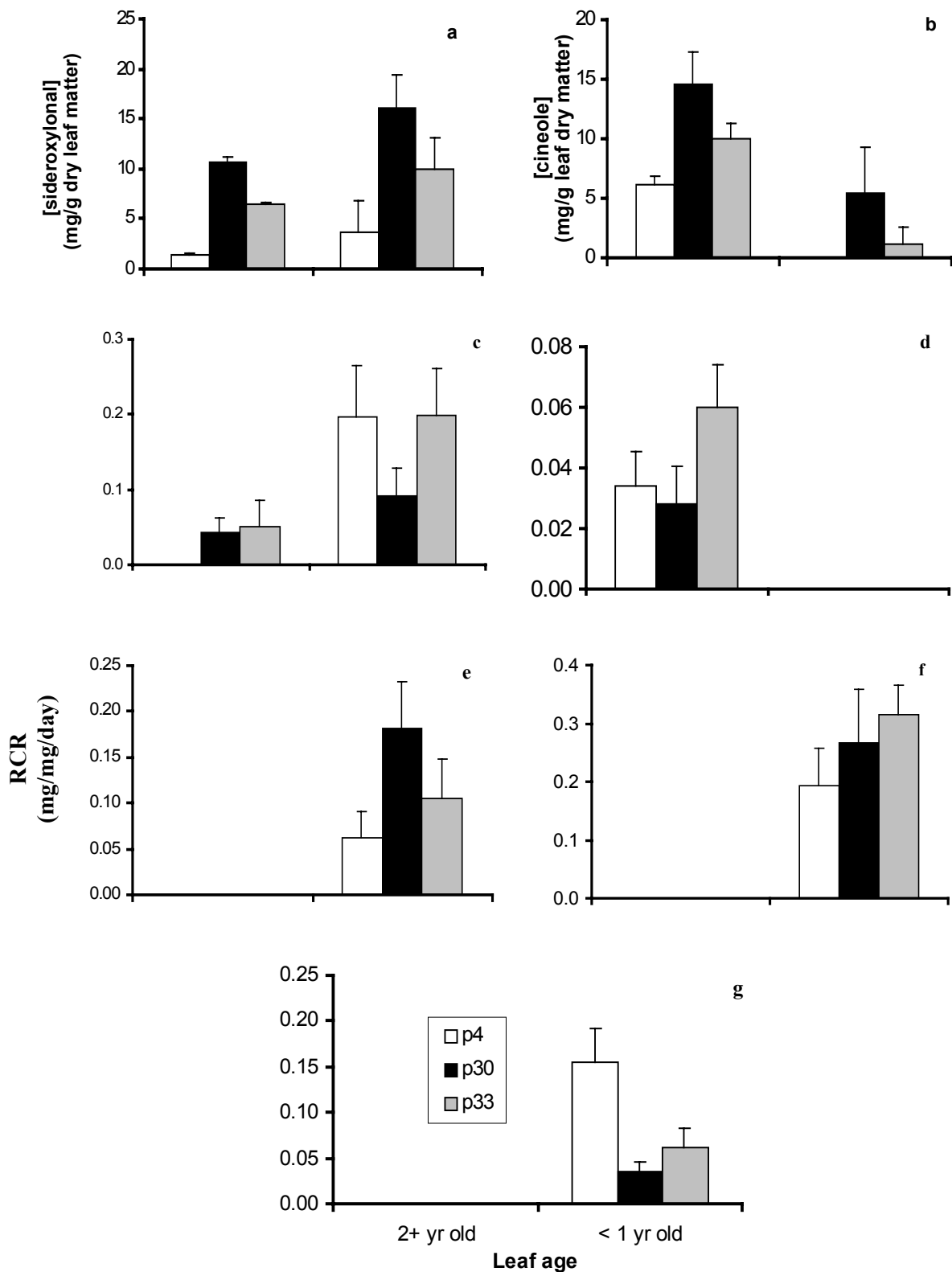


Figure 4.5 Concentrations of a) sideroxylonal and b) 1,8-cineole of *E. polyanthemus* trees and relative consumption rates (RCR) of the Christmas beetles c) *A. chloropyrus*, d) *A. montanus*, e) *A. pallidicollis*, f) *A. sutularis* and -g) *A. viriditarsis*. Experiments with 2+ yr old leaves were conducted in the 1997 - 98 season, and those with < 1 yr old leaves were conducted in the 1998 - 99 season. Mean + SE, $n = 9 - 15$ beetles per tree.

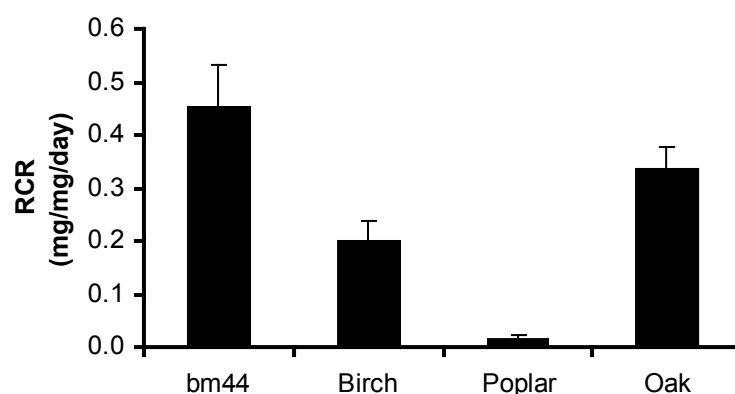


Figure 4.6 Relative consumption rates (RCR) of *A. chloropyrus* on exotic plants (*Quercus*, *Betula*, and *Populus*) compared with the susceptible *E. melliodora* (bm44). Mean + SE, $n = 9 - 15$ beetles per tree.

Effects of sideroxylonal and 1,8-cineole on leaf consumption

There was a negative dosage-dependent effect of sideroxylonal on leaf consumption by *A. suturalis*, *A. viriditarsis*, *A. chloropyrus* and *A. pallidicollis* (Figure 4.7). Relative consumption rates (RCR) in high sideroxylonal concentrations (40 and 80 mg g⁻¹ leaf dry mass) were reduced to approximately one third of those in the control. In *A. chloropyrus*, *A. pallidicollis*, and *A. suturalis*, there was no effect of increased sideroxylonal concentrations at least up to one half of the maximum concentration found naturally in leaves of *E. sideroxylon* and *E. melliodora* (i.e., 20 mg / g leaf dry mass).

When droplets of pure 1,8-cineole were applied onto leaves of *Eucalyptus* branches on which Christmas beetles were feeding, the beetles immediately ceased feeding and quickly moved as far away from the droplets as they could within a cage. Artificial increase in concentration of 1,8-cineole reduced leaf consumption by the five species of Christmas beetles (Figure 4.7). The beetles did not feed on 1,8-cineole painted leaves until 1,8-cineole evaporated off the leaves. Once 1,8-cineole evaporated off, the treatment leaves were consumed just as control leaves. Therefore, the observed reduction in consumption is due to effective reduction in feeding time caused by evaporating 1,8-cineole.

Artificial 50% reduction in the concentration of 1,8-cineole by steaming leaves increased leaf consumption by *A. chloropyrus* when the natural concentration of sideroxylonal was moderate (i.e., <20 mg / g leaf dry mass) and that of 1,8-cineole was high (mm4 in Figure 4.8). However, steaming leaves did not affect consumption of leaves when the natural concentrations of both sideroxylonal and 1,8-cineole were high (bm20) or when the natural concentrations of both sideroxylonal and 1,8-cineole were low (bm44).

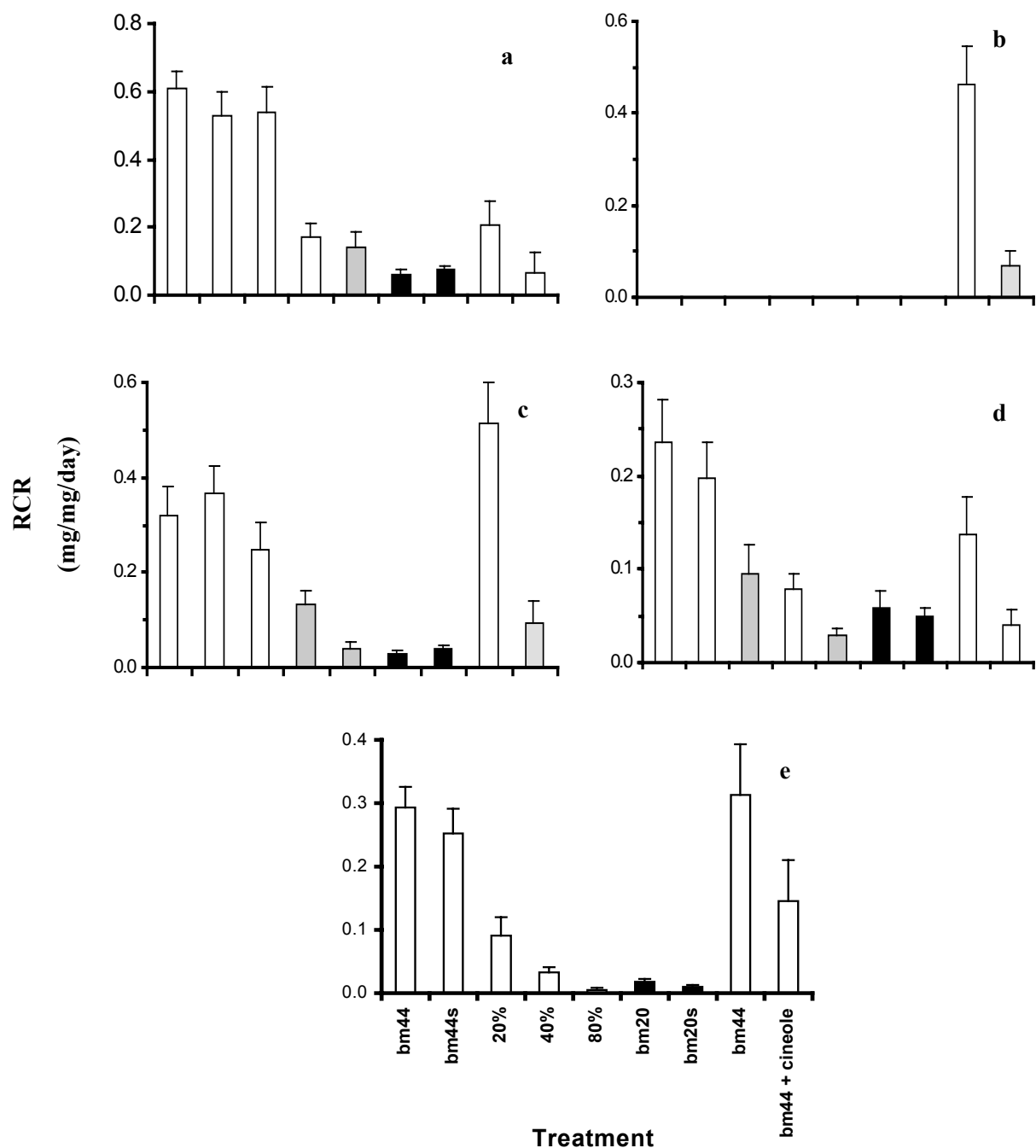


Figure 4.7 Effects of artificially increased concentration of sideroxylonal and 1,8-cineole on relative consumption rate (RCR) of *Eucalyptus melliodora* leaves by five species of Christmas beetles (a) *A. chloropyrus*, b) *A. montanus*, c) *A. pallidicollis* d) *A. suturalis* and e) *A. viriditarsis*). The solvent control is indicated by 's' after each tree number. 20, 40, and 80 indicate the amounts of sideroxylonal painted on leaves of bm44 (mg g⁻¹ leaf dry matter). Mean + SE, $n = 9 - 15$ beetles per tree.

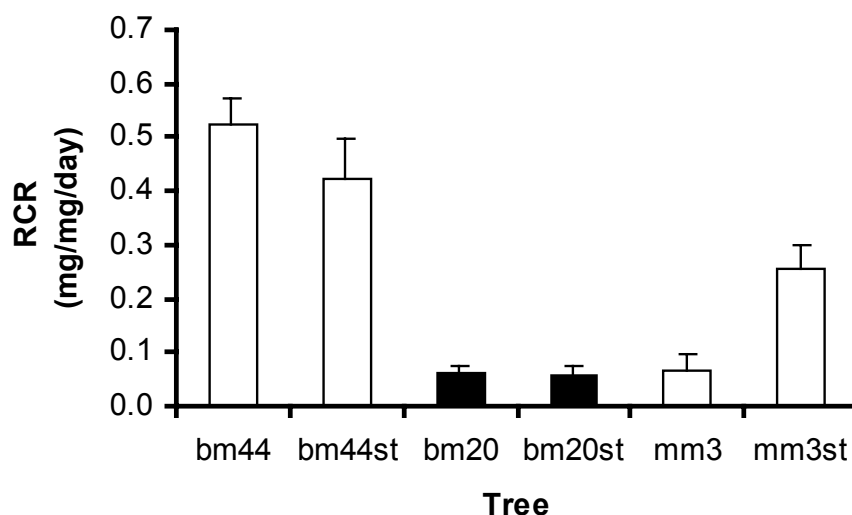


Figure 4.8. Effects of artificially decreased concentration of 1,8-cineole by steaming leaves on relative consumption rate (RCR) of *Eucalyptus melliodora* leaves by *A. chloropyrus*. The steamed treatment is indicated by 'st' after each tree number. Steaming reduced the 1,8-cineole concentration by 50%. Mean + SE, $n = 9 - 15$ beetles per tree.

Discussion

Major findings of this study are that: (1) Leaves of the three *Eucalyptus* species studied showed large intra-specific variation in concentrations of sideroxylonal and 1,8-cineole; (2) Leaves of *E. melliodora* and *E. sideroxylon* trees with low concentrations of both sideroxylonal and 1,8-cineole were eaten more than those of trees with high concentrations of sideroxylonal and 1,8-cineole by all six species of Christmas beetles studied; and (3) Artificial increase in concentrations of sideroxylonal or 1,8-cineole reduced leaf consumption by the five species of Christmas beetles. However, low concentrations of both sideroxylonal and 1,8-cineole did not necessarily result in high leaf consumption in *E. polyanthemus*, a non-host species.

Observed intra-specific variation in leaf chemistry in the three *Eucalyptus* species studied is likely to be at least partly controlled by genetic differences among trees. In *E. melliodora* and *E. sideroxylon*, some trees show intra-individual variation in herbivory by Christmas beetles that are correlated with intra-individual variation in leaf chemistry (Edwards *et al.* 1990; P. Edwards and W. Wanjura, *personal communication*). Somatic mutations in branches are hypothesised to be responsible for the intra-individual variation in leaf chemistry and herbivory. At least in *E. camaldulensis* (a species closely related to *E. melliodora* and *E. sideroxylon*), variation in leaf chemistry has been shown to be more strongly controlled by genetics than the environment (Boland *et al.* 1991, Stone and Bacon 1994, Doran and Matheson 1994: also see Pryor and Bryant 1958 for other *Eucalyptus* species).

Choice vs no-choice experiments

Results of no-choice experiments reveal the physiological abilities of herbivores to deal with leaves with different chemistry. In this study, natural variation in concentrations of sideroxylonal and some terpenes such as 1,8-cineole in *E. sideroxylon* and *E. melliodora* were negatively correlated with consumption by Christmas beetles in no-choice experiments. Moreover, artificial increase in concentrations of sideroxylonal and 1,8-cineole decreased leaf consumption (Figure 4.7).

Results of choice experiments may indicate behaviour of herbivores and the extent of herbivory on plants with different leaf chemistry in the field, where herbivores encounter leaves with a wide variety of chemistry. Christmas beetles tended to discriminate leaves of *E. melliodora* more strongly than those of *E. sideroxylon* or *E. polyanthemos* (Table 4.4). The three trees of *E. melliodora* (bm9, bm20, and bm44) showed greater differences in leaf chemistry than the three trees of *E. sideroxylon* (s42, s45, and s47) (Figure 4.2). Thus, the difference in the pattern of feeding preference observed between *E. sideroxylon* leaves and *E. melliodora* leaves may be a reflection of effects of leaf chemistry on the ability of beetles to discriminate leaf quality. Christmas beetles may be able to discriminate leaves of different quality less effectively in *E. polyanthemos* compared with *E. melliodora* perhaps because *E. polyanthemos* is not a typical host species. Also, it is possible that the index *D* used to estimate feeding preference was not able to show differences in preference among *E. polyanthemos* leaves because the beetles ate much smaller quantities of *E. polyanthemos* leaves compared with *E. melliodora* leaves or *E. sideroxylon* leaves.

In this study, leaves of the susceptible trees (with low sideroxylonal concentrations) were always preferred over leaves of intermediate trees or resistant trees (with high sideroxylonal concentrations), and leaves of the intermediate trees were almost always preferred over leaves of the resistant trees. These results indicate that the fate of leaves of intermediate quality depends on availability of high quality leaves. Consequently, leaves of intermediate quality are less likely to be damaged when Christmas beetles are at low density than when the beetles are at high density. In this study, two leaves were placed side by side in vials, and therefore, there was little cost associated with a beetle moving from one leaf to the other leaf. A preliminary result showed that *A. montanus* still preferred leaves of the susceptible trees over the resistant trees even when the cost of moving between branches were introduced (M. Matsuki, *unpublished data*). Also, the ratio of mass of the two leaves was held constant at approximately unity. In the field, Christmas beetles encounter variation in leaf quality at within tree and between tree levels. In order to understand Christmas beetle feeding behaviour and natural variation in herbivory by the beetles, we may need to consider how, in addition to the mean, variation in leaf quality affects leaf consumption by the beetles. One of the next steps may be to introduce search costs in choice experiments by presenting different ratios of high to low quality leaves.

Role of sideroxylonal and 1,8-cineole on Christmas beetle herbivory

We found that an artificial increase in sideroxylonal concentrations resulted in decreased leaf consumption by Christmas beetles. Sideroxylonal and related acylphloroglucinol derivatives have also been shown to have negative effects on various organisms and biochemical reactions: inhibiting Epstein-Barr virus activation, aldol reductase, photosynthetic electron transport, growth of *Staphylococcus aureus*, *Bacillus subtilis*, and HeLa cells (reviewed in Ghisalberti 1996) and reducing food consumption by arboreal marsupial folivores (Lawler *et al.* 1998a, 1998b, 1999, in press).

When leaves with high concentrations of sideroxylonal are given, Christmas beetles take only one to several bites. Also, when high concentrations of sideroxylonal is patchily painted onto leaves with low concentrations of sideroxylonal, patches with painted sideroxylonal are not consumed by Christmas beetles. These observations suggest that, when sideroxylonal concentrations are high, Christmas beetles detect presence of sideroxylonal using sensory organs in their mouth parts (cf. Frazier 1992, Chapman 1995). When leaves with moderate concentrations of sideroxylonal are given, Christmas beetles continue feeding for a while, but never consume entire leaves, while leaves with low concentrations of sideroxylonal are consumed entirely. Therefore, it appears that even when sideroxylonal is ingested at low concentrations, there is another mechanism to stop Christmas beetles from ingesting large amounts of sideroxylonal.

Unlike sideroxylonal, high concentration of 1,8-cineole appears to act as pre-ingestive deterrent. Christmas beetles did not feed on leaves with increased concentrations of 1,8-cineole. Only after 1,8-cineole has evaporated off the leaves did Christmas beetles start feeding. Terpenes (e.g., 1,8-cineole) have harmful effects on insects post-ingestively, and some insects possess biochemical mechanisms to reduce harmful effects of terpenes such as 1,8-cineole. For instance, increased concentrations of some

monoterpenes commonly found in *Eucalyptus* oils have been shown to increase activity levels of the mixed function oxidases in a generalist insect herbivore *Spodoptera eridania* (Lepidoptera). However, *A. montanus* and *Paropsis atomaria* (Chrysomelidae: Coleoptera) feeding on *Eucalyptus* leaves did not appear to detoxify monoterpenes, and these insects may simply “tolerate” monoterpenes (Morrow and Fox 1980). It is not clear from these biochemical studies how, for example, the observed covariation between herbivory by Christmas beetles and quality and quantity of *Eucalyptus* oils (Edwards *et al.* 1993) can be explained by the ability of beetles to detoxify or tolerate monoterpenes.

Christmas beetles may use 1,8-cineole, or other volatile compounds in *Eucalyptus* oils, as a negative cue for selecting host trees in the field. In the laboratory, Christmas beetles quickly moved away from sources of highly concentrated 1,8-cineole. In the field, Christmas beetles often fly around the canopy at a close range (within 30 cm) after approaching a tree from a distance. If the tree is resistant, many beetles fly away without landing on a leaf or land on a leaf but take off without feeding (M. Matsuki, *personal observation*). High concentration of 1,8-cineole may be present in the atmosphere immediately surrounding resistant trees because a small number of Christmas beetles do feed on some leaves. In fact, sensory organs on the antennae of *A. chloropyrus* have been shown to respond to 1,8-cineole and other monoterpenes (limonene, α -pinene, *p*-cymene, and α -phellandrene) in the atmosphere (F. Schiestle and M. Matsuki, *unpublished data*). Since Christmas beetles seem to detect high concentrations of sideroxylonal using sensory organs in their mouthparts, the observed host selection behaviour of the beetles in the field is not likely to be driven by sideroxylonal in leaves.

That Christmas beetles may use 1,8-cineole as a negative cue for high concentrations of sideroxylonal may have an important theoretical implication for evolution of plant - herbivore interactions mediated by secondary metabolites. Guilford and Cuthill (1991) have suggested that evolution of unpalatability is not a likely result of synergistic selection (on individuals sharing the same phenotype) because unpalatability or “defence as such does not provide predators with a signal over which to generalise”. By this, the authors mean that, unlike aposematic coloration in animals, palatable and unpalatable plants appear identical except for the difference in palatability. However, results of present study suggest that volatile compounds such as 1,8-cineole may act as a signal for presence and the concentration of compounds such as sideroxylonal (cf. Eisner and Grant 1981, Launchbaugh and Provenza 1993, Augner 1994, Augner and Bernays 1998, Bernays and Lee 1988, Blaney *et al.* 1987, Dethier 1980, Dethier *et al.* 1979). An interesting twist in the *Eucalyptus* - herbivore system is that some trees of *E. sideroxylon* seem to have only moderate to low concentrations of sideroxylonal but high concentrations of 1,8-cineole (e.g., mm⁴ in this study). If these trees suffer low levels of herbivory in the field, as is observed in the laboratory experiments, then it may be possible that high concentrations of 1,8-cineole in these trees acts as a false signal.

Tuomi and Augner (1993) and Leimar and Tuomi (1998) showed, using game theory, that evolution of plant unpalatability might result from synergistic selection, even without a cue, if herbivores take samples from a population of plants and make generalisations about the population. The three species of *Eucalyptus* used in the present study show striking variation in leaf chemistry among neighbouring individuals (Edwards *et al.* 1993). Therefore, if synergistic selection has been involved in evolution of chemical defences using sideroxylonal in *E. sideroxylon* and related species, presence of volatile compounds such as 1,8-cineole, which can be used as a cue (Guilford and Cuthill 1991), is a more likely mechanism than leaf sampling and subsequent generalisation by herbivores (Tuomi and Augner 1993, Leimar and Tuomi 1998).

So far, we have discussed secondary metabolites that negatively affect tree choice and feeding, but some compounds may act as positive cues for Christmas beetles. Under natural conditions, Christmas beetles rarely feed on non-*Eucalyptus* species. Christmas beetles however, can feed on some exotic plants under no-choice conditions. Leaves of these exotic species do not contain high concentrations of sideroxylonal or 1,8-cineole. Therefore, lack of feeding of those exotic species under natural conditions can not be explained by presence of high concentrations of sideroxylonal or 1,8-cineole. Moreover, *A. chloropyrus* showed neutral responses to α -phellandrene and *p*-cymene that are found in higher concentrations in trees with low concentrations of 1,8-cineole than trees with high

concentrations of 1,8-cineole. Thus, Christmas beetles may use both positive and negative cues to select trees.

Future research directions

In this study, we showed that leaf consumption by Christmas beetles was negatively affected by sideroxylonal. Also, we showed that sideroxylonal reduced leaf consumption by Christmas beetles when its concentration was greater than 10 mg g⁻¹ dry leaf mass. Of 21 trees with sideroxylonal, 10 trees showed sideroxylonal concentration less than 10 mg g⁻¹ dry leaf mass. We observed variation in consumption of leaves from the 10 trees with low sideroxylonal concentration. These observations on natural variation in the sideroxylonal concentration and presence of variation in leaf consumption by Christmas beetles when leaves contain low concentrations of sideroxylonal beg two questions: (1) What causes variation in leaf consumption when the sideroxylonal concentration is low; and (2) Why is such a large proportion of the population inadequately defended by sideroxylonal? Results of this study suggest that some trees may be defended by the false signal of volatile compounds such as 1,8-cineole even when the sideroxylonal concentration is less than 10 mg g⁻¹ dry leaf mass. However, since concentrations of sideroxylonal and 1,8-cineole are positively correlated with each other, the majority of trees with low concentrations of sideroxylonal are likely to have low concentrations of 1,8-cineole.

Perhaps one of the next steps in the study of intra-specific variation in *Eucalyptus* - herbivore interactions is to examine effectiveness of herbivores in reducing survivorship and fitness of trees with different leaf chemistry. Although severe defoliation by Christmas beetles reduces growth of young trees, even trees less than four years old can withstand complete defoliation by Christmas beetles (R. Floyd and M. Matsuki, *unpublished results*; also see Carne *et al.* 1974). Thus, selection pressure by Christmas beetles and other herbivores may be strongest during seedling and sapling stages (cf. Bryant *et al.* 1992). In many *Eucalyptus* species, seedling recruitment occurs after fire and in very wet years. Consequently, seedling recruitment is spatially and temporally variable. Population size of adult Christmas beetles also show marked spatial and between year variation (Carne *et al.* 1974). Therefore, it is possible that inadequately defended genotypes are able to persist in populations.

Although we examined primarily intra-specific variation in leaf chemistry and herbivory, we would also like to discuss briefly inter-specific variation in herbivory by Christmas beetles. One of the three *Eucalyptus* species examined in this study, i.e., *E. polyanthemos*, is not a typical host of Christmas beetles, while the other two species, *E. sideroxylon* and *E. melliodora*, are typical host species. However, *E. polyanthemos* did not differ significantly from *E. sideroxylon* and *E. melliodora* in terms of leaf physical characteristics, i.e., water content and specific mass, and chemistry, i.e., terpenes and sideroxylonal. Also, leaf consumption by Christmas beetles was not negatively correlated with concentration of sideroxylonal or 1,8-cineole in *E. polyanthemos*. These results suggest that we may need to examine compounds other than terpenes and sideroxylonal and leaf characteristics such as size and shape to understand why *E. polyanthemos* is not a typical host of Christmas beetles.

There is a scope for looking for generality from specific results of this study. There are more than 700 species of *Eucalyptus* in Australia (Williams and Brooker 1997). Aside from Christmas beetles, there are numerous other important insect herbivores on *Eucalyptus* (Ohmart and Edwards 1991). Sideroxylonal is one of many compounds collectively known as acylphloroglucinol derivatives (Ghisalbarti 1996). Leaves of many *Eucalyptus* species contain one to several different acylphloroglucinol derivatives (Ghisalbarti 1996) as well as many terpenes (Boland *et al.* 1991). Moreover, there is qualitative as well as quantitative variation in leaf chemistry among species (Boland *et al.* 1991, Ghisalbarti 1996) and among individuals within a species (Edwards *et al.* 1993, Stone and Bacon 1994, this study). Further studies using carefully selected sets of *Eucalyptus* and herbivore species based on phylogenies, geographic distribution ranges, and leaf chemistry may allow us to extend our understanding of the *Eucalyptus* - herbivore interactions mediated by acylphloroglucinol derivatives, terpenes, and other aspects of leaf chemistry.

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5. Individualistic responses of insect herbivores to three species of *Eucalyptus* in southeast Australia⁴

Introduction

Plant genus *Eucalyptus* (Myrtaceae) provides an interesting challenge to understanding the nature of plant - herbivore interactions. Some species of *Eucalyptus* show marked intra-specific variation in susceptibility to insect herbivores (Edwards *et al.* 1990, 1993; Farrow *et al.* 1994, Floyd *et al.* 1994, ms; Matsuki *et al.* manuscript-a; Raymond 1995; Stone and Bacon 1994). Some *Eucalyptus* spp. also show marked intra-specific variation in genetically based leaf chemistry (e.g., Boland *et al.* 1991), and at least in some species, there are relationships between variation in leaf chemistry and variation in herbivory.

Leaves of *Eucalyptus* spp. contain essential oils that are primarily consisted of terpenes (Boland *et al.* 1991). Moreover, it has been shown that the proportion of 1,8-cineole (a monoterpene) in essential oils is higher (60 – 80%) in trees resistant to Christmas beetles (*Anoplognathus* spp., Scarabaeidae, Coleoptera) than susceptible trees (< 40%) (Edwards *et al.* 1993). A series of other studies has revealed some *Eucalyptus* spp. also show intra-specific variation in susceptibility to mammalian folivores (Lawler *et al.* 1998a, 1998b; 1999, in press; Lawler and Foley 1999). Susceptibility to mammalian folivores, however, is shown to be affected by a group of compounds called acylphloroglucinol derivatives. These compounds are hybrids between phenolics and terpenes/isoprenes (Boland *et al.* 1992, Ghisalberti 1996), and leaves of many *Eucalyptus* spp. contain one to several different acylphloroglucinol derivatives (Ghisalberti 1996).

In some *Eucalyptus* spp., such as *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis*, variation in leaf chemistry and susceptibility to herbivores is observed between individuals within populations (Edwards *et al.* 1993, Matsuki *et al.* manuscript-b). Concentrations of 1,8-cineole and sideroxylonal (an acylphloroglucinol derivative) range from less than 1 to greater than 25 mg g⁻¹ leaf dry matter within a population. These differences in leaf chemistry are responsible for up to 40-fold difference in leaf consumption by common ringtail possum (Lawler *et al.* in press) and 25-fold difference in Christmas beetles (Matsuki *et al.* manuscript-b). The susceptible individuals were completely defoliated by Christmas beetles, while the resistant individuals were hardly damaged (Edwards *et al.* 1993). With such a strong relationship between leaf chemistry and susceptibility of individual trees to herbivores, it is surprising to observe persistence of susceptible individuals in populations of these *Eucalyptus* spp.

One of the hypothesised mechanisms to maintain genetic variation is variable selection by different agents of selection. Any one of many *Eucalyptus* spp. is typically a host to a wide range of insect herbivores (Ohmart and Edwards 1991). If trees resistant to Christmas beetles and marsupial herbivores are not resistant to other herbivores, then this might explain the observed variation in leaf chemistry. The purpose of this study was to examine cross-resistance of individual trees of *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis* to a wide range of insect herbivores on *Eucalyptus*.

Study organisms

The three *Eucalyptus* spp. used in this study (*E. sideroxylon*, *E. melliodora*, and *E. camaldulensis*) are similar in many aspects of their natural history. They are medium-sized trees (typically to 20 m in height) and are found in pastures and woodlands throughout southeast Australia (Boland *et al.* 1984).

⁴ Based on a manuscript developed by M. Matsuki, W. Foley and R. B. Floyd

Eucalyptus melliodora is found in large continuous populations at low to moderate density, while *E. sideroxylon* is found in small isolated populations at high density. *Eucalyptus camaldulensis* is found along rivers and watercourses. The three *Eucalyptus* spp. exhibit two distinct types of leaf chemistry (Edwards *et al.* 1993). Individuals with small amounts of 1,8-cineole in essential oils are susceptible to *Anoplognathus* spp., while individuals with large amounts of 1,8-cineole are resistant to *Anoplognathus* spp. (Edwards *et al.* 1993). The resistant individuals of *E. melliodora* and *E. sideroxylon* also contain higher concentrations of sideroxylonal, than the susceptible individuals (Matsuki *et al.* manuscript-b).

Adult Christmas beetles (*Anoplognathus* spp.) feed on a wide range of *Eucalyptus* species, including *E. sideroxylon*, *E. camaldulensis*, and *E. melliodora*, while larvae feed on roots of grass and crops. We used five species of Christmas beetles that were commonly found around Canberra, Australian Capital Territory (*A. montanus*, *A. viriditarsis*, *A. chloropyrus*, *A. pallidicollis*, and *A. suturalis*). Adults of *A. montanus* and *A. suturalis* emerge from early to late December, *A. pallidicollis* and *A. chloropyrus* emerge from early December to mid January, and *A. viriditarsis* emerges from early to mid January. Adult *Anoplognathus* spp. live for less than one month. There is a considerable spatial and between-year variation in abundance of *Anoplognathus* spp. Population densities tend to be low in years with droughts in November and December (Carne *et al.* 1981).

A species of eucalypt leaf-beetle (*Chrysophtharta variicollis*, Chrysomelidae, Coleoptera) and a species of eucalypt weevil (*Gonipterus scutellatus*, Curculionidae, Coleoptera) are both found in southeast Australia. These two species are typically bivoltine, and adults and/or larvae are found throughout the year in small numbers. Both adults and larvae of these two species feed on young leaves of a number of *Eucalyptus* spp. including *E. sideroxylon* and *E. melliodora*. There is little evidence to suggest these two species discriminate between individuals within a host species (R. A. Farrow, *personal communication*).

Gum Leaf Skeletoniser (*Uraba lugens*, Noctuidae, Lepidoptera) is distributed along the East Coast of Australia from tropics to cool temperate regions and southwest Australia. This species is found on a wide range of *Eucalyptus* spp. but is especially common on *E. camaldulensis*. Larvae are commonly found in late spring to summer (one to two generations), and periodic outbreaks have been recorded (Campbell 1962). The 1st and 2nd instar larvae leave vascular tissues untouched (hence the common name “skeletoniser”), while later instars feed on all parts of leaf lamina. There is little evidence to suggest that *U. lugens* discriminate between individuals within a host species (R. A. Farrow, *personal communication*).

Emperor gum moth (*Opodiphthera helena* and *O. eucalypti*, Saturniidae, Lepidoptera) are found on a wide range of *Eucalyptus* spp. in eastern Australia. Eggs were found in clusters of one to six. They are univoltine, and larvae are found during summer months. A species of case moth (*Hyalarcta huebneri*, Psychidae, Lepidoptera) is also found on a wide range of *Eucalyptus* spp. in eastern Australia. Larvae live inside a silken case with fragments of dead leaves and are found throughout the year. Females pupate, eclose, and lay eggs in their cases.

A species of spitfire sawfly (*Pergagraptia latreillei* (Westwood), Pergidae, Hymenoptera) is found on a wide range of *Eucalyptus* spp. in eastern Australia. This and other related species of spitfire sawflies are bivoltine, and larvae are found throughout the year. Larvae are found in clusters (10 to 50+ individuals per cluster). When moving off the leaf, the last larva often removes the partly consumed leaf by girdling the petiole (Edwards and Wanjura 1989). Larvae of spitfire sawflies use liquid regurgitate to deter vertebrate predators when threatened. The liquid regurgitate is stored in a special pouch and, at least in one species, *Perga affinis*, it contains essential oils (Morrow *et al.* 1976) and high concentrations of sideroxylonal (B. Clarke and M. Matsuki, *personal observation*) from *Eucalyptus* leaves.

Methods

Choice of trees

Trees used in this study were selected based on records of herbivory by *Anoplognathus* spp. and leaf consumption by marsupial folivores (Table 5.1). We defined susceptible, resistant, and intermediate trees of the *E. sideroxylon* and *E. melliodora* based on leaf consumption by marsupial folivores. Among eight *E. melliodora* trees, bm44 supported the highest leaf consumption, and bm20 supported the lowest leaf consumption by koala (*Phascolarctos cinereus*: B. Moore, unpublished results) and common brushtail possum (*Trichosurus vulpecula*: Watson 1998). Among 12 *E. sideroxylon* trees, s45 supported the highest leaf consumption, and s47 supported the lowest leaf consumption by common brushtail possum (Watson 1998) and common ringtail possum (*Pseudocheirus peregrinus*: Lawler 1999). We chose some susceptible (trees c8, c25, & c35) and resistant (trees c3, c12, & c37) trees of *E. camaldulensis* based on records of herbivory by *Anoplognathus* spp. (mostly *A. montanus*) in the field (P. B. Edwards and W. J. Wanjura, unpublished data). However, leaf consumption by koala was higher on trees resistant to *Anoplognathus* spp. than those susceptible (B. Moore, unpublished results).

Table 5.1 Summary of information about trees used in this study.

Tree #	Species	Susceptibility	Herbivore species	% defoliation or leaf consumption
bm9	<i>E. melliodora</i>	intermediate	koala	23 g kg ⁻¹ day ⁻¹
			brushtail possum	--
bm20	<i>E. melliodora</i>	resistant	koala	9 g kg ⁻¹ day ⁻¹
			brushtail possum	16 g kg ⁻¹ day ⁻¹
bm44	<i>E. melliodora</i>	susceptible	koala	35 g kg ⁻¹ day ⁻¹
			brushtail possum	34 g kg ⁻¹ day ⁻¹
s42	<i>E. sideroxylon</i>	intermediate	brushtail possum	21 g kg ⁻¹ day ⁻¹
			ringtail possum	32 g kg ⁻¹ day ⁻¹
s45	<i>E. sideroxylon</i>	susceptible	brushtail possum	27 g kg ⁻¹ day ⁻¹
			ringtail possum	68 g kg ⁻¹ day ⁻¹
s47	<i>E. sideroxylon</i>	resistant	brushtail possum	10 g kg ⁻¹ day ⁻¹
			ringtail possum	2 g kg ⁻¹ day ⁻¹
c3	<i>E. camaldulensis</i>	resistant	Christmas beetle	20%
			koala	--
c8	<i>E. camaldulensis</i>	susceptible	Christmas beetle	95%
		resistant	koala	19 g kg ⁻¹ day ⁻¹
c12	<i>E. camaldulensis</i>	resistant	Christmas beetle	30%
		susceptible	koala	37 g kg ⁻¹ day ⁻¹
c25	<i>E. camaldulensis</i>	susceptible	Christmas beetle	100%
		intermediate	koala	26 g kg ⁻¹ day ⁻¹
c36	<i>E. camaldulensis</i>	susceptible	Christmas beetle	98%
		resistant	koala	20 g kg ⁻¹ day ⁻¹
c37	<i>E. camaldulensis</i>	resistant	Christmas beetle	30%
		susceptible	koala	38 g kg ⁻¹ day ⁻¹

Physical and chemical characteristics of leaves

A sub-sample of leaves from a leaf sample for nearly all growth/consumption trials was used to characterise leaf physical and chemical traits. For the physical traits, we measured leaf water content and specific mass. Because *Eucalyptus* leaves contain volatile monoterpenes, leaves were dried at 40°C for 2 days. Specific mass of a leaf was measured by weighing a small disk of a unit area cut out from the leaf blade. For the chemical traits, we estimated concentrations of sideroxylonal and 1,8-cineole using near-infrared reflectance spectrometry (NIRS). The method of estimation using NIRS has been described in details elsewhere (Foley *et al.* 1998, Matsuki *et al.* manuscript-b).

Growth/consumption trials

Insect herbivores were collected from southeast Australia between December 1997 and January 2000 whenever opportunities arose (Table 5.2). *Anoplognathus* spp. were kept in cages (37 × 28 × 55 cm each), and freshly cut branches of *E. leucoxydon* in small vases were placed in the cages each day. *Anoplognathus* spp. readily feed on leaves of certain trees of *E. leucoxydon*. Other species were kept in tightly sealed plastic containers, and freshly cut branches of *E. camaldulensis* (for *U. lugens*), *E. sideroxylon*, *E. melliodora*, and *E. leucoxydon* (for *P. latreillei*, *C. variicollis*, *O. helena*, *O. eucalypti*, *H. huebneri*, and *G. scutellatus*) were placed in the containers.

Table 5.2 Summary of insect species used in this study.

Species	Origin	Date of collection	Life Stage	Leaves naturally eaten	Leaves given
<i>Anoplognathus montanus</i>	Holbrook, NSW	Dec-98	adult	expanded	expanded
<i>A. viriditarsis</i>	Boorowa, NSW	Jan-99	adult	expanded	expanded
<i>A. chloropyrus</i>	Gunning, NSW	Jan-99	adult	expanded	expanded
<i>A. pallidicollis</i>	Gunning, NSW	Jan-99	adult	expanded	expanded
<i>A. suturalis</i>	Gunning, NSW	Jan-99	adult	expanded	expanded
<i>Gonipterus scutellatus</i>	Gunning, NSW	Feb-99	adult	expanding/ expanded	expanded
<i>Chrysophtharta variicollis</i>	Shepparton, Vic	Mar-99	adult	expanding/ expanded	expanded
<i>C. variicollis</i>	Gunning, NSW	Dec-99	4th (final) instar	expanding	expanding
<i>Uraba lugens</i>	Howlong, NSW	Nov-98	4th & 5th (final) instar	expanding/ expanded	expanded
<i>Opodiphthera helena</i>	Ginninderra, NSW	Dec-99	5th (final) instar	expanding/ expanded	expanded
<i>O. eucalypti</i>	Ginninderra, NSW	Jan-00	5th (final) instar	expanding/ expanded	expanded
<i>Hyalarcta huebneri</i>	Boorowa, NSW	Dec-99	3rd - 5th (final) instar	expanding/ expanded	expanded
<i>Pergagraptia latreillei</i>	Canberra, ACT	Dec-99	3rd (penultimate) instar	expanding	expanding

For all experiments, insects were individually weighed and placed with a leaf or leaves in clear plastic screw-top vials (250 ml). Each vial had moist plaster of paris to maintain leaf turgor pressure. Experiments were terminated after 5 to 60 hr when some leaves were nearly completely consumed. Each insect was weighed again at the end of each experiment.

For each experiment, several small branches were taken from each tree so that leaves used in our experiments were representatives of leaves on the experimental trees. Size and age of leaves used in

the experiments in a given season were carefully standardised in order to minimise effects of age and size on leaf consumption. We used leaves of six months to two years old in the summer of 1998 - 1999 (except for *A. montanus*). Leaves of two months to one year old were used in the summer of 1999 - 2000.

Since adult beetles (*Anoplognathus* spp. *C. variicollis*, and *G. scutellatus*) grew very little, we calculated only relative consumption rates (RCR). For larvae, however, we calculated relative growth rates (RGR), relative consumption rates (RCR), and relative rates of frass production (RFP), and estimated relative assimilation rates (RAR) (Gordon 1968, Waldbauer 1968):

$$\begin{aligned} \text{RGR} &= [\ln(M_f) - \ln(M_i)] / (T_f - T_i), \\ \text{RCR} &= \text{consumption} / [M \times (T_f - T_i)], \\ \text{RFP} &= \text{frass} / [M \times (T_f - T_i)], \text{ and} \\ \text{RAR} &= \text{RCR} - \text{RFP}. \end{aligned}$$

where

frass = dry mass of frass produced during the experiment,
consumption = $L_{iw} \times (L_{cw} / L_{cd}) - L_{id}$,
 L_{iw} = initial fresh mass of consumed leaf,
 L_{cw} = initial fresh mass of control leaf,
 L_{cd} = final dry mass of control leaf,
 L_{id} = final dry mass of consumed leaf,
 $M = (M_f - M_i) / \ln(M_f / M_i)$ for larvae,
 $M = (M_f - M_i) / 2$ for adult insects,
 M_i = initial dry mass of beetle measured at time T_i , and
 M_f = final dry mass of beetle measured at time T_f .

Since adult beetles did not grow exponentially, we used the mean dry mass of adult beetles to calculate RCR as suggested by Waldbauer (1968), while for larvae of *C. variicollis*, *O. helena*, *O. eucalypti*, *P. latreillei*, *H. huebneri* and *U. lugens*, we used the ratio of the mean dry mass to the mean of natural log dry mass as suggested by Gordon (1968). Using a subset of each species, dry mass was estimated from a wet mass: dry mass ratio for each species. Control leaves were individually placed in a plastic vial with moist plaster of Paris. Within each block, location of vials with control leaves and those with experimental leaves and insects was randomised.

Experimental design and statistical analyses

For, *Anoplognathus* spp., *C. variicollis*, *G. scutellatus*, *P. latreillei*, and *U. lugens*, we used 5 blocks $\times$ 2 to 6 treatments (trees) $\times$ 3 replicates. Blocks in the experiments represented rooms in three different buildings; however, the same five rooms were used in all experiments. There were only three larvae of *O. helena*. In a given experimental period, a larva was given leaves of one of three *E. melliodora* trees, but leaves from different trees were given to different larvae. A larva was given leaves of different trees in successive experimental periods. Hence, all three larvae were given leaves of all three *E. melliodora* trees in three successive experimental periods (i.e., Latin square design). Similarly, all three larvae were given leaves of all three *E. sideroxylon* trees in the following three successive experimental periods. This process was repeated twice. There was only one *O. eucalypti* larva available, and the larva was given leaves of the six trees twice.

We analysed data on leaf physical and chemical characteristics, and insect performance using ANOVAs (when the data set was balanced) or restricted maximum likelihood analyses (when the data set was unbalanced). We used GENSTAT (Genstat 5 Committee 1993) and SAS (SAS Institute 1989) for statistical analyses.

Results

Leaves more than 2 year-old had lower water content and higher specific leaf weight (SLW) than leaves less than 1 yr old (Tables 5.3 and 5.4). However, there was no inter- or intra-specific variation in leaf water content or SLW in a given leaf age.

Different insect species showed different responses to the same trees of eucalypts. Two species of Christmas beetles (*Anoplognathus montanus* and *A. viriditarsis*) showed markedly higher consumption rates on trees with lower concentrations of 1,8-cineole than those with higher concentrations of 1,8-cineole in *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis* (Figure 5.1). *Eucalyptus melliodora* and *E. sideroxylon*, trees with high concentrations of 1,8-cineole also had high concentration of sideroxylonal, while there was no sideroxylonal in *E. camaldulensis*. Three other species of Christmas beetles (*A. chloropyrus*, *A. pallidicollis*, and *A. suturalis*) showed higher consumption rates of *E. melliodora* and *E. sideroxylon* trees with low concentrations of sideroxylonal and 1,8-cineole. *Anoplognathus chloropyrus*, *A. pallidicollis*, and *A. suturalis* showed individualistic responses to the six *E. camaldulensis* trees.

Table 5.3. Water content (%) of leaves used in various experiments. Mean (SE), $n = 5$ or 10. Water content of leaves used in the experiment with *U. lugens* was not measured.

	2+ yr old	< 1 yr old	< 1 yr old	< 1 yr old	1 yr old	< 1 yr old
	<i>A. montanus</i>	<i>A. viriditarsis</i>	<i>G. scutellatus</i>	<i>C. variicollis</i>	<i>O. helena</i>	<i>C. variicollis</i>
	<i>A. viriditarsis</i>	<i>A. chloropyrus</i>			<i>O. eucalypti</i>	<i>P. latreillei</i>
	<i>A. chloropyrus</i>	<i>A. pallidicollis</i>			<i>H. huebneri</i>	
	<i>A. pallidicollis</i>	<i>A. suturalis</i>				
Tree	<i>A. suturalis</i>					
c3	54.7 (0.4)	63.0 (0.6)				
c8	52.2 (0.5)	53.0 (0.9)				
c12	52.6 (1.6)	58.2 (1.4)				
c25	53.8 (1.0)	62.7 (0.9)				
c35	55.2 (0.7)	50.9 (1.6)				
c37	51.7 (0.9)	52.2 (0.6)				
bm9		55.7 (0.5)	53.0 (0.4)	54.0 (0.6)	48.0 (0.3)	
bm20		53.2 (0.5)	54.5 (0.3)	53.7 (0.3)	48.7 (0.3)	61.2 (0.4)
bm44		57.0 (1.3)	57.5 (0.3)	59.6 (0.5)	52.7 (0.4)	63.5 (0.4)
s42		55.9 (0.5)	53.7 (0.6)	50.7 (0.5)	46.2 (0.2)	
s45		55.9 (0.6)	52.3 (0.5)	55.2 (0.5)	47.5 (0.2)	
s47		56.0 (0.4)	49.4 (0.5)	50.1 (1.1)	43.7 (0.5)	

Adult eucalypt weevils (*G. scutellatus*) and eucalypt leaf beetles (*C. variicollis*) showed no corresponding variation in consumption rates with concentrations in sideroxylonal and 1,8-cineole (Figure 5.2). The same tree (s45) supported the highest consumption rates in *G. scutellatus* and the lowest in *C. variicollis*. Larvae of *C. variicollis* showed no difference in growth rates, consumption rates, frass production, and assimilation rates between an *E. melliodora* tree (bm20) with high concentrations of sideroxylonal and 1,8-cineole and another *E. melliodora* tree (bm44) with low concentrations of those compounds (Figure 5.3). In contrast, larvae of spitfire sawfly (*P. latreillei*) performed better on the *E. melliodora* tree (bm44) with low concentration of sideroxylonal and 1,8-cineole than the *E. melliodora* tree (bm20) with high concentrations of those compounds.

Larvae of gumleaf skeletoniser (*U. lugens*) showed no difference in growth rates, consumption rates, frass production, and assimilation between 12 trees of *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis* (Figure 5.4). Larvae of emperor gum moth (*O. helena* and *O. Eucalypti*) and a case moth (*H. huebneri*) also showed no difference in their performance on the six trees of *E. melliodora* and *E. sideroxylon* (Figure 5.5).

Table 5.4. Specific Leaf Weight (SLW) (mg mm^{-1}) of leaves used in various experiments. Mean (SE), $n = 1 - 10$. SLW of leaves used in the experiment with *U. lugens* was not measured.

	2+ yr old	< 1 yr old	< 1 yr old	< 1 yr old	1 yr old	< 1 yr old
	<i>A. montanus</i>	<i>A. viriditarsis</i>	<i>G. scutellatus</i>	<i>C. variicollis</i>	<i>O. helena</i>	<i>C. variicollis</i>
	<i>A. viriditarsis</i>	<i>A. chloropyrus</i>			<i>O. eucalypti</i>	<i>P. latreillei</i>
	<i>A. chloropyrus</i>	<i>A. pallidicollis</i>			<i>H. huebneri</i>	
	<i>A. pallidicollis</i>	<i>A. suturalis</i>				
Tree	<i>A. suturalis</i>					
c3	18.0 (0.7)	13.8 (0.8)				
c8	16.2 (0.3)	14.9 (0.5)				
c12	16.7 (0.8)	12.6 (0.3)				
c25	14.6 (0.4)	10.9 (0.2)				
c35	17.8 (0.4)	19.7 (0.9)				
c37	17.4 (0.3)	20.1 (0.8)				
bm9		14.8 (1.0)	17.4 (0.6)	17.1 (0.5)	19.4 (0.4)	
bm20		13.9 (0.5)	15.9 (0.5)	15.2 (0.3)	17.6 (0.3)	11.6 (0.4)
bm44		13.1 (0.4)	13.6 (0.7)	11.8 --	17.8 (0.5)	11.8 (0.3)
s42		12.6 (0.2)	13.1 (0.3)	13.0 (0.4)	19.3 (0.5)	
s45		12.9 (0.3)	14.7 (0.4)	12.5 (0.2)	17.3 (0.5)	
s47		15.1 (0.5)	18.4 (0.6)	17.1 (0.3)	23.9 (0.5)	

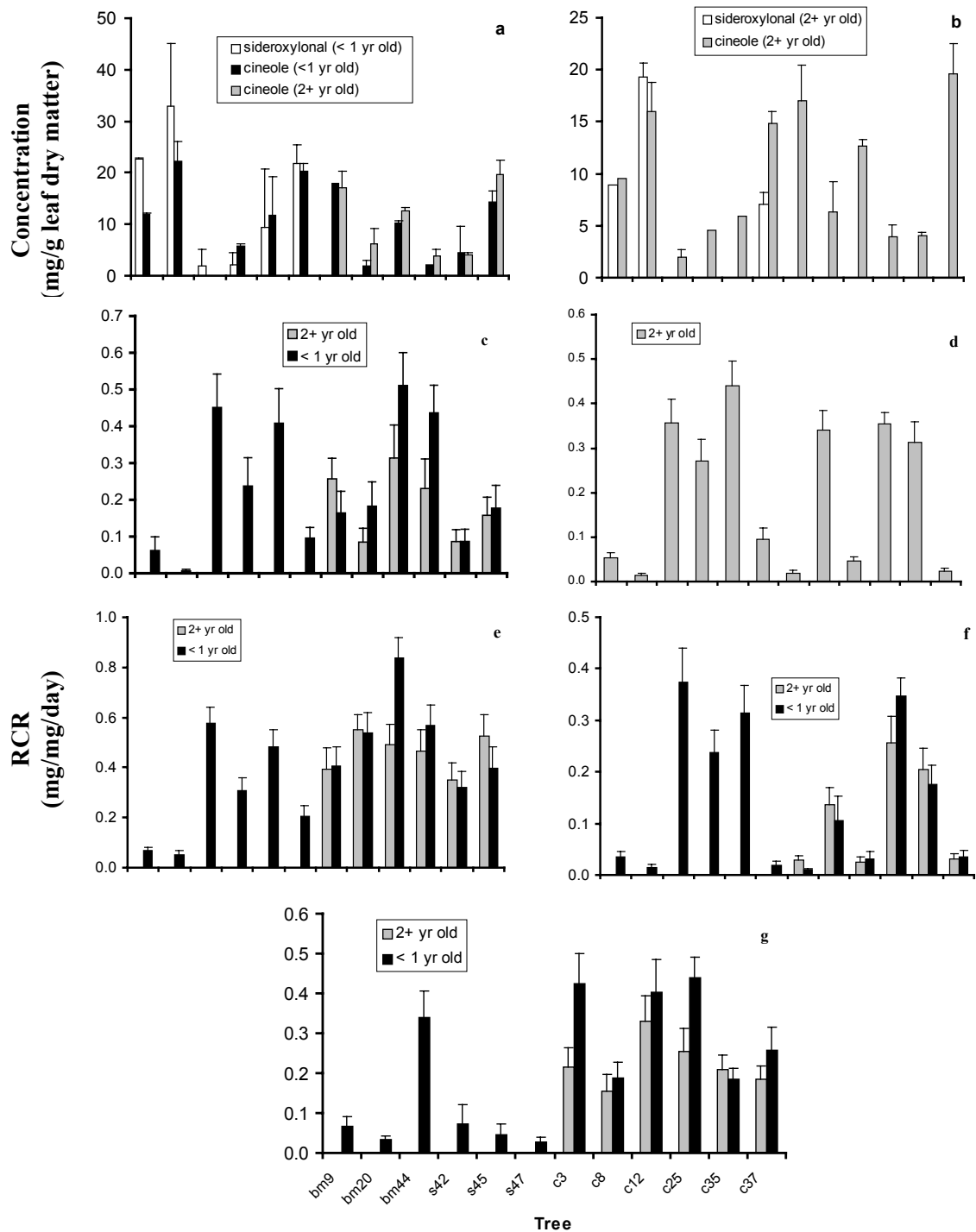


Figure 5.1. Concentrations of sideroxylonal and 1,8-cineole of *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis* trees (a, b) and relative consumption rates (RCR) of Christmas beetles (*Anoplognathus* spp.), (c) *A. chloropyrus*, d) *A. montanus*, e) *A. pallidicollis*, f) *A. viriditarsis* and g) *A. suturalis*. Mean + SE, $n = 9 - 15$ beetles per tree. RCR of *A. montanus* was measured with leaves of 2+yr old only. RCR of other species was measured using leaves of the same age. Leaves of *E. camaldulensis* do not contain sideroxylonal.

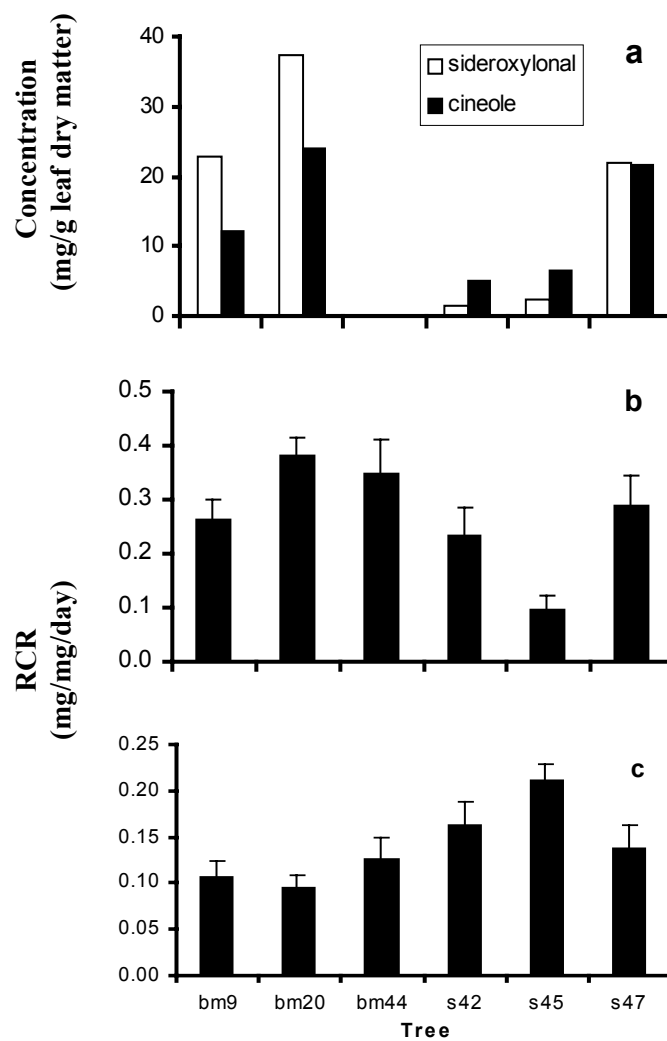


Figure 5.2 a) Concentrations of sideroxylonal and 1,8-cineole of *E. melliodora* and *E. sideroxylon* trees and relative consumption rates (RCR) of adult b) *Chrysophtharta variicollis* and c) *Gonipterus scutellatus*. Mean + SE, $n = 15$ beetles per tree.

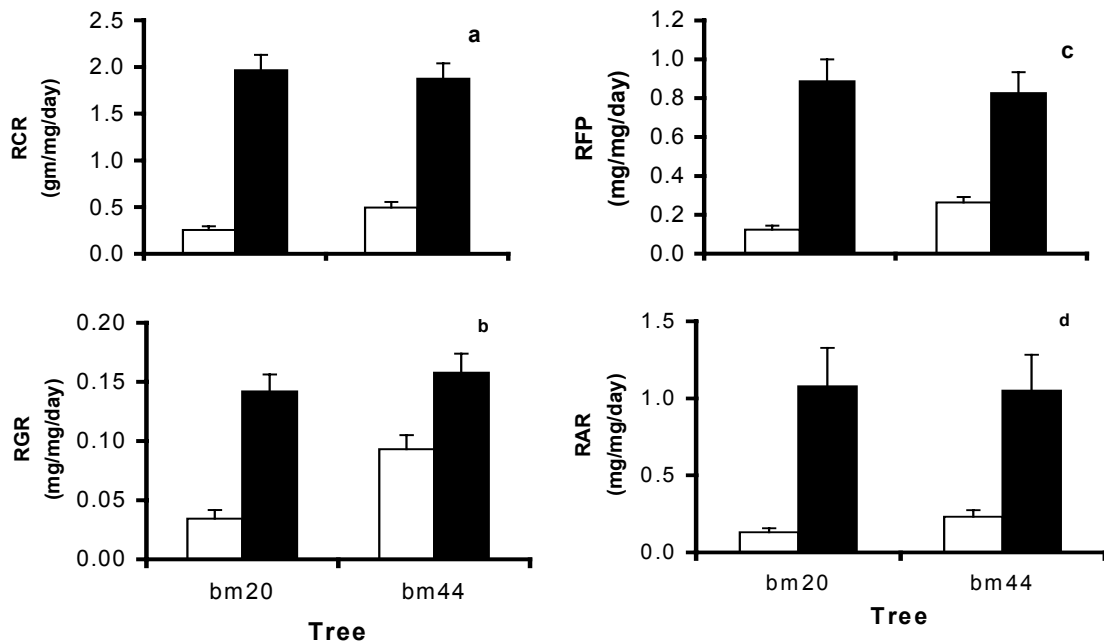


Figure 5.3. a) Relative consumption rates (RCR), b) relative growth rates, (RGR), c) relative rates of frass production (RFP), and d) relative assimilation rates (RAR) of larvae of *Chrysophtharta variicollis* (□) and *Pergagraptia latreillei* (■). Mean + SE, $n = 8 - 15$ larvae per tree.

Discussion

Leaf chemistry of the six trees of *E. sideroxylon* and *E. melliodora* is shown to be consistent for at least three years and at different leaf age (Matsuki *et al.* manuscript-b). Also, the marked variation in leaf chemistry of *E. sideroxylon* and *E. melliodora* is found in other populations of these species (Matsuki *et al.* manuscript-b). Therefore, insect herbivores on these *Eucalyptus* spp. are likely to encounter between-individual variation in leaf chemistry consistently.

Previous studies showed that leaf consumption by *Anoplognathus* spp. was positively correlated with concentrations of 1,8-cineole in leaves (Edwards *et al.* 1993, Matsuki *et al.* manuscript-b). However, additional results of this study showed that *A. chloropyrus*, *A. pallidicollis*, and *A. suturalis* did not discriminate *E. camaldulensis* trees with different concentrations of 1,8-cineole. Leaf consumption rates observed for these species in this study were not consistent with the historical record of defoliation of the six *E. camaldulensis* (Table 5.1). Severe defoliation of the *E. camaldulensis* population was observed in years when population density of *A. montanus* was particularly high (W. Wanjura, *personal communication*). *Anoplognathus montanus* and *A. viriditarsis* did discriminate the *E. camaldulensis* trees in this study, and these two species had previously been shown to be more sensitive to leaf chemistry than the three species that did not discriminate the trees (Matsuki *et al.* manuscript-b). It is possible that concentrations of 1,8-cineole in at least some of the historically "resistant" *E. camaldulensis* trees are not high enough for *A. chloropyrus*, *A. pallidicollis*, and *A. suturalis*.

There is at least one other possible reason for the different response of *Anoplognathus* spp. to *E. camaldulensis* compared with *E. sideroxylon* and *E. melliodora*. The main acylphloroglucinol derivative in *E. sideroxylon* and *E. melliodora* is sideroxylonal, while *E. camaldulensis* has several acylphloroglucinol derivatives all belong to a group called macrocarpals (W. Foley, *unpublished*

data). Unfortunately, we are not yet able to purify and quantify macrocarpals. Thus, we are not able to examine effects of macrocarpals on feeding by *Anoplognathus* spp.

Individual trees of *E. melliodora* and *E. sideroxylon* that are resistant to Christmas beetles (*Anoplognathus* spp.) are not resistant to other insect herbivores on *Eucalyptus* spp. The main ecological difference between *Anoplognathus* spp. and other insect species used in this study is that larvae of *Anoplognathus* spp. do not feed on *Eucalyptus* leaves, while larvae of other insect species do.

Anoplognathus spp. may fly several kilometres to find trees to feed on. There is some evidence that *Anoplognathus* spp. use volatile monoterpenes as preingestive cues to discriminate *E. melliodora* and *E. sideroxylon* trees (Matsuki *et al.* manuscript-b). The same study has also shown that *Anoplognathus* spp. are able to detect high concentrations of sideroxylonal in leaves before digesting food (Matsuki *et al.* manuscript-b). These abilities of *Anoplognathus* spp. are useful perhaps because *Anoplognathus* spp. are able to move between trees.

In contrast, larvae have a limited capability to move between trees, and it is probably necessary for larvae to be able to feed and grow on the tree that they hatched. Thus, there must have been a strong selection pressure on ovipositing females (especially of monophagous and oligophagous species) to be able to select right host plants. There must also have been a strong selection pressure on larvae (of polyphagous species) to be able to feed and grow on a wide range of plants with different physical and chemical traits.

The results of this study are still surprising because some acylphloroglucinol derivatives are found to act as inhibitors of various biochemical processes affecting viruses and organisms in at least three different Kingdoms. For example, sideroxylonal and closely related compounds have been shown to act as inhibitors of Epstein – Barr virus activation (Takasaki *et al.* 1990), to inhibit seed germination (Bolte *et al.* 1985) and electron transport in plants (Yoneyama *et al.* 1990), to have antibacterial property against *Staphylococcus aureus* and *Bacillus subtilis*, to inhibit aldol reductase and growth of HeLa cells (Sato *et al.* 1992), and to act as an antifouling agent against *Mytilus edulis* (Singh *et al.* 1997). Recent studies using arboreal marsupial herbivores also show negative effects of sideroxylonal and a related compound on food consumption (Lawler *et al.* 1998a, 1998b, in press). The formyl groups of some acylphloroglucinol derivatives have been shown to have negative effects on leaf consumption by the marsupial herbivores (Lawler *et al.* 1998a).

Acylphloroglucinol derivatives are found in a plant family Myrtaceae, and many biologically active acylphloroglucinol derivatives are found only in *Eucalyptus*, which is native to Australia (Ghisalberti 1996). In contrast, the negative biological effects of acylphloroglucinols are found in a wide range of organisms from all over the world. Therefore, it is tempting to hypothesise that the ability of the generalist insect herbivores on *Eucalyptus* spp. to feed and grow on leaves with high concentrations of sideroxylonal is a derived trait evolved independently in many taxa. Many disparate insect taxa have rapidly evolved to deal with the same insecticide because insecticides have acted as strong selection agents. Sideroxylonal and related compounds affect serotonin receptors in marsupial folivores (Lawler *et al.* 1999). In insects, one of the roles of serotonin is to control feeding. Disrupted feeding by sideroxylonal and related compounds might have acted as a strong selection pressure on generalist herbivores on *Eucalyptus* spp. If generalist herbivores that have never encountered sideroxylonal in their evolutionary history show reduced consumption and growth when sideroxylonal is added to their diet, then the ability of the herbivores to consume and grow on leaves with high concentrations of sideroxylonal is likely to be results of evolution facilitated by sideroxylonal in leaves of eucalypts.

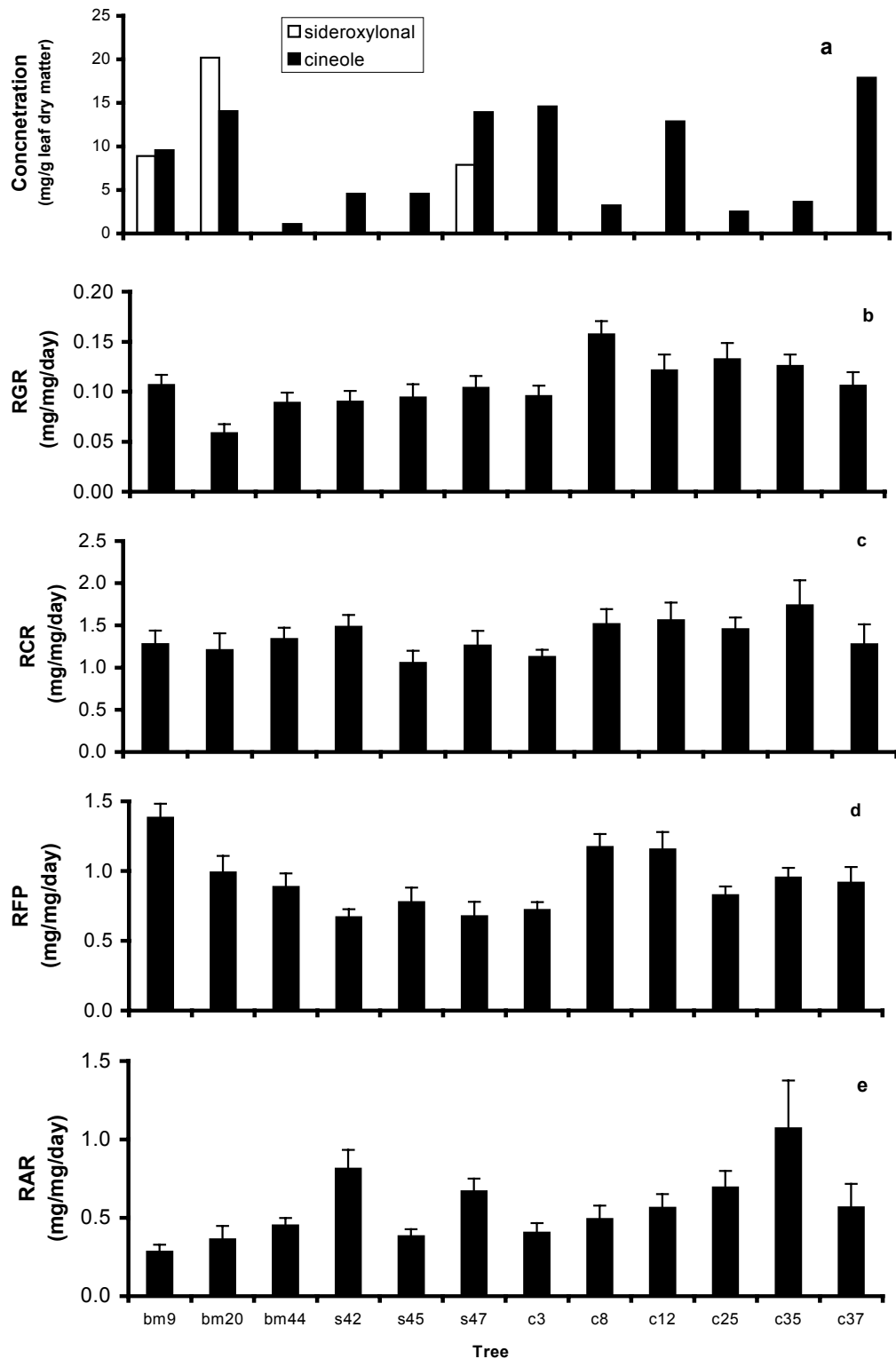


Figure 5.4. a) Concentrations of sideroxylyl and 1,8-cineole of *E. melliodora*, *E. sideroxylyl*, and *E. camaldulensis* trees and b) relative consumption rates (RCR), c) relative growth rates (RGR), d) relative rates of frass production (RFP), and e) relative assimilation rates (RAR) of *Uraba lugens* larvae. Mean + SE, $n = 15$ larvae per tree. Leaves of *E. camaldulensis* do not contain sideroxylyl.

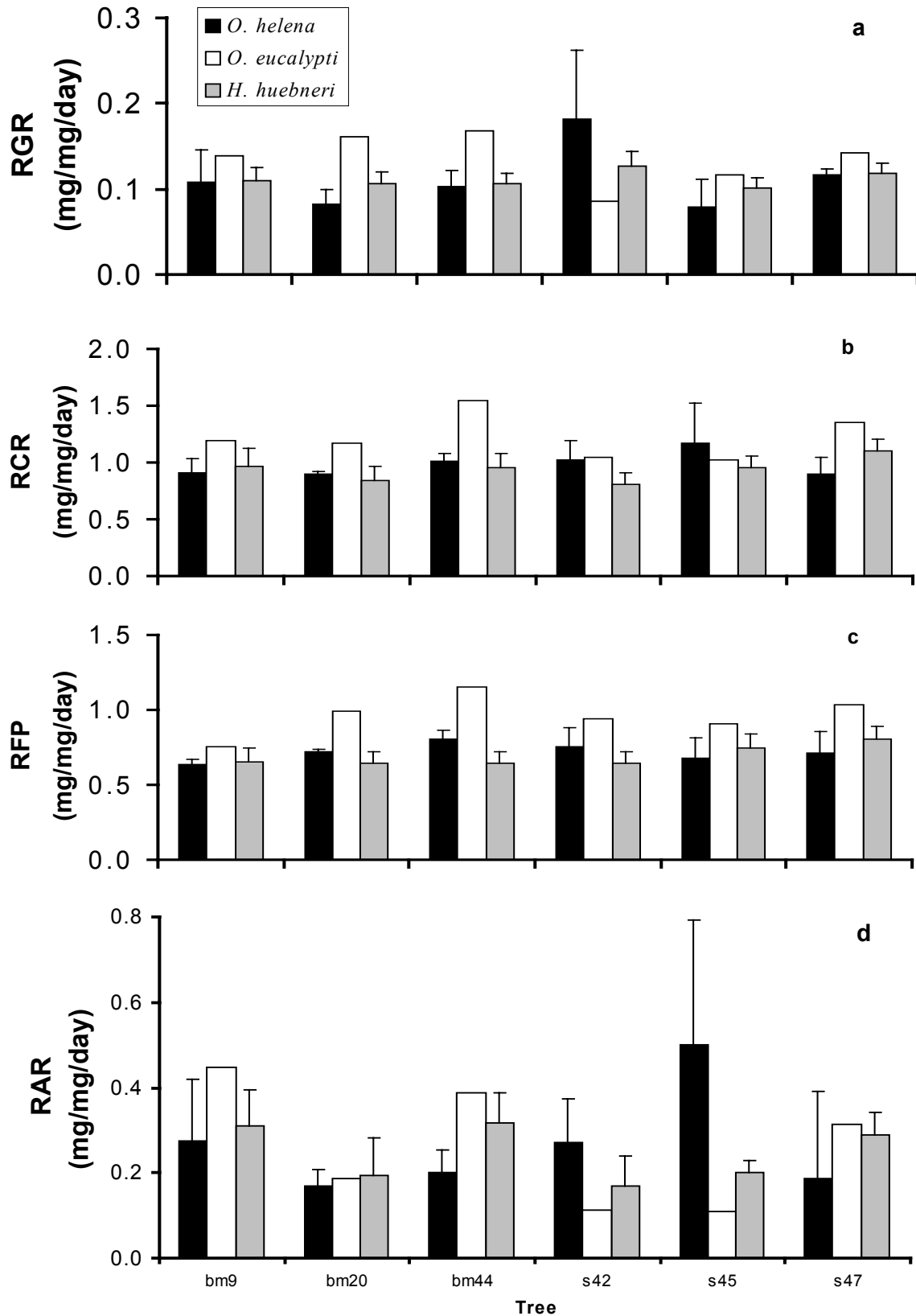


Figure 5.5 a) Relative consumption rates (RCR), b) relative growth rates (RGR), c) relative rates of frass production (RFP), and d) relative assimilation rates (RAR) of larvae of *Opodiphthera helena*, *O. eucalypti*, and *Hyalarcta huebneri*. Mean + SE, $n = 3$ for *O. helena*, $n = 1$ for *O. eucalypti*, and $n = 15$ for *H. huebneri*.

Alternatively, larvae of generalist herbivores may already possess a standard mechanism of modifying properties of various chemical compounds in the digestive system, and acylphloroglucinol derivatives are modified and excreted from the digestive system just as other secondary metabolites with similar chemical properties (cf. Morrow and Fox [1980] for the same argument with monoterpenes). This hypothesis may be supported if generalist herbivores that have never encountered sideroxylonal in their evolutionary history did not show reduced consumption and growth when sideroxylonal is added to their diet. A preliminary study using *Spodoptera* sp. (Noctuidae, Lepidoptera) showed that growth rates of larvae feeding on artificial diet coated with sideroxylonal were not different from larvae on the control diet (M. Matsuki, *unpublished data*), but further studies are needed to draw a more definite conclusion.

Many *Eucalyptus* spp. show marked within- and between-population variation in leaf chemistry (e.g., Boland *et al.* 1991). Leaf chemistry is considered to be genetically controlled in at least some *Eucalyptus* spp. (Boland *et al.* 1991, Stone and Bacon 1994, Doran and Matheson 1994, Pryor and Bryant 1958). Maintenance of chemically variable populations in *E. sideroxylon*, *E. melliodora*, and *E. camaldulensis* suggests that selection pressure by herbivores have not been strong enough to eliminate genotypes with very low concentrations of acylphloroglucinol derivatives and monoterpenes. Consistent with this, larvae of the generalists used in this study did not show lowered consumption and growth on trees with high concentrations of sideroxylonal and 1,8-cineole. Although *Anoplognathus* spp. did show marked reductions in leaf consumption on trees with high concentrations of sideroxylonal and 1,8-cineole, population densities of *Anoplognathus* spp. vary considerably in space and time (Carne *et al.* 1974). Thus, seedlings of susceptible genotypes may have opportunities to grow into large enough trees that can withstand repeated defoliation events.

Also, assuming that there are costs of producing high concentrations of acylphloroglucinol derivatives and monoterpenes, there must be strong enough selection pressure by herbivores to maintain genotypes with high concentrations of these compounds in populations. As discussed above, however, we did not find strong evidence for the insect species used in this study acting as selection agents. Further studies are needed to examine costs of producing high concentrations of acylphloroglucinol derivatives and monoterpenes and differences in survivorship and reproduction of genotypes with markedly different leaf chemistry.

Acknowledgment

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6. The basis of intraspecific differences in feeding on *Eucalyptus melliodora* foliage by common brushtail possums (*Trichosurus vulpecula*)⁵

Introduction

The density of arboreal folivores tends to be low, relative to the amount of potential food that exists. Much of this foliage is never eaten, regardless of forest structure and age and the frequency of tree hollows. This suggests that factors like the availability of nutrients in the foliage or the presence of plant secondary metabolites (PSM) determine feeding behaviour of herbivores (Freeland and Janzen, 1974) and thus partly control the size of animal populations. Lawler et al (1998; 2000) have argued that these effects are best studied at the level of individual trees but analytical limitations force most studies to focus on interspecies differences even when it is clear that individuals of some species are preferred over others. In this paper we focus on intraspecific differences in the palatability of *Eucalyptus melliodora* foliage to common brushtail possums (*Trichosurus vulpecula*).

Eucalyptus is a highly variable genus and dominates more than 90% of Australian forests and woodlands yet only four marsupial species eat significant amounts of the foliage. These marsupials cover a range of browsing herbivore niches from the koala (*Phascolarctos cinereus*) and the greater glider (*Petauroides volans*), which are highly specialized eucalypt feeders (Martin and Handasyde 1999; Foley and Hume 1987) to the ringtail possum that eats eucalypt leaves over much of its range but also eats the leaves and flowers of other canopy and understorey trees (Pahl, 1987). At the other end of the continuum lies the common brushtail possum (*Trichosurus vulpecula*), a generalist herbivore (Kerle, 1984), that feeds mainly on leaves from a variety of plants, including eucalypts, but which also eats fruits and flowers and understorey plants (MacLennan, 1984; Statham, 1984).

Maintenance of these species in captivity confirms the reluctance of common brushtails to feed exclusively on a diet of *Eucalyptus* leaves (Freeland and Winter 1975) and they are almost invariably fed diets of fruits and cereals together with some foliage. There are several possible explanations of the different reliance on eucalypt foliage among these marsupials including differences in digestive physiology (Foley and Hume 1987) and in the capacity to detoxify terpenes and phenols (Dearing and Cork 1999; Boyle et al 1999; 2000). A third explanation may be the differential susceptibility of the marsupial species to the effects of plant defence compounds. This is of interest because of the discovery of a new group of secondary plant compounds called formylated phloroglucinol compounds (FPCs). In common ringtail possums (*Pseudocheirus peregrinus*) a single FPC, Sideroxydonal-A, explained about 75 - 85% of the variation in food intake amongst foliage from 36 individual *E. polyanthemos* trees. In addition, Lawler et al (1998) argued that koalas had a significantly higher tolerance of foliar FPCs, in particular macrocarpals than common ringtails but at that time it was not possible to accurately measure FPC concentrations.

Here we asked whether common brushtails have a lower tolerance of foliar sideroxydonals than other marsupial species, in particular the more specialized feeders, the common ringtail possum and the koala by measuring the degree to which foliar sideroxydonal A deterred feeding on different *E. melliodora* trees. In addition we measured the distribution of sideroxydonals in a natural stand of *E. melliodora* so that we could assess the relevance of intraspecific variation to the scale of feeding choices encountered by free-ranging animals.

⁵ Based on a manuscript developed by I. Wallis, W. Foley, M. Matsuki and R. B. Floyd

Materials and methods

Animals and housing

Four male and two non-lactating common brushtail possums were caught in cage traps on the campus of the Australian National University over a three-week period in February 1999. They were placed immediately in metabolism cages measuring 130 x 60 wide x 85 cm, fitted with a nesting box (32 x 27 wide x 18 cm), a tree branch for a perch, a water container and a polythene pipe (30 x 10 cm) filled with water in which to stand foliage. These cages were housed in a room with a 12:12 hour light: dark cycle with a temperature set at 20°C (range 18-21°C). Four 60W incandescent bulbs provided the daytime lighting while the minimal lighting at night came from a single 40W red bulb. In the wild brushtail possums are strictly nocturnal so food was offered in the evening (ca 1700h) and removed at 0900h the next day.

Upon capture we fed the possums a mixed diet of apple, carrot, banana and *E. melliodora* foliage cut from a tree that possums are known to eat. Ten days before the experiment started the fruit was removed from the diet and the amount of foliage increased so that the possums were feeding *ad libitum*. We weighed the possums weekly from capture.

Experimental design

In this experiment we used a six by six Latin square design run twice. Thus, each of six animals was offered foliage from each of six *E. melliodora* trees in the first period and from each of another six trees in the second period. Likewise, in each period every tree was fed to a possum on every night. The experimental trees were chosen by collecting samples of leaf from 30 individual trees and then predicting the Sideroxylonal A content using near infrared reflectance spectroscopy (see below)(NIR Systems 6500 Scanning Spectrophotometer with a spinning cup attachment) and a previously determined model (Lawler et al 2000). We then chose 12 of these trees to give a range of foliage Sideroxylonal-A concentrations which, in ringtail possums, determines largely how much food they eat (Lawler et al 2000). The risk in experiments like this is that a possum might be offered unpalatable foliage on consecutive nights to the point that it is essentially fasted. To avoid this situation we interspersed each experimental day with a “rest” day when we offered the possums *E. melliodora* foliage from a tree known to be palatable. Branches of foliage were cut from mature trees (5 to 20 m) and were chosen to be of similar maturity and quality, determined visually. The branches were immediately placed in a large plastic bag with the stems protruding. Upon returning to the laboratory (ca 2-3 h) these bags were stored in a cool room (ca 5°C) with the stems of the branches standing in water. When stored in this way foliage stays fresh for about a week so we made two collections from each tree during the course of the experiment.

On the experimental days a bundle of foliage weighing approximately 400 g was placed in the cage at 1700 h. A smaller (150 g) but otherwise identical bundle of foliage (termed “control foliage”) was placed in a similar tube in front of each cage. Starting at 0900 h the following day we weighed the uneaten intact foliage and collected the spilled foliage into a paper bag, which was dried at 40°C to constant mass. The control foliage was used to monitor changes in hydration during the feeding period but was also used to obtain a representative sample of the foliage offered. A 10 g sample was placed in a paper bag and dried in a forced draught oven at 40°C for 48 h to determine the dry matter of the foliage offered and the dry mass of the foliage refused. These data together with the dry mass of spilt foliage enabled us to calculate the dry matter intake of the possums. A separate sample (ca 20 g) of the control leaves was frozen in a plastic bag for later freeze-drying and chemical analysis.

Distribution of sideroxylonals amongst a natural Eucalyptus melliodora population

Once we knew that sideroxylonals were a major determinant of the feeding of brushtail possums we wanted to know how the compound was distributed amongst trees of a natural population.

Accordingly we collected foliage from 150 individuals of *E. melliodora* growing in natural woodland in the Canberra Nature Park. These individuals were distributed over an area of 4 ha which approximates the home-range size for an adult male common brushtail in this region. Where possible we collected specimens from the mid crown in the north-eastern quadrant of the tree but the asymmetrical nature of some crowns made this impossible. Each sample was placed in a polythene bag and frozen in the field. They were later freeze-dried and the Sideroxylonal-A concentration determined by near-infrared spectroscopy.

Analyses

The nitrogen content of freeze dried, ground foliage was determined on duplicate 250 mg samples using a semi-micro Kjeldahl technique with a Tecator 2012 digester, selenium catalyst and a Gerhardt Vapodest-5 distillation and titration apparatus.

Sideroxylonals were extracted from 2 g of freeze-dried, ground leaf for 13 h using a Soxhlet apparatus containing 125 mL of a 20:80 (v:v) mixture of acetone and light petroleum spirit (40-60°C boiling point). After extraction the solvent was evaporated at 40°C and the crude extract was quantitatively transferred with about four 5 mL aliquots of a transfer solvent (20:80 mixture of methanol and dichloromethane) to a 30 mL pre-weighed glass vial. The contents of the vial were then dried under air for 24 h after which the vial and contents were weighed. A known amount of extract (10 ± 1 mg) was dissolved in 10.0 mL of HPLC grade methanol, placed in a sonicating bath for 30 s and then 1 mL was filtered through a 13 mm syringe filter with a pore size of 0.45 µm. Within one hour of dissolving the extract in methanol, 15 µL of solution was injected onto a Waters Nova-Pak® C18 HPLC column (3.9 x 150 mm) with an eluent (95 % methanol, 4.9 % water and 0.1 % trifluoroacetic acid) flowing at 1.0 mL per minute. Absorbance was measured at 275nm with a UV detector. The Sideroxylonal-A concentration was then computed from a standard curve of peak area versus Sideroxylonal-A concentration. Pure Sideroxylonal A, for standard curves, was prepared as previously described (Lawler et al 2000; Eschler and Foley 1999).

Cineole concentration was determined by gas-liquid chromatography. Cineole was extracted from small samples of fresh leaf by immersion in hexane for 24 h and quantified relative to an internal standard of tridecane as previously described (Lawler et al 2000).

Near-infrared spectroscopy

We collected the spectra of whole freeze dried samples of leaf using an NIRSystems 6500 Scanning Spectrophotometer with a transport attachment and a large rectangular sample cell. We modeled the relationship between spectral features and the Sideroxylonal A concentration of a subset of samples using the methods previously described (Lawler et al 2000). When combined with samples from other studies reported elsewhere, this resulted in a model that predicted foliar Sideroxylonal A concentration in whole freeze-dried *E. melliodora* foliage with a correlation co-efficient of 0.97, a standard error of cross validation of 1.94 and an N of 125. Accordingly we applied this model to the spectra of all the field samples collected and predicted their concentration of Sideroxylonal A.

Statistical analyses

Relationships between DMI and the nitrogen, Sideroxylonal A and cineole contents of the foliage were investigated with stepwise linear regression with a rejection level of $\alpha=0.05$.

Results and Discussion

The foliage from the twelve *E. melliodora* trees varied substantially in their concentrations of both Sideroxylonal A (ca 1 to 56 mg per g of leaf DM) and cineole content (ca 1 to 31 mg per g of leaf

DM). There was a strong positive correlation between the concentrations of the two substances ($R^2 = 0.91$). One tree had extremely high concentrations of both Sideroxylonal A (56 mg per g dry leaf) and cineole (31 mg per g dry leaf) whereas two trees contained negligible amounts of these substances. The remaining trees had intermediate concentrations of Sideroxylonal A (15 to 32 mg per g of leaf DM) and of cineole (14 to 25 mg per g dry leaf).

The amount eaten by possums varied substantially between individual trees (12.1 to 61 g DM per animal) (Figure 6.1). The mean dry matter intake over all trees also varied widely between possums (19.7 to 38.2 g DM per animal). Importantly there was a significant negative correlation between dry matter intake and foliar sideroxylonal concentration (Figure 6.1) and cineole concentration (Figure 6.2), the latter by virtue of the correlation between sideroxylonal and cineole (Figure 6.3).

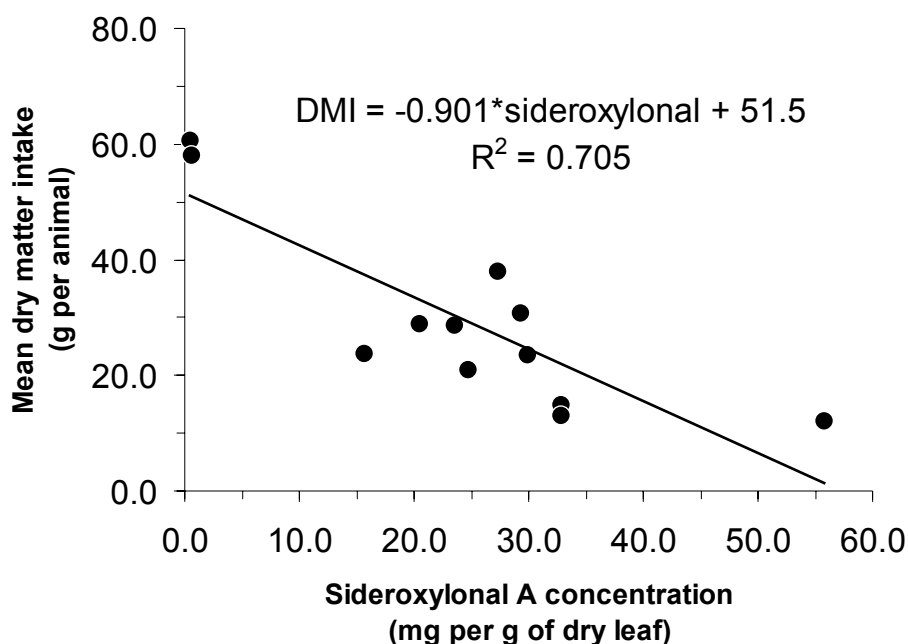


Figure 6.1. The relationship between food intake and sideroxylonal A concentration in foliage for common brushtail possums fed twelve *E. melliodora* trees.

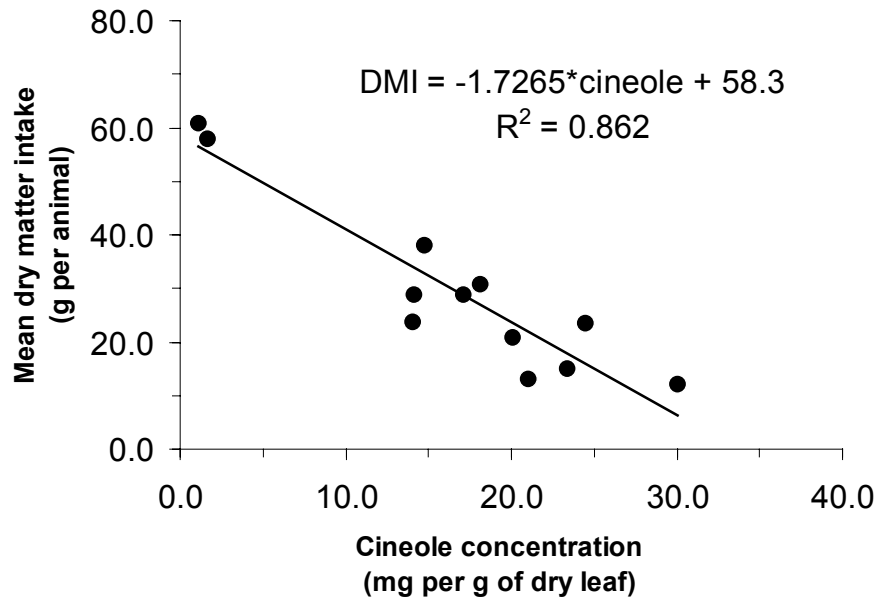


Figure 6.2. The relationship between food intake and cineole concentration in foliage for common brushtail possums fed twelve *E. melliodora* trees.

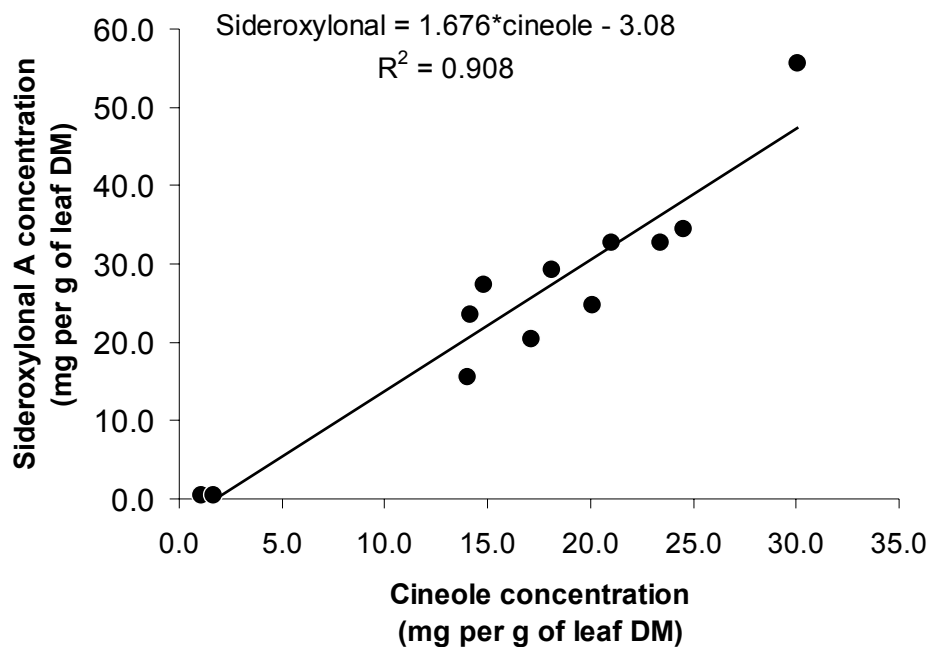


Figure 6.3. The relationship between the concentrations of sideroxylonal A and cineole in the foliage from twelve *E. melliodora* trees.

Sideroxylonal and cineole concentrations may be correlated because these compounds probably share some common biosynthetic pathways. There are many different kinds of FPCs in *Eucalyptus* but they all share common features including a fully substituted aromatic ring with aldehyde and phenol functionalities (Ghisalberti 1995) and a lipophilic sidechain. In the macrocarpals and euglobals the sidechain is a full mono or (more usually) sesquiterpene whereas sideroxylonals and simple FPCs such as jensenone contain only isoprene (C5 units). Although no studies of the biosynthesis of FPCs have been performed, it seems likely that increased flux through pathways important in the biosynthesis of terpenes such as the mevalonic acid pathway may provide substrate for an enhanced biosynthesis of FPCs. Understanding these interactions is important because the concentration of terpenes appears to be an important cue that animals use to detect FPCs.

Earlier studies in common ringtail and brushtail possums have shown that cineole itself is not deterrent at the concentrations measured here and so we do not regard the association between cineole concentrations and dry matter intake as causative. For example, Lawler et al 1998b showed that common brushtails could consume up to 12% DM of cineole with no diminution of food intake. However, FPCs could condition an aversion to cineole in which the animals use the smell or taste of cineole as a guide to the likely concentrations of FPCs that they will encounter. We argue that the same conditioned aversion is responsible for the reduced feeding on some *E. melliodora* foliage recorded in these experiments and that the association between Sideroxylonal concentrations and dry matter intake is causative. Support for this comes from studies by Watson and Foley (unpublished) in which common brushtails were offered a choice between a basal diet treated with sideroxylonals and one treated with solvent alone. Above a concentration of 0.05% the sideroxylonal-treated diets were significantly aversive. In contrast the role of terpenes in interactions between *Eucalyptus* and common brushtail possums is secondary, as a cue to deterrence rather than acting as a primary deterrent in their own right.

In our experiments common brushtails reduced their intake of *E. melliodora* foliage by 50% from their maximum intake at a sideroxylonal concentrations of about 25 mg/g. In *E. polyanthemos*, the maximal sideroxylonal A concentration is only about 16 mg/g and at these concentrations, common ringtails in Lawler et al's (2000) experiments ate less than 10% of their maximal intake. This suggests that contrary to our suggestion, the differences between these marsupial species in their susceptibility to foliar sideroxylonals are not consistent with their different reliance on *Eucalyptus* as a food resource. Studies in which both common ringtails and brushtails are fed exactly the same diet could help to resolve the role of sideroxylonals in patterning dietary niches.

The field study in a natural stand of *E. melliodora* showed that the concentrations of foliar sideroxylonal was extremely variable over an area of about one possum home range and that the distribution of values was normal ($\chi^2 = 7.15$) (Figure 6.4). Given that environmental factors are likely to be relatively constant, this suggests that there is a strong genetic basis to the variations in foliar sideroxylonal and the normal frequency distribution suggests a multi-allele system. Little is currently known about the fine-scale genetic structure of *Eucalyptus* but one study of *E. globulus* (Skabo et al 1998) shows small patches of genetically similar material. However, our preliminary analyses of the spatial patterns of occurrence of palatable and unpalatable individuals in our field study did not reveal any pattern of clumping. Clearly studies of fine-scale genetic structure in *E. melliodora* could be useful in explaining the basis of the patchiness in foliar sideroxylonal concentrations.

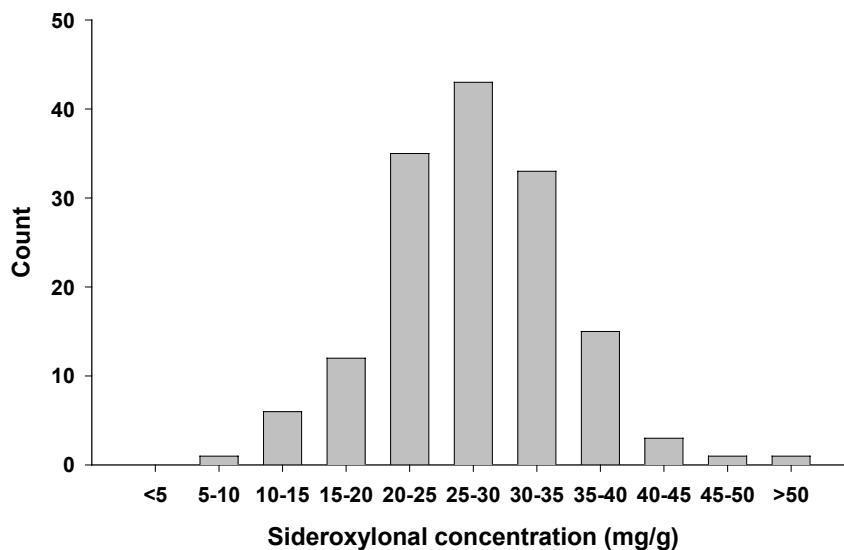


Figure 6.4. Frequency distribution of sideroxylonal concentrations in foliage from *E. melliodora* collected from a natural stand at Canberra Nature Park.

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7. Spectrometric prediction of the chemical composition of fresh *Eucalyptus* foliage⁶

Introduction

Trees of the genus *Eucalyptus* dominate more than 90% of Australian forests and woodlands. Associated with these forests is a unique and diverse fauna including species such as the koala (*Phascolarctos cinereus*) that are totally dependent on *Eucalyptus* leaves for food. Conservation of this fauna depends to a large degree on resolving conflicts between forestry activities and the reservation of areas of high habitat quality for the animals. However, it has proven difficult to identify such sites reliably because until recently, we had little idea of what made some *Eucalyptus* trees suitable as food for koalas and related marsupials.

Studies in the past three years have shown that the nutritional quality of *Eucalyptus* for marsupial herbivores is set largely by the presence of a group of plant secondary metabolites called formylated phloroglucinols. For example, Lawler et al (2000) showed that 86% of the variance in feeding rates by common ringtail possums in the laboratory was due to the presence of a single compound called Sideroxylonal A. These laboratory findings have been partly transferred to the field by using near infrared spectrophotometry as a means of rapidly analysing foliar samples for sideroxylonal, as well as other nutritional attributes such as total nitrogen. Although this has been largely successful (McIlwee et al 2000; Lawler et al 2000), the scale at which these analyses can be made is not broad enough to provide useful information for management of animal populations.

Our previous spectroscopic work has been conducted with dried and finely ground samples of foliage. Drying *Eucalyptus* foliage reduces the concentration of the volatile oils that function in part as cues in diet selection by marsupials (Lawler et al 1999a). Grinding the foliage produces a very homogeneous material that reduces light scattering, but is very time consuming. However, if we are to increase the scale of spectrometric measurements, we have to be able to acquire spectra that allow the prediction of specific foliar attributes from the tree canopy.

The success of studies in other systems to predict chemistry of tree canopies remotely encouraged us to consider this possibility in *Eucalyptus* forests. Although the complexities introduced by atmospheric interference and canopy architecture must be addressed in the longer term, we believe a necessary first step is to ensure that spectrometric predictions of specific aspects of foliar chemistry that describe the nutritional quality of the foliage for herbivores are not compromised by either loss of data in the water absorbing parts of the spectrum or by the collection of spectra from whole-leaf. Accordingly we tested whether we could predict the concentration of total nitrogen, sideroxylonal A and the volatile terpenoid 1,8 cineole in fresh and dried whole leaves of *Eucalyptus melliodora* - a widely distributed woodland species and in *Eucalyptus globulus*, a major commercial forest species.

Materials and methods

We collected foliage from 50 different trees of *E. melliodora* growing in open woodland within 20km of Canberra. All samples were of fully-expanded adult foliage from the mid to upper canopy. The samples were kept in polythene bags and held at 4°C for a maximum of 2h before transport to the laboratory. The near-infrared spectra (400– 500 nm) of each sample was collected using a NIR

⁶ Based on a manuscript developed by W. J. Foley, M. Ebberts, M. Matsuki and R. B. Floyd

Systems 6500 spectrometer (details below). We measured the concentration of 1,8 cineole in hexane extracts of two leaves from each sample by gas chromatography using tridecane as an internal standard. Within 4h of collection, we measured the dry matter content of a second subsample by oven drying (3 d at 40°C) so that we could express the cineole concentrations in terms of dry leaf weight.

We then freeze-dried all the samples that had been scanned when fresh and then collected near-infrared spectra of the whole dried leaves. Those samples were then ground to pass a 1 mm screen in a Cyclotec 1093 Sample Mill (Tecator, Sweden) and we again collected near infrared spectra of the ground samples. Thus for each of the 50 samples collected we had near-infrared spectra of whole leaves when both fresh and dried and also the ground leaf.

We measured foliar cineole concentration on the whole dried leaf samples as described above and sideroxylonal A concentration on samples of the dried, ground material using HPLC as previously described (Lawler et al 2000). Sideroxylonal A is the major antifeedant chemical active against koalas, ringtail possums and Christmas beetles (*Anoplognathus* spp) (Lawler et al 1998; Lawler et al 2000; Matsuki unpublished).

***Eucalyptus globulus* foliage samples**

We conducted a second series of experiments with foliage from *Eucalyptus globulus* because this species of eucalypt carries morphologically different adult and juvenile foliage. In addition juvenile foliage is often highly glaucous and we wanted to know whether that would affect the quality of the spectra that we were able to collect. *E. globulus* does not contain sideroxylonals and so we restricted these experiments to the estimation of cineole and total nitrogen. Accordingly, we investigated adult foliage from 30 *E. globulus* trees and juvenile foliage from 15 trees. We collected spectra in the same way as described for *E. melliodora* with the exception that we explored whether presenting the glaucous surface to the spectrometer had any impact on the predictions derived from the spectra.

Spectra of purified compounds

We collected spectra of purified cineole and sideroxylonal so that we could relate specific features of the spectra from foliage samples back to authentic spectra. Pure cineole (99.9 % by GLC) was re-distilled from a commercial supply (Sigma). It is oil at room temperature. We applied approximately 1 ml to a glass fibre filter (Whatman) and placed the filter in the spinning cup sample holder of the spectrometer and collected spectra as described below. Sideroxylonal A was extracted and purified as previously described (Lawler et al 2000) and about 1g of the white crystals placed directly into the spinning cup sample holder.

Spectral measurement and data analysis

All spectra were collected with a NIRSystems Model 6500 scanning monochromator. This instrument measures spectral reflectance at wavelengths between 400 nm and 2498 nm at 2 nm intervals. Whole (fresh and dried) leaf samples were scanned using the NIRSystems Transport Module with a large rectangular sample cell. Dried, ground samples were scanned using the Spinning Cup Module and small sample cup. The reflectance (R) values of each spectrum was converted to absorbance (A) values using the following equation: $A = \log(1/R)$ and each spectrum was converted to a first-derivative spectrum in order to emphasize subtle changes in slope.

We modelled the relationship between cineole, total nitrogen and sideroxylonal concentrations and spectral characteristics of whole fresh leaves, whole dry leaves and ground dried leaves using modified partial least squares regression (Shenk and Westerhaus 1991) and following the standard principles described by the American Society of Testing and Materials (Anon 1995).

Results and discussion

Eucalyptus melliodora foliage

Cineole content ranged from 1.3 mg/(g dm) to 20.0 mg/(g dm) in *E. melliodora* leaf samples. The freeze-dried specimens of *E. melliodora* foliage lost between 26 and 84% cineole (mean 65%) of the cineole measured in the fresh leaves.

Figure 7.1 shows the (average) first derivative absorbance spectra of *E. melliodora* foliage for fresh leaves, freeze-dried whole leaves and freeze-dried ground leaves. The greatest difference between the dried sample spectrum and the fresh sample spectrum is as expected, around the water region in the spectra (between 1350 and 1450 nm and between 1850 and 1950 nm). However, the reflectance spectrum of pure cineole (Figure 7.2) does not show any absorbance at these wavelengths. Major absorbances of pure cineole were between 2250 and 2300 nm.

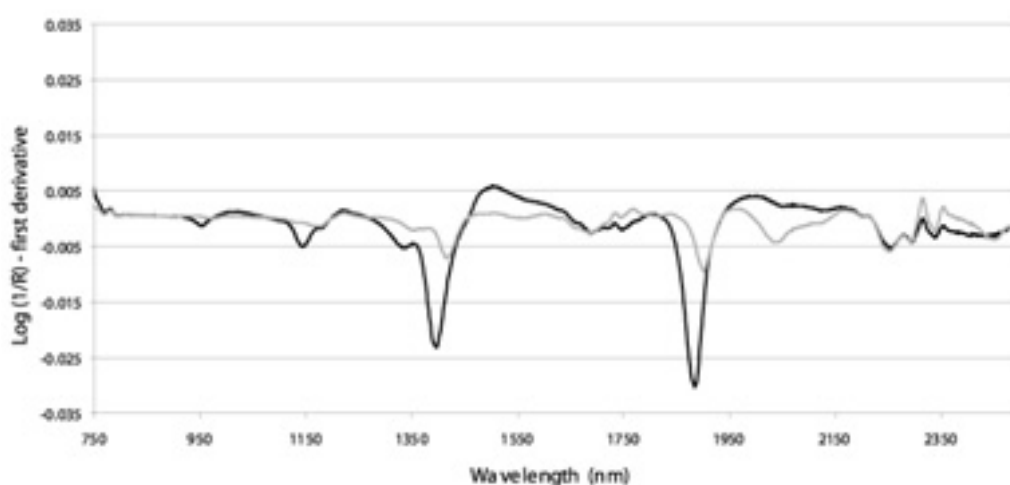


Figure 7.1. Average first derivative NIR absorbance spectra of adult *E. melliodora* foliage samples, scanned fresh (N = 50) (heavy line) and freeze-dried whole leaves (N = 49) (light line).

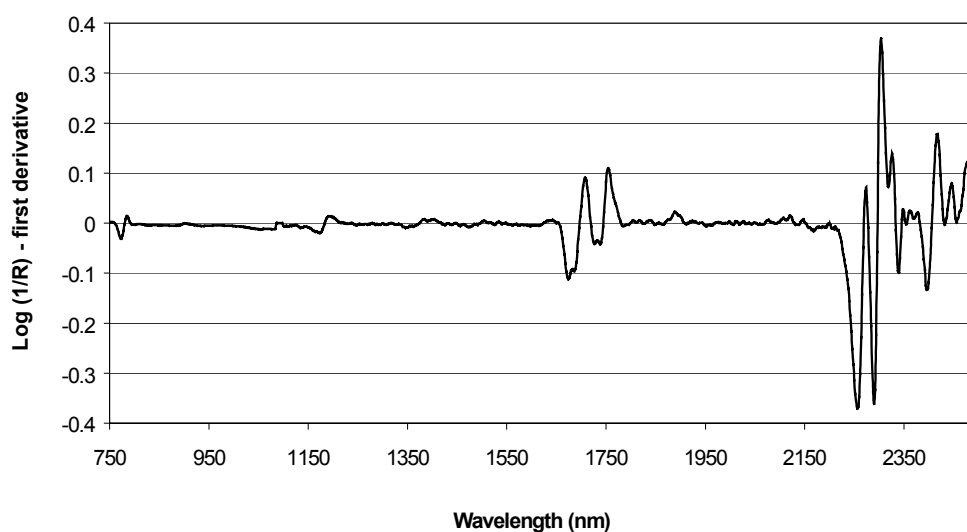


Figure 7.2. First derivative NIR transmission spectrum of pure 1, 8 cineole.

This suggests that the major features of the absorbance spectrum of cineole is unlikely to be obstructed by absorbances arising from water and that cineole concentration should be able to be directly derived from the spectra of whole fresh leaf. The absorption spectrum of sideroxylonal (Figure 7.3) was more complex than that observed for 1,8 cineole. In particular there were significant absorptions in the region 1850-1950 nm suggesting the potential for water derived features to overlap when predicting sideroxylonal concentrations in whole fresh leaves.

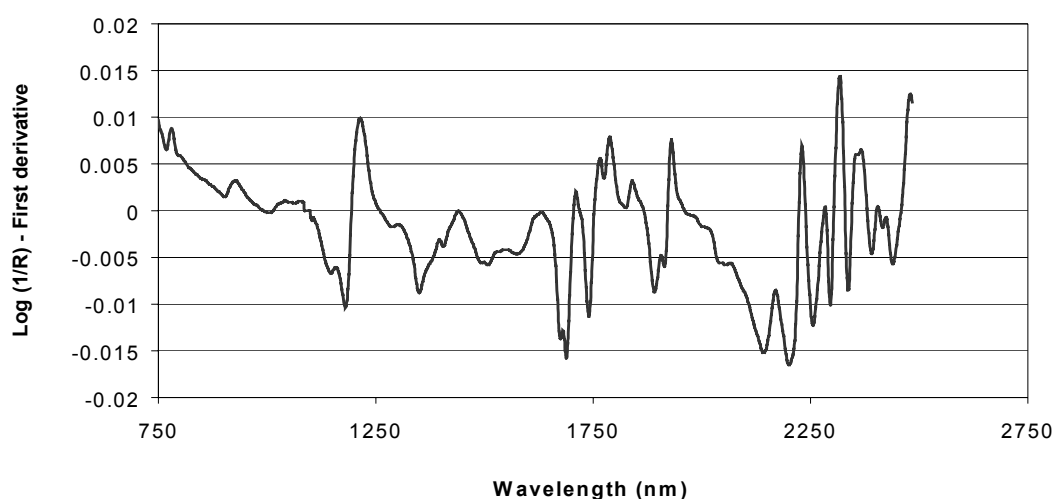


Figure 7.3. First derivative NIR reflectance spectrum of pure sideroxylonal.

Nonetheless we were able to model the relationship between absorbance spectra and foliar concentration successfully for all three-leaf constituents in both fresh and dried whole leaf. Although we explored a range of alternative procedures including changing the derivative, the number of data points and the smoothing functions for generating these models, we report here only those with the lowest standard error on cross validation, but in all instances the modified partial least squares regression approach proved the most useful (Table 7.1). In all cases the r^2 value refers to the correlation between the measured values for each constituent and the value predicted by the model.

Our predictions of both cineole and sideroxylonal concentration depended strongly on wavelengths that were important features of the absorbance spectra for purified compounds. This is shown by the correlelograms in Figures 7.4 and 7.5. Correlelograms allow assessment of the wavelengths that have the greatest correlation with the constituent values. Arrows in those figures highlight the important absorbance features of the purified compounds. For both cineole and sideroxylonal there is excellent agreement between the absorbance features of the purified compounds and the importance of those wavelengths to the models predicting those compounds in both fresh and dried whole leaf. This suggests that the models are detecting unique chemical attributes of the foliage.

Table 7.1. Summary of results from modelling (using partial least squares procedures with cross-validation) the relationship between spectral characteristics of *E. melliodora* foliage and the foliar concentration of 1, 8 cineole, sideroxylonal A and total nitrogen.

Component/ Sample	r^2	SECV	Mathematical Treatment	Wavelength range	Scatter correction	<i>N</i>
1, 8 Cineole						
Fresh leaf	0.97	1.60	2441	750-2492	None	49
Freeze dried whole leaf	0.94	1.52	2441	1108-2492	None	46
Freeze-dried ground leaf	0.88	1.81	2441	750-2492	None	48
Sideroxylonal						
Fresh leaf	0.90	2.69	1441	1108-2492	SNV/ Detrend	48
Freeze dried whole leaf	0.95	2.10	1441	1108-2492	None	45
Freeze-dried ground leaf	0.94	2.05	1441	1108-2492	None	48
Total Nitrogen						
Fresh leaf	0.92	0.07	2441	1108-2492	SNV/ Detrend	50
Freeze dried whole leaf	0.97	0.04	2441	1108-2492	SNV/ Detrend	47
Freeze-dried ground leaf	0.98	0.04	2441	1108-2492	SNV/ Detrend	48

Notes: The r^2 value indicates the degree of correlation between the predicted values and the actual measured values, the SECV is the standard error of the cross-validated predictions, the column labelled “Mathematical Treatment” the derivatives and range of data points over which the derivative and smoothing functions were calculated (see text for further detail), the wavelength range over which the model was derived as well as if a statistical scatter correction was applied. The SNV/Detrend scatter correction procedure is that described by Barnes *et al.* (1989).

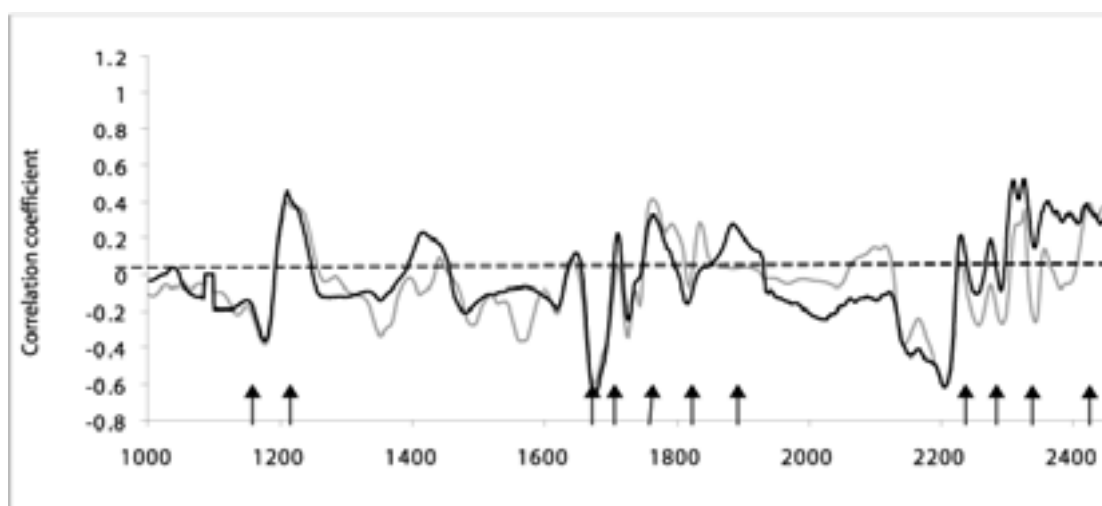


Figure 7.4. Wavelength dependence of correlation between first-derivative absorbance spectra and (fresh leaf) cineole concentration for fresh leaf (heavy line) and freeze-dried whole leaf (light line) *E. melliodora* samples. Arrows indicate the most important wavelengths that are absorbed by pure cineole.

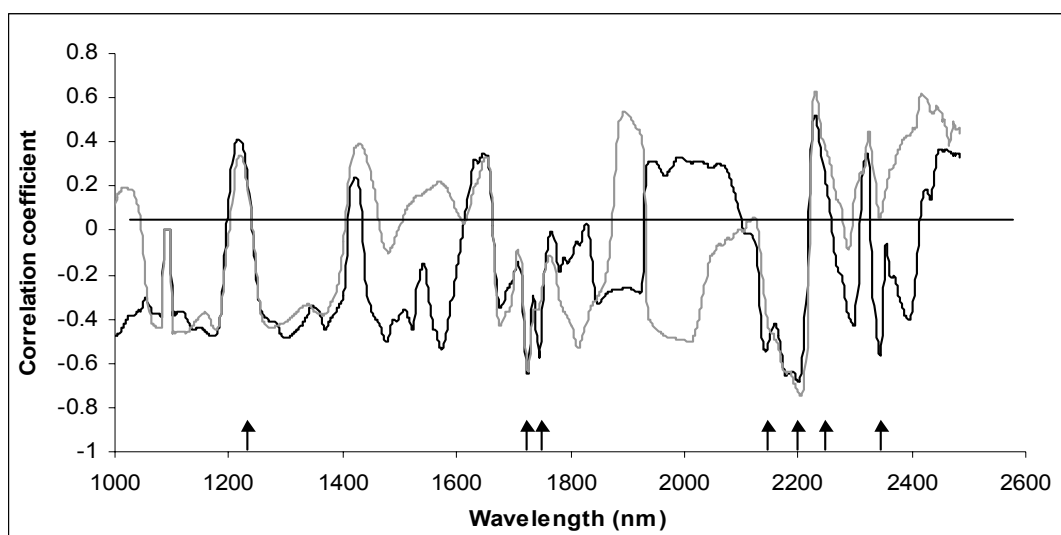


Figure 7.5. Wavelength dependence of correlation between first-derivative absorbance spectra and (dried leaf) sideroxylonal content for fresh leaf (grey line) and freeze-dried whole leaf (black line) *E. melliodora* samples. Arrows indicate the most important wavelengths that are absorbed by pure sideroxylonal.

Although one of the strengths of NIRS is its ability to robustly model attributes without a unique chemical signal, the strong influence of actual wavelengths associated with pure cineole demonstrated here means that scaling up these models to other platforms such as airborne spectrometers may be feasible. Airborne spectrometers can record only limited numbers of spectral bands in contrast to the full spectrum coverage provided by laboratory instruments. The close association between absorption features of the purified compounds of interest and the important features of the spectra-based models suggests that we can assign particular diagnostic wavelengths to detecting these important biological molecules in this key woodland eucalypt.

Eucalyptus globulus foliage

Cineole content ranged from 7.5 mg/(g dm) to 24.6 mg/(g dm) in adult *E. globulus* leaf samples and from 15.1 mg/(g dm) to 48.2 mg/(g dm) in juvenile *E. globulus* leaf samples. The same pattern in absorbance spectra is found for adult fresh and freeze-dried whole leaves (Figure 7.6) as in the *E. melliodora* samples, with the greatest differences around the water absorbance wavelength range. Similarly, there is a high correlation between features of the absorbance spectrum of pure cineole and those parts of the spectrum, which are most highly correlated with cineole concentration.



Figure 7.6. Average first derivative NIR absorbance spectra of adult *E. globulus* foliage samples, scanned fresh (N = 30) (heavy line) and freeze-dried whole leaves (N = 30) (light line).

The models built with the *E. globulus* adult and juvenile leaf samples do not predict the actual cineole or nitrogen concentrations as well as those using *E. melliodora* samples (Table 7.2) even though there was good agreement between the cineole spectrum and the wavelengths that contributed most to the predictive models (Figures 7.8 and 7.9). This may be partly due to the lower number of samples used in the studies. In addition, the agreement between the values predicted by the models and the actual measured values are better in the dried material than in the fresh leaf samples. This suggests that there are other spectral features apart from water content which may affect the estimation of cineole in *E. globulus* but not in *E. melliodora*. One possible candidate is surface waxes (Li et al 1997) and this was investigated further with juvenile *E. globulus* leaves.

The absorbance spectra for juvenile (fresh and freeze-dried) samples scanned with the waxy side up were slightly different from the spectra for samples scanned with the waxy side down (Figure 7.7). This was especially the case for the fresh leaf samples. The wavelength ranges where the differences appear, included some of the important absorbance ranges for pure cineole, suggesting that some components of the leaf waxes may well have interfered with the precision of the cineole estimates in *E. globulus*. However, the predictions from models relating spectral features to actual concentrations were slightly better when juvenile *E. globulus* foliage was presented to the spectrometer with the obviously glaucous side facing the light beam rather than the opposite (Table 7.3). Waxes occur on both sides of the leaf but are more pronounced on the abaxial side. As expected a relatively good model was obtained from freeze-dried ground leaf samples.

Table 7.2. Summary of results from modelling (using partial least squares procedures with cross-validation) the relationship between spectral characteristics of different samples of *E. globulus* foliage and the foliar concentration of 1, 8 cineole, and total nitrogen.

Component/ Sample	r^2	SECV	Mathematical treatment	Wavelength range	Scatter correction	<i>N</i>
1,8 cineole						
Fresh adult	0.74	3.57	2441	750-2492	None	30
Freeze-dried whole adult	0.91	2.71	1441	1108-2492	SNV	29
Freeze-dried ground adult	0.75	3.76	2441	750-2492	SNV/Detrend	30
Fresh juvenile	0.76	6.79	2441	1108-2492	SNV/Detrend	15
Freeze-dried juvenile	0.83	6.85	1881	1108-2492	SNV	15
Freeze-dried ground juvenile	0.77	6.65	1441	1108-2492	SNV	15
Total Nitrogen						
Fresh adult	0.90	0.74	2641	1108-2492	SNV/Detrend	29
Freeze-dried whole adult	0.93	0.6	1441	1108-2492	SNV/Detrend	28
Freeze-dried ground adult	0.99	0.4	2441	1108-2492	SNV/Detrend	30
Fresh juvenile	0.71	2.38	2441	1108-2492	SNV/Detrend	15
Freeze-dried juvenile	0.93	1.36	2441	1108-2492	SNV/Detrend	15
Freeze-dried ground juvenile	0.97	0.88	2441	1108-2492	SNV/Detrend	15

Note: Columns descriptions as for Table 7.1.

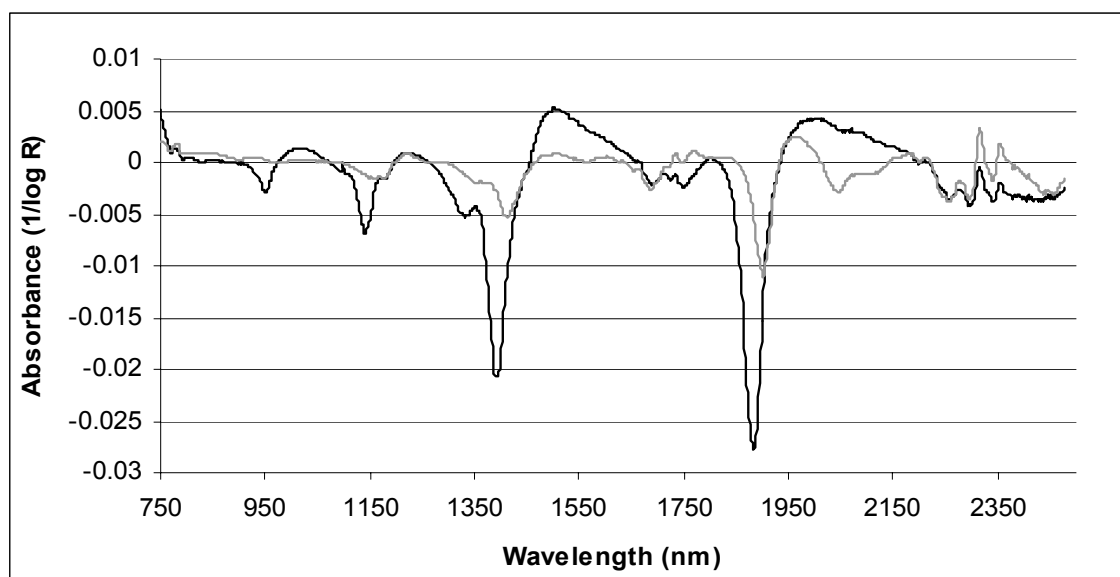


Figure 7.7. Average first derivative NIR absorbance spectra of juvenile *E. globulus* foliage samples, scanned fresh (N = 15) (black line) and freeze-dried whole leaves (N = 15) (grey line).

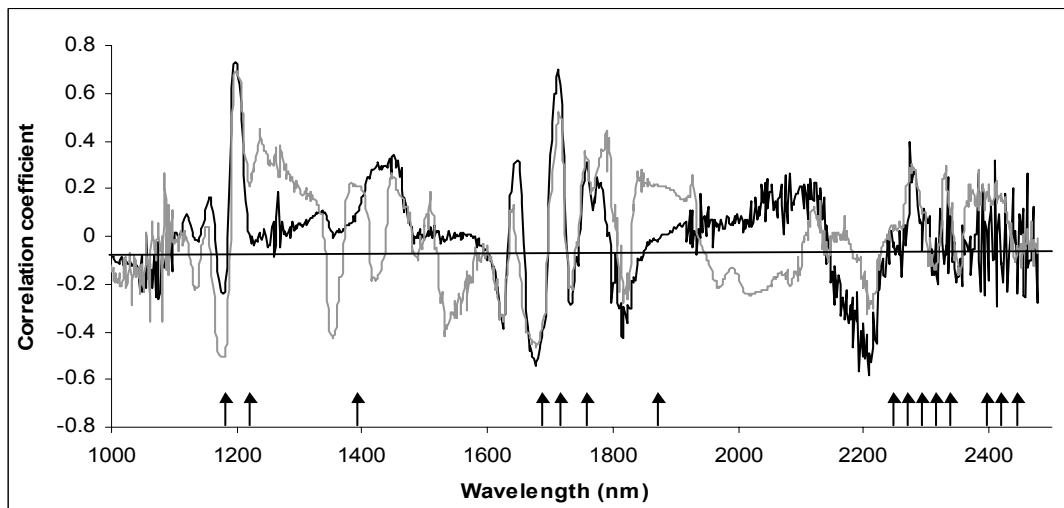


Figure 7.8. Wavelength dependence of correlation between first-derivative absorbance spectra and (fresh leaf) cineole content for fresh leaf (black line) and freeze-dried whole leaf (grey line) adult *E. globulus* samples. Arrows indicate the most important wavelengths that are absorbed by pure cineole.

Table 7.3. Summary of results from modelling (using partial least squares procedures with cross-validation) the relationship between spectral characteristics of juvenile leaves of *E. globulus* presented with either the glaucous or waxy side towards the light source (“wax up”) or away from the light source (“wax down”) and the foliar concentration of 1, 8 cineole, and total nitrogen.

Component/ Sample	R ²	SECV	Mathematical treatment	Wavelength range	Scatter correction	N
Fresh juvenile leaves wax up	0.64	6.83	1441	750-2492	SNV and detrend	15
Fresh juvenile leaves wax down	0.76	6.79	2441	1108-2492	SNV and detrend	15
Freezedried juv. Leaves wax up	0.58	8.55	0881	1108-2492	SNV and detrend	15
Freezedried juv. Leaves wax down	0.83	6.85	1881	1108-2492	SNV	15
Fresh juvenile leaves wax up	0.85	2.13	1441	1108-2492	SNV and detrend	15
Fresh juvenile leaves wax down	0.71	2.38	2441	1108-2492	SNV and detrend	15
Freeze-dried juv. Leaves wax up	0.82	1.97	2441	1108-2492	SNV and detrend	15
Freeze-dried juv. Leaves wax down	0.93	1.36	2441	1108-2492	SNV and detrend	15

Notes: Column descriptions as for Table 7.1.

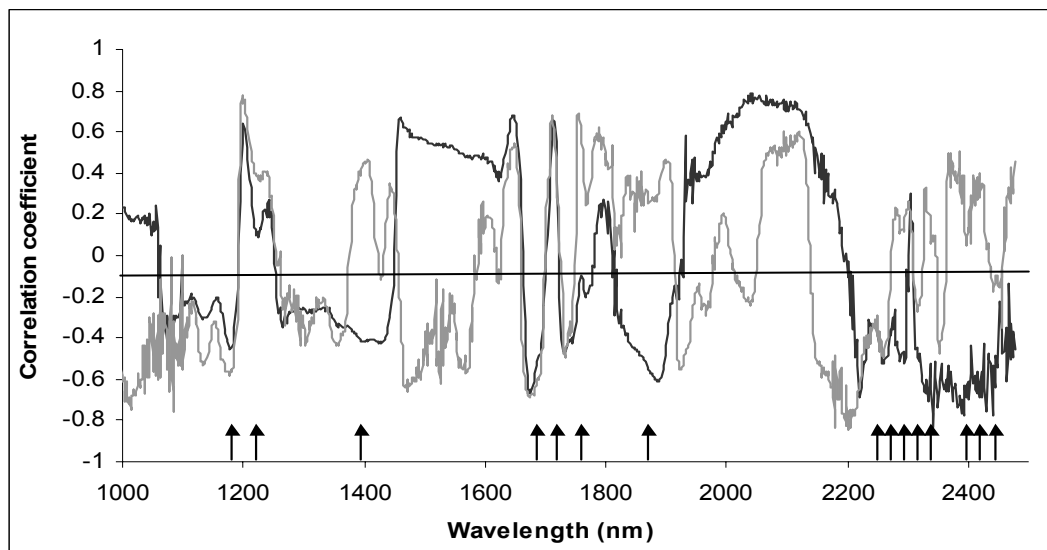


Figure 7.9. Wavelength dependence of correlation between first-derivative absorbance spectra and (fresh leaf) cineole content for fresh leaf (black line) and freeze-dried whole leaf (grey line) juvenile *E. globulus* samples. Arrows indicate the most important wavelengths that are absorbed by pure cineole.

Overall this work has shown that ecologically important aspects of the chemical composition of *Eucalyptus* foliage are detectable in whole dried leaf specimens as well as in whole fresh foliage. Using whole dried leaf in place of whole ground leaf in broad scale spectral studies will result in significant savings since grinding is the major cost of collecting NIR spectra.

Secondly, our success in obtaining spectra from whole fresh leaves suggests that it is worth exploring the possibility of remotely sensing canopy chemistry of *Eucalyptus*. That this is a realistic possibility, is shown by the strong correlations between NIR spectral data from an aerial platform and foliar nitrogen ($r^2 = 0.87$; Martin and Aber (1997), cellulose ($r^2 = 0.79$; Gastelluetchegorry *et al.* (1995) and lignin ($r^2 = 0.77$; Martin and Aber (1997). Modelling the effects of canopy versus laboratory derived spectra of leaf samples showed that leaf biochemical information was transmitted virtually unchanged from the leaf to the canopy in the near-infrared wavelengths (Kupiec and Curran 1995). However most of these studies have been undertaken in forests dominated by conifers but *Eucalyptus* canopies are usually much more open than are conifer canopies. This introduces significant difficulties in separating reflectance spectra of foliage from bark and ground and resolving that factor is the next challenge to scaling up spectral measurements of *Eucalyptus* forests.

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8. General discussion and conclusions

Natural variation in resistance

We examined the extent of natural variation in growth and resistance to insect herbivores in 18 provenances representing all four subspecies of southern blue gum (*Eucalyptus globulus*) in an existing plantation trial. The growth of 432 plants was determined from annual measurements of height and stem diameter, and resistance was estimated from damage assessments carried out at least twice a year for five years. This provenance trial is different from most others in that half the trees were sprayed and the other half were left unsprayed. Therefore, we were able to examine growth of trees with and without damage by insects.

There was variation in the growth and resistance to insect feeding of *E. globulus* grown in a common garden, and the variation was observed between and within provenances (Figures 8.1 and 8.2). Reduction in volume due to insect damage after five years was significant in many provenances (Figure 8.1a). Christmas beetles affected all except one out of 185 plants in the unsprayed treatment. Zero to 88% of the total damage, and 0 to 100% of annual damage was caused by Christmas beetles. Provenances that were damaged by Christmas beetles in one year tended to be damaged by Christmas beetles in the following year. Provenances from Tasmania and Bass Strait Islands were most resistant to Christmas beetles. A previous study has shown that provenances from Tasmania and Bass Strait Islands are also resistant to autumn gum moth (*Mnesampela privata*) and leafblister sawfly (*Phylacteophaga froggatti*) (Floyd and Farrow, 1994). Furthermore, seedlings from King Island and Flinders Island showed resistance to common brushtail possum (Figure 8.3).

Based on the mean plant volume of sprayed and unsprayed treatments, we calculated a predicted ranking of plant volume under different probabilities of occurrence of insect damage. Predicted mean plant volume of a provenance when the probability of occurrence of insect damage was p , was calculated as:

$$\text{Volume } (p) = [\text{mean volume of unsprayed plants} \times p + \text{mean volume of sprayed trees} \times (1 - p)] / 2.$$

Regardless of the probability of occurrence of insect damage, the provenance from Geeveston is predicted to be the best provenance in terms of plant volume after 5 yr (Table 8.1). Provenances from Taralgon, Flinders Island, and St. Mary's are predicted to be good provenances when the probability of occurrence of insect damage is low. In contrast, provenances from King Island, Cape Barren Island, and Black Range are predicted to be good provenances when the probability of occurrence of insect damage is high.

Natural variation in resistance to marsupial and insect herbivores was also examined in yellow box (*E. melliodora*) and red ironbark (*E. sideroxylon*). First, at least six trees of each species were fed to marsupial folivores (koala, common brushtail possum, and common ringtail possum) which allowed us to select the most resistant, the most susceptible, and an intermediate tree to the marsupial folivores. Cross-resistance of these trees with insect herbivores was examined using three trees of *E. melliodora* and three trees of *E. sideroxylon*. We used *E. melliodora* and *E. sideroxylon*, rather than commercially important species such as *E. globulus* because we had already developed methods of quantifying leaf chemistry in *E. melliodora* and *E. sideroxylon* but not that in *E. globulus*.

There was up to a 40-fold difference in leaf consumption between the most resistant and the most susceptible tree by common ringtail possum. Christmas beetles showed up to 26-fold difference in leaf consumption. Trees of *E. melliodora* and *E. sideroxylon* showed cross-resistance to marsupial folivores and Christmas beetles (Figures 8.4 and 8.5).

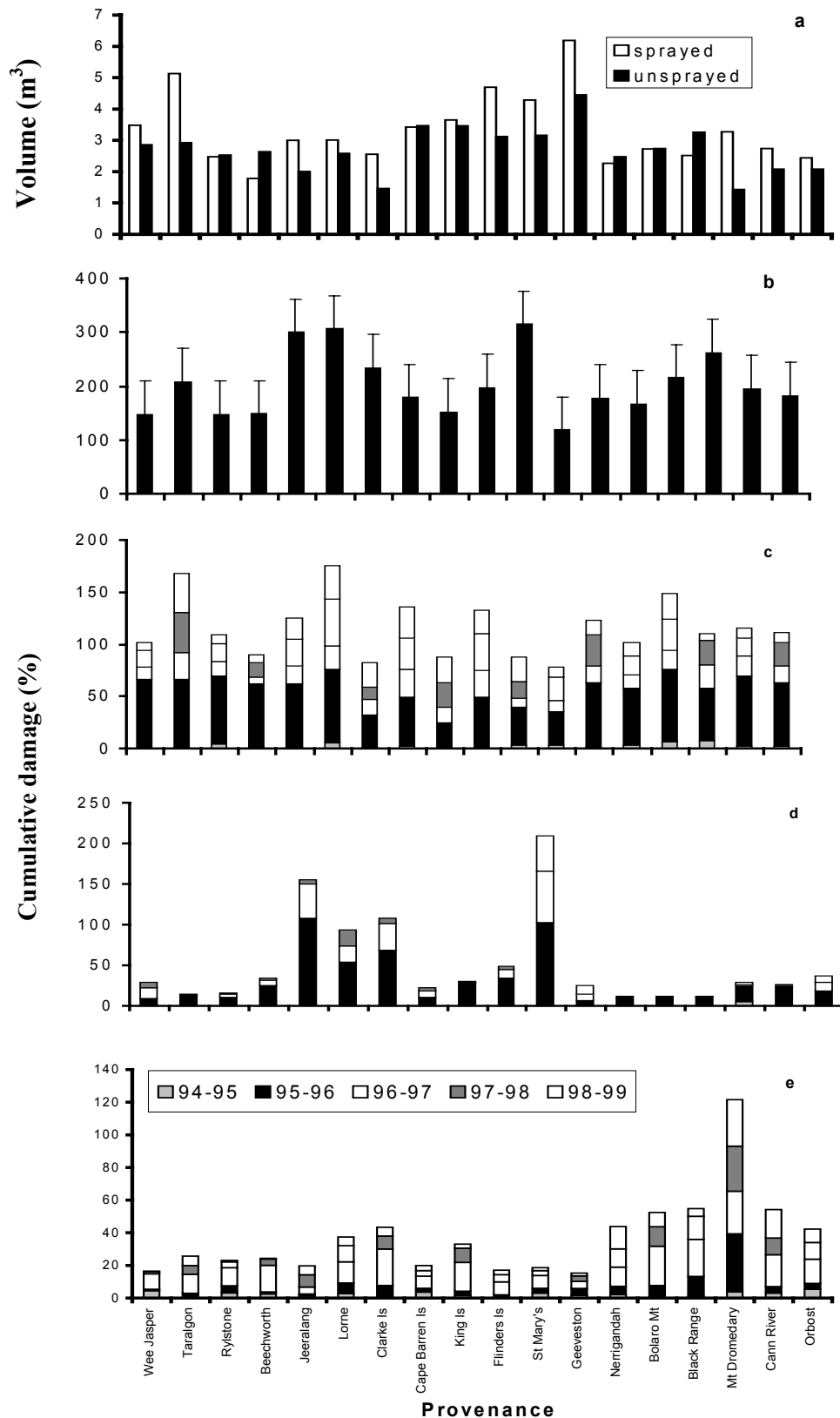


Figure 8.1. Between-provenance variation in a) mean tree volume and cumulative insect damage over five years (b - e) in 18 provenances of blue gum (*E. globulus*) in a trial at Lyneham Ridge, Canberra, ACT. $n = 9 - 12$ trees. Sources of damage shown are b) total damage (mean + se), c) Christmas beetles (*Anoplognathus* spp.), c) scale insects and e) other herbivores.

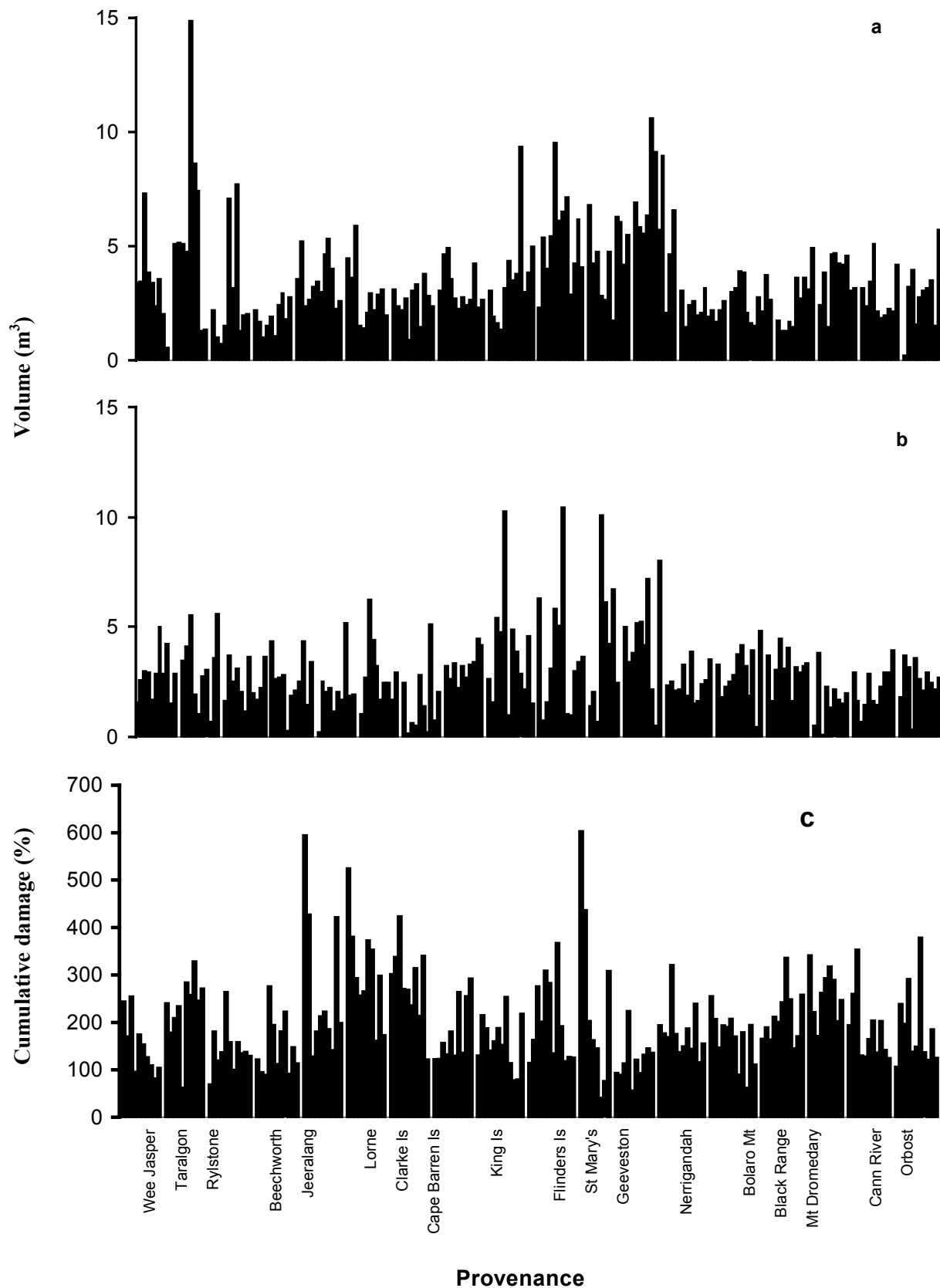


Figure 8.2. Within-provenance variation in tree volume of a) sprayed and b) unsprayed after five years and in c) total cumulative damage in 18 provenances of blue gum (*E. globulus*) in a trial at Lyneham Ridge, Canberra, ACT

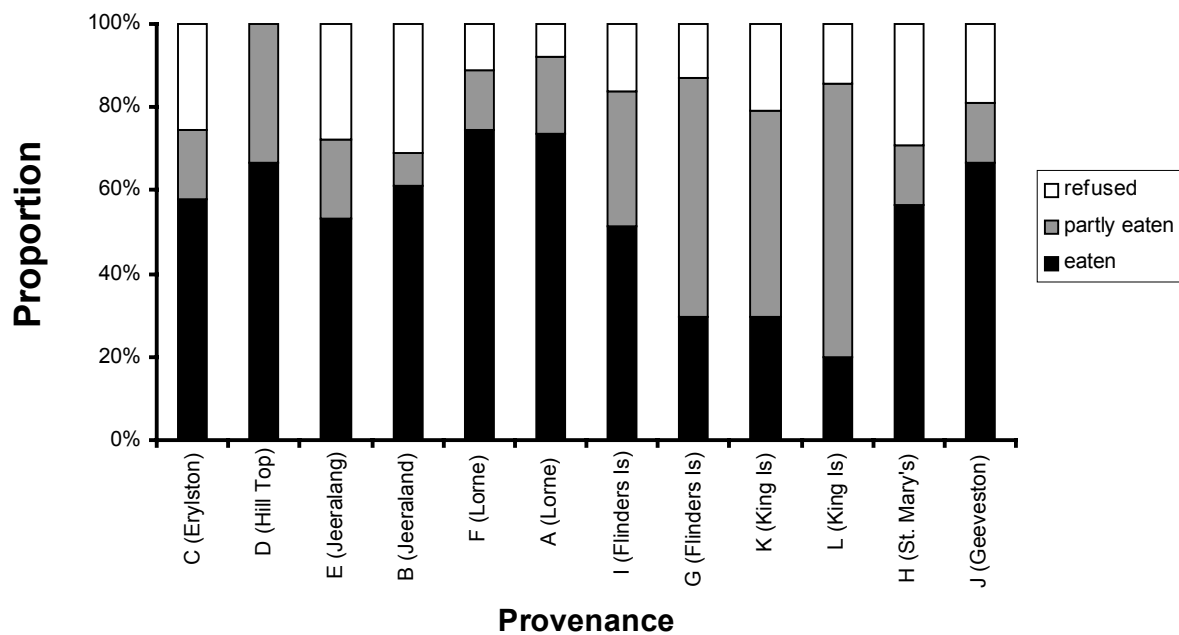


Figure 8.3. Mean proportion of *Eucalyptus globulus* seedlings mainly eaten, partly eaten, and mainly refused by common brushtail possums (*Trichosurus vulpecula*). Each possum was given nine seedlings from the same parent tree each night. Letters A – L represent parent trees, and provenances are indicated in parentheses. Mean of six possums.

Table 8.1. Predicted rank order of plant volume for 18 provenances of *E. globulus* at different probability of insect attack. A rank of 1 represents the provenance with greatest volume.

Provenance	Probability of insect attack										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Wee Jasper	6	7	7	7	7	7	7	7	8	8	8
Taralgon	2	2	2	2	2	2	2	3	6	7	7
Rylstone	15	14	14	14	12	11	11	11	11	12	12
Beechworth	18	18	18	18	17	17	15	13	12	11	10
Jeeralang	10	10	10	11	11	12	13	14	15	16	16
Lorne	9	9	8	8	8	9	9	10	10	10	11
Clarke Is	13	15	16	17	18	18	18	18	18	18	17
Cape Barren Is	7	6	6	6	6	6	6	6	3	3	2
King Is	5	5	5	5	5	5	5	4	2	2	3
Flinders Is	3	3	3	3	3	3	3	2	4	5	6
St Mary's	4	4	4	4	4	4	4	5	5	4	5
Geeveston	1	1	1	1	1	1	1	1	1	1	1
Nerrigundah	17	17	17	16	15	14	12	12	13	13	13
Bolaro Mt	12	11	11	9	10	10	10	9	9	9	9
Black Range	14	13	12	10	9	8	8	8	7	6	4
Mt Dromedary	8	8	9	12	14	16	17	17	17	17	18
Cann River	11	12	13	13	13	13	14	15	14	14	15
Orbost	16	16	15	15	16	15	16	16	16	15	14

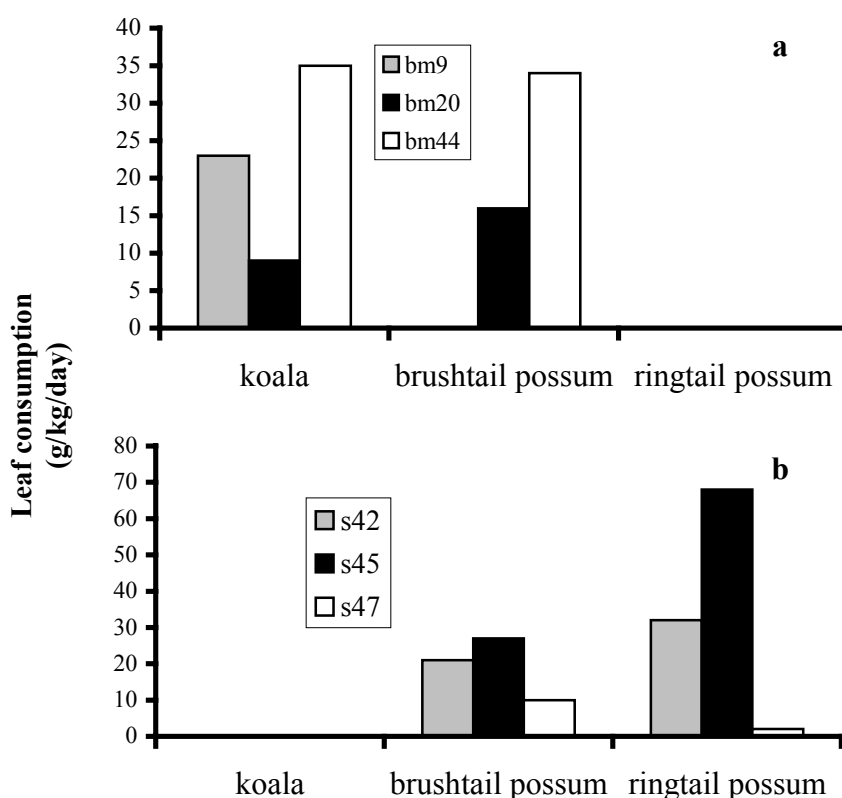


Figure 8.4. Mean leaf consumption of common brushtail possum (*Trichosurus vulpecula*), common ringtail possum (*Pseudocheirus peregrinus*), and koala (*Phascolarctos cinereus*) with leaves of yellow box (*E. melliodora*) and/or red ironbark (*E. sideroxylon*) trees. The tree bm9 was not used in the experiment with common brushtail possum. $n = 6 - 8$ animal per species.

Cross-resistance amongst a broader range of insect herbivores was not always evident. We examined cross-resistance of selected trees of *E. melliodora*, *E. sideroxylon*, and *E. camaldulensis* to a wide range of insect herbivores. We used trees with high concentrations of defensive compounds and those with low concentrations of the same compounds. Five species of Christmas beetles showed markedly high consumption of *E. melliodora* and *E. sideroxylon* trees with high concentrations of defensive compounds compared with trees with low concentration of the compounds. However, leaf consumption of three of the five species of Christmas beetles (*Anoplognathus* spp.: Coleoptera) were not related to concentrations of a defensive compound in *E. camaldulensis*. Trees of *E. melliodora* and *E. sideroxylon* that were resistant to Christmas beetles did not show cross-resistance to six other species of insect herbivores on *Eucalyptus* (Coleoptera: *Chrysophtharta variicollis* and *Gonipteris scutellatus*, Lepidoptera: *Uraba lugens*; *Opodiphthera helena*; *O. eucalypti*; and *Hyalarcta huebneri*). *Pergagraptia latreillei* (Hymenoptera) showed higher leaf consumption and growth on the *E. melliodora* tree that is susceptible to Christmas beetles than the *E. melliodora* tree that is resistant to Christmas beetles. We concluded that other species of insect herbivores did not usually show cross-resistance with the marsupial folivores and Christmas beetles (Figure 8.6).

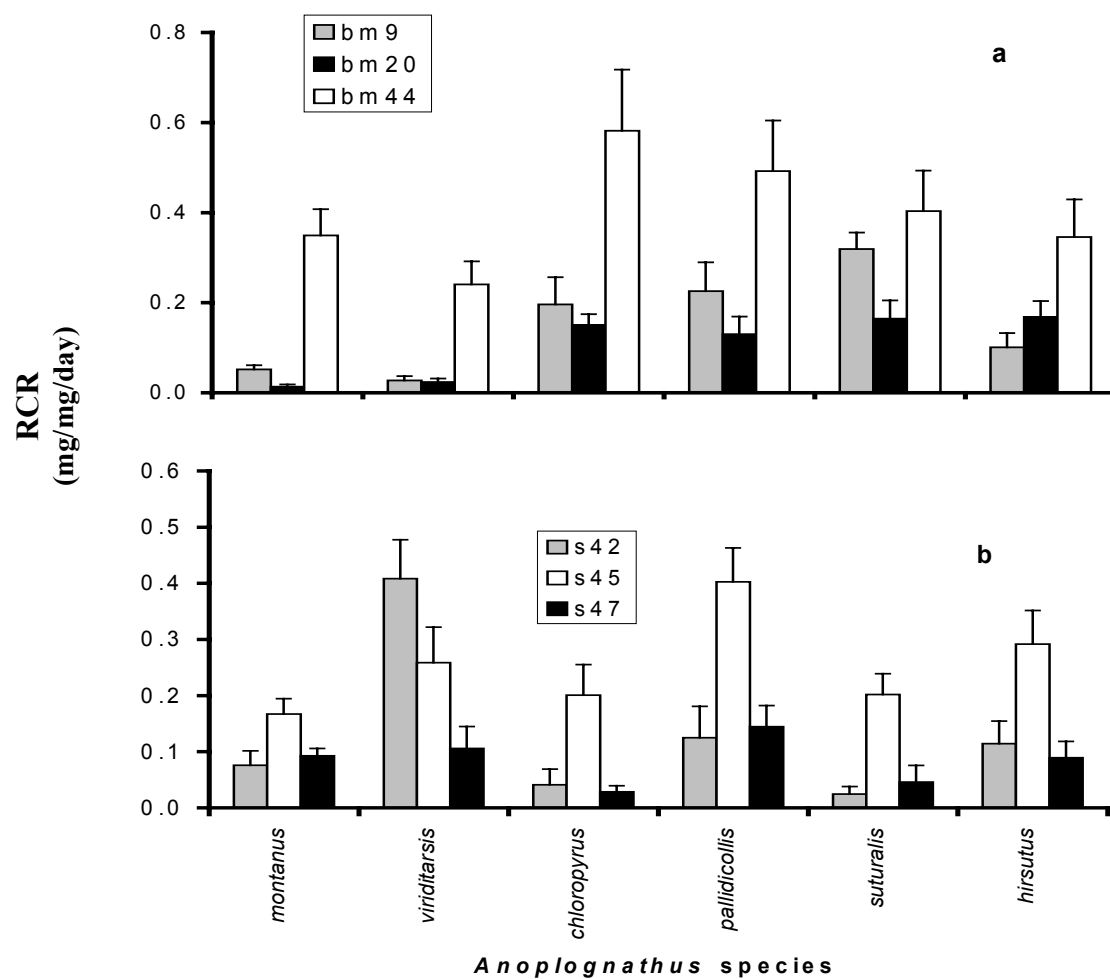


Figure 8.5. Relative leaf consumption rates (RCR) of Christmas beetles (*Anoplognathus* spp.) with leaves of a) *E. melliodora* and b) *E. sideroxylon* trees. Mean + SE, $n = 9 - 15$ beetles.

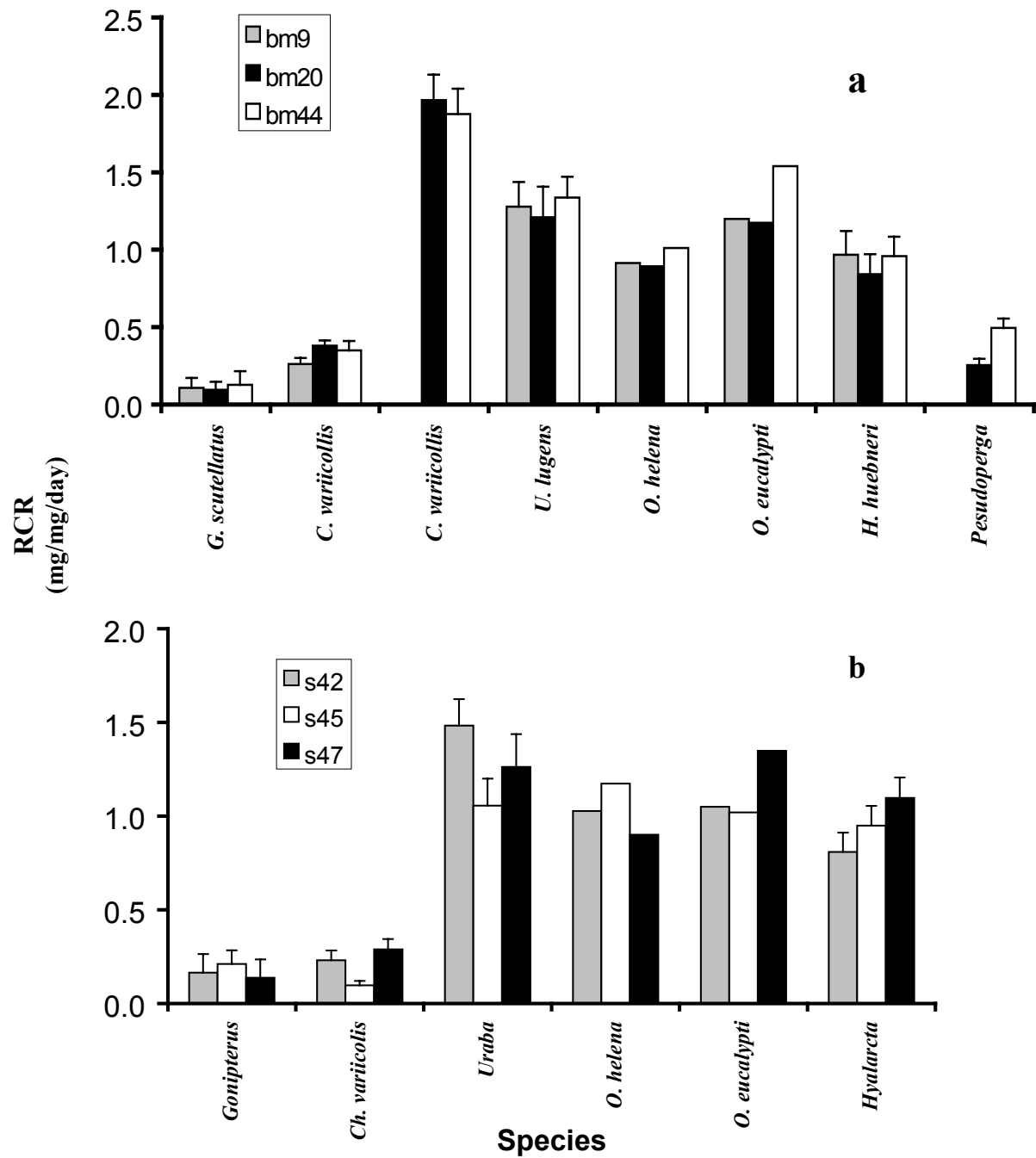


Figure 8.6. Relative leaf consumption rates (RCR) of other insect herbivores (*Gonipterus scutellatus*, *Chrysophtharta variicollis*, *Uraba lugens*, *Opodiphthera helena*, *O. eucalypti*, *Hyalarcta huebneri*, *Pseudoperga* sp.) with leaves of a) *E. melliodora* and b) *E. sideroxylon* trees. Mean + SE, $n = 9 - 15$ insects.

Examining mechanisms of resistance

We quantified some components of leaf chemistry of *E. melliodora* and *E. sideroxylon* trees used in this study. Leaves of many *Eucalyptus* species have three main secondary chemical components; phenolics, terpenes, and formylated phloroglucinol compounds (FPCs) as well as leaf surface waxes. Tannins are the most common example of phenolics whereas volatile components of essential oils are examples of terpenes. Formylated phloroglucinol compounds are structurally hybrids between phenolics and terpenoids. A number of *Eucalyptus* species show within-species variation in terpene contents in their essential oils (Boland *et al.*, 1991), and that variation is considered to be genetically, not environmentally, determined (e.g., Doran and Matheson, 1994). Previous studies have shown that cineole, an oxygenated monoterpene, is correlated with resistance of a number of *Eucalyptus* species to Christmas beetles (Edwards *et al.*, 1990, 1993). Other studies have shown that a number of FPCs, including jensenone, macrocarpal-G, and sideroxylonal (Ghisalberti, 1996), reduce leaf consumption by marsupial folivores (Pass *et al.*, 1998; Lawler *et al.*, 1998a, 1998b, 2000; Stapley *et al.* 2000). Thus, we focused our efforts on examining monoterpenes and sideroxylonal which is the major FPC derivative in *E. melliodora* and *E. sideroxylon*. We then artificially altered concentrations of sideroxylonal and cineole and examined independent effect of each compound on leaf consumption by Christmas beetles.

Concentrations of sideroxylonal and cineole (Figure 8.7) were markedly higher in leaves of trees of *E. melliodora* and *E. sideroxylon* with low leaf consumption by marsupial and Christmas beetles than those of trees with high leaf consumption by herbivores (Figures 8.4 and 8.5). Concentrations of sideroxylonal and cineole were artificially manipulated to explore the independent effects of these compounds. There was a negative dosage-dependent effect of sideroxylonal on leaf consumption by *A. suturalis*, *A. viriditarsis*, *A. chloropyrus* and *A. pallidicollis* (Figure 8.8). Relative consumption rates (RCR) in high sideroxylonal concentrations (40 and 80 mg g⁻¹ leaf dry mass) were reduced to approximately one third of those in the control. In *A. chloropyrus*, *A. pallidicollis*, and *A. suturalis*, there was no effect of increased sideroxylonal concentrations at least up to one half of the maximum concentration found naturally in leaves of *E. sideroxylon* and *E. melliodora* (i.e., 20 mg / g leaf dry mass).

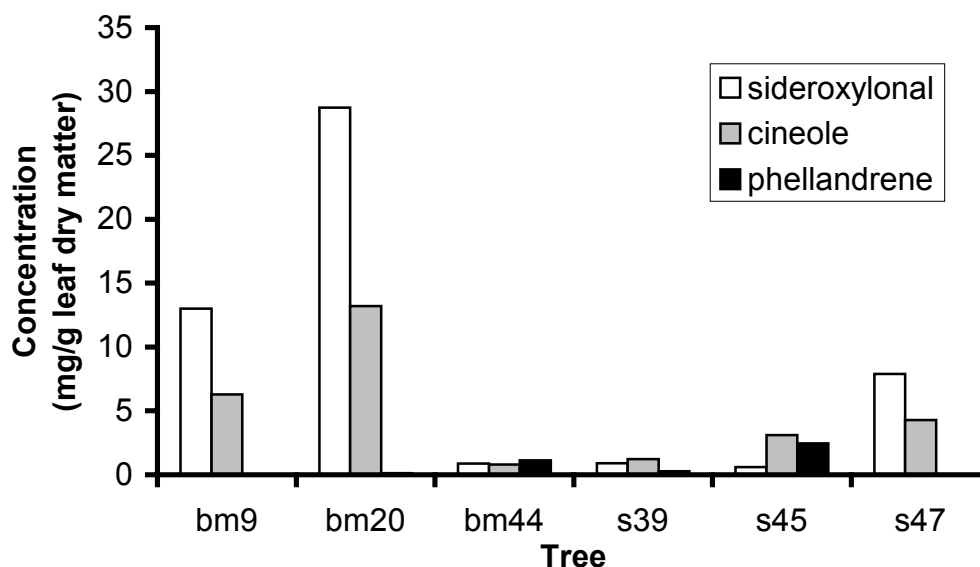


Figure 8.7. Mean concentrations of sideroxylonal, cineole, and phellandrene in leaves of *E. melliodora* (bm9 - bm44) and *E. sideroxylon* (s39 – s47) trees used in consumption experiments with insect and marsupial folivores. *n* = 2 samples per tree.

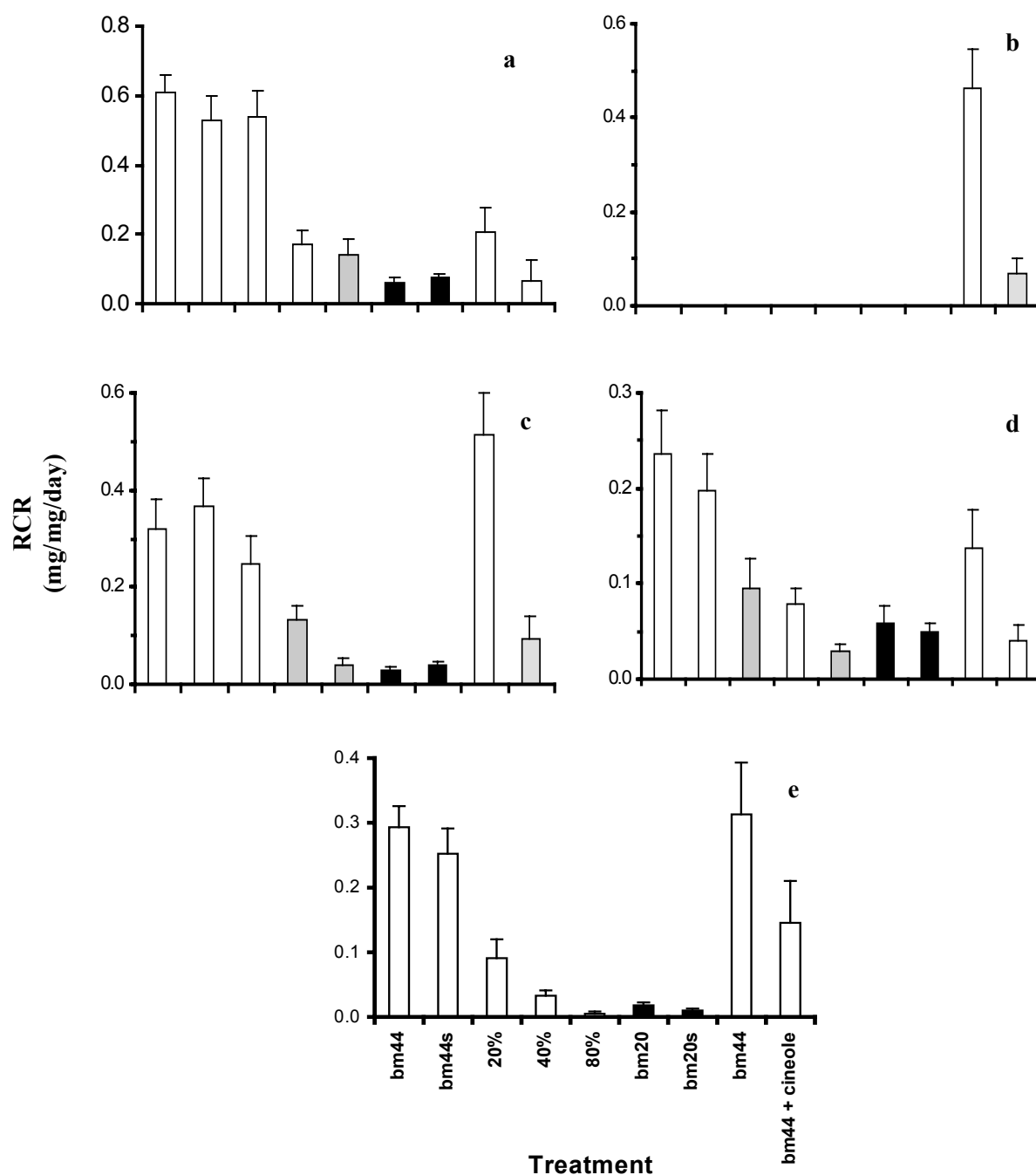


Figure 8.8 Effects of artificially increased concentration of sideroxylonal and 1,8-cineole on relative consumption rate (RCR) of *Eucalyptus melliodora* leaves by five species of Christmas beetles (a) *A. chloropyrus*, b) *A. montanus*, c) *A. pallidicollis* d) *A. suturalis* and e) *A. viriditarsis*). The solvent control is indicated by 's' after each tree number. 20, 40, and 80 indicate the amounts of sideroxylonal painted on leaves of bm44 (mg g^{-1} leaf dry matter). Mean + SE, $n = 9 - 15$ beetles per tree.

When droplets of pure 1,8-cineole were applied onto leaves of *Eucalyptus* branches on which Christmas beetles were feeding, the beetles immediately ceased feeding and quickly moved as far away from the droplets as they could within a cage. Artificial increase in concentration of 1,8-cineole reduced leaf consumption by the five species of Christmas beetles (Figure 8.8). The beetles did not feed on 1,8-cineole painted leaves until 1,8-cineole evaporated off the leaves. Once 1,8-cineole

evaporated off, the treatment leaves were consumed just as control leaves. Therefore, the observed reduction in consumption is due to effective reduction in feeding time caused by evaporating 1,8-cineole.

Artificial 50% reduction in the concentration of 1,8-cineole by steaming leaves increased leaf consumption by *A. chloropyrus* when the natural concentration of sideroxylonal was moderate (i.e., <20 mg / g leaf dry mass) and that of 1,8-cineole was high. However, steaming leaves did not affect consumption of leaves when the natural concentrations of both sideroxylonal and 1,8-cineole were either high or low.

Although there is now substantial evidence that marsupials and some Christmas beetles will not eat leaves containing high concentrations of sideroxylonals the mechanisms by which they detect and avoid these plant compounds are not clear. It is important to understand the mechanism because of the danger that tree-breeding programs could focus on associated compounds that are used by animals as cues rather than on the actual factor conferring resistance, an idea first presented by Dr Mamoru Matsuki.

For vertebrates, the key factor is learning through experience in much the same way that we mould our own likes and dislikes of different foods. Koalas and ringtail and brushtail possums will all eat leaves containing FPCs. Therefore, the focus should be on how they regulate their intake rather than whether they avoid the compounds.

Studies with captive ringtail and brushtail possums have shown that they regulate their intake of FPCs so as not to exceed a threshold dose. This threshold is closely monitored, probably by feedback from the nausea centre of the brain. A possible mechanism is as follows: FPCs appear to damage cells in the small intestine, which in turn release serotonin that may initiate nauseous responses. The evidence for this comes from experiments with drugs that block one part of this pathway, which leads to animals eating more food. In particular, a drug (ondansetron) that blocks a serotonin (5HT3) receptor and which is used in human cancer therapy as an anti-nausea drug, partially counters the antifeedant effects of FPCs. This link is particularly important because nausea is the most potent way of mediating learning in animals.

Animals can learn to avoid most poisonous plants by associating unpleasant effects such as nausea with the smell or taste of the plant. In the case of FPCs, there is a strong correlation between the concentration of volatile oils (monoterpenes) in the leaf and FPCs. This arises because the compounds share a common biosynthetic pathway. The monoterpenes have a strong and distinctive odour but are not in themselves aversive. In fact, both ringtail and brushtail possums can eat large amounts of these monoterpenes without ill-effect. However, feeding monoterpenes with nausea-inducing FPCs, even for a short time, will stop animals eating large amounts of monoterpenes in the future - even when the FPC is no longer present. In other words, aversions to monoterpenes are conditioned by the presence of FPCs and can be extinguished and re-established. It is not the monoterpene that is toxic but the animal has learnt to associate the smell of that monoterpene with an unpleasant consequence.

Difficulties may arise, however, if this resistance is then attributed to the wrong compound and selection is based on that criterion rather than resistance per se. However, our knowledge of the biosynthesis of FPCs, and their relationship with the biosynthesis of terpenoids, is sorely lacking at present. There is a distinct danger that at some point in the artificial selection process the relationship between concentrations of the two groups of compounds may break down, and that high essential oil genotypes may no longer contain high acylphloroglucinol concentrations. This danger is especially significant where genetic engineering may be used to manipulate a single factor, such as cineole production, unless flow-on effects on related compounds are also assessed.

Developing NIRS methods for identifying resistant trees

Little work had been done using near-infrared spectroscopy and tree leaves prior to this work and our first aim was to ensure that we developed and used standard methods for all spectroscopic measurements. Previous work (Foley *et al.*, 1998) had used dried, finely ground material but grinding many leaf samples is a time-consuming and laborious process. Initially we investigated whether we could collect valid spectral information from dried whole (unground) leaves. This proved to be the case (See Chapter 7) and so all NIR spectra in this study were collected from whole unground samples of foliage. We showed also that important chemical information could also be collected from whole fresh leaves. We considered that fresh material was harder to work with in the laboratory but recognised that this finding would allow the future use of small hand-held or airborne spectrometers to be used for a range of measurements on plantation trees in the future.

We acquired spectra of all samples using a near-infrared reflectance spectrophotometer (NIRSystems 6500: Silver Springs MD) equipped with a spinning cup sample holder. This instrument was housed in a room maintained at constant temperature 22 ± 1 °C and relative humidity $55 \pm 5\%$. We collected at least two replicate spectra of reflected monochromatic light between 400 and 2498 nm at 2 nm intervals. We averaged these replicates only if the standard error of the difference between the two spectra (expressed as $\log(1/R)/10^6$) was less than 50. This ensured the quality of the spectral data collected (Shenk and Westerhaus 1993a). The reflectance (R) readings were converted to absorbance (A) values using the following equation: $A = \log(1/R)$ (data analysis was conducted using the ISI software System (Shenk and Westerhaus 1991a)

Many alternative procedures have been published for modelling the relationship between NIR spectra and measured attributes of a sample - be they chemical components or functional responses such as relative defoliation. We used modified partial least squares regression (MPLS) (Martens and Jensen 1982, Shenk and Westerhaus 1991b). The MPLS method uses all the spectral data in contrast to alternative approaches such as multiple linear regression that use a small subset of the spectral data. We applied several transformations (calculation of first derivative (Osborne *et al.* 1993) and detrending (Barnes *et al.* 1989)) to the spectral data in order to remove the effect of particle size and to minimise autocorrelations between spectral measurements. These procedures are part of the recommended standard procedures (Anon 1995).

The MPLS approach requires cross validation to prevent overfitting (i.e. using too many terms in the equation) and to select the optimum number of terms for each calibration equation (Osborne *et al.* 1993). Cross validation involves dividing the sample set into "n" groups and performing the calibration on "n-1" groups with the remaining group being used as an independent validation set. This exercise is repeated until all samples have been cross-validated and the residuals of each prediction are pooled to provide a standard error of cross-validation (SECV). A final calibration procedure is then performed on all samples using the number of factors determined by the cross validation procedure. Cross validation is an efficient procedure because all samples are used for both calibration and validation, and avoids the need to set aside samples for a validation set (Osborne *et al.* 1993; Shenk and Westerhaus 1993). Another advantage of cross validation is that outliers from the prediction residuals are identified readily (Shenk and Westerhaus 1991a, 1993).

Once we had established the relationship between the spectra of foliage from various *E. melliodora*, *E. sideroxylon*, and *E. globulus* trees, and the concentration of various chemical components as measured using conventional analytical procedures (e.g. High Performance Liquid Chromatography and Gas Chromatography) we were able to predict the concentration of those compounds by applying the model to a new group of spectra for which we had no chemical data. We also collected spectra from representative samples of the foliage that was used in marsupial feeding experiments, and used these models to predict leaf consumption by marsupial herbivores on untested trees. To validate NIRS-based predictions, we first selected untested trees and predicted concentrations of foliar chemicals such as sideroxylonal and cineole or leaf consumption by common ringtail possum, and then independently

measured actual concentrations of the chemical compounds or leaf consumption by common ringtail possum, respectively, on those trees. Finally, we developed NIR models for predicting leaf chemistry of seedlings of *E. sideroxylon* and *E. globulus*.

There was a strong positive correlation between NIRS predicted concentrations of sideroxylonal and actual concentrations of sideroxylonal (Figure 8.9). Also, there was a strong positive correlation between NIRS predicted leaf consumption by common ringtail possum and actual leaf consumption (Figure 8.10).

Concentrations of sideroxylonal in leaves of *E. sideroxylon* seedlings did not show expected differences in concentrations of sideroxylonal observed in their mothers (Figure 8.11a). Similarly, concentrations of cineole in leaves of *E. globulus* seedlings did not equate with differences in concentrations of cineole in provenances with different mean resistance to insect herbivores (Figure 8.11b). These trials need to be continued through to an older age to determine when adult chemical profiles become evident. The end of this project has restricted the length of these trials.

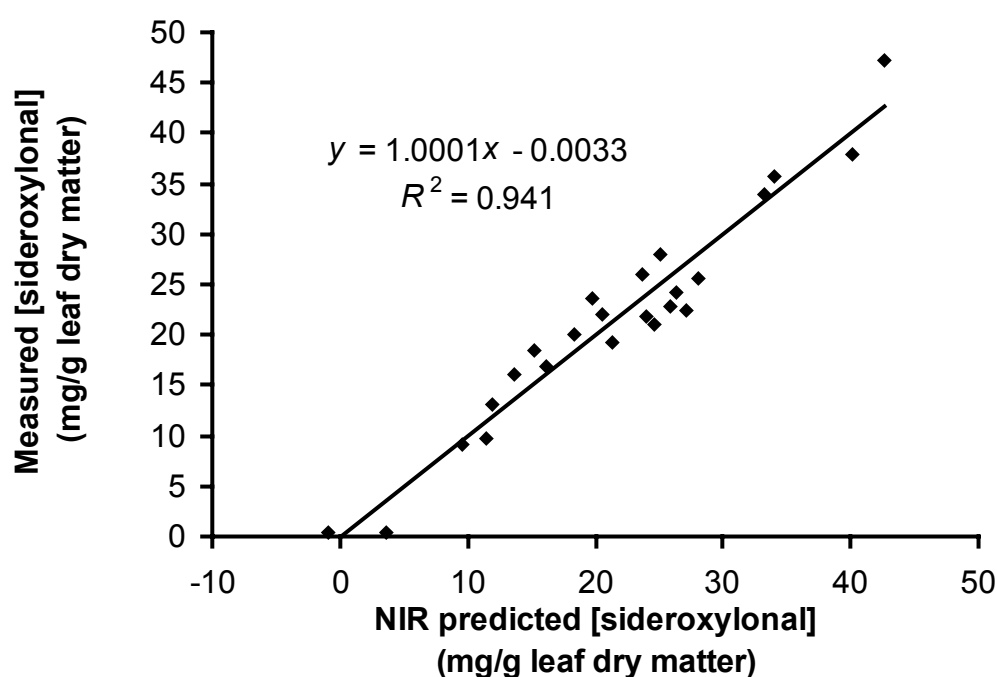


Figure 8.9. Relationship between actual and NIRS predicted concentrations of sideroxylonal in leaves of 23 *E. melliodora* trees.

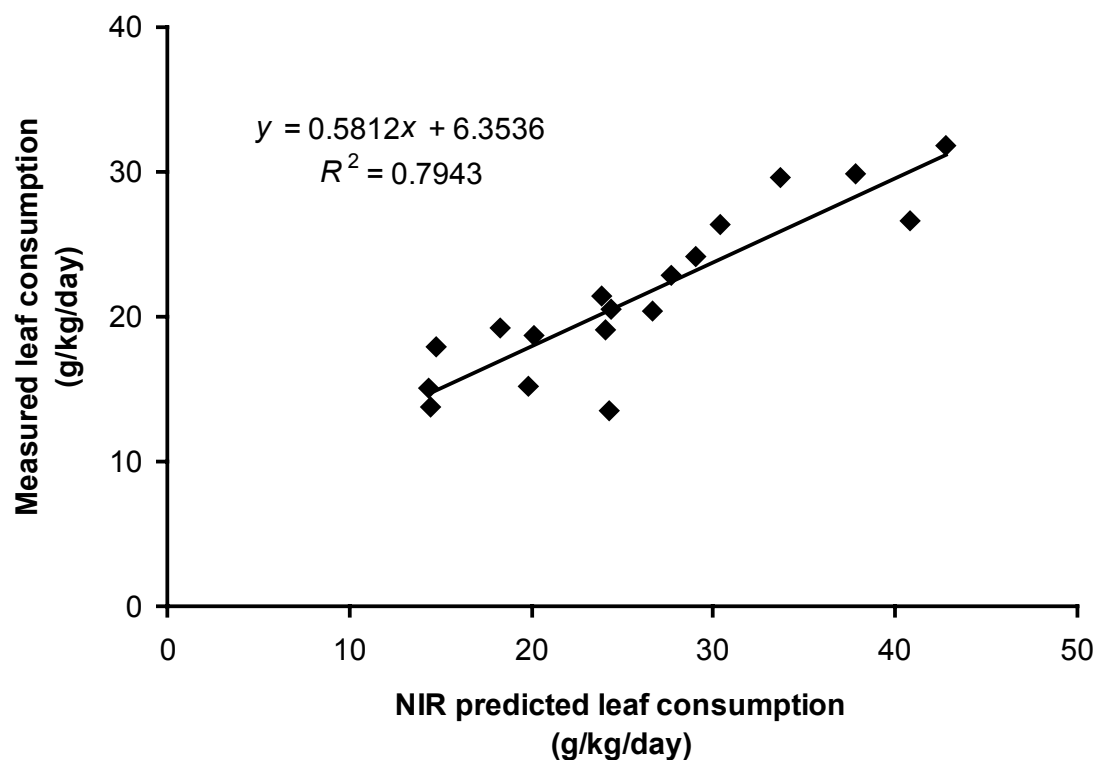


Figure 8.10. Relationship between actual and NIRS predicted consumption of leaves of red box (*E. polyanthemus*) by common ringtail possum (*Pseudocheirus peregrinus*). Each point represents the mean intake of one tree by six possums. Leaves of red box contain sideroxylonal and cineole, and leaf consumption of common ringtail possum is also strongly negatively correlated with concentration of sideroxylonal in red box leaves (data not shown).

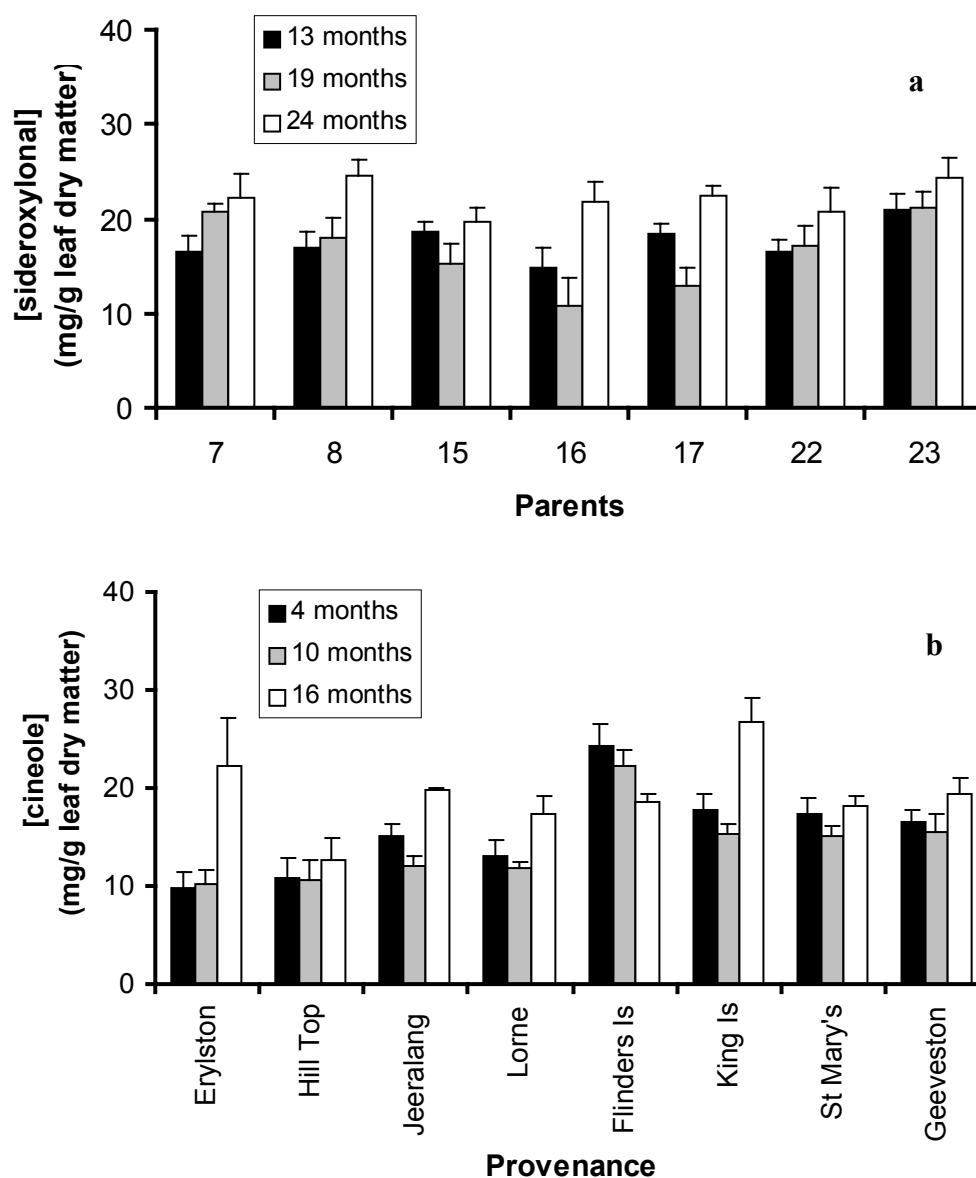


Figure 8.11. (a) Changes in concentrations of sideroxydonal in seedlings of *E. sideroxydon*. Parents 8 and 16 are susceptible, while all others are resistant. $n = 12$ seedlings per parent. (b) Changes in concentrations of cineole in seedlings of *E. globulus*. Provenances from King Island, St. Mary's and Geeveston have been shown to be resistant to insect pests in the field, while other, particularly provenances from Jeeralang and Lorne have been shown to be susceptible. We first calculated mean concentrations for each parent tree based on 7 seedlings per parent. Then calculated mean concentrations for each provenance based on 3 to 5 parents per provenance.

Conclusions and implications

The objective of this study was to develop our understanding of the mechanisms that confer resistance of eucalypts to both vertebrate and invertebrate herbivores and to assess the potential of near-infrared reflectance spectroscopy as a method of rapid identification of resistant trees for use in agroforestry. We were able to determine the chemical basis for resistance to insect and marsupial folivores in farm forestry. Very little was known about either of these two subjects prior to the commencement of this study and hence our work was very much the pioneering study in this area. To that end we are now in the position of having an excellent understanding of important resistance mechanisms and of the application of near-infrared spectroscopy to identifying plants that express key resistance traits.

Near-infrared spectroscopy proved to be a highly suitable tool for rapidly assessing components of natural herbivore resistance in eucalypts. In one sense, near-infrared spectroscopy is an entirely empirical technique in that we seek a statistical model between spectra of a leaf and a component or attribute of interest. In theory it should not matter whether or not we know what the underlying reasons for this is so long as the NIRS models are robust and accurately identify resistant individuals.

However, given the poor understanding of the basis of resistance to herbivores in eucalypts and the large number of inconclusive studies that preceded the current work, we felt that it was important to show that the wavelengths that contributed most to the resistance models that we developed were in fact based on known traits. The use of NIRS in measuring and predicting pulp yield in eucalypts is relatively successful but there is little evidence that the models are describing wood properties that are understandable in terms of current knowledge about pulping properties. One of the strengths of this work is that the resistance traits that we have demonstrated (e.g. sideroxylonal and cineole) are strongly represented in the NIRS models that we have developed. Accordingly we are in the enviable position of being able to use NIRS to predict resistance rapidly but also of being able to verify that the resistance traits are being correctly identified.

Different insect and vertebrate pests attack *Eucalyptus* plantings in farm forestry and we recognised from the outset the need to improve our understanding of this issue. It was encouraging that we were able to demonstrate that there is substantial cross-resistance between marsupial herbivores and one major group of insect defoliators, the Christmas beetles (*Anoplognathus* spp). This is the first time that cross-resistance has been demonstrated between invertebrate and vertebrate herbivores of *Eucalyptus* and the first time that the chemical basis of cross-resistance between vertebrate and invertebrate herbivores has been identified. Although all the marsupial species examined are susceptible to the effects of sideroxylonal (albeit at different concentrations), we found that a range of different insect defoliators did not respond the same way as Christmas beetles. Given the wide variety of Orders represented and the different evolutionary histories of these species, the lack of full cross-resistance is perhaps not surprising. Nonetheless it is evident from other studies that there are uncharacterised resistant traits against some of the species that we studied (e.g. *Uraba lugens*). In our study we were able to examine cross resistance in one direction only (ie between material which was resistant to Christmas beetles and other species. We did not have field material to examine which showed clear resistance against these other species and so we cannot judge the possibility that there is cross resistance in the other direction (e.g.. between *U. lugens* and *Anoplognathus* sp).

We have demonstrated that there are major differences between the chemical composition of seedlings of some eucalypt species. This important trait of eucalypts has only been studied to a limited extent in the past yet is of major ecological importance. We believe it likely, based on the little available data (Doran and Matheson 1994), that eucalypts undergo major chemical changes at about the time that they finally acquire their adult-type foliage. In this study we have followed a group of *E. sideroxylon* seedlings from parents with distinct chemical profiles for 24 months but have yet to detect any significant change in the concentrations of defensive chemistry. Nonetheless we believe that it is worthwhile to continue to chart the progress of this material since all available evidence suggests that

these resistance traits are determined predominantly by genetic makeup rather than environmental conditions (Lawler et al. 2000; Adamson, Foley and Woodrow unpublished).

As we anticipated, there was considerable work required to develop NIRS based models of resistance and resistance traits. We made considerable improvements to the methods of acquiring spectra and of building models once those spectra had been acquired but the measurement of resistance itself through feeding experiments is time consuming. However, in all cases where we did this, the value of those experiments was proved since we were able to show that traits that others had claimed as resistant (e.g. cineole for Christmas beetles; Edwards *et al.* 1993) were in fact not causally associated with resistance.

In the case of vertebrates, we believe that there is sufficient understanding of resistance to be able to recommend that trees expressing high concentrations of sideroxylonal will confer partial resistance to herbivorous marsupials. However, the presence of sideroxylonal (unless in very high concentrations) is not in itself an absolute deterrent. As would be expected, animals can eat some of the compound but the nausea that results if a threshold dose is exceeded is a very strong promoter of future avoidance. Animals in the field will always have choices between seedlings and other plants and McArthur (unpublished data: CRC for Sustainable Production Forestry) has shown that the range of choices available can have a big impact on the consumption of seedlings irrespective of the inherent resistance of those seedlings.

It is important to recognise that not all eucalypt species contain sideroxylonal and it is absent from several commercially important species such as *E. globulus*, *E. camaldulensis* and *E. dunnii* (Eschler *et al.* 2000). In these species, there is a related group of compounds called macrocarpals that are also believed to confer resistance against marsupial herbivores. Although these are closely related to sideroxylonals they are a more complex group of compounds that are poorly resolved chromatographically. Consequently there is as yet no reliable way of assaying the concentration of the compounds but we do know that NIRS can detect and rank plants containing these compounds (Eschler, Moore and Foley unpublished).

Implications of selecting and deploying resistant trees

This study has made substantial progress towards the development of a rapid identification method of resistant trees based on resistance mechanisms. This section will discuss some of the important implications of selecting and deploying resistant trees in agroforestry and farm forestry.

The advantage of the rapid identification method is that, once one develops NIRS methods for discriminating between trees for resistance, it is possible to select individual trees with greater resistance. If the result of this technology was to establish plantations of uniformly high resistance, some potentially serious issues need to be considered. These issues are growth - defence trade-offs and counter-adaptation of pests. Both issues can be easily managed if understood. We will discuss each issue in some detail.

Growth-defence trade-offs

Plants show considerable variation in growth and susceptibility to herbivores within and between genotypes (Bryant *et al.*, 1983; Denno and McClure, 1983; Coley *et al.*, 1985; Herms and Mattson, 1992). It has been hypothesised that variation in susceptibility to herbivores is largely determined by variation in amounts and types of plant secondary metabolites (Fraenkel, 1959). Moreover, it is widely accepted that there is a trade-off between plant growth and chemical defence (ie resistance) because these functions compete for limiting resources (Bazzaz *et al.*, 1987; Chapin *et al.*, 1990). For example, carbohydrates, which are produced by photosynthesis, are required for both growth and defence. Building materials of plants, such as cellulose and lignin, are made from simple carbohydrates such as glucose. Similarly, many chemical compounds that act as a defence against herbivores (ie secondary

metabolites) are also made from simple carbohydrates. Thus, fast growing plants (species, populations, or individuals) are hypothesised to be more susceptible to herbivores than slow growing plants (species, populations, or individuals). The growth-defence trade-off may be best managed by selecting trees with a moderate increases in resistance which might result in equivalent or enhanced growth when compared with susceptible trees if reduced growth due to resistance in resistant trees were less than reduced growth due to damage in susceptible trees.

Counter-adaptation of pests

The strength of selection for counter-adaptation by pests to cope with host-plant resistance is (1) positively related to strength of plant resistance to pests and (2) negatively related to heterogeneity of plant resistance to pests. Managing counter-adaptation in forestry also needs to take account of the long rotation length of the crop, when compared to agriculture, and the large number of generations of pest species per rotation. However, one of the aspects of forestry that will normally slow down the rate of counter-adaptation is the genetic mixing between pest populations in plantations and those in surrounding vegetation. When plantations are the dominant landuse, as is happening in SW Western Australia and the Green Triangle, genetic mixing is low and the risk of counter adaptation is higher.

Both these issues can be easily managed in farm forestry by avoiding deploying large areas of uniformly resistant trees expressing the same resistance trait and considering the relative benefits of moderate levels of resistance rather than strong resistance. Given the current nature of the forest industry in Australia and the moderate strength of insect resistance conferred by sideroxylonal and cineole, these risks can be effectively and efficiently minimised.

Future research directions

The incorporation of resistant trees into plantations is a highly desirable option for pest management and this project has made significant progress in the understanding of mechanisms of resistance against insect and marsupial herbivores as well as proof of concept of the use of NIRS as a rapid method of identifying resistance. Before NIRS screening for resistance becomes an operational reality, there are several areas that need further work.

It is necessary to link seedling NIRS profiles to the ultimate level of resistance of more advanced plants. It is clear that we need to understand more about the chemical changes that occur in the transition from seedling to adult plants particular if we are to use seedlings as predictors of resistance in adult plants. In particular this is important if we are seeking cross-resistance between vertebrate and invertebrate herbivores because the two groups may attack plants at different stages. For example, high concentrations of sideroxylonals in seedlings may confer resistance against wallaby browsing but not against chrysomelid beetles when the tree has grown to adult form.

Recent advances in the analysis of *Eucalyptus* defence metabolites will allow the concepts developed here to be expanded and applied to a much wider range of eucalypts. In this project we concentrated our efforts on species of *Eucalyptus* that express sideroxylonals (which is one of the main groups of formylated phloroglucinol compounds) present. However, many important commercial species contain a closely related group of compounds called macrocarpals that have proven very difficult to quantify. However, application of two new analytical techniques together with the efforts of a Japanese scientist visiting the ANU have essentially solved the difficulties and will allow the quantification of the major macrocarpals in *Eucalyptus* foliage. Accordingly, we believe that the next research goal should be to apply the approaches we have pioneered to a specific eucalypt that is of interest to industry such as *E. globulus*. That work would involve developing NIR based predictors of resistance for the major pest species (including autumn gum moth, chrysomelid leaf beetles and weevils) for a much wider range of material, developing a wider data bank of chemical characterisation of defence compounds for *E. globulus* and a direct evaluation of any defence and

growth trade-offs. These goals have the support of industry in several states, particularly in Western Australia where the industry is encountering substantial opposition to aerial spraying of insecticides – currently their only effective and efficient method of pest management.

In addition to the above extension to this projects work on NIRS techniques for screening for resistance, there are two other avenues for using pest resistant eucalypts to reduce reliance on pesticides.

Firstly, past research has been focussed on discouraging pests using pesticides or naturally occurring chemicals that confer pest resistance. A result of this study indicates, however, that there is a possibility that at least some insect species may be using positive cues as well as negative cues (suggested by Dr Mamoru Matsuki). Christmas beetles can feed on oak and birch leaves, but they do not normally feed on these plant species. This is probably because Christmas beetles do not recognise oak and birch trees as their food. There are some chemical compounds (e.g., phellandrene) that are found in higher concentrations in leaves of susceptible trees than in leaves of resistant trees of *E. melliodora* and *E. sideroxylon*. If there were some plants lacking positive cues, then those plants would be ignored by insects. Further studies in this area may prove fruitful.

Secondly, we need to gain better understanding of the role of heterogeneity and complexity in reducing pest problems in agroforestry and farm forestry plantations. Pest resistant trees may be deployed as long as there is enough heterogeneity and complexity in plant resistance to pests present in plantations. However, we do not know the threshold level of heterogeneity or complexity to allow this. Also, heterogeneity and complexity in natural habitats have been hypothesised to have important roles in regulating pest populations. For developing ecologically and evolutionarily sound pest management strategies, more research effort should be directed towards understanding roles of heterogeneity and complexity in regulating pest populations and evolution. In particular, selecting resistant populations, rather than individuals, using provenance trials deserves more attention because by selecting resistant populations, at least some variation in resistance present in the selected populations will be maintained in plantations. Moreover, we need to gain better understanding of natural variation in leaf chemistry and pest resistance. Techniques in NIRS developed during this study can be applied to remotely assess spatial variation in leaf chemistry using sensors on board an aircraft. Continued research on understanding mechanisms of resistance in a wider range of *Eucalyptus* species and on developing NIRS techniques for remote sensing will substantially advance our capability to study natural variation in pest resistance and to develop more effective pest management strategies.

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