Chemoecology 17: 235–243 (2008)
0937-7409/07/040235-9

DOI 10.1007/s00049-007-0382-z

CHEMOECOLOGY

Constitutive or induced defences - how does *Eucalyptus globulus* defend itself from larval feeding?

Luke P. Rapley[1*], Geoff R. Allen[1], Brad M. Potts[2] and Noel W. Davies[3]

Cooperative Research Centre for Forestry, Hobart, Tasmania, Australia
[1]School of Agricultural Science/TIAR, University of Tasmania, Hobart, Tasmania, Australia
[2]School of Plant Science, University of Tasmania, Hobart, Tasmania, Australia
[3]Central Science Laboratory, University of Tasmania, Hobart, Tasmania, Australia
[*]Current address: School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, P.O. Box 6811, Cairns, Qld, 4870

Summary. Following herbivory, induced responses involving plant secondary metabolites have been reported in a number of tree species. Although a wide range of plant secondary metabolites appear to operate as constitutive plant defences in trees belonging to the *Eucalyptus* genus, no induced responses have as yet been reported following foliar-chewing insect damage. We empirically tested whether branch defoliation (artificial and larval) of 2-year-old *Eucalyptus globulus* Labill. trees altered the abundance of specific plant secondary metabolites immediately (3 months after initial larval feeding) and 8 months after the cessation of larval feeding. Metabolites assayed, included essential oils, polyphenolic groups and foliar wax compounds and in all cases their abundance was not significantly altered by defoliation. However, the level of foliar tannins after 3 months of larval feeding did display a trend that suggested elevated levels as the result of defoliation, though this trend was not evident 8 months later, indicating that, if real, the response was a rapid and not a delayed induced response. The level of foliar tannins was also negatively correlated to both average larval survival and average percentage branch defoliation, suggesting that foliar tannins may operate as toxins and/or anti-feedants to *M. privata* larval feeding.

Key words. Plant-insect interactions – leaf chemistry – induced and constitutive plant defences – Lepidoptera – Geometridae – *Mnesampela privata* – Myrtaceae – *Eucalyptus globulus*

Introduction

Induced responses have been recorded for many plant secondary metabolites in different tree species including; phenolic compounds in birches induced by *Epirrita autumnata* larval feeding (Kaitaniemi *et al*. 1998) and artificial leaf damage (Hartley and Lawton 1991), phenolic compounds in quaking aspen following larval feeding by *Choristoneura conflictana* (Clauson *et al*. 1991), tannins in oak after larval damage by *Lymantria dispar* (Schultz and Baldwin 1982; Rossiter *et al*. 1988) and terpenoids and phenolics in conifers after attack by bark beetles (Raffa 1991). However, although changes in plant secondary metabolites have been recorded as a response to, for example, wounding and disease in the phloem and xylem of eucalypts (Eyles *et al*. 2003a; 2003b), induced responses as a result of foliar-chewing insect herbivory have yet to be demonstrated (Haukioja *et al*. 1994). Nevertheless, Edwards and Wanjura (1989) have reported that several species of eucalypt feeding insects chew off and discard partially eaten leaves and proposed that these insects are sabotaging a feedback loop from the damaged leaf to the host tree that may confer resistance. We also might expect to find induced resistance in eucalypts, because the foremost hypothesis explaining inducible plant defences suggests it is of particular benefit for plants, such as eucalypts, that experience variable herbivory (Herms and Mattson 1992). This is because defending a plant against herbivory is assumed to have a cost, since resources allocated to defence cannot be simultaneously allocated to other functions (Zangerl and Bazzaz 1992; Karban *et al*. 1997; Heil 2002). Therefore, inducible defences economise the plants expenditure by allowing the plant to invest in defence only when necessary, avoiding costly allocations when herbivores are absent (Agrawal 2000). Further, Agrawal and Karban (1999) identified alternate reasons why inducible defences might be preferred over constitutive strategies. These included improved resistance against differing herbivores due to the variability it creates in food quality, which may inhibit feeding and slow adaptation and cause greater dispersal of damage by increasing herbivore movement. Also, induced defences may reduce autotoxicity and host finding by specialist herbivores and limit deleterious effects on natural enemies and pollinators (Agrawal and Karban 1999).

In the present study the larvae of autumn gum moth, *Mnesampela privata* Guenée, were used to achieve defoliation of experimental trees. These larvae are a destructive defoliator of many *Eucalyptus* species (Elliott and Bashford 1978; Neumann and Collett 1997; Lukacs 1999), including

Correspondence to: Luke P. Rapley, e-mail- Luke.Rapley@jcu.edu.au

the most commercially significant hardwood species grown in Australia, *Eucalyptus globulus* Labill. (Farrow *et al*. 1994; Jones *et al*. 2002; Rapley *et al*. 2004a). The study focused on empirically testing *E. globulus* foliar plant secondary metabolites, that are known to affect eucalypt herbivory, to determine if they are mobilised either during larval feeding in a rapid induced response (little time lag, affecting the generation of herbivores that did the damage), or 8 months later in a delayed induced response (with time lag, affecting subsequent generations), or simply operate as constitutive defences uninfluenced by herbivore feeding (Klemola *et al*. 2003; Haukioja 2005). The time-frame tested was designed to determine the appropriateness of a retrospective approach used for identifying putative constitutive chemical differences in the foliar waxes between proximal trees which differ in observed levels of field herbivory (Jones *et al*. 2002; Rapley *et al*. 2004b). The compounds investigated were foliar wax constituents (Edwards 1982; Jones *et al*. 2002; Rapley *et al*. 2004b), essential oils (Larsson and Ohmart 1988; Li 1993; Steinbauer and Matsuki 2004) and polyphenolic compounds (Larsson and Ohmart 1988; Floyd and Foley 2001; Steinbauer and Matsuki 2004). In the present study the foliar wax abundance of the even carbon length benzyl alkanoates, and in particular benzyl n-tetracosanoate, were of foremost interest as increased abundance of these compounds have been associated with reduced *E. globulus* defoliation by *M. privata* larvae (Jones *et al*. 2002; Rapley *et al*. 2004b).

Materials and methods

Experimental site

A 2-year-old *E. globulus* plantation near the Russell River (42º54'S, 146º43'E) in southern Tasmania was selected as the experimental site. Site selection involved locating an *E. globulus* plantation in which trees were displaying homoblastic juvenile foliage and had no previous history of herbivory or current damage. In July 2002, 40 trees of similar height (ca. 3m) and form were selected and marked for experimental usage. No experimental trees were neighbours to each other, with experimental trees at least separated by a distance of 10m, the greatest distance between trees being approximately 300m. On each of these trees eight branches were marked in order to identify branches to which treatments were to be applied. This represented between 15 to 20% of branches present on trees.

Replicates and treatments

Field replicates were designed so that the four trees in each replicate had a similar abundance of benzyl n-tetracosanoate in their foliar wax prior to treatment application (see pre and post treatment foliage sampling). This was achieved by ranking the 40 experimental trees according to the abundance of benzyl n-tetracosanoate present in their foliar waxes and then allocating field replicates to the highest ranked four trees sequentially down to the lowest ranked four trees. One month (7^{th}-9^{th} of August) later experimental treatments were randomly allocated to each of the four trees within a replicate. The four treatments used, to control for each other, were (i) uncaged branches (control); (ii) caged branches (cage control); (iii) caged branches, plus artificial defoliation of branches within cages and (iv) caged branches, plus *M. privata* larval defoliation of branches within cages. Each cage enclosed the six expanded leaf pairs located directly behind the second expanded leaf pair (sampled leaf pair) back from the terminal bud. Cages were tubular sleeves (60×20cm) made from white mesh cloth, which were then tied at both ends on to branches to enclose the desired leaves and larvae. For the artificial defoliation treatment, three quarters of each leaf to be caged was removed by hand ripping. Larval defoliation was achieved by releasing 40 second and third instar larvae in to each cage at the time of securing the cage on to the branch.

Foliage sampling pre and post treatments

Approximately 2 weeks (24^{th} of July) prior to the commencement of the experiment foliage samples were collected from each tree. Foliage samples were comprised of eight leaves, with one leaf removed from each marked branch. Each leaf sampled was randomly chosen from the second expanded leaf pair back from the terminal bud and removed from the branch by cutting the petiole with scissors. All eight leaves from each tree were pooled in a single sample bag, which was transported to the laboratory in a car fridge. In the laboratory, the eight leaves in each foliage sample were cut into 1.0 to 1.5cm^2 squares to provide approximately 8 grams of cut foliage, with care taken to remove the midrib of the leaf, as well as any necrotic material. Cut foliage of each sample was placed in a sealed plastic container, which was shaken to randomise the sample. The foliage samples were then split by weight for use in the extractions of foliar wax compounds (2g), essential oils (2g) and polyphenolic compound groups (1g).

Approximately 3 months after the application of treatments to trees (4^{th} of November), at the cessation of larval feeding, cages, larvae and foliage samples were removed from marked branches of each tree. Foliage samples at this time were obtained by cutting the remaining leaf from the leaf pair previously sampled from each of the eight marked branches. The number of surviving larvae and the percentage of branch defoliation caused by larval feeding were also recorded. Approximately 8 months after cessation of larval feeding (24^{th} of July), to coincide with the time of year in which *M. privata* oviposition occurs in southern Tasmania, foliage samples were again removed from marked branches of each tree. Foliage samples at this time were obtained by randomly cutting a leaf from the leaf pair located at the next node out from the node formerly used for the first two foliage samples. However, as leaves sampled at this time were smaller then previously obtained there was not enough foliage mass to quantify essential oils. Foliage samples that were removed from trees after 3 months of larval feeding as well as 8 months later were prepared for chemical analysis as previously described.

Extraction of foliar waxes, essential oils and polyphenolics

Foliar waxes were obtained by saturating the cut foliage sample (2g) in a 10ml aliquot of high performance liquid chromatography (HPLC) grade hexane containing the internal standard n-tetradecane (0.1mL of n-tetradecane in 1L of hexane) for 50 minutes. The extraction method used was developed by Li *et al*. (1997) and modified by Jones *et al*. (2002) for small foliage samples. The wax extracts were then analysed using gas chromatography and mass spectrometry (GC-MS) with instrument hardware and settings the same as used by Jones *et al*. (2002). The compounds were identified by both their relative retention time and full mass spectra. The relative abundance of each wax compound was then expressed as the ratio of the area of a diagnostic ion to the total ion current area of the internal standard. The use of diagnostic ion areas rather than total ion current areas enables more precise determinations in otherwise overlapping GC peaks. A standardised measure of the wax yield was also obtained from the mass spectra by calculating the total ion current for the complete profile and correcting this value by subtracting the total ion current of the internal standard.

Essential oils were obtained by saturating the cut foliage sample (2g) in a 10ml aliquot of HPLC grade dichloromethane containing the internal standard heptadecane (100mg of heptadecane in 1L of dichloromethane) for 1 hour. Essential oil extracts were analysed by combined GC-MS on a Hewlett-Packard 5890 Gas Chromatograph coupled to a Hewlett-Packard 5970B Mass Selective Detector using a 25m×0.32mm HP-1 column with a 0.17 micron film. The interpretation of mass spectra was the same as previously described for foliar wax compounds.

Polyphenolic compounds were obtained by adapting a methodology developed by Close *et al*. (2001). Using scissors the foliage sample (1g) was finely cut before being homogenised with a 20 ml aliquot of acidified methanol in a tissue homogeniser. Samples were then immersed in a water bath at 90°C for 1.5 minutes and left to extract in the dark at 5°C for 24 hours. Extracts were centrifuged at 2500rpm for 7 minutes and from the resultant supernatant a small volume was pipetted in to an HPLC vial. Polyphenolic extracts were analysed within 24 hours of being obtained by HPLC-UV with instrument & column the same as used by Close *et al*. (2001). The mobile phases were A: 2% acetic acid in methanol, B: 2% acetic acid in water, C: hexane. Flow rate was 0.8mL/min, and the gradient was 15:85 A:B to 100% A at 6 minutes, to 80:20 A:C at 7 minutes and this was held till 12 minutes. Re-equilibration was 1 minute at 100%, A then programmed to 15:85 A:B at 14 minutes, with 3 minutes hold before the next injection. Measurement of tannins was made at 280nm for all peaks prior to 7 minutes retention time, and FPCs were measured at 280nm as all peaks from 7 to 11 minutes (based on LC-MS data on previous Eucalypt samples). Flavonoids were measured at 370nm as all peaks between 4.5 and 7 minutes. A small correction to the tannin measurement was made based on 80% of the absorbance of flavonoids at 370nm, since these fell within the same time window.

Data analysis

The abundance of each chemical compound or group assayed was log transformed following Jones *et al*. (2002). To test for treatment effects on the abundance of each compound a one-way ANOVA using the PROC GLM procedure of SAS (SAS Institute, 1999) was undertaken on the difference between pre-treatment and post-treatment log transformed abundance measures. Tree rank on the level of benzyl n-tetracosanoate (see replicates and treatments) was included in the model as a covariate along with the fixed treatment effect. The significance of treatment effects was tested for each chemical compound or group assayed, totals of even carbon length phenylethyl and benzyl alkanoates ($C^{20\text{-}26}$) as well as wax and oil yield, both after 3 months of larval feeding (test for a rapid induced response) and 8 months later (test for a delayed induced response). Probability values for treatment effects were Bonferroni adjusted for multiple comparisons between each chemical compound or group within each secondary metabolite class (i.e. foliar wax compounds, polyphenolic groups and essential oils) before a significant response was declared.

To allow for the possibility of interactive effects between individual chemical compounds or groups, a multivariate approach was also undertaken. We therefore tested for significant treatment effects of various classes of secondary metabolites using a discriminant analysis. This analysis was again based on the change in log transformed abundance of the various chemical compounds or compound groups within each secondary metabolite class and was achieved using PROC DISCRIM procedure of SAS (SAS Institute 1999). The discriminant analyses were undertaken both for abundance changes after 3 months of larval feeding and 8 months later.

For the 10 trees subjected to larval defoliation the PROC CORR procedure of SAS (SAS Institute 1999) was used to calculate Pearson's correlation coefficients of larval survival with the abundance of each chemical compound or group, both prior to caging and after 3 months of larval feeding. Further, Pearson's correlations of the foliar tannin abundances with average branch defoliation were also calculated. Finally, we investigated the stability of the variation between trees of each compound or group across time for a 3 month (pre-treatment until cessation of larval feeding), 8 month (cessation of larval feeding until final sample) and 11 month (pre-treatment until final sample) duration. This was achieved by calculating Pearson's correlation coefficients between all sampling periods for chemical abundance. Probability values calculated from all correlations were Bonferroni adjusted for multiple comparisons as described previously.

Results

Across the three classes of secondary metabolites investigated, no individual compound or group showed a significantly different change in abundance between the four treatments after Bonferroni adjustment, either after 3 months of larval feeding or 8 months later (Table 1). As no significant treatment effects were found we investigated the stability of the variation between trees over the 11 month period for each chemical compound or group. The strong correlation between the levels of the chemicals in each tree across the duration of the experiment (Table 1) indicated a strong stability of the *E. globulus* chemical profile. It was only the levels of the two wax ketones, heptadecan-2-one and n-pentatriacontan-16, 18-dione, and three monoter-

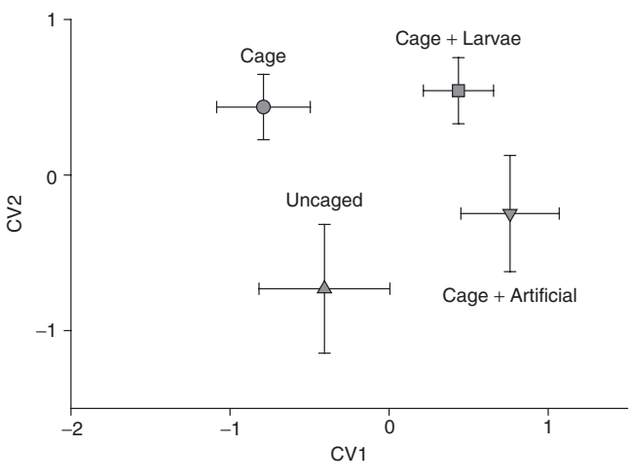

Fig. 1 The ordination of defoliation and control treatments (mean ± SE) on the first (CV1, 56%) and second (CV2, 39%) axes derived from discriminant analysis of changes in the three polyphenolic groups assayed (tannins, flavonoids and FPCs).

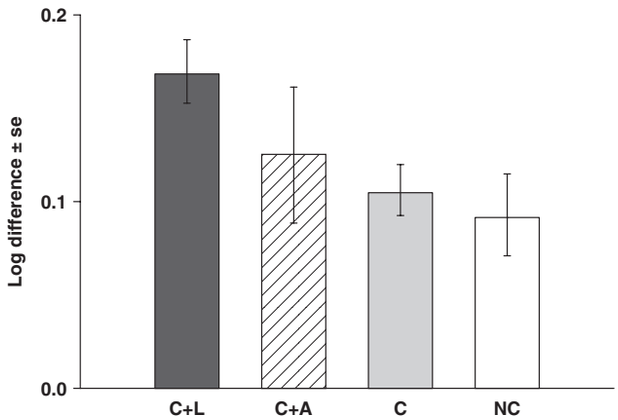

Fig. 2 Mean (± SE) increase in foliar tannins at the three months sampling period, calculated by subtracting the log abundance at the time of sampling from the log abundance prior to treatment application for the treatments: caging larvae on branches (C+L), caging branches following artificial damage (C+A), caging branches (C) and uncaged branches (NC).

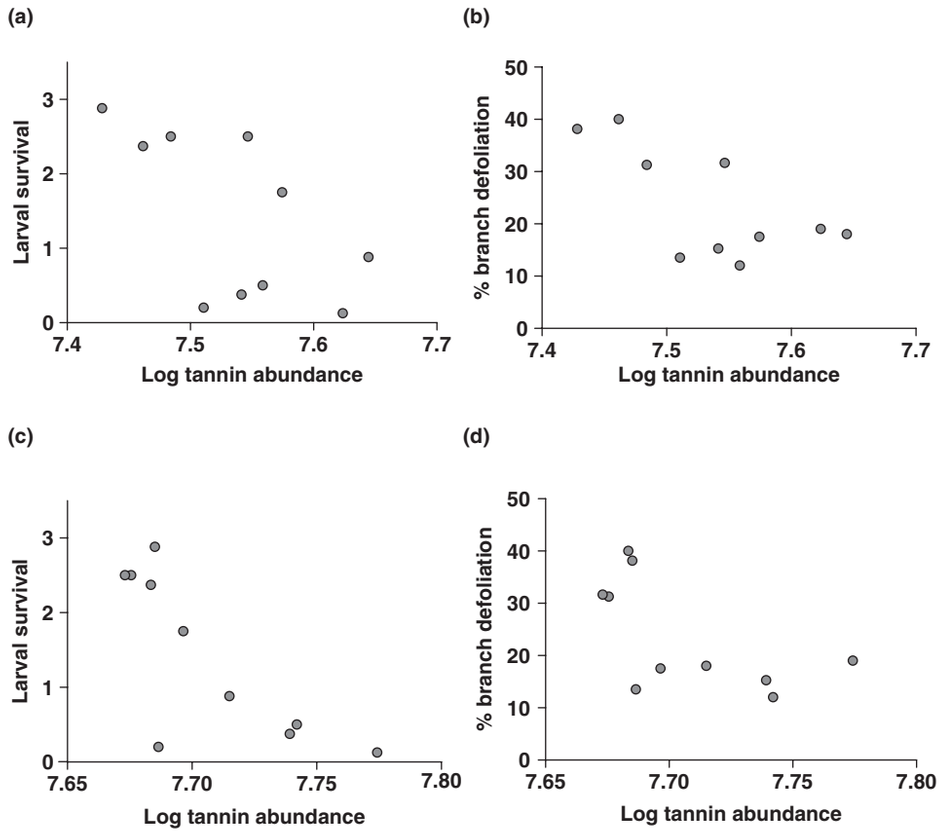

Fig. 3 The average survival of *M. privata* larvae and average percentage defoliation of caged branches for each of the 10 trees subjected to larval defoliation plotted against the foliar tannin abundance (logged). Plots are shown for tannin abundance prior to caging (a and b) and after 3 months of larval feeding (c and d).

penes, α-pinene, 1,8 cineole and limonene as well as oil yield, which changed between trees at one or other time interval (Table 1). Interestingly the abundance of wax alkanoates, including benzyl n-tetracosanoate, was the most highly correlated across time, indicating that the differences in these wax compounds between trees are extremely stable (Table 1).

The only suggestion of a treatment effect on the plant chemistry that we detected involved the polyphenolic compounds (FPCs, flavanoids and tannins), and this was only significant at the multivariate level. A difference between the three caged treatments was evident in the discriminant analysis involving the three polyphenolic compound groups at the cessation of larval feeding (CV1 $P = 0.005$, CV2 $P = 0.030$; Fig. 1). At the cessation of larval feeding, both defoliation treatments were separated from both the uncaged and the caged controls along CV1 and this separation was associated with a trend for tannins to increase in the defoliation treatments after 3 months of larval feeding (Fig. 2). This trend was not evident 8 months later. At the cessation of *M. privata* larval feeding, high mortality was evident with the average number of larvae surviving on branches per tree being 1.41 ± 0.35 (range: 0.13 to 2.88) out of 40 and average defoliation of branches per tree was $26.3\% \pm 3.34$ (range: 10 to 40 %). At this time larval survival was significantly positively correlated to defoliation (Pearson's correlation, $n = 10$, $r = 0.89$, $P < 0.001$). Average survival of *M. privata* larvae on branches and average defoliation of branches per tree was also negatively correlated to foliar tannin abundance prior to caging (Pearson's correlation, $n = 10$, survival: $r = -0.63$, $P = 0.05$, defoliation: $r = -0.68$, $P = 0.03$) (Fig. 3a and b) and after 3 months of larval feeding (Pearson's correlation, $n = 10$, survival: $r = -0.77$, $P = 0.009$, defoliation: $r = -0.61$, $P = 0.06$) (Fig. 3c and d). Levels of the other foliar compounds assayed were not significantly correlated to survival prior to caging or after 3 months of larval feeding (Table 1).

Discussion

Jones *et al*. (2002) reported that variable *E. globulus* defoliation in a genetics trial was correlated with the variability of several aliphatic phenylethyl and benzyl alkanoates found in juvenile foliage waxes. For specific even carbon length benzyl alkanoates and in particular benzyl n-tetracosanoate this correlation was found to be robust in other germplasm and across different locations (Rapley *et al*. 2004b). However, as the quantification of foliar wax compounds of both studies was undertaken post tree defoliation, we could not be certain that the alkanoates were operating as a constitutive resistance mechanism rather then being induced as the result of larval defoliation. Until this study, the evidence that these foliar wax alkanoates were not induced was that their levels did not significantly differ between undamaged and damaged foliage of the same plant, or between undamaged and damaged ramets of the same genotype grown in a common field environment (Jones *et al*. 2002). In the current study

Table 1. F-values and their probability for rapid (3 months) and delayed (11 months) differences between the four experimental treatments for the change in abundance of foliar wax compounds, polyphenolic groups and essential oils. Also presented are the r-values and their probability for compound stability measured by calculating Pearson's correlation coefficients between chemical abundance prior to caging with levels 3 and 11 months later and between levels at 3 months and 11 months (listed as 8 month in table) for all trees (n = 40). Further r-values and their probability were calculated for the Pearson's correlations between the average larval survival (n = 10) and the chemical abundance prior to caging (0 months) and immediately after the cessation of larval feeding (3 months).

	Treatment (F / Prob.)		Stability (r / Prob.)			Larval survival (r / Prob.)	
Wax Compounds	**3 months**	**11 months**	**3 months**	**8 months**	**11 months**	**0 months**	**3 months**
Aliphatic alkanoates							
Benzyl n-eicosanoate (C_{20})	2.63 ns	0.22 ns	0.90 ***	0.60 ***	0.56 ***	–0.03 ns	0.04 ns
Benzyl n-heneicosanoate (C_{21})	2.16 ns	–	0.62 ***	–	–	0.45 ns	0.43 ns
Benzyl n-docosanoate (C_{22})	3.98 *[a]	1.20 ns	0.90 ***	0.67 ***	0.71 ***	–0.29 ns	–0.10 ns
Benzyl n-tetracosanoate (C_{24})	0.28 ns	0.75 ns	0.92 ***	0.86 ***	0.92 ***	–0.60 ns	–0.58 ns
Benzyl n-hexacosanoate (C_{26})	2.09 ns	0.60 ns	0.79 ***	0.76 ***	0.82 ***	–0.49 ns	–0.38 ns
Benzyl n-octacosanoate (C_{28})	0.31 ns	0.35 ns	0.76 ***	0.58 ***	0.54 ***	–0.10 ns	–0.31 ns
Benzyl alkanoates ($C_{20\text{-}26}$)	2.26 ns	0.73 ns	0.93 ***	0.77 ***	0.79 ***	–0.55 ns	–0.44 ns
Phenylethyl n-eicosanoate (C_{20})	1.21 ns	1.31 ns	0.90 ***	0.85 ***	0.84 ***	0.57 ns	0.60 ns
Phenylethyl n-heneicosanoate (C_{21})	1.95 ns	–	0.85 ***	–	–	0.53 ns	0.46 ns
Phenylethyl n-docosanoate (C_{22})	1.14 ns	0.64 ns	0.86 ***	0.82 ***	0.78 ***	0.54 ns	0.62 ns
Phenylethyl n-tetracosanoate (C_{24})	0.43 ns	0.73 ns	0.88 ***	0.77 ***	0.84 ***	0.31 ns	0.37 ns
Phenylethyl n-hexacosanoate (C_{26})	0.56 ns	0.30 ns	0.84 ***	0.76 ***	0.79 ***	–0.21 ns	–0.15 ns
Phenylethyl n-octacosanoate (C_{28})	0.29 ns	0.73 ns	0.90 ***	0.69 ***	0.73 ***	0.38 ns	0.31 ns
Phenylethyl alkanoates ($C_{20\text{-}26}$)	1.09 ns	0.80 ns	0.90 ***	0.86 ***	0.83 ***	0.55 ns	0.60 ns
Aliphatic β-diketones							
n-Hentriacontane-14, 16-dione (C_{31})	0.97 ns	2.07 ns	0.84 ***	0.62 ***	0.60 ***	0.53 ns	0.51 ns
n-Tritriacontane-16, 18-dione (C_{33})	0.53 ns	1.71 ns	0.76 ***	0.60 ***	0.51 ***	0.30 ns	0.25 ns
n-Pentatriacontane-16, 18-dione (C_{35})	1.16 ns	3.26 *[a]	0.34 *[a]	0.52 ***	0.25 ns	0.03 ns	–0.35 ns
Flavonoids							
Desmethyl eucalyptin	1.28 ns	1.70 ns	0.47 **[a]	0.71 ***	0.56 ***	–0.30 ns	–0.39 ns
Eucalyptin	0.55 ns	1.14 ns	0.55 ***	0.59 ***	0.51 ***	–0.06 ns	0.08 ns
Ketones							
Heptadecan-2-one	0.07 ns	2.69 ns	0.64 ***	0.19 ns	0.24 ns	0.01 ns	0.25 ns
Total Ion Current							
Wax yield	0.67 ns	0.69 ns	0.76 ***	0.65 ***	0.63 ***	0.15 ns	0.17 ns
Polyphenolic Compounds							
FPC's	1.98 ns	3.16 *[b]	0.77 ***	0.76 ***	0.55 ***	–0.37 ns	–0.32 ns
Flavonoids	0.47 ns	1.73 ns	0.59 ***	0.62 ***	0.45 **	–0.35 ns	0.05 ns
Tannins	1.93 ns	0.85 ns	0.69 ***	0.49 **	0.49 **	–0.63 *[b]	–0.77 **
Essential oils							
Sesquiterpenes							
α-gurjunene	0.30 ns	–	0.76 ***	–	–	0.00 ns	–0.22 ns
Eudesmol acetate	3.00 *[c]	–	0.72 ***	–	–	–0.19 ns	0.24 ns
Bicyclogermacrene	0.78 ns	–	0.68 ***	–	–	0.37 ns	0.38 ns
Viridiflorol	0.61 ns	–	0.71 ***	–	–	0.00 ns	0.39 ns
Aromadendrene	0.66 ns	–	0.63 ***	–	–	0.27 ns	0.39 ns
Alloamroadendrene	0.37 ns	–	0.70 ***	–	–	0.03 ns	0.41 ns

	Treatment (F / Prob.)		Stability (r / Prob.)			Larval survival (r / Prob.)	
Wax Compounds	**3 months**	**11 months**	**3 months**	**8 months**	**11 months**	**0 months**	**3 months**
Globulol	0.41 ns	–	0.70 ***	–	–	–0.03 ns	0.39 ns
β-eudesmol	1.60 ns	–	0.77 ***	–	–	0.16 ns	0.19 ns
α-eudesmol	1.57 ns	–	0.74 ***	–	–	0.15 ns	0.17 ns
Monoterpenes							
α-pinene	1.22 ns	–	0.43 **[c]	–	–	0.33 ns	0.31 ns
1, 8 cineole	0.39 ns	–	0.41 *[c]	–	–	0.11 ns	–0.18 ns
α-terpineol	0.68 ns	–	0.77 ***	–	–	–0.01 ns	–0.39 ns
Terpinyl acetate	1.21 ns	–	0.76 ***	–	–	0.33 ns	0.33 ns
Limonene	0.41 ns	–	0.30 ns	–	–	0.16 ns	–0.17 ns
Total Ion Current							
Oil yield	0.62 ns	–	0.36 *[c]	–	–	0.16 ns	0.06 ns

[a]Compounds not significant after Bonferroni's adjustment for multiple comparisons between wax compounds (P > 0.002 = ns)
[b]Compounds not significant after Bonferroni's adjustment for multiple comparisons between polyphenolic groups (P > 0.017 = ns)
[c]Compounds not significant after Bonferroni's adjustment for multiple comparisons between essential oils (P > 0.003 = ns)
– Compound not sampled 8 months after cessation of larval feeding

we found no empirical evidence indicating branch defoliation induced any tree level changes in the abundance of any foliar wax alkanoates including benzyl n-tetracosanoate. It therefore appears these alkanoates are operating as a constitutive plant defence. Interestingly the phenotypic differences in the abundance of benzyl n-tetracosanoate between trees was extremely stable over time and a large proportion of these differences are thought to be under strong genetic control as Jones *et al*. (2002) reported a broad-sense heritability of 0.82 for benzyl n-tetracosanoate. Rapley *et al*. (2004a) has found that *E. globulus* trees most resistant to *M. privata* defoliation are least preferred for oviposition of gravid female moths. It has therefore been hypothesised by Jones *et al*. (2002) and Rapley *et al*. (2004b) that benzyl n-tetracosanoate may be antixenotic in its function, acting as a deterrent to *M. privata* oviposition.

Significantly the present study found evidence that foliar tannins were operating as a chemical defence in *E. globulus* against larval feeding. Trees with increased foliar tannins prior to treatment application recorded the greatest larval mortality and the correlation after 3 months of larval feeding was strengthened by what appears to be the inducement of tannins. Condensed and hydrolysable tannins are both powerful precipitants of protein forming indigestible complexes that lower the efficiency of conversion of food to animal biomass and elevate the amount of nitrogen excreted (Reese 1979). Tikkanen and Julkunen-Tiitto (2003) found the level of condensed tannins in oak foliage was negatively correlated to the growth and survival of *Operophtera brumata* larvae, while Kopper *et al*. (2002) found condensed tannins in paper birch increased stadium duration, decreased relative growth rates and decreased food conversion efficiencies of *Orgyia leucostigma* larvae. Interestingly, however, Ayres *et al*. (1997) demonstrated that condensed tannins are specific in antiherbivore activity, recording variable survival or development when six species of lepidopteran larvae were reared on condensed tannins from 16 woody plant species depending on the specific species combinations (insect and woody plant) used. The positive correlation between larval survival and branch defoliation indicates that larval mortality must have happened quite soon after larvae were introduced to experimental trees, so that low mortality could lead to high foliar damage and vice versa. This suggests that foliar tannins or another unidentified compound was an important determinant for mortality early in larval feeding.

After 3 months of larval feeding, there was a trend for a greater abundance of tannins in tree defoliation treatments compared to control treatments. Similarly, Schultz and Baldwin (1982) and Rossiter *et al*. (1988) both reported increased tannin levels in oak foliage following defoliation by *L. dispar* larval feeding and induced increases in wood tannins have also been reported following artificial wounding and fungal infection in *Eucalyptus globulus* and *Eucalyptus nitens* (Barry *et al*. 2001; Eyles *et al*. 2003b). The trend for increased foliar tannins in tree defoliation treatments was not evident in foliage sampled 8 months later suggesting that foliar tannin induction in *E. globulus* is a rapidly induced response affecting feeding larvae rather than a lasting delayed induced response affecting future generations of larvae (Haukioja and Neuvonen 1987; Stevens and Lindroth 2005). Furthermore, increased levels of foliar tannins have also been hypothesised to be a response to photodamage (Close and McArthur 2002), but in our experiments the plantation studied did not have a closed canopy and the position of the defoliation would not have altered light penetration levels to the sampled leaves.

Excluding one possible exception (foliar tannins), over the time-frame studied, we found no evidence for Edwards and Wanjura's (1989) hypothesis for the existence of induced chemical resistance to herbivores within eucalypts. This includes the even carbon length benzyl alkanoates identified by Jones *et al*. (2002) and Rapley *et al*. (2004b) to be associated with reduced *E. globulus* defoliation by *M. privata*. It is possible, that the trees with the damage treatments might have induced a defensive response in the control trees so that no chemical differences were detectable. This phenomenon is known as inter-plant communication and occurs when a plant damaged by herbivory elicits a defensive response in a neighbouring conspecific. This type of communication has been demonstrated over shorter distances than used in our experiment, in poplars and maples in the laboratory (Baldwin and Scultz 1983) and alders in the field (Dolch and Tscharntke 2000, Tscharntke *et al*. 2001), but is highly unlikely in the present study due to the greater distances between damaged and undamaged trees. Although again unlikely, it may be that in establishing baseline levels of secondary metabolites in experimental trees we induced a defensive response in all trees prior to defoliation. However, we only removed a very small fraction of the leaves on a tree (eight leaves) for baseline chemical analysis, and all by a clean abscission at the petiole. Therefore, we cannot completely reject the idea of induced resistance in *E. globulus*. Indeed the trend for increased foliar tannins in defoliation treatments and the evidence indicating their abundance are a determinant of larval survival lead us to suspect that with increased tree defoliation or sampling foliage during larval feeding or more specific compound sampling (eg. analysing levels of hydrolysable and condensed tannins separately) a compound induced by herbivory may be identified. Also, future studies should incorporate primary metabolites as the nutritive status of the leaves may influence the effects of defensive secondary compounds on insect feeding behaviour (Haukioja 2003). It is feasible though, that in young *E. globulus* trees displaying only juvenile foliage induced responses during and/or post herbivory are non-existent as carbon allocation remains principally dedicated to plant metabolites essential for the continuation of rapid growth and constitutive resistance mechanisms, such as the foliar wax alkanoate benzyl n-tetracosanoate.

Acknowledgements

The field assistance Vinu Patel is gratefully acknowledged. Forestry Tasmania and in particular Craig Wilson is thanked for identifying and allowing access to an appropriate *E. globulus* plantation. We thank Dr. Mamoru Matsuki, Dr. Martin Steinbauer and Dr. David Ratkowsky for advice and comments. The study was funded by University of Tasmania, CRC for Sustainable Production Forestry and Helsham.

References

Agrawal AA (2000) Benefits and costs of induced plant defence for *Lepidium virginicum* (Brassicaceae). Ecology 81: 1804–1813

Agrawal AA, Karban R (1999) Why induced defenses may be favoured over constitutive strategies in plants. Pp 45–61in Tollrian R, Harvell CD (eds) The Ecology and Evolution of Inducible Defenses.

Ayres MP, Clausen TP, MacLean Jr SF, Redman AM, Reichardt PB (1997) Diversity of structure and antiherbivore activity in condensed tannins. Ecology 76: 1696–1712

Baldwin IT, Schultz JC (1983) Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plants. Science 221: 277–279

Barry KM, Davies NW, Mohammed C (2001) Identification of hydrolysable tannins in the reaction zone of *Eucalyptus nitens* wood by high performance liquid chromatography-electrospray ionisation mass spectrometry. Phytochemical Analysis 12: 120–127

Clausen TP, Reichardt PB, Bryant JP, Werner RA (1991) Long-term and short-term induction in quaking aspen: related phenomena? Pp 71–83 in Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. John Wiley and Sons, New York.

Close DC, McArthur C (2002) Rethinking the role of many plant phenolics - protection from photodamage not herbivores? Oikos 99: 166–172

Close DC, Davies NW, Beadle CL (2001) Temporal variation of tannins (galloylglucoses), flavonols and anthocyanins in leaves of *Eucalyptus nitens* seedlings: implications for light attenuation and antioxidant activities. Aust. J. Plant Physiol. 28: 269–278

Edwards PB (1982) Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? Aust. J. Ecol. 7: 347–352

Edwards PB, Wanjura WJ (1989) Eucalypt-feeding insects bite off more than they can chew: sabotage of induced defences? Oikos 54: 246–248

Elliott HJ, Bashford R (1978) The life history of *Mnesampela privata* (Guen.) (Lepidoptera: Geometridae) a defoliator of young eucalypts. J. Aust. Entomol. Soc. 17: 201–204

Eyles A, Davies NW, Yuan ZQ, Mohammed C (2003a) Host responses to natural infection by *Cytonaema* sp. in the aerial bark of *Eucalyptus globulus*. Forest Pathology 33 (5): 317–331

Eyles A, Davies NW, Mohammed C (2003b) Wound wood formation in *Eucalyptus globulus* and *Eucalyptus nitens*: anatomy and chemistry. Can. J. For. Res. 33 (12): 2331–2339

Dolch R, Tscharntke T (2000) Defoliation of alders (*Alnus glutinosa*) affects herbivory by leaf beetles on undamaged neighbours. Oecologia 125: 504–511

Farrow RA, Floyd RB, Neumann FG (1994) Inter-provenance variation in resistance of *Eucalyptus globulus* juvenile foliage to insect feeding. Aust. For. 57: 65–68

Floyd RB, Foley WJ (2001) Identifying pest resistant eucalypts using near-infared spectroscopy. Rural Industries Research and Development Corporation, Canberra.

Hartley SE, Lawton JH (1991) Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. Pp 105–132 in Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. John Wiley and Sons, New York.

Haukioja E (2005) Plant defenses and population fluctuations of forest defoliators: mechanism-based scenarios. Ann. Zool. Fennici 42: 313–325

Haukioja E (2003) Putting the insect into the birch-insect interaction. Oecologia 136: 161–168

Haukioja E, Hanhimäki S, Walter GH (1994) Can we learn about herbivory on eucalypts from research on birches, or how general are general plant-herbivore theories? Aust. J. Ecol. 19: 1–9

Haukioja E, Neuvonen S (1987) Insect population dynamics and induction of plant resistance: the testing of hypotheses. Pp 411–432 in Barbosa P, Schultz JC (eds) Insect Outbreaks. Academic Press, San Diego.

Heil M (2002) Ecological costs of induced resistance. Current Opinion in Plant Biology 5: 1–6

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q. Rev. Biol. 67: 283–335

Jones TH, Potts BM, Vaillancourt RE, Davies NW (2002) Genetic resistance of *Eucalyptus globulus* to autumn gum moth defoliation and the role of cuticular waxes. Can. J. For. Res. 32: 1961–1969

Kaitaniemi P, Ruohomäki K, Ossipov V, Haukioja E, Pihlaja K (1998) Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. Oecologia 116: 182–190

Karban R, Agrawal AA, Mangel M (1997) The benefits of induced defenses against herbivores. Ecology 78: 1351–1355

Klemola T, Hanhimäki S, Ruohomäki K, Senn J, Tanhuanpää M, Kaitaniemi P, Ranta H, Haukioja E (2003) Performance of the cyclic autumnal moth, *Epirrita autumnata*, in relation to birch mast seeding, Oecologia 135: 354–361

Kopper BJ, Jakobi VN, Osier TL, Lindroth RL (2002) Effects of paper birch condensed tannin on whitemarked tussock moth (Lepodoptera: Lymantriidae) performance. Physiological and Chemical Ecology 31: 10–14

Larsson S, Ohmart CP (1988) Leaf age and larval performance of the leaf beetle *Paropsis atomaria*. Ecol. Entomol. 13: 19–24

Li H (1993) Phytochemistry of *Eucalyptus* spp. and its role in insect host tree selection. PhD thesis, School of Plant Science, University of Tasmania, Hobart, p 475.

Li H, Madden JL, Potts BM (1997) Variation in leaf waxes of the Tasmanian *Eucalyptus* species - I. Subgenus *Symphyomyrtus*. Biochem. Syst. Ecol. 25: 631–657

Lukacs Z (1999) Phenology of autumn gum moth *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae). PhD thesis, School of Agricultural Science, University of Tasmania, Hobart, p 252.

Neumann FG, Collett NG (1997) Insecticide trials for control of the autumn gum moth (*Mnesampela privata*), a primary defoliator in commercial eucalypt plantations prior to canopy closure. Aust. For. 60: 130–137

Raffa KF (1991) Induced defensive reactions in conifer-bark beetle systems. Pp 246–276 in Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. John Wiley and Sons, New York.

Rapley L, Allen GR, Potts BM (2004a) Oviposition by autumn gum moth (*Mnesampela privata*) in relation to *Eucalyptus globulus* defoliation, larval performance and natural enemies. Agric. For. Entomol. 6: 205–213

Rapley L, Allen GR, Potts BM (2004b) Susceptibility of *Eucalyptus globulus* to *Mnesampela privata* defoliation in relation to a specific foliar wax compound. Chemoecology. 14: 157–163

Reese JC (1979) Interactions of allelochemicals with nutrients in herbivore food. Pp 309–326 in Rosenthal GA, Janzen DH (eds) Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Rossiter M, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology 69: 267–277

Schultz JC, Baldwin IT (1982) Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217: 149–151

SAS Institute Inc. (1999) Proprietary Software Version 8. SAS Institute, Cary, NC, USA

Steinbauer MJ, Matsuki M (2004) Suitability of *Eucalyptus* and *Corymbia* for *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae) larvae. Agric. For. Entomol. 6: 323–332

Stevens MT, Lindroth RL (2005) Induced resistance in the intermediate growth of aspen (*Populus tremuloides*). Oecologia 145: 298–306

Tshcharntke T, Thiessen S, Dolch R, Boland, W (2001) Herbivory, induced resistance, and interplant signal transfer in *Alnus glutinosa*. Biochem. Syst. Ecol. 29 (10): 1025–1047

Tikkanen O, Julkunen-Tiitto R (2003) Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*. Oecologia 136: 244–251

Zangerl AR, Bazzaz FA (1992) Theory and pattern in plant defence allocation. Pp 363–391 in Fritz RS, Simms EL (eds) Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics. The University of Chicago Press, Chicago.

Received 18 January 2007; accepted 17 July 2007.
Published Online First 17 September 2007.