Forest Ecology and Management xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets

Ian R. Wallis[a,*], Helen J. Smith[b], Martin L. Henery[a,c], Michael Henson[b], William J. Foley[a]

[a] Ecology, Evolution and Genetics, Research School of Biology, Australian National University, Canberra, ACT 0200, Australia
[b] Grafton Forest Technology Centre, Forests NSW, Grafton, NSW 2460, Australia
[c] CSIRO Entomology, Black Mountain Laboratories, GPO Box 1700, Canberra ACT 2601, Australia

ARTICLE INFO

Article history:
Received 5 March 2010
Received in revised form 19 May 2010
Accepted 24 May 2010

Keywords:
Defence
Eucalypt
Clone
Herbivory
FPC

ABSTRACT

The tendency for managers of eucalypt plantations to plant large, homogeneous (i.e. clonal) stands presents a new suite of problems. Perhaps foremost among them is the elevated risk of disease or predation. One way to counter this risk is to select material with high natal resistance, such as material with high concentrations of plant secondary metabolites. This would be much simpler if we could predict future defences from the chemistry of juvenile plants. The present study aimed to determine the relationship between the concentrations of formylated phloroglucinol compounds (FPCs) in the leaves of newly established *Eucalyptus grandis* (Hill ex Maiden) clones in the nursery and those in the same genetic material in experimental plantations. There was almost no relationship between the concentrations of defensive chemicals in the leaves of newly established *E. grandis* clones growing in pots in the nursery and of those in the same genetic material growing in plantations. This implies that age effects and probable gene × environment interactions prevent evaluating the defensive qualities of clones until they are several years old and even then the results are site-specific.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Producers of plantation timber typically aim to develop homogeneous stands with a very high productivity, natural resistance to pests and diseases and uniform raw material for their production processes. The selection of favourable wood traits along with improved silviculture has contributed to massive increases in productivity. The results are dramatic. For example, in Brazil—one of the largest producers of eucalypt products, the yield has risen from 12 to 40 $m^3\ ha^{-1}\ year^{-1}$ in 30 years (Campinhos, 1999). Perhaps the greatest advance was the development, in the 1970s, of asexual propagation (cloning) that found commercial use for the first time in Brazil in 1979 (Eldridge et al., 1994). This has radically changed the industry from the 1960s and 1970s when sowing of seed was the only method used to propagate *Eucalyptus* plants, to now, when most new eucalypt plantations in Brazil are monoclonal plantings (Stape et al., 2001).

The turn to large, homogeneous stands, however, presents a new suite of problems. Perhaps foremost among them is the risk of disease or predation causing colossal losses due to the genetic similarity of the planted material (Wingfield et al., 2001) and the interaction between animal species richness and plant species richness (Wright and Samways, 1998). Even outside Australia, particularly in Brazil and South Africa, native insects, predominantly Lepidoptera and Coleoptera, now cause problems (Zanuncio et al., 1994, 1998, 2001; Guedes et al., 2000). There is consensus that widespread chemical control is too costly, damages the environment and invokes rapid resistance in the target pests. But, there seems little agreement on the best approach for managing pests and diseases. One school of thought believes that DNA based technologies hold all of the answers (e.g., Wingfield et al., 2001). In contrast, one of the best control methods seems to be biological: in Brazil mixing native vegetation with the eucalypts favours the maintenance of local bird populations that help to control the insect population (Campinhos, 1999). In Australia pests pose a greater challenge to eucalypt plantations, simply because there are far more herbivorous species capable of doing damage. Accordingly, plantation managers in Australia will likely benefit from planting material that deters herbivores, including that which confers chemical protection.

Abbreviations: FPCs, formylated phloroglucinol compounds; DM, dry matter; s, standard deviation of the mean; CV, coefficient of variation; df, degrees of freedom.

* Corresponding author at: Research School of Biology, Australian National University, Building 116, Daley Road ANU, Acton, ACT 0200, Australia.
Tel.: +61 2 6125 2058; fax: +61 2 6125 5573.
E-mail addresses: ian.wallis@anu.edu.au (I.R. Wallis), helens@sf.nsw.gov.au (H.J. Smith), Martin.Henery@csiro.au (M.L. Henery), MichaelHe@sf.nsw.gov.au (M. Henson), william.foley@anu.edu.au (W.J. Foley).

0378-1127/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.foreco.2010.05.034

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

This poses two problems. First, there may be a trade-off between growth rates and resistance to herbivory, such that the most productive genotypes are those most likely to suffer damage from herbivores (Coley et al., 1985; Stone, 2001). In this case, the likelihood of attack by herbivores should influence the choice of planted material. The second problem is to understand the interaction between the genetic and environmental components of chemical resistance. Researchers have searched extensively for feeding deterrents in eucalypts. However, the only compounds eucalypts produce that deter feeding by mammalian and some invertebrate herbivores in controlled feeding studies using eucalypt leaves as well as isolated compounds are the formylated phloroglucinol compounds (FPCs) (Moore et al., 2004a). Unfortunately, the extent of their role in resistance is unknown because there are many individual compounds within several FPC classes – the simple FPCs, macrocarpals, sideroxylonals, and euglobals – making it difficult to assess their individual importance (Eschler et al., 2000). That said, increasing concentrations of the simple FPCs (e.g. jensenone), various macrocarpals and the sideroxylonals all depress food intake by ringtail and brushtail possums and koalas (Lawler et al., 1998; Wallis et al., 2002; Moore et al., 2004a). Likewise, the sideroxylonals have a similar effect on scarab beetles (Andrew et al., 2007). The effect of euglobals on feeding is unknown, but they do show wide-ranging biological activity (Konoshima and Takasaki, 2002). As expected from traits under constant selection pressure, the concentrations of the FPCs have significant heritability (Andrew et al., 2005). If high concentrations of defensive compounds confer resistance to pests it would be useful to be able to select material for planting based on chemical markers at the seedling stage, thus eliminating the need for long-term trial plantations. We have no understanding, however, of the relationship between the concentrations of chemical compounds in the foliage of young plants, growing under ideal conditions in a nursery, and the concentrations in the same genetic material growing in a plantation. The advent of clonal material allows us to determine this more easily than we could with conventional seedlings, because there is no genetic variation between the clonal plant in the nursery and that in the plantation. The present study had one main aim: to determine the relationship between the concentrations of FPCs in the leaves of newly established *Eucalyptus grandis* (Hill ex Maiden) clones in the nursery and those in the same genetic material planted in experimental plantations. A secondary aim was to examine the relationship between the concentrations of the various FPCs in *E. grandis*, with a view to understanding how the selection for one might influence the concentrations of others. This information is necessary to assess the prospects of clonal selection for enhancing the chemical defences of *E. grandis*.

2. Materials and methods

2.1. Site descriptions

Foliage of *E. grandis* (and from two *E. grandis* × *E. urophylla* (S. T. Blake) and one *E. urophylla* × *E. grandis* hybrids), was collected over 2 days in winter in collaboration with employees of Forests NSW. Three sites were sampled in the vicinity of Coffs Harbour and Grafton, on the north coast of NSW, where clonal material is growing in randomised field trials, with replicates (blocks) within trials. The trees were 18–30 months old. We collected 28 samples at the first site, about 25 km northwest of Coffs Harbour, where the trees were 2–3 m high and in good condition. The trees at the second site, 5 km west of Woolgoolga, were highly variable in both height (0.1–4 m) and in the foliar invertebrate damage they had sustained. Here we collected 37 samples. The third site is about 20 km northwest of Grafton where the trees were in excellent condition and were 3 m tall ($N = 16$). Finally, we collected as many samples as possible ($N = 42$) to match the plantation trees from newly established clonal cuttings (from potted hedge plants or in-ground archive hedges) maintained in pots under shadecloth at the Grafton Forest Technology Centre. These plants were approximately 4 months old. Chemical analyses of the samples gave us two sets of data. The first was all of the samples ($N = 134$) from which we established the chemical profile of the species and the relationship between the chemicals of interest. The second set of data was pairs of samples: those collected from established trees at various sites and the same clonal material growing in pots at the nursery. We could not pair all of the samples collected in plantations. The clones were not related so each of the 42 pairs represents an independent comparison of chemistry in a plantation versus that in a young nursery ramet.

2.2. Origin of clones

The clones used in the study originated from several sources. These were bulked family seedlots from Wedding Bells Seed Orchard, individual tree seedlots from the same orchard, plus trees selected in native forests and old plantations, and field selects in young (3–5-year-old) plantations. The clones were initially propagated by cuttings from seedlings or coppice, depending on their origin, between 1996 and 1999. Ramets of each clone were established as in-ground hedges in the Forests NSW Eucalypt Clonal Archive on the central coast of NSW during 1999–2001. Cuttings were set from these hedges to propagate plants for field trials and for potted hedge plants located at the Grafton Forest Technology Centre. The three hybrids came from material imported from Brazil.

Material was not collected from the parent trees from which the genotypes originated as many of the trees were not locatable or no longer existed, or for the Wedding Bells Seed Orchard bulked families, it was not clear which tree in the family had produced that particular seed. In the case of seedling-based clones, the parent trees were likely to have some different characteristics to the progeny due to outcrossing.

2.3. Plant material

The collection procedure involved locating the tree in the plantation and then stripping about 60 g of adult leaves from it. We did not assess herbivore damage. Also, there was no statistical benefit in sampling more than one tree of the same clone (i.e. ramets) in the trial. Instead, we collected all material from the same replicate of the trial and from the first vigorous tree in the plot. The leaves, collected from the same position in the canopy, were placed in paper bags in a portable freezer. They were kept frozen until freeze-drying and grinding (Tecator cyclone mill with a 1 mm screen) 2 weeks after collection. The size of the newly established clones limited the amount of foliage we could collect. In most instances, we took 6–10 small (ca 40 mm × 25 mm) juvenile leaves. These were frozen soon after collection and freeze-dried with the remaining samples 2 weeks later. The small amount of sample, however, precluded us grinding them in a cyclone mill so instead we used an SDI Ultramat 2 amalgam mixer (Henry Schein Regional, Rosebery NSW), which vibrates a small plastic capsule containing the sample (ca 200 mg) and a stainless steel rod or pestle (ca 10 mm × 2 mm). This vibrating mill reduces the sample to a fine powder in about 6 s. To ensure that we analysed a representative sample of the collected leaves, we stacked the leaves and cut them into fine strips, which we placed in the capsule. A comparison of leaves processed in the cyclone grinder or the capsule revealed no significant difference in the concentration of the compounds of interest.

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

2.4. Chemical analysis

We analysed the samples using the rapid extraction method developed for the FPC, sideroxylonal (Wallis and Foley, 2005), sonicating 100 mg of freeze-dried ground leaf in a known mass of diluent (ca 4.5 g of 7% MilliQ water in HPLC grade acetonitrile containing 0.1% trifluoroacetic acid and 0.3000 g L^{-1} of the internal standard 2-ethylphenol). Fifteen μL of the filtered sample was then injected onto a Wakosil 250 mm × 4 mm GL 3C18RS (SGE) column maintained at 37 °C with a flow rate of 0.75 mL min^{-1} on a Waters Alliance Model HPLC. The FPCs were eluted under gradient conditions with 0.1% TFA acid in acetonitrile (A) and 0.1% TFA in water (B) as follows: 60% A/40% B for 5 min, linear gradient to 90% A/10% B at 60 min, hold for 10 min and return to starting conditions over 10 min. We measured the peak response at 275 nm. The specific FPC compounds present were quantified using authentic standards purified in the laboratory. For easy comparison with other papers on FPCs, we express concentrations as mg g^{-1} dry matter (DM) rather than mmoles. In this paper, the two measures equate because the most prevalent FPCs—the sideroxylonals (MW = 500), grandinal (MW = 500) and the euglobal that elutes at 66 min (MW = 474) have similar molecular weights. The exception is grandinol (MW = 252).

2.5. Statistical analysis

All data were analysed using GenStat Release 6.2 Lawes Agricultural Trust (Rothamsted Experimental Station). We examined the relationships between the concentrations of individual compounds using correlation and partial correlation. Using both statistics allowed us to eliminate spurious correlations—those that appeared significant with correlation but disappeared with partial correlation. The procedure is sensitive to the number of variables, which we minimised by choosing the main peaks (the 66-min euglobal, grandinal, the sideroxylonals and grandinol) and by using the combined concentrations of sideroxylonals A and C. We assumed that the concentrations of two substances were linked when the correlation and partial correlation coefficients were both significant and in the same direction, either both positive or both negative.

The paired data, from young plants growing in the nursery and from the same clones growing in field trials, were analysed in two ways. First, we used a multivariate analysis of variance (MANOVA) testing the hypothesis that the leaves of nursery plants and trees in plantations had similar FPC concentrations. We chose MANOVA, rather than multiple univariate ANOVA, to avoid incorrect conclusions caused by close correlations between the concentrations of some of the compounds. Genstat, however, provides the results for univariate ANOVAs as part of the results for MANOVA, so we included the results for both analyses. Distributions of residuals and normal quantile–quantile plots with 95% confidence intervals showed that the data for most of the constituents satisfied the requirements for MANOVA. However, certain constituents (e.g. macrocarpal I and J) were either in very low concentrations or were absent in many plants, resulting in skewed quantile–quantile plots. Thus, we repeated the MANOVA on the difference between chemical concentrations in leaves collected from the nursery and the plantations with the hypothesis that the difference is zero. This procedure did not improve the interpretation and, along with results for the minor constituents, is not reported.

The second approach we used for the paired data was simple linear regression to examine whether the chemical concentrations in the leaves of young trees growing in the field were indicative of those in the same genetic material growing in pots in the nursery. We used regression rather than correlation because we assumed that the chemistry of the trees growing in plantations depended on the chemistry of the original genetic material (Andrew et al., 2007). This made clear the choice of dependent and independent variables. It is conceivable that the ranking of the clones, in terms of chemical concentrations, remains the same between the laboratory and the field but that the actual concentrations differ. Because we were interested in whether or not a relationship exists, rather than the shape of the relationship, we did not force the regression through the origin or to a slope of one. Of the 42 different clones sampled, we collected many at both the nursery and at more than one plantation site. One randomly selected site for each of these clones was used in the above regression.

Table 1
The formylated phloroglucinol compounds and other compounds from HPLC chromatograms of extracts of leaves from all the *Eucalyptus grandis* sampled ($N = 134$).

Compound FPCs	Retention time (min)	Concentration (mg g^{-1} dry matter)	
		Mean	Range
Macrocarpal I	13	1.0	0.0–3.0
Grandinol	16	1.2	0.0–8.4
Macrocarpal J	17	0.1	0.0–1.2
Unknown macrocarpal	34	0.6	0.0–2.4
Sideroxylonal A	44	5.5	0.7–11.0
Sideroxylonal C	45	2.0	0.3–4.0
Grandinal	43, 53[a]	4.6	1.6–10.0
Euglobal	66	7.5	3.0–18.6
Total FPCs	–	23.0	8.0–47.0
Other compounds			
Unknown	21		Not quantified
Eucalyptin[b]	24		Not quantified
Unknown	25		Not quantified

[a] Grandinal is a tautomer and thus has two peaks.
[b] Eucalyptin is a flavonoid common in eucalypts (Horn and Lamberton, 1963).

3. Results

3.1. The defensive chemistry of E. grandis

Based on retention time and our experience in identifying eucalypt compounds (see Eschler et al., 2000; Moore et al., 2004a), the HPLC chromatogram of *E. grandis* contained at least eight formylated phloroglucinol compounds (Table 1). The concentrations of the sideroxylonals, grandinal and the euglobal in all samples accounted on average for 87% of the total FPC concentration (range = 70–96%), regardless of whether this was expressed as a mass (8–47 mg g^{-1} DM) or as a molar concentration (0.018–0.104 mmoles g^{-1} DM). Nearly all samples contained grandinol and macrocarpal I but typically in low concentrations. Fewer samples contained macrocarpal J and the macrocarpal that elutes at 34 min and often at negligible concentrations. Therefore, we focused our interpretation on four compounds: the 66-min euglobal, grandinal, the combined concentration of sideroxylonals A and C, grandinol and the sum of these four entities. These compounds are prominent and we know from experience that FPCs deter certain herbivores (Lawler et al., 2000; Wallis et al., 2002; Moore et al., 2004a). We are less certain about the biological effects of the euglobal, but evidence suggests that the formyl group may be a prerequisite for the antifeedant effects of FPCs (Lawler et al., 1999).

Frequency plots of the focal compounds, using all samples ($N = 134$) show that their concentrations follow approximate normal distributions (Fig. 1), with the exception of grandinol (Fig. 1a). The distribution of grandinol concentrations in this population has a long tail, exacerbated by a few samples with relatively high concentrations. One sample (Clone 039, Grafton Forest

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

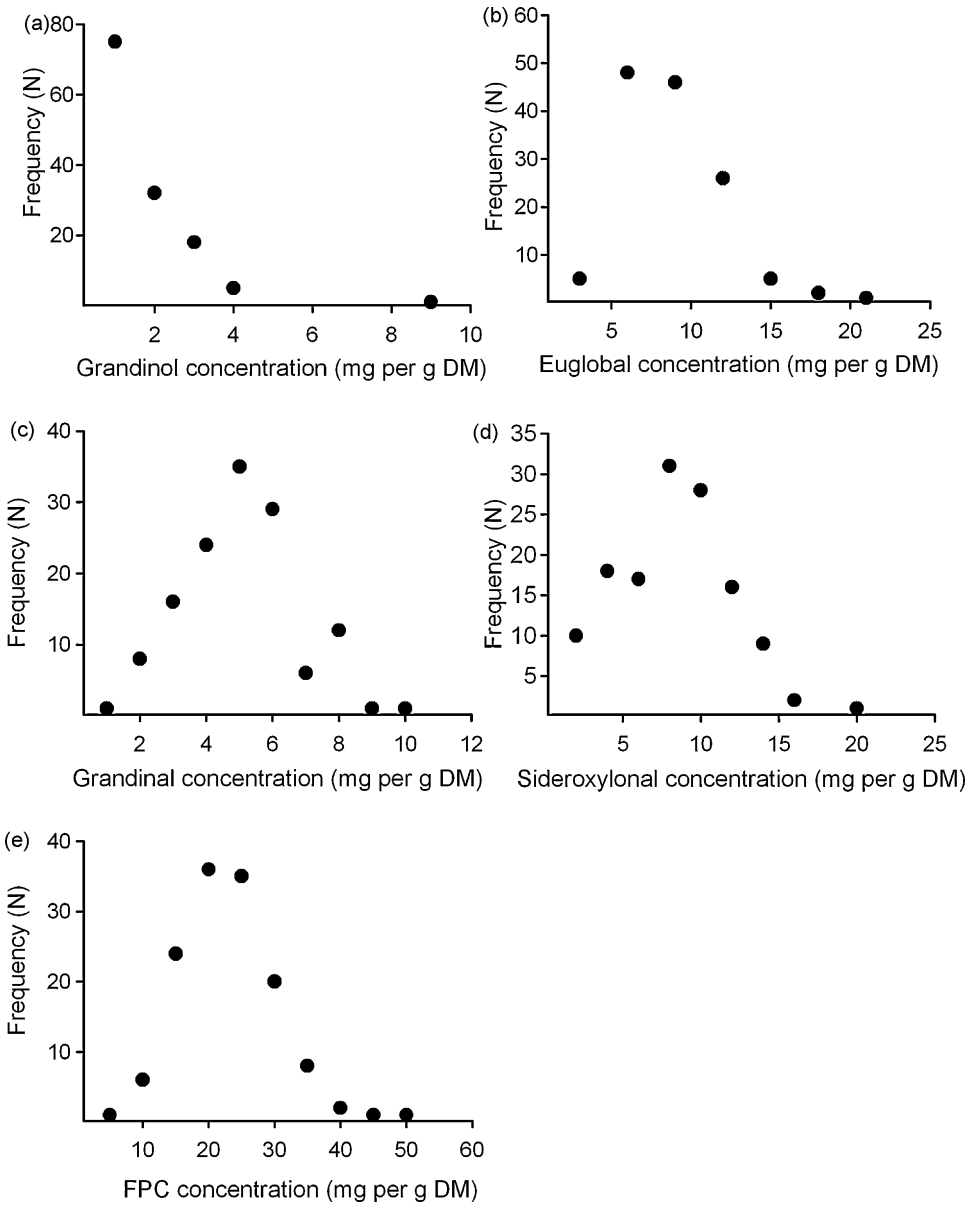

Fig. 1. The frequency distributions of the foliar concentrations of grandinol (a), the 66-min euglobal (b), grandinal (c), sideroxylonals (d) and the sum of these components (e) using all samples collected ($N = 134$).

Technology Centre), in particular, contributed to this skewed distribution. Foliage from this young clone contained 8.3 mg g^{-1} DM of grandinol—some six standard deviations away from the overall mean for grandinol (1.2 mg g^{-1} DM) and five standard deviations from the mean grandinol concentration of the nursery plants (1.75 mg g^{-1} DM) (Fig. 1a). A repeat analysis of this plant confirmed the high concentrations. The same plant had one of the highest concentrations of sideroxylonals (14.8 mg g^{-1} DM) and one of the highest total concentrations of the four constituents of interest (37 mg g^{-1} DM). The sample from the same clone collected from the plantation at Coffs Harbour contains about three times the average grandinol for the site (2.7 mg g^{-1} DM versus 0.9 mg g^{-1} DM) but is otherwise ordinary. Clone 4, an *E. urophylla* × *E. grandis* hybrid, also was unique, being the only clone that contained none of the 66-min euglobal. It had among the lowest total concentration of FPCs, made up almost entirely of sideroxylonal and grandinal. In contrast, there was nothing unique about the chemistry of the two *E. grandis* × *E. urophylla* hybrids and it would be difficult to separate them from any of the pure *E. grandis*.

There were significant correlations between the concentrations of the 66-min euglobal and grandinal ($r = 0.41$; $P < 0.01$) and between grandinal and the sum of sideroxylonals A and C ($r = 0.77$; $P < 0.01$) (Table 2). Both correlation coefficients remained virtually unchanged in the partial correlation, with the concentrations of the two other constituents controlled. These correlations suggest a weak link between the concentration of the 66-min euglobal and that of grandinol. The correlation coefficient was significant ($r = 0.24$; $P < 0.05$) whereas the coefficient from partial correlation was not significant ($r = 0.17$; $P = 0.08$) (Table 2). In contrast, the correlation between the concentrations of the 66-min euglobal and the sideroxylonals was positive but insignificant while the partial correlation coefficient was negative and highly significant ($r = -0.300$; $P = 0.011$), implying a spurious link (Table 2).

There were also conspicuous qualitative differences in chemistry. Most obvious was an unknown compound (21.7 min) that was usually present in the young clones collected at the nursery but often absent or in very low concentrations from samples collected in the field.

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

Table 2
Correlation (probability) and partial correlation coefficients between concentrations of the four main formylated phloroglucinol compounds (FPCs) in all clones sampled ($N = 134$).

	66-Min euglobal	Grandinal	Grandinol
Correlations			
Grandinal	0.41 ($P < 0.001$)		
Grandinol	0.24 ($P = 0.034$)	0.21 ($P = 0.073$)	
Sideroxylonals	0.14 ($P = 0.20$)	0.77 ($P < 0.001$)	0.13 ($P = 0.024$)
Partial correlations			
Grandinal	0.46		
Grandinol	0.17	0.08	
Sideroxylonals	−0.30	0.79	0.003

Table 3
Comparisons of the concentrations of formylated phloroglucinol compounds (FPCs) (mg g^{-1} dry leaf) in clones of *E. grandis* and hybrids grown as potted nursery hedges and in a field trial along with results of univariate (ANOVA) and multivariate analyses (MANOVA) $N = 134$.

Variate	Nursery mean (CV)[a]	Field mean (CV)	Sed[b]	Probability
ANOVA				
66-Min euglobal	9.1 (43)	6.8 (35)	0.74	$P < 0.001$
Grandinal	3.8 (49)	5.1 (26)	0.36	$P < 0.001$
Sideroxylonal A + C	5.5 (63)	8.5 (31)	0.69	$P < 0.001$
Grandinol	2.0 (72)	0.76 (86)	0.25	$P < 0.001$
Sum of the four FPCs	20.4 (42)	21.0 (24)	1.78	$P = 0.61$
MANOVA tests				
Wilk's lambda	0.351			
Approximate χ^2	77.5 (5 df[c]) ($P < 0.001$)			
Approximate $F_{5,72}$	26.7 ($P < 0.001$)			
Pillai-Bartlett trace	0.649			
Lawley-Hotelling trace	1.850			

[a] CV coefficient of variation (%).
[b] Sed standard error of a difference between means.
[c] Degrees of freedom.

Table 4
Simple linear regression describing the relationship between the concentration (mg g^{-1} DM) of formylated phloroglucinol compounds (FPCs) in the foliage from *E. grandis* and *E. grandis* hybrids growing in the field and from the corresponding clone growing in the nursery. ($N = 42$).

Variate	Constant (se)	Coefficient (se)	R^2 (%)	Probability
66-Min euglobal	4.36 (1.74)	0.694 (0.241)	16.1	0.007
Grandinal	1.78 (1.15)	0.404 (0.225)	5.5	0.081
Sideroxylonal A + C	2.91 (1.83)	0.309 (0.206)	3.2	0.14
Grandinol (all data)	1.134 (0.305)	1.092 (0.306)	23.6	0.001
Grandinol (one value removed)[a]	1.472 (0.235)	0.449 (0.256)	5.5	0.088
Sum of the four FPCs	13.66 (5.79)	0.319 (0.269)	1.1	0.243

[a] Excluding one grandinol clone that contained 8.3 mg g^{-1} DM of grandinol—some six standard deviations away from the overall mean for grandinol (Fig. 1a).

3.2. Does the chemistry of young clones predict future chemistry in plantations?

The results for the MANOVA showed highly significant differences in the FPC concentrations in foliage from potted plants in the nursery and young trees growing in plantations (Table 3). The univariate analyses, which should be treated with caution, due to correlations between the concentrations of individual FPCs, provide clues about these differences (Table 3). While the total concentration of the four main constituents was similar in foliage from nursery and plantation trees, the concentrations of individual components differed. Concentrations of grandinal and the sideroxylonals were higher in plantation trees whereas those of the 66-min euglobal and of grandinol were higher in the nursery plants. The FPC concentrations tended to be more variable in the nursery plants than they were in the plantation trees. This was most noticeable for the sideroxylonals and for grandinal and these effects carried through to the sum of the four FPCs of interest. Grandinol provided the exception, giving high coefficients of variation for both nursery and plantation material.

Simple linear regression of the field concentrations of the four FPCs on the nursery values reveals either weak relationships of little predictive value (e.g. the 66-min euglobal $R^2 = 16\%$; $F_{1,37} = 8.3$; $P = 0.007$) or no relationship at all (e.g. grandinal, sideroxylonal and the sum of the four FPCs (Table 4). Grandinol shows a spurious relationship ($R^2 = 23\%$; $F_{1,37} = 12.7$; $P = 0.001$) that disappears ($R^2 = 5.5\%$; $F_{1,37} = 3.1$; $P = 0.08$) when we remove the data for one clone (#039; see above), which had a distinct chemistry compared to other individuals. The data for the combined concentration of the four FPCs were interesting because they show the poorest relationship between nursery and field concentrations.

4. Discussion

4.1. The chemistry of young clones of E. grandis provides no indication of the defences of older plants

The main finding from this study was that there was almost no relationship between the concentrations of defensive chemicals in the leaves of young *E. grandis* clones growing in pots in the nursery and of those in the same genetic material growing in plantations (Table 3). Published data suggest that the concentrations of cineole, total terpenes and FPCs in foliage are under moderate to strong genetic control (individual heritability = 0.27–0.85) (see Barton et

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

al., 1991; Doran and Matheson, 1994; Doran, 2002; Andrew et al., 2005). Apart from the obvious environmental variation, which presumably was high between and within plantations but was largely controlled in the nursery, the other noteworthy disparity was in the age and development of the material sampled. The young trees in the plantations were 15–30 months old, 2–3 m tall and were growing vigorously. In contrast, the nursery clones were small potted specimens, up to 30 cm high and with few leaves for sampling and of a different physiological age ("juvenile leaves"). We know little about the changes in concentration of FPCs with ontogenetic development. Some of our unpublished results from "juvenile" and "adult" leaf forms collected from the same tree indicate that leaves of different ontogeny typically contain highly correlated concentrations of the same FPCs. Even if this variation affected foliar concentrations of individual FPCs it would still be expected that the rankings of clones on total FPCs would be positively correlated. There was no such relationship and, instead, the results suggest that the environmental variance completely masked the genetic variance. Nevertheless, the aim of the study was to resolve whether it is feasible to predict elements of the defensive chemistry of *E. grandis* from early clonal material. The results show that it is not.

This is not the first time that plant secondary chemicals that are thought to be under strong genetic control are found to vary widely with the environment. Laitinen et al. (2004) found large variation within clones for several secondary chemicals in birch bark. They attributed this to the fact that resin glands, phenolics and triterpenoids in birch are often affected by environmental factors, such as nutrients and UV light. Likewise, in a study of *E. nitens*, Close et al. (2003) minimised genetic variation and varied environmental conditions and by doing so altered concentrations of foliar sideroxylonals. They argued that sideroxylonals or their precursors originally provided an antioxidant function but have since been selected for by herbivore pressure. In other words, they serve at least two roles in the plant. Perhaps more relevant is the finding of Barton et al. (1991), that environmentally-derived variation in cineole concentration decreases (as indicated by increased heritability) as young trees develop a mature canopy. In all species examined in our laboratory, there is a strong relationship ($R^2 = 0.70–0.95$) between the concentrations in foliage of total FPCs and of cineole (e.g., Lawler et al., 2000; Wallis et al., 2002; Moore et al., 2004a). Thus we might expect the environmentally-induced variation in the concentration of FPCs to similarly decrease during this early phase of maturation. Sampling and analysis of foliage from multiple ramets within sites during their early development would be useful in elucidating the influence of environment and ontogeny on these compounds.

In this study, it was beyond our means to measure the variation within and between clones because there were insufficient ramets of the various clones among the nursery stock. Nevertheless, it is a subject deserving of comment. The concentrations of the compounds of interest had a roughly normal distribution in *E. grandis*, as do FPCs in other eucalypts. The present results, however, suggest that environmental factors may have influenced the production of these compounds. Concentrations of the 66-min euglobal and of grandinol were conspicuously higher in the nursery plants, while those in plantations produced leaves with more sideroxylonals. Also, according to the coefficient of variation, concentrations of grandinal and sideroxylonal were twice as variable in the nursery plants as they were in the plantation trees. This led to a similar finding for the four FPCs of interest. Concentrations of grandinol provided the exception, being extremely variable in both environments. A partial explanation is that analytical variation was higher for grandinol due to its inherently low concentrations.

We chose to study *E. grandis* because it is a key plantation species, we are familiar with some of the FPCs it produces and because clonal trials are already underway, making it possible to sample a suitable number of clones. The life history of the species, however, detracts from its initial appeal as a study organism. In its native habitat, *E. grandis* typically grows in fertile valleys at low altitudes on deep soils (Boland et al., 1984). The vegetation associations in these places—often on the edge of rainforest, are usually complex so that *E. grandis* mostly occurs with several other eucalypts, including *E. intermedia* (R. T. Baker), *E. resinifera* (Smith), *E. microcorys* (F. Mueller), *E. pilularis* (Smith) and *E. saligna* (Smith), as well as other large trees such as *Syncarpia glomulifera* (Smith), *Lophostemon conferta* (R. Brown) and *Allocasuarina torulosa* (Aiton) (Boland et al., 1984). *E. grandis* intolerates shading and so, in this environment, fast growth is important. Thus, *E. grandis* fits the mould of a fast-growing species in a resource-rich habitat (Coley et al., 1985), a point emphasised by Stone (2001). The appeal to commercial forestry of these fast-growing species that produce canopies with high rates of leaf area development and turnover is obvious. What makes them of questionable value for this study and sometimes for commercial forestry, is that many fast-growing species typically invest little in defensive chemicals and, instead, counter herbivory with higher growth rates (Coley et al., 1985). There are no studies of this phenomenon in eucalypts but the total concentrations of FPCs in *E. grandis* were similar to those reported for other eucalypts (e.g., Wallis et al., 2002; Moore et al., 2004a, b).

4.2. The need for innate chemical defences

Many of the plant herbivore defence theories predict that fast-growing species counter increased herbivory by quickly replacing the destroyed tissues. De Jong (1995) expressed this most eloquently by pointing out that a doubling of growth rate equates with a 50% reduction in herbivore pressure. This is understandable in a natural environment where a herbivore has a finite amount of material available and where predators are likely to play a role. But, what relevance is this to plantations, especially those established with few clones (i.e. minimal genetic variation), selected for fast growth rates (and perhaps minimal chemical defence), and planted over vast areas and with growth rates enhanced by fertilisers? In our experience, the foliage of fertilised *E. grandis* contains extraordinarily high concentrations of nitrogen (more than 3.5%) and presumably high concentrations of available N (DeGabriel et al., 2008), making them appealing for most eucalypt herbivores. As Stone (2001) concedes, this situation can dramatically alter the carrying capacity of some exploitive insects to the point that the rate of foliage destruction greatly exceeds the rate of replacement. In Australia, there is also the risk of colossal damage from vertebrates, such as brushtail possums and various macropods. Controlling either of these outbreaks invites criticism from the public, who object to the spraying of chemicals and the culling of native fauna. This suggests that where the species may be challenged by a wide array of herbivores, large, homogeneous plantations based on very limited genetic material are prone to disaster. Furthermore, this situation may occur after a prolonged period of little herbivory (Gruppe et al., 1999). Innate chemical defences are likely to elicit an arms race with herbivores, while a better alternative seems silvicultural practices that grow a mixture of species—a forest rather than a monoculture, although this view requires stringent testing.

Acknowledgements

We thank the Rural Industries Research and Development Corporation for funding the work, Chris Moran of Forests NSW for assistance with collecting the samples and David Kleinig for allowing us use of the hybrid material. Gavin Moran made several suggestions to improve the manuscript. We thank Dr Ann Cowling, Head, Statistical Consulting Unit, for statistical advice. The comments of two anonymous reviewers improved the manuscript.

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034

References

Andrew, R.L., Peakall, R., Wallis, I.R., Wood, J.T., Knight, E.J., Foley, W.J., 2005. Marker-based quantitative genetics in the wild?: the heritability and genetic correlation of chemical defenses in *Eucalyptus*. Genetics 171, 1989–1998.

Andrew, R.L., Wallis, I.R., Harwood, C.E., Henson, M., Foley, W.J., 2007. Heritable variation in the foliar secondary metabolite sideroxylonal in *Eucalyptus* confers cross-resistance to herbivores. Oecologia 153, 891–901.

Barton, A.F.M., Cotterill, P.P., Brooker, M.I.H., 1991. Short Note—heritability of cineole yield in *Eucalyptus kochii*. Silvae Genet. 40, 37–38.

Boland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A., Turner, J.D., 1984. Forest Trees of Australia. Nelson-CSIRO, Melbourne.

Campinhos, E.J., 1999. Sustainable plantations of high-yield *Eucalyptus* trees for production of fiber: the Aracruz case. New Forest 17, 129–143.

Close, D., McArthur, C., Paterson, S., Fitzgerald, H., Walsh, A., Kincade, T., 2003. Photoinhibition: a link between effects of the environment on eucalypt leaf chemistry and herbivory. Ecology 84, 2952–2966.

Coley, P.D., Bryant, J.P., Chapin, F.S., 1985. Resource availability and plant antiherbivore defense. Science 230, 895–899.

De Jong, T.J., 1995. Why fast growing plants do not bother about defence. Oikos 74, 545–548.

DeGabriel, J.L., Wallis, I.R., Moore, B.D., Foley, W.J., 2008. A simple, integrative assay to quantify nutritional quality of browses for herbivores. Oecologia 156, 107–116.

Doran, J.C., 2002. Genetic improvement of eucalypts: with special reference to oil-bearing species. In: Coppen, J.J.W. (Ed.), Eucalyptus: The Genus *Eucalyptus*. Taylor and Francis, London, pp. 75–101.

Doran, J.C., Matheson, A.C., 1994. Genetic parameters and expected gains from selection for monoterpene yields in Petford *Eucalyptus camaldulensis*. New Forest 8, 155–167.

Eldridge, K.G., Davidson, J., Harwood, C., van Wyk, G., 1994. Eucalypt Domestication and Breeding. Clarendon Press, Oxford.

Eschler, B.M., Pass, D.M., Willis, R., Foley, W.J., 2000. Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. Biochem. Syst. Ecol. 28, 813–824.

Gruppe, A., Fusseder, M., Schopf, R., 1999. Short rotation plantations of aspen and balsam poplar on former arable land in Germany: defoliating insects and leaf constituents. Forest Ecol. Manage. 121, 113–122.

Guedes, R.N.C., Zanuncio, T.V., Zanuncio, J.C., Medeiros, A.G.B., 2000. Species richness and fluctuation of defoliator Lepidoptera populations in Brazilian plantations of *Eucalyptus grandis* as affected by plant age and weather factors. Forest Ecol. Manage. 137, 179–184.

Horn, D.H.S., Lamberton, J.A.C., 1963. Nuclear magnetic resonance (NMR) study of a new flavonoid. Chem. Ind. (Lond.), 691–692.

Konoshima, T., Takasaki, M., 2002. Chemistry and bioactivity of the non-volatile constituents of *Eucalyptus*. In: Coppen, J.J.W. (Ed.), Eucalyptus: The Genus *Eucalyptus*. Taylor and Francis, London, pp. 269–290.

Laitinen, M.-L., Julkinen-Tiitto, R.K.Y., Heinonen, J., Rousi, M., 2004. Variation in birch bark chemistry between and within clones: implications for herbivory by hares. Oikos 104, 316–326.

Lawler, I.R., Eschler, B.M., Schliebs, D.M., Foley, W.J., 1999. Relationship between chemical functional groups on *Eucalyptus* secondary metabolites and their effectiveness as marsupial antifeedants. J. Chem. Ecol. 25, 2561–2573.

Lawler, I.R., Foley, W.J., Eschler, B., 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. Ecology 81, 1327–1338.

Lawler, I.R., Foley, W.J., Eschler, B., Pass, D.M., Handasyde, K., 1998. Intraspecific variation in secondary metabolites determines food intake by folivorous marsupials. Oecologia 116, 160–169.

Moore, B.D., Wallis, I.R., Palá-Paúl, J., Brophy, J.J., Willis, R.H., Foley, W.J., 2004a. Antiherbivore chemistry of *Eucalyptus*—cues and deterrents for marsupial folivores. J. Chem. Ecol. 30, 1743–1769.

Moore, B.D., Wallis, I.R., Wood, J.T., Foley, W.J., 2004b. Foliar nutrition, site quality, and temperature influence foliar chemistry of tallowwood (*Eucalyptus microcorys*). Ecol. Monogr. 74, 553–568.

Stape, J.L., Gonçalves, J.L.M., Gonçalves, A.N., 2001. Relationship between nursery practices and field performance for *Eucalyptus* plantations in Brazil. New Forest 22, 19–41.

Stone, C., 2001. Reducing the impact of insect herbivory in eucalypt plantations through management of extrinsic influences on tree vigour. Aust. Ecol. 26, 482–488.

Wallis, I.R., Foley, W.J., 2005. The rapid determination of sideroxylonals in *Eucalyptus* foliage by extraction with sonication followed by HPLC. Phytochem. Anal. 16, 49–54.

Wallis, I.R., Watson, M.L., Foley, W.J., 2002. Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. Aust. J. Zool. 50, 507–519.

Wingfield, M.J., Roux, J., Coutinho, T., Govender, P., Wingfield, B.D., 2001. Plantation disease and pest management in the next century. S. Afr. Forest J. 190, 67–72.

Wright, M.G., Samways, M.J., 1998. Insect species richness tracking plant species richness in a diverse flora: gall insects in the Cape floristic region, South Africa. Oecologia 115, 427–433.

Zanuncio, J.C., Guedes, R.N.C., Zanuncio, T.V., Fabres, A.S., 2001. Species richness and abundance of defoliating Lepidoptera associated with *Eucalyptus grandis* in Brazil and their response to plant age. Aust. Ecol. 26, 582–589.

Zanuncio, J.C., Nascimento, E.C., Garcia, J.F., Zanuncio, T.V., 1994. Major lepidopterous defoliators of eucalypts in southeast Brazil. Forest Ecol. Manage. 65, 53–63.

Zanuncio, T.V., Zanuncio, J.C., Miranda, M.M.M., Medeiros, A.G.B., 1998. Effect of plantation age on diversity and population fluctuations of Lepidoptera collected in *Eucalyptus* plantations in Brazil. Forest Ecol. Manage. 108, 91–98.

Please cite this article in press as: Wallis, I.R., et al., Foliar chemistry of juvenile *Eucalyptus grandis* clones does not predict chemical defence in maturing ramets. Forest Ecol. Manage. (2010), doi:10.1016/j.foreco.2010.05.034