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Predicting crown damage to *Eucalyptus grandis* by *Paropsis atomaria* with direct and indirect measures of leaf composition

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ABSTRACT

A genetic basis for variation in resistance to defoliating insects within *Eucalyptus* species has been identified in many studies. This variation has frequently been ascribed to variation in secondary metabolites but studies investigating variation in resistance to defoliation by paropsine chrysomelids have failed to correlate foliar chemistry with resistance. We found that the extent of crown damage due to defoliation by *Paropsis atomaria* (Chrysomelidae: Colepoptera) in two matched progeny trials of *Eucalyptus grandis* was a heritable trait that exhibited a strong correlation with provenance latitude. Despite this, neither foliar nitrogen or concentrations of a recently discovered group of compounds, formylated phloroglucinol compounds, could account for significant variation in defoliation. We also investigated whether defoliation in the field could be predicted from foliar near-infrared reflectance spectra. Such an approach takes into account all compositional variation simultaneously rather than relying on a restricted number of measured traits. Modified partial least squares regression models performed poorly in predicting variation in crown defoliation between trees within a site primarily due to the high level of variability and coarseness of the calibration data. Discriminant analyses however demonstrated a consistent difference between spectra from trees in families suffering low level defoliation from families more susceptible to defoliation by *P. atomaria* suggesting chemical differences between the two groups are important in determining resistance to this insect.

1. Introduction

The chemical composition of leaves within a species is highly variable both temporally and spatially. This is due primarily to the diversity of plant secondary metabolites generated by genetic differences between individuals (Hwang and Lindroth, 1997; Laitinen et al., 2000; Donaldson and Lindroth, 2004; Andrew et al., 2005) and changes in either the overall or the relative concentrations of individual compounds due to ontogenetic (Zou and Cates, 1995; Gleadow and Woodrow, 2000b; Riipi et al., 2004) or environmentally induced (Havill and Raffa, 1999; Osier and Lindroth, 2001; Cronin and Lodge, 2003; Laitinen et al., 2005) changes to biochemical pathways controlling their production. Such intraspecific variation in leaf composition is acknowledged as being critical in insect–herbivore interactions as it affects a given host plant's attractiveness and its nutritional status which in turn affects both spatial variation in densities of insects, and consequently their interactions with predators and parasitoids (Hunter, 1997).

Foliar secondary chemistry is known to vary significantly both between (Fox and Macauley, 1977; Moore et al., 2004a) and within (Gleadow and Woodrow, 2000a; Moore et al., 2004a,b) *Eucalyptus* species. Such chemical variation is likely to underpin the considerable variation in susceptibility to some insect pests that has been identified in eucalypts. This includes between and within species variation in *E. melliodora*, *E. conica*, *E. sideroxylon*, *E. viminalis*, *E. blakelyi*, *E. calignosa* and *E. camaldulensis* (Lowman and Heatwole, 1987; Edwards et al., 1990, 1993); intraspecific 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; Floyd et al., 2002; Rapley et al., 2004b), *E. grandis* (Shepherd et al., 2000) and *E. delegatensis* (Ohmart et al., 1984). In a few cases this variation in susceptibility to a particular insect species has been linked to variation in a chemical component of the leaves (e.g. Edwards et al., 1990, 1993; Jones et al., 2002; Rapley et al., 2004b). Most of the

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aforementioned studies involve commercially important plantation tree species that have been damaged by insect pests.

Paropsine leaf beetles (Coleoptera: Chrysomelidae) are eucalypt folivores that are monophagous within the genus *Eucalyptus* and both larvae and adults consume eucalypt foliage. In common with most other insect herbivores, paropsine growth and fecundity are strongly influenced by leaf protein concentration (Fox and Macauley, 1977; Ohmart et al., 1985a,b) and leaf toughness (Ohmart et al., 1987; Larsson and Ohmart, 1988). Most species within the genus *Eucalytpus* have tough sclerophyllous leaves that are an effective deterrent to leaf chewing insects, particularly young larvae (Ohmart et al., 1985a, 1987). Paropsine chrysomelid larvae, and to a lesser extent adults, are therefore restricted to consuming immature and expanding foliage which, although of greater nutritional quality than mature leaf, often contains high concentrations of putative toxins and digestibility reducers in the form of surface waxes, tannins, other phenolics and terpenes. Despite considerable investigation however, variation in paropsid larval performance either within or between eucalypt species has not been shown to be influenced by any secondary compounds (Fox and Macauley, 1977; Morrow and Fox, 1980; Patterson et al., 1996; Lawler et al., 1997). In addition, it appears that host selection is primarily influenced by the availability of oviposition sites on suitable young foliage rather than variation in leaf chemistry (Steinbauer et al., 1998; Howlett and Clarke, 2003). This suggests that intraspecific variation in severity of herbivory on individual trees in the field may be due to as yet unquantified factors in the leaves or subtle synergistic effects of multiple compounds (e.g. Berenbaum and Zangerl, 1993; Hay et al., 1994; Guillet et al., 2000; Hummelbrunner and Isman, 2001; Calcagno et al., 2002; Dyer et al., 2003) on feeding larvae that cannot be detected by quantifying a limited number of individual chemical components as has been done in the past.

In recent years a distinct group of plant secondary compounds, the formylated phloroglucinol compounds (FPCs) have been isolated from eucalypts of the subgenus *Symphyomyrtus* (Eschler et al., 2000), and have been found to deter feeding by folivorous eucalypt-feeding marsupials (Lawler et al., 1998, 2000; Moore and Foley, 2005; Moore et al., 2005). These compounds also appear to act as antifeedants against Christmas beetles (*Anoplognathus* spp.) (Coleoptera: Scarabidae) (Floyd and Foley, 2001), another insect group that feeds on eucalypts in the adult phase, but as yet have no known effects on other phytophagous insects.

Our objective in this study was to examine whether variation in FPCs could account for any variation in canopy defoliation by *Paropsis atomaria* (Ol.), a paropsine chrysomelid beetle, in replicated progeny trials of *E. grandis* whilst simultaneously accounting for leaf *N*, a known correlate of insect performance. We did this by initially identifying the least defoliated and the most defoliated open-pollinated families (OPFs) from field experiments and then collecting leaf material from these for further analyses. These analyses were carried out using near-infrared reflectance spectroscopy (NIRS), which is a secondary method of chemical analysis that enables large numbers of samples to be examined relatively quickly (Foley et al., 1998). This large-scale sampling is important in a field progeny experiment where the response of the insect to variation in leaf composition cannot be measured precisely and environmental variation increases the number of factors affecting insect activity.

In addition to predicting quantified leaf chemistry we wished to determine if NIRS could be used to model quantified levels of insect damage directly against spectral variation and thus indirectly relate variation in leaf composition to insect herbivory. Whilst NIRS is unsuited to pinpointing which chemical component of the leaves is important, it has the advantage of being able to account for variation in nutritional quality of food concurrently with plant secondary metabolites that might negatively affect the digestibility of the leaves since the spectra captures variation in chemistry holistically (McIlwee et al., 2001). This technique has previously been used successfully to discriminate between red cedar (*Toona* sp.) saplings varying in damage by an insect herbivore (Cunningham and Floyd, 2004). Similarly NIRS was able to predict 54% of the variation in larval performance of the sugarcane borer on 35 sugarcane clones (Rutherford and van Staden, 1996) and has been used to build spectra-based models of foliage consumption rates by vertebrate herbivores feeding on *Eucalyptus* (McIlwee et al., 2001; Wallis and Foley, 2003).

The plantations we used, like other forestry genetic trials, are generally established using seed collected from trees that exhibit a growth form that suits forestry ideals. No other characteristics are considered. Consequently variation in resistance to a plantation pest within an experimental plantation may be lower than that present within the entire tree species. It follows that this variation will most likely be a consequence of subtle differences (not necessarily chemical differences) between host trees. Ability to model *P. atomaria* defoliation against leaf spectra in a genetically diverse *E. grandis* progeny trial, whilst not essential for identification of resistant genotypes, may help indicate to what degree resistance is related to leaf composition. This, in turn may suggest to what extent this will affect insect performance, and may ultimately, with improvement to the calibration data, be a tool by which tree breeders can identify and select suitable genetic material.

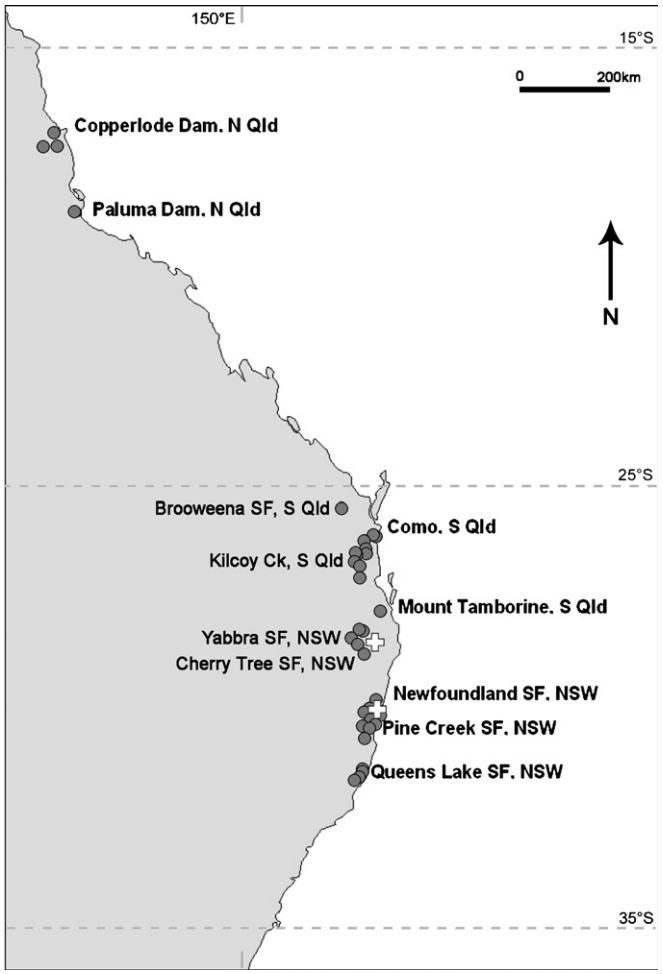

Fig. 1. The locations of provenances of ***Eucalyptus grandis*** along the east coast of Australia with families included in the trial populations and location of the two progeny trials (white crosses).

2. Materials and methods

2.1. Canopy defoliation assessment of progeny trials

A field assessment of overall canopy damage was conducted in April 2002 in two replicated progeny experiments of *Eucalyptus grandis* W. Hill ex Maiden. These trials were established by Forests NSW near Kyogle (Lat 28°37 S Long 153°03 E) (referred to as 'Hills' and established April 1999) and Coffs Harbour (Lat 30°09 S Long 153°06 E) ('Crabtrees' established February 2000) (Fig. 1). Each experiment contained 210 seedlots with 204 of those seedlots being single open-pollinated "families" where trees within a family were derived from seed collected from a single open-pollinated parent tree. These OPFs included a number of second-generation select OPFs or seed from "improved" material (selected on both favourable growth and form characteristics) sourced from other trial plantations, but also contained collections of non-improved material from 42 provenances (151 and 164 families at Crabtrees and Hills, respectively). There were 182 OPFs common to both sites. The design of the trials was for five replicates of 14 rows × 15 columns comprising five-tree plots planted along rows in a resolvable alpha lattice design. Where available, the canopy damage for three trees per plot was quantified using the crown damage index (CDI) (Stone et al., 2003; Stone and Coops, 2004) by classifying each tree, using a visual quantification of the damage to the whole tree crown, into a damage category. Damage was assessed as the percentage (classed into eight categories; 1 = 0–3%, 2 = 3–6%, 3 = 6–21%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–95%, 8 > 95%) of defoliation (partial or entire leaf missing), necrosis (dead tissue) or discolouration, caused by insects, fungi or abiotic agents.

As the trials were assessed in late summer, the measured CDI included cumulative crown damage from throughout the current season and previous seasons. The herbivore damage in the two plantations was predominantly derived from leaf chewing by *P. atomaria* (Coleoptera: Chrysomelidae) larvae and adults, although larvae remove the vast majority of leaf material. The major defoliator was identified by the presence of this species in high numbers during the assessment and characteristic feeding patterns for this species on the trees. The putative identification of this species as being the primary defoliator was confirmed by reports from forest health survey officers (Forests NSW, Forest Health Surveillance Unit) who had visited the trials monitoring pest populations prior to the date of the canopy damage assessment.

All trees in the progeny trial at Hills were assessed at 41 months for growth (diameter at breast height and height). These data were converted to an estimate of wood volume and an analysis of genetic correlation was performed to examine the relationship between this trait and CDI.

2.2. Statistical analysis of the field trials

The OPFs were assigned to 42 separate provenances largely on the basis of collection locality throughout the native distribution of *E. grandis*, which extends along the east coast of Australia (Fig. 1). For the purposes of our analysis a further nine seedlots sourced from various seed orchards consisting of improved material plus one mixed seed lot from South Africa, were assigned to provenance classes determined by which trial planting site or breeding orchard they had been collected from. CDI data from both trials were analysed using a mixed-effects individual tree model with a restricted maximum likelihood (REML) approach to estimate random effects in ASReml (Gilmour et al., 1999). In this model, Provenance (Prov) and Replicate (Rep) were treated as fixed effects and Tree, Row, Column and Plot as random effects. The model was as follows:

$$y = \mu + \text{Rep} + \text{Prov} + \text{Tree} + \text{Rep.Col} + \text{Rep.Row} + \text{Plot} + \text{residual}$$

Spatial analysis using the methods outlined in Silva et al. (2001) and Dutkowski et al. (2002) was also used to analyse data from the Crabtrees trial as the replicates were contiguous and layout of the trial was amenable to this approach. Narrow-sense heritability was calculated from the estimated variance components, assuming an average relatedness of 0.25 within families and no inbreeding or dominance effects (Lynch and Walsh, 1998). Genetic correlations between stem volume and CDI at the Hills site and between CDI at both sites (measured on different individuals from the same OPF) were estimated by fitting a bivariate model where each trait followed the above model and covariances were unconstrained. The mean CDI values for OPFs were also analysed using a family REML model (Lynch and Walsh, 1998) to determine the rankings of families at both sites and thus identify families consistently more prone or less prone to defoliation. This model was as follows with Rep.Row.Col as a random factor (synonymous with Plot in the previous analysis):

$$y = \mu + \text{Rep} + \text{Prov} + \text{Prov.OPF} + \text{Rep.Row.Col} + \text{residual}$$

2.3. Leaf sampling

Leaf samples of mature foliage were collected from the 14 least defoliated and 10 most defoliated OPFs across both sites based on mean CDI score. These families were selected to maximise any foliar compositional differences between these groups that might underlie variation in canopy damage and thus maximise variation in predictive variables and the likelihood of detecting significant regression relationships. In addition, ten OPFs (intermediate defoliation) were randomly selected from the centre of the family mean CDI ranking as well as two particularly resistant families located only at the Hills site. Eight individuals per OPF (where possible) across four of the five replicate plantings per site were sampled resulting in a total of 16 trees per OPF and a total of 575 foliage samples. The sampling procedure for each tree consisted of collecting approximately 100 g fresh weight of fully-expanded leaf from the current season on a single branch, which was bagged and immediately frozen. This sampling strategy was adopted primarily to minimise ontogenetic variation between trees as leaf composition is known to change rapidly during leaf expansion (Southwell and Stiff, 1989; Laitinen et al., 2002; Lambdon and Hassall, 2005) and secondarily because expanding leaves were unavailable on many trees. The leaf samples were freeze-dried and then ground to pass a 1 mm sieve in a Tecator Cyclotec mill. The resulting powder was used for the collection of NIR spectra and a sub-sample for analyses of the total C, total N and FPC concentrations. All concentrations are expressed per unit of dry leaf mass.

2.4. Quantification of foliar chemistry

Near-infrared spectroscopy (NIRS) was used to predict concentrations of chemical constituents in foliage samples from their near-infrared spectra. This technique (reviewed by Foley et al., 1998) uses multivariate statistical models to relate characteristics of the near-infrared spectra to attributes of a subset of samples (n values in Table 1) that have been determined using traditional laboratory techniques. As NIR spectra are holistic representations of the underlying chemical composition of samples, variations in spectra can be related statistically to a component of interest (Shenk et al., 1992; Shenk and Westerhaus, 1994). Where possible, regression models were developed to describe the relationship between NIR leaf spectra and laboratory measures of foliar constituents obtained from subsets of the *E. grandis* foliage samples using the methods

Table 1
NIRS modified partial least squares regression models developed to predict foliar content of *E. grandis* leaf components and mean CDI values within and across two sites

Constituent	*n*	Mean	S.D.	SEC[a]	r^2	Wavelength range (nm)	Bands[b]	Maths treatment[c]
CDI (combined)	240	3.40	1.22	0.78	0.64	750–2492	216	1,4,4,1
CDI (Crabtrees)	270	4.40	0.10	0.99	0.05	750–2492	142	1,8,8,1
CDI (Hills)	283	2.59	0.77	0.64	0.38	1108–2492	173	1,4,4,1
C:N	56	26.37	4.23	1.13	0.96	750–2492	213	2,6,4,1
N%	57	1.86	0.34	0.057	0.99	750–2492	429	2,4,4,1
Total extract[d]	68	128.3	20.1	6.57	0.96	1108–2492	172	2,4,4,1
Total grandinal[d]	66	6.19	3.87	1.84	0.94	1108–2492	172	2,4,4,1
Total sideroxylonal[d]	68	5.20	3.93	1.58	0.95	1108–2492	171	2,6,4,1
Eucalyptin[d]	65	2.05	1.91	0.72	0.94	1108–2492	171	2,4,4,1
Euglobal[d]	67	8.31	3.56	2.68	0.92	750–2492	858	2,4,4,1
RT74	37	1.89	0.77	0.58	0.81	750–2492	286	2,4,4,1

[a] Standard error of calibration.
[b] Number of wavelength bands.
[c] Describes the mathematical treatment applied to the spectra (stored as log[1/reflectance]). The first number describes the derivative used; the third and fourth numbers indicate the degrees of primary and secondary smoothing performed on the derivative. Thus, 2,4,4,1 indicates that the second derivative was calculated with a gap size of 4 nm and that a maximal primary smooth (4) but no secondary smooth (1) was used.
[d] mg/g leaf dry weight.

described below. These constituents included foliar nitrogen concentration (nitrogen), C:N ratio and the concentrations of a variety of FPCs.

Foliar nitrogen was determined by a semi-micro Kjeldahl procedure using a selenium catalyst performed on a Gerhardt Vapodest nitrogen analyzer. Foliar carbon and a second comparable nitrogen value were determined for each sample using a CHN analyzer (Model CHN 900, LECO, St Joseph, MI). Standards were used throughout both analyses in to validate the results.

Foliar FPC composition was assayed using the high performance liquid chromatography (HPLC with photo diode array detector) technique described by Wallis et al. (2003) with minor modifications to the chromatographic analysis. The column was maintained at 37 °C with a flow rate of 0.75 mL/min 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. The peak response was measured at 275 nm. The area under the major peaks from the resulting chromatographs was measured. Grandinol, sideroxylonals A and C, grandinal, eucalyptin (a non FPC methyl flavanone) and a euglobal were identified on the basis of their elution times by comparison with other HPLC data analysed under identical conditions (Moore et al., 2004a). Several previously unidentified peaks that were of significant size relative to that of the sideroxylonals, which commonly comprised the largest HPLC peaks in *E. grandis*, were present in many (but not all) of the genotypes. To determine whether these unidentified compounds could account for any variation in CDI, peak area per unit dry weight of leaf was used as an explanatory variable in statistical analyses.

2.5. Near-infrared spectroscopy

The dried, ground leaf samples were prepared for scanning by placing them in a 40 °C oven for at least 1 h to remove residual moisture that might otherwise interfere with the NIR spectra. After allowing the samples to cool to room temperature in a desiccator, the reflectance spectrum of each sample between 400 and 2500 nm was obtained using an NIRSystems 6500 scanning spectrophotometer with spinning cup attachment (NIRSystems, Silver Spring, Maryland, USA). Duplicate spectra of each sample were collected until the root mean square of two scans (stored as log (1/reflectance)) was less than 3.0×10^{-4}, and the two spectra averaged.

Calibrations were produced using modified partial least squares (MPLS) regressions with cross validation to prevent overfitting of models (Shenk and Westerhaus, 1991; Anonymous, 1995). For all calibrations various mathematical transformations on the raw spectra (including first and second derivatives of wavelength segments and smoothing) and selected models based on high r^2 and low standard error of calibration were iteratively tested. Scattering effects due to variation in particle size were reduced using standard normal variate scatter correction and detrend baseline transformations (Barnes et al., 1989). All calibrations were performed using the software WinISI II v. 1.02a (Infrasoft International, LLC). The NIRS models are summarized in Table 1. Where the range of predicted values exceeded the scope of the equation, the number of samples falling outside the range of the models was very small. The range of values used to develop the equation for nitrogen included 98.8% of predicted sample values, for total extract 99.7%, for sideroxylonal 99.3%, eucalyptin 99.8%, euglobal 96.7% and for total grandinal and C:N 100%.

In addition to models predicting foliar constituents an attempt was made to explain variation in field-determined CDI using NIR spectra as a holistic measure of leaf composition without *a priori* knowledge of which particular chemical component or components are influencing insect response. For predicting CDI across both sites, this model was developed using a random sub-sample of spectra from approximately half the trees.

Whole leaf spectra were used to discriminate each sample into one of three groups differing in susceptibility to defoliation by *P. atomaria*. These groups included trees from least defoliated, most defoliated and intermediate defoliated OPFs (as defined above in "*Leaf sampling*"). Two partial least squares models were developed to discriminate groups and subsequently assign all samples to a group with the nearest spectral mean. The first model was developed using a random subset of 100 leaf spectra from trees belonging to "least defoliated" and "most defoliated" families ($n = 200$) and the second was developed using a random subset of 100 leaf spectra from trees in each of the three groups ($n = 300$).

2.6. Statistical analyses

Multiple linear regression was used to investigate the relationship between concentrations of the various leaf components for which NIR calibrations could be produced and CDI within and between the two progeny trials. The ability of any component to account for variation in CDI was tested by both forward and backward stepwise regression and the significant terms were used in a final model. In analyses where both trials were included, an ordinal variable denoting site was used to account for site differences.

Table 2
Estimated variance components (±S.E.) for random effects in REML analyses and calculated narrow-sense heritabilities of CDI at two field sites

Source	Tree	Repl.Col	Rep.Row	Plot	Residual	h^2
Crabtrees	0.190 ± 0.036	0.009 ± 0.004	0.007 ± 0.003	0.064 ± 0.008	0.107 ± 0.028	0.53 ± 0.09
Hills	0.070 ± 0.043	0.027 ± 0.009	0.238 ± 0.045	0.146 ± 0.018	0.511 ± 0.036	0.10 ± 0.06

3. Results

3.1. Analysis of growth and CDI data by site

CDI values were normally distributed within each of the sites with relatively few trees exhibiting either minimal or almost complete defoliation. Overall defoliation at Hills was much lower with a mean CDI of 3.1 (6–12% leaf removal) compared to 6.3 (50–75% leaf removal) at Crabtrees. The distribution of mean CDI for all OPFs at Crabtrees was in the range of 4.5–7.3, with 95% of mean values between 6.1 and 6.3. This indicates the relatively low variability in degree of defoliation in *E. grandis* despite the potentially large genetic variation amongst families in the experimental trial. Narrow-sense heritability estimates demonstrated that CDI was under significant genetic control (Table 2). The higher heritability estimate at Crabtrees was partly derived from a higher additive genetic variance and partly from a lower environmental variance at the plot and tree level (Table 2). This in turn, was derived primarily from higher intensity and more even distribution of insect defoliation at Crabtrees and concomitant greater environmental heterogeneity at the Hills site. This was confirmed by the Crabtrees spatial analysis which made only minor alterations to the heritability estimates obtained with the alternate model that included terms for row and column effects (results not shown). There were significant differences between provenances and replicates at both Crabtrees (Provenance $F_{(44,188)} = 5.22$, $P < 0.001$ and Replicate $F_{(4,188)} = 6.98$, $P < 0.001$) and Hills (Provenance $F_{(42,168)} = 3.81$, $P < 0.001$ and Replicate $F_{(4,168)} = 17.95$, $P < 0.001$). A genetic correlation of 0.64 (S.E. 0.17) was estimated for CDI between sites demonstrating that the family ranking of resistance to defoliation is relatively stable between locations. A correlation of 0.66 was found for estimated Provenance effects on CDI between Hills and Crabtrees.

Comparing the ranking of families at the two sites showed that seed sourced from the northernmost provenances was commonly the least defoliated by *P. atomaria*. Indeed provenance latitude explained a large proportion of variation in CDI at Crabtrees ($F = 41.25$, d.f. = 39, $r^2 = 0.51$) (Fig. 2). CDI and latitude were similarly positively correlated at Hills but did not account for significant variation in simple linear regression ($F = 3.76$, d.f. = 37, $r^2 = 0.09$) (Fig. 2). However, this relationship is heavily influenced by low susceptibility to defoliation of the most northerly provenances which, due to the disjunct distribution of *E. grandis* along the east coast of Australia, are a significant distance from the nearest provenance to the south.

The analysis of growth data and CDI at the Hills progeny trial detected a genetic correlation of −0.73 (S.E. 0.15) between wood volume and insect defoliation.

3.2. Predicting CDI from foliar spectra

A randomly selected subset of 250 spectra from the original dataset (36 families, 575 trees across both sites) was used to develop the MPLS regression for CDI. This approach was used because all trees had been directly scored by CDI and we wished to test how effectively CDI could be predicted for the remaining set of trees. Performance of the calibration equation across the two sites was good, given the coarseness of the dependent variable CDI (Table 1). However, when trees from the same set of OPFs were examined within sites the performance of the calibration equation decreased dramatically (Hills) or the development of a calibration equation was unsuccessful (Crabtrees) (Table 1 and Fig. 3). This suggested that differences in mean CDI between the sites accounted for most of the ability of the regression model to predict CDI.

3.3. Relationships between CDI and foliar chemistry

The success of using N concentration, C:N ratio, and concentrations of various FPCs as explanatory variables for variation in CDI in a multiple regression model was limited. Using a categorical site variable in an analysis of data from both sites accounted for the majority of the variation in CDI ($F = 475.8$, d.f. = 1, 573, $P < 0.001$, $r^2 = 0.453$) due to the overall difference in the intensity of defoliation between locations. Fitting other variables in this model explained very little additional variation (5.8%). We considered it therefore more appropriate to fit variables using separate regression models for each site (Table 3). At Crabtrees, grandinal and the unidentified peak RT74 were the only variables that were significant in explaining variation in CDI although the percentage of variation accounted for overall by the model was minimal ($r^2 = 0.058$). At Hills, a larger proportion of variance was accounted for by the model ($r^2 = 0.219$), although a different set of variables were identified as important. C:N ratio which in general was relatively higher at this site than Crabtrees (and non-significant at that site in explaining variation) accounted for proportionally more variation than other variables as indicated by its larger sum of squares. Variation in C:N ratio was found to be predominantly due to variation in %N rather than %C, which varied relatively little. This suggests leaf quality for insect herbivores at Crabtrees was higher than at Hills due to higher nitrogen concentrations (mean 2.0% at Crabtrees compared to 1.7% at Hills). Total foliar grandinal concentration was found to have a small but significant positive relationship with CDI across both sites.

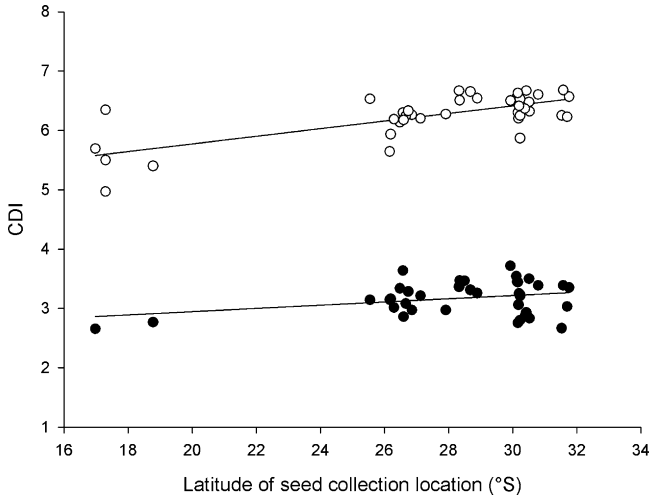

Fig. 2. Regression relationships between estimated CDI for provenance and provenance latitude for the two ***Eucalyptus grandis*** progeny trials at Hills (●) and Crabtrees (○).

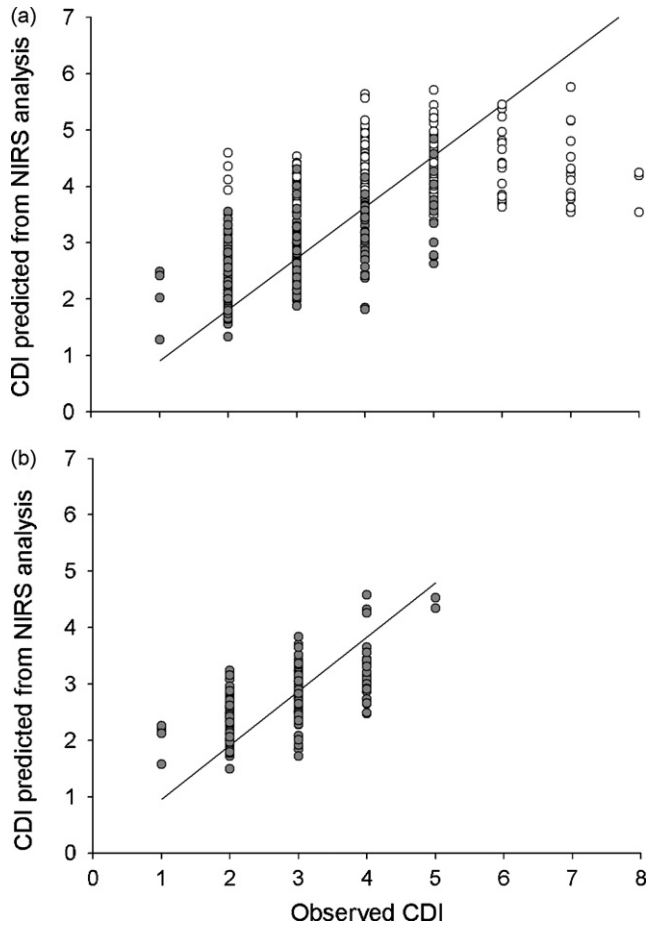

Fig. 3. Performance of NIRS leaf spectra equations with predicted versus observed CDI (a) across both progeny trials for all trees sampled (n = 575) and (b) at Hills only. The regression lines shown are forced through the origin. Symbols indicate trees from Hills (●) and Crabtrees (○).

3.4. Discriminant NIR PLS regression

Analysis of NIR spectra collected from a subset of *E. grandis* trees within both progeny trials suggests that underlying compositional differences between the most resistant and susceptible OPFs could be used to discriminate between these groups (Table 4). When the analysis included only trees from least defoliated and most defoliated OPFs a PLS regression model correctly classified 86% of trees into their respective groups. However, when the group of trees of intermediate defoliation was introduced into the discriminant model the proportion of trees correctly identified as most defoliated and least defoliated declined to 58% and 80%, respectively. The OPFs assigned to the intermediate category were sourced from within the centre of the distribution of mean OPF CDI values and consequently only 57% were correctly assigned to this group with the remainder assigned as either resistant or susceptible trees. The ability to differentiate between resistant and susceptible phenotypes using NIR spectral variation appears to be a based on relatively minor differences between the two mean absorbance spectra (Fig. 4a). The maximum difference appears near 1100 nm where resistant genotypes have around 5% greater mean absorbance values relative to susceptible trees (Fig. 4b).

Table 3
Results from multiple linear regressions for CDI against foliar formylated phloroglucinol concentrations and leaf C:N ratio at two sites. Each regression model was fitted using variables selected by forward stepwise method

Source	d.f.	m.s.	F	P	r^2
Crabtrees					
Whole model	2	11.71	9.52	<0.001	0.058
Residual	277	1.23			
Total grandinal	1	11.69	9.58	0.002	
RT 74	1	11.73	9.62	0.002	
Total extract	1	3.08	2.53	0.113	
Grandinal Pk2	1	2.13	1.75	0.187	
Residual	275	1.22			
Hills					
Whole model	4	12.58	21.62	<0.001	0.219
Residual	290	0.58			
C:N	1	34.89	61.72	<0.001	
Euglobal	1	6.09	10.78	0.001	
Total grandinal	1	4.65	8.23	0.004	
Total extract	1	4.69	8.30	0.004	
SideroxylonalC	1	1.42	2.52	0.114	
SideroxylonalA	1	4.53	8.01	0.005	
Residual	287	0.57			

4. Discussion

This study has demonstrated that significant variation between provenances in susceptibility to defoliation by *P. atomaria* exists in *E. grandis*. The susceptibility of OPFs to defoliation by *P. atomaria* appears to have a strong genetic component as indicated by both the heritability estimate for CDI at Crabtrees and the consistency of OPF rankings by CDI across sites. This ranking correlation was obtained despite the fact that the range of mean CDI for OPFs was relatively small at each site and overall the level of defoliation at Crabtrees was significantly higher than that experienced by the trees at Hills. Previous findings from other *Eucalyptus* plantation species have shown strong negative affects on growth resulting from defoliation by insects (Candy et al., 1992; Elliott et al., 1993; Floyd et al., 2002; Jordan et al., 2002). The strong negative genetic correlation between wood volume and CDI at Hills could be interpreted as a reduction in growth with an increasing susceptibility to defoliation. However, other studies have found a negative correlation between oviposition rate of paropsine chrysomelids and tree height (Ohmart et al., 1984; Raymond, 1995; Rapley et al., 2004a,b). This has been interpreted as being behavioural selection by chrysomelid adults for slower growing trees due to a greater proportion of preferred leaf age class present in the slower growing crowns (Raymond, 1995). Thus, in this instance, cautious interpretation of cause and effect in the relationship between resistance to insect defoliation and growth rate is required.

We also detected an overall increase in susceptibility to defoliation from north to south in terms of geographic origin of *E. grandis* in the progeny trials. Based on compiled data from published studies of herbivory rates, herbivory has been reported to be generally greater in tropical latitudes than at more temperate latitudes (Coley and Aide, 1991; Coley and Barone, 1996). Andrew and Hughes (2005) tested this latitudinal trend by directly assessing herbivory damage within a species across its latitudinal range on the east coast of Australia but failed to detect such a predicted relationship. This hypothesised global cline in herbivory is attributed to the more stable temperatures and rainfall in the tropics relative to temperate climes where seasonality limits the annual duration of insect activity. In eucalypt plantations in more temperate parts of its range *P. atomaria* has been shown to complete two generations per year (Carne, 1966). In plantations in warmer subtropical parts of its range, *P. atomaria* beetles are active for much longer, complete up to four generations per year and the mild climate enables late-instar larvae to survive through winter thus supplementing the overwintering adult population (Carnegie et al., 2005). Thus, *E. grandis* could conceivably suffer greater amount of defoliation from insect herbivores in the northern extremes of its distribution.

Table 4
NIRS discriminant equations based on *a priori* groupings of susceptibility to defoliation with evaluation of the equations performance via classification of *E. grandis* into defoliation categories

Groups[a]	*n*	S.E. cross validation	r^2	Wavelengths range (nm)	Number wavelength bands	Maths treatment[b]
L–M	201	0.082	0.67	408–2492	256	2,4,4,1
L–M–I	301	0.129	0.42	408–2492	256	2,4,4,1

Performance of discriminant equations

Equation	Initial groupings	Trees assigned by discriminant equation			Total
		Least	Most	Intermediate	
L–M	Least defoliated	205	36	–	241
	Most defoliated	18	140	–	158
L–M–I	Least defoliated	178	23	40	241
	Most defoliated	12	118	28	158
	Intermediate	34	49	93	176

[a] See Section 2 for origin of groupings. Groups are least defoliated (L), most defoliated (M), intermediate defoliation (I).
[b] See Table 1 for explanation.

Such pressure from herbivores may explain the concomitant trend for leaves in the tropics having a greater diversity of defences as well as higher levels of defence than have leaves in temperate latitudes (Coley and Aide, 1991). However, this generalisation was derived from summaries of published results on taxonomically diverse species from a great range of habitats rather than inferred from intraspecific variation in plant defence. In an experiment akin to testing latitudinal gradient effects, Salmore and Hunter (2001) detected a decrease in defensive alkaloid concentrations in *Sanguinaria canadensis* (Papaveraceae) with increasing elevation. If increased levels of defence are a response to increasing herbivore pressure then our results suggest that *E. grandis* from northern provenances suffers greater pressure from herbivores. Such evolutionary shifts towards increased resistance to insect herbivores with decreasing latitude may be extremely subtle and easily disguised by large variation in resistance within provenances. This is particularly true if, as is the case in both experimental plantations, many of the provenances are represented by less than three OPFs (This applies to 16 and 11 provenances at Crabtrees and Hills, respectively). Our ability to detect this relationship may only have been made possible by the large latitudinal range of *E. grandis* (approximately 15°) and the statistical power of large-scale progeny trials. Large, replicated trials enable many families from multiple locations within a species range to be grown in a common environment and exposed to a relatively consistent level of herbivory at site thus maximising genetic variation whilst minimising confounding environmental variation.

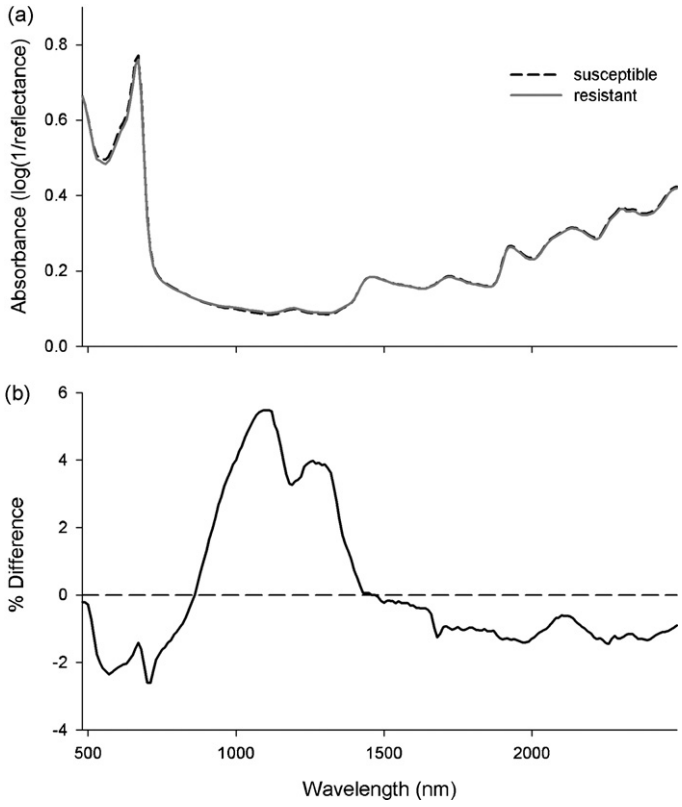

Fig. 4. (a) Mean NIR leaf spectra from groups of ***Eucalyptus grandis*** varying in susceptibility to defoliation by ***P. atomaria*** and (b) the percentage difference in absorbance between the two groups calculated as $100 \times (Abs_{(least\ defoliated)} - Abs_{(most\ defoliated)})/Abs_{(least\ defoliated)}$.

The relationship between leaf composition and CDI proved elusive in this study. Using individual FPCs or total FPC content as independent explanatory variables accounted for little variation in multiple linear regression models of CDI at either of the sites. This suggests *P. atomaria* feeding or oviposition is relatively unaffected by variation in FPC content between hosts in *E. grandis* which is consistent with all other putative chemical defences previously examined in relation to paropsines on eucalypts (Fox and Macauley, 1977; Morrow and Fox, 1980; Patterson et al., 1996; Lawler et al., 1997). Concentrations of the main FPC compounds from *E. grandis* recorded from the progeny trials show moderate concentrations when compared to other species from the subgenus *Symphyomyrtus* (Foley and Lassak, 2004) although the intraspecific variability is still high (e.g. total sideroxylonal 0–17 mg/g dry matter). It is possible that FPCs are not present in high enough concentrations in *E. grandis* to negatively affect *P. atomaria* feeding significantly. However, it is equally possible that trees with negligible amounts of FPCs may be defended by factors (not necessarily chemical) not measured in this study.

Based on findings from previous *P. atomaria* feeding studies (Morrow and Fox, 1980; Ohmart et al., 1985a,b), we concluded that increased defoliation at Crabtrees may be due in part to relatively high N content of the leaves which, all other factors being equal, would both increase female fecundity and improve larval performance and survival rates. Ohmart et al. (1985a) demonstrated that *P. atomaria* larval growth on *E. blakelyi* was at optimum rates above a certain threshold of N concentration in the range 1.2–1.7%. In the current study measurements of leaf N from a subsample of fully-expanded leaf from *E. grandis* saplings at Crabtrees suggest that 95% of the trees in the trial have foliar nitrogen

concentrations higher than 1.7% and possibly all trees would exceed this threshold if only expanding foliage was measured. This provides an explanation as to why C:N ratio as a explanatory variable accounted for very little variation in CDI at this site but did account for some variation in CDI at Hills where overall the levels of N were lower and consequently larval survival and performance may have been influenced more strongly by this variable.

Undoubtedly some NIR leaf spectral variation in the samples is a consequence of variation in leaf nitrogen concentration. This variation due to N, in combination with overall site differences in CDI, most likely underpins the ability to successfully predict CDI directly from NIR leaf spectra across two sites. Conversely the inability of an individual site model to successfully predict CDI from spectra at Crabtrees results from a combination of a decrease in the range of CDI values being regressed within a site with the reduction in importance of spectral variation due to N in accounting for defoliation differences. It may also indicate that large quantitative variation in leaf secondary chemistry (such as found in FPCs) does not strongly influence variation in CDI. In a related study, we found that significant variation in resistance to *P. atomaria* in *E. grandis* appeared to be derived from unidentified constitutive defences located in expanding leaf tissues that greatly reduced first instar survival (Henery et al., unpublished manuscript). If such defences are only present in expanding leaves and are not strongly correlated with other variable leaf components that are present in fully-expanded leaves (and consequently variation in these defences will not captured by NIR spectral variation), this could provide a reason for our inability to predict CDI from foliar spectra.

In addition, MPLS regression is not improved by the coarseness of the CDI measure itself which is based on broad percentile groupings that, by necessity, condense finer scale variation in defoliation into a few discreet categories. Such methods, however, are the only viable approach for assessing the large numbers of trees examined here. Although variation in canopy damage due to herbivory by *Paropsis* was detected, the degree of difference in mean level of defoliation between the most heavily defoliated and the least defoliated families within the Crabtrees trial was small as damage was relatively evenly spread across all individuals, with even the least defoliated provenances sustaining considerable damage. This indicates that compositional or other differences between host trees are likely to be subtle; consequently establishing a relationship between foliar composition and CDI damage in *E. grandis* was difficult. Interestingly, the model derived from spectra from both sites does not accurately predict trees that are almost completely defoliated (CDI = 8). One possible explanation is that once defoliation has occurred the resulting flush of regrowth makes the tree more susceptible to further defoliation. This positive feedback mechanism has been implicated as being important in the phenomenon of rural dieback of eucalypts. Repeated cycles of defoliation and regrowth can result in higher overall damage to individual trees because the regrowth foliage is of higher nutritional quality and insect performance is enhanced (Landsberg, 1990a, b). The presence of young regrowth foliage would also be attractive to ovipositing female paropsids (Steinbauer et al., 1998).

In contrast to MPLS regression, discriminant analysis proved to be successful in using spectral variation to separate trees from least defoliated and most defoliated OPFs independent of differences between the two sites. This classification into either group was based on relatively minor differences in the mean absorbance spectra of the two groups (Fig. 4). One of the primary reasons for the success of this approach versus regression against CDI is that leaf spectra directly reflect genetically-based variation in leaf composition and provides no indication of the highly variable insect response (CDI) within OPFs, which is susceptible to environmental influences. In addition, the initial classification into the defoliation categories was based on OPF mean CDI rankings from both sites that were supported by large sample sizes of trees for each OPF. This classification system was robust because of the expansive scale of the trials and repeatable between sites, which minimised the confounding effect of genotype × environment interactions on ranking OPFs by susceptibility to defoliation.

Improvement to the NIR approach to predicting resistance from leaf spectra could be made by directly measuring some aspect of insect response (e.g. oviposition frequencies) and using this as calibration data. This would, however, be worthwhile only if it were known beforehand that this aspect of insect behaviour was an important contributor to the observed pattern. Heritable variation in CDI could be derived from factors such as host preference by ovipositing adult beetles, variation in larval mortality on different hosts, the presence and strength of induced responses in some host genotypes or tritrophic interactions. Thus, detailed studies are required in order to determine which aspect of the insect's life history is affected by less defoliated OPFs before progress can be made using this technique.

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