VARIATION AND INHERITANCE OF RESISTANCE TO DEFOLIATION BY CHRISTMAS BEETLES, *ANOPLOGNATHUS* SP (LEACH) IN EUCALYPTS

Mervyn Shepherd[1*], Jose X Chaparro[2] & Robert Teasdale

ForBio Inc, 52 Douglas St, Milton, Brisbane, Queensland 4064, Australia
[1)] Current Address, Cooperative Research Centre for Sustainable Production Forestry, Centre for Plant Conservation Genetics, Southern Cross University, Military Rd, Lismore 2480, New South Wales, Australia. phone: +61 2 66203412, fax: +61 2 66222080, e-mail: mshepher@scu.edu.au
[2)] Current Address, USDA Horticulture Research Laboratories, 2120 Camden Rd Orlando, Florida, USA
[*)] Corresponding author

Received March 9, 1999; accepted October 16, 1999

ABSTRACT

Natural populations of eucalypts exhibit variation to defoliation by adult Christmas beetles at the species, subspecies and intra-tree levels. In this study we examine experimental populations of eucalypts in order to establish the origins of variability and the importance of genetic factors in host-insect interactions. A simple laboratory bio-assay was developed to overcome difficulties with measurement of defoliation in the field. Leaf area consumed (LAC) by beetles was used to estimate variation within and between several families of interspecific hybrid eucalypts. Extreme susceptibility to defoliation resulted from interspecific hybridisation where individuals from non-adapted parental species introduced susceptibility into a family. Host genotype, at the family and individual level were found to be significant in determining the amount of foliage consumed in bio-assays. Ranking of resistance classes in bio-assays was shown to correspond with field assessments of defoliation.

Key words: *Eucalyptus, Anoplognathus*, insect resistance, genetic variation, bio-assay

INTRODUCTION

Christmas beetles of the genus *Anoplognathus* (Leach) (Coleoptera: Scarabaeidae) inhabit the coastal and sub-coastal regions of eastern Australia (OHMART & EDWARDS 1991). The adult beetles feed on the leaves of trees, mostly eucalypts, while the larvae are soil dwelling and feed on soil organic matter, the roots of grasses, and the finer roots of eucalypts (CARNE 1957). Damage by the adults can be severe in some eucalypt species, restricting the use in Australia of some potentially valuable forestry species such as *Eucalyptus dunnii* (HILLIS & BROWN 1984, OHMART & EDWARDS 1991). Repeated canopy defoliation by adults has been found to be an important factor in rural dieback, a syndrome of premature, rapid decline of eucalypts and other Australian native species (STONE *et al.* 1998, KILE 1981).

Defoliation by adult Christmas beetles has attracted attention for study as a model of plant-insect interactions as the adults have shown a marked preference for certain species and individuals within populations of *Eucalyptus* (PRYOR 1953; EDWARDS *et al.*, 1993, personal observations).

A preference for feeding on some species of eucalypts by Christmas beetles was observed in young plantations in the Coffs Harbour region (CARNE *et al.*, 1974). Plantations of *E. saligna* were rarely attacked and plantings of *E. pilularis* were never attacked by the two major species of beetle found in this investigation, *A. porosus* and *A. chloropyrus*. Stands of *E. grandis*, however, a close relative of *E. saligna* were often attacked by both species of beetles but the extent of herbivory by *A. chloropyrus* was dependant upon the availability of a preferred food source, *E. dunnii*, nearby.

Interspecific hybridisation was thought to be the cause of variation in resistance to defoliation in ornamental plantings of *E. rubida,* and *E. macarthuri* (PRYOR 1953). Trees with unusual resistance properties for each species were determined to be of hybrid origin and had inherited immunity from *E. maculosa* in the case of *E. rubida*, and susceptibility from *E. viminalis* or *E. rubida* in the case of *E. macarthuri*. As resistance or susceptibility of the hybrids was equivalent to resistance or susceptibility in the donating parent, PRYOR suggested resistance were probably inherited in a simple dominant mode (PRYOR 1953).

Intraspecific variation to defoliation by Christmas beetles was reported in six species of eucalypts growing in forest remnants and in isolated stands in paddocks and roadsides in south-east Australia (EDWARDS *et al.*

1993). Trees of the same species growing side by side were observed to sustain different degrees of herbivory. An association was found between foliar terpene composition and herbivory levels, indicating a possible biochemical mechanism mediating the interaction between beetles and their hosts.

Within-tree variation to Christmas beetle defoliation has also been observed (EDWARDS 1990). The authors suggested differences in susceptibility between branches of the same individual plant probably arose through somatic mutations early in the development of a branch. This observation suggests a mechanism for the source of variation underlying intra- and inter-specific variation in which a single locus may be sufficient to confer resistance or susceptibility.

The current investigation was stimulated by observations of extreme variation in resistance to Christmas beetle defoliation amongst individuals of an open-pollinated interspecific hybrid eucalypt family in an experimental planting at Gympie, Australia (SHEPHERD 1998). In this experiment, each individual was replicated by clonal propagation and planted out at randomised locations within plots. Trees were assessed visually for defoliation and categorised according to the proportion of canopy removed. It was found that some clones consistently experienced very high levels of defoliation, with almost complete canopy removal, whereas other clones remained largely undamaged. This suggested the beetles were not selecting trees randomly, but rather their choice was determined by tree genotype.

To advance our understanding of the genetics of resistance to Christmas beetle defoliation in eucalypts, we explored the use of laboratory bio-assays. It was hoped bio-assays would overcome many of the problems of assessing defoliation in the field due to difficulties in objectively quantifying defoliation, and fluctuations in environmental conditions (SHEPHERD 1998). Furthermore, a more detailed understanding of the host-population genetic structure was required. In this paper we report on a field study and a series of laboratory bio-assays to investigate the significance of genic effects upon variation to herbivory and to establish the origin of susceptibility in two generations of eucalypts. We use genetic markers to examine the genetic structure of an open-pollinated family of eucalypts and relate within-family genetic structure with levels of foliage consumption in a bio-assay.

METHODS

Plant material

Plant material consisted of an open-pollinated (OP) family from *E. grandis* and four second generation controlled-cross families (Table 1). The OP family was derived from a seed orchard based on a single *E. grandis* clone (Coffs Harbour selection) and 25 *E. urophylla* clones (M7–1 Orchard Aracruz Forestal S.A. Brazil) (GRATTAPAGLIA *et al.* 1996). Two seedlots collected from the *E. grandis* clone were imported into Australia by Queensland Forest Service (QFS) in 1986. Rooted cuttings (ramets) from seedlings (ortets) from the first importation were planted in QFS field experiment 363 (QFS 363) at Toolara, Queensland, Australia in January 1989. Seedlings from the second seedlot were planted in pots and maintained as hedges at QFS nursery until transfer to a shadehouse at Bureau of Sugar Experimental Stations at Brisbane (Queensland,

Table 1. Second generation controlled-cross families used in laboratory feeding trials.

Family	Cross[1]	Female parent[2]	Male parent[2]	Seedlot No. &/or provenance of male parent	Source of foliage for bio-assay	No. of individuals with coppice	No. of individuals with adult foliage
	OP	G44[3]	25 U[3]	Aracruz selects	QFS 363[4]	0	41
	OP	G44	25 U	Aracruz selects	Potted seedlings	0	70
7	BC to *E. urophylla*	GU11	U16	14531 Mt Egon, Flores Is.	DMSF[5] 50 A&B	8	33
9	BC to *E. grandis*	GU11	G22	Mt Lewis	DMSF 50 A&B	5	37
10	BC to *E. grandis*	GU11	G17	13289 Mt Lewis	DMSF 50 A&B	11	21
68	F2 self	GU11	GU11	Dongmen select	DMSF 50 A&B	12	17

[1] OP = open pollination; BC = backcross; Self = self pollination

[2] A G or U followed by a number indicates a selection of *E. grandis* or *E. urophylla*; GU represents an *E. grandis* × *E. urophyla* hybrid

[3] Seed was obtained from ramets of a single *E. grandis* selection, G44, planted in a ratio of 1 : 3 with ramets of 25 *E. urophylla* selections (GRATTAPAGLIA *et al.* 1996).

[4] QFS 363 = Queensland Forest Service Experiment 363, Gympie, Australia

[5] DMSF = Dongmen State Forest Farm Experiment 50 A & B, Guangxi Province, China

Australia) in 1995. Seventy surviving ortets were maintained as hedges in pots in Brisbane, Australia.

Four second generation controlled-cross families were grown at Dongmen, Southern China. These four families consisted of a self and three families which were backcrossed (BC) to select individuals of the pure parental species (Table 1). The four families shared as a common parent, GU11 (*E. grandis* × *E. urophylla* selection 11), which is a select interspecific-hybrid from the M7–1 seed orchard. Foliage from each individual from all four families was collected from two contiguous trials. Fifteen individuals from each family were obtained from a single-tree plot experiment testing 70 families. The remaining samples were collected from a randomised complete block (RCB) design trial in which the trees were arranged in five replicates of seven-tree row-plots. Only juvenile foliage was available for some progeny in each family as some trees had been felled to induce basal coppice.

In selecting foliage for feeding trials, newly emerged or older leaves from the previous growth season were avoided. Foliage was collected just prior to use or maintained as fresh as possible by refrigeration. Foliage from the self and backcross families were collected on the 7–8th December 1995 in China and shipped to Australia chilled in plastic bags for laboratory feeding trials on the 13th December 1995.

RAPD genotyping and pedigree verification

DNA preparation and the generation and analysis of RAPD markers is given in SHEPHERD (1998). Essentially, methodology followed that of BOUSQUET *et al.* (1990) for DNA preparation and GRATTAPAGLIA & SEDEROFF (1994) for RAPD assays. In order to identify non-hybrid progeny in the open pollinated family, OP material from the field trials and ortets maintained as hedges were screened with four co-dominant RAPD marker pairs, known to be heterozygous in the maternal parent (GRATTAPAGLIA & SEDEROFF 1994). Four co-dominant markers gave ca. 94% probability of identifying the maternal genotype. Ie If selfing occurred, the probability of a maternal heterozygote genotype in the progeny was 0.5, hence with 4 markers $(1 - 0.5^4)$ of detecting all self progeny. OP progeny were also screened for the presence of a *E. urophylla* specific RAPD marker to confirm their interspecific status (RAPD data provided by Forbio Research P/L, Brisbane, Australia, [SHEPHERD 1998]).

Ramets from QFS 363 were genotyped for 30 RAPD markers to verify which were genetically identical. The markers selected for analysis were reliable markers which segregated in Mendelian ratio's in a mapping population based on the same family (SHEPHERD *et al* 1999). A genetic distance matrix was generated based on a simple matching coefficient (SNEATH & SOKAL 1973). Individuals sharing greater than 95% similarity for marker genotypes were considered identical.

The parentage of GU11 was verified by testing GU11 self progeny for the presence of 4 co-dominant RAPD makers known to be present in the putative maternal parent of GU11 (see above). It was only possible to identify an allele for two of the 4 co-dominant RAPD marker pair in the self progeny of GU11. These markers were expected to segregate in 3:1 presence to absence ratio in this family.

Field assessment of insect resistance

Field assessment of resistance on the ramets from the OP family was carried out at QFS 363 over two insect seasons (Nov–Jan 1994 & 1995). Ramets were randomly located in 9 plots of 48 trees with a 4.2 × 2.5m spacing. Two plots were located within a RCB design trial with nine other species of eucalypts. The remaining seven plots were planted as a block contiguous with the RCB trial. Trees were classified as (1) resistant, (2) intermediate or (3) susceptible based on a visual assessment of beetle damage to the tree canopy (SHEPHERD 1998). In the second year of assessment, classification was carried out by two assessors independently.

Collection of adult Christmas beetles

Christmas beetles were captured from the ground or from an elevating platform using insect nets at QFS field experiment 363. A sample of 892 adult beetles was captured in 1994. Beetles belonged predominantly to the single species *Anoplognathus porosus* (90%), however, there was also a small proportion of *A. boisduvali* (8%) and *A. pallidicollis* (2%). Beetles were kept in holding cages and fed foliage of either *E. dunnii* or *E. grandis* × *E. urophylla* prior to laboratory feeding trials.

Implementation of laboratory feeding trials
General implementation

To obtain an estimate of leaf area consumed (LAC), detached leaves were digitised to quantify surface area prior to and after feeding trials. Consumption was expressed as the difference in the two areas in units of pixels per cage. Detached leaves were first photocopied to give greyscale images. Greyscale images were converted to binary images and quantified using image analysis software (Image v 4.1 shareware from National

Super Computing Centre America) following scanning on a flat bed scanner at a resolution of 100 dpi. Cages were constructed from opaque, round plastic food containers (ca. 70 mm dia × 50 mm height) by puncturing the lids for ventilation. Experiments used a RCB design, where three blocks of treatments were run on three consecutive days or nights. Sufficient foliage was supplied so that beetles could not consume all the available foliage when left to feed overnight (14 hrs). This was found to be a ratio of six leaves to five beetles for susceptible foliage when food was withdrawn from beetles ca. eight hrs prior to the start of each trial. At the completion of a trial, beetles were returned to a large pool from which a fresh random selection was made for following trials.

Statistical analysis

A mixed model ANOVA was used to test family and individual within-family effects amongst the four second generation families (Model3 in Harvey, WR 1990 LSMLMW and MIXMDL Software, PC2 version). Family was treated as a fixed affect whereas individual within-family and block were considered random. The model also included a covariate, number of surviving beetles. Where the covariate explained a significant proportion of the variation in LAC, adjusted means were calculated prior to multiple comparison testing. All other ANOVA or independent t tests were performed using Statistica v4 for Windows software (Statsoft, Tulsa OK). Multiple comparison testing followed the guidelines of MILLIKEN and JOHNSON (1984) where Scheffe's test was applied to unplanned comparisons and least significant difference tests were applied to planned comparisons where F tests were significant. Two sided p-values are reported for t tests and were considered highly significant if <0.01 and significant if >0.01 but <0.05.

RESULTS

Paternity testing and verification of identity of field material

To minimise the possibility of error due to mis-identified material in field plantings, 192 ramets derived from OP ortets planted in the field experiment QFS 363 were typed for 30 RAPD markers. Five percent of the ramets were incorrectly identified in the trial and were re-grouped on the basis of genetic marker identity to give a total of 53 unique clones. The number of representatives for each clone ranged from one to 11.

A representative from 41 of 53 clones as well as each of the 70 OP ortets maintained in pots, were genotyped for a set of four co-dominant RAPD markers to establish which genotypes were non-hybrids. This gave around a 94% probability (see methods) of detecting all individuals with a maternal genotype. The co-dominant RAPD genotype of the maternal *E. grandis* parent for at least one of the four markers was identified in 25 of the 111 individuals. This indicated these individuals were not interspecific hybrids but probably selfs of the maternal parent or progeny of an outcross to another *E. grandis*.

The parentage of GU11, the interspecific parent of the second-generation controlled-crosses, was also verified with genetic markers. Only two of the four co-dominant markers heterozygous in the *E. grandis* parent of GU11 were identified with certainty in a sample of 12 offspring from the GU11 self-family (Family 68). The presence of one allele from each of the two loci segregating in an approximate 3:1 presence to absence ratio was consistent with the expected segregation ratio for a dominant marker. Several of the progeny possessed a marker specific to *E. urophylla*, supporting the presumption that GU11 was an interspecific hybrid.

Open-pollinated family-structure and field resistance to Christmas beetle defoliation

Previous field studies had indicated Christmas beetles sought out particular clones to feed upon in the field (SHEPHERD 1998). Field resistance ratings from the 1993/1994 season were re-examined with ramets re-classified on the basis of genetic marker identity into sub-family populations (Figure 1). Resistance ratings for some clones were variable, however, the repeatibility for other clones was high. For example, Groups 7 and 8 had 11 and 6 ramets respectively, all were rated as resistant. Groups that were resistant appeared to largely comprise those clones identified as *E. grandis*

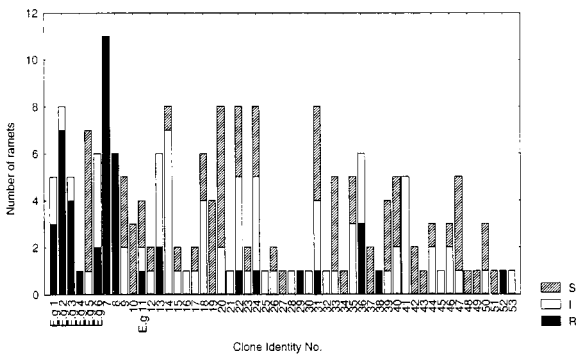

Figure 1. Field resistance ratings for ramets QFS 363 field trial arranged into groups on the basis of genetic marker identity. Groups of ramets that were determined to be *E. grandis* are prefixed within an E. g. Groups without a prefix were *E. grandis* × *E. urophylla* hybrids.

(putative selfs). Hybrid clones on the other hand, appeared more variable, and consisted largely of susceptible or intermediate ramets.

Group 7 appeared to be an exception, as all of its representatives were resistant but it was not possible to confirm that Group 7 was an *E. grandis* clone using co-dominant RAPD markers. The probability of detecting non-hybrids using this screen was 94%, hence, in a sample of 111 individuals it was expected that ca. five would go undetected. It is likely that Group 7 is an *E. grandis* clone but was not detected in the screen using co-dominant RAPD markers. This was supported by the grouping of a representative of Group 7 upon a branch containing *E. grandis* individuals in a cluster analysis based on 30 RAPD markers (data not shown). Nonetheless, to be conservative in further hypothesis testing, Group 7 was treated as hybrid.

To test whether sub-family structure significantly influenced resistant ratings, an average field rating was determined for 28 of the 53 groups of ramets that contained three or more representatives. The average field ratings for the seven *E. grandis* groups were compared in a t test to those of 21 hybrid groups (Independent t test: Mean field resistance rating for a sub-family population ± SD: *E. grandis* 1.64 ± 0.68, Hybrids 2.36 ± 0.50, t-value = –3.01, df = 26, p-value < 0.01). This indicated that *E. grandis* clones were significantly less defoliated than hybrids.

Laboratory feeding trials comparing *E. grandis* and interspecific hybrid progeny

Foliage of 70 OP ortets was tested in a bio-assay to evaluate the importance of family and sub-family population structure on resistance. On the basis of RAPD genotyping, 17 progeny were *E. grandis* whereas 53 were interspecific hybrids (data not shown). The means for each individual were averaged over three replicates and sub-family populations compared (Mean LAC ± SD for *E. grandis* 73416 ± 23441 and hybrids 98724 ± 33817, t-value = –2.87, df = 68, p-value < 0.01). Hybrids were significantly more susceptible than *E. grandis* individuals, which was consistent with the field observations for the equivalent sub-family populations. Hybrids and *E. grandis* were treated as separate populations in further analysis of OP progeny.

Consumption of *E. grandis* and hybrid foliage was analysed separately for individual within-family and replication effects (Table 2). The individual within sub-family population effect was highly significant for both *E. grandis* and hybrid populations but replicate effect was not. Multiple comparison testing of the means indicated that both *E. grandis* and hybrids consisted of several overlapping sub-groups (data not shown).

Table 2. Analysis of variance of LAC in a bio-assay for two sub-family populations of a open pollinated eucalypt family.

Population	Source	df	MS Effect	MS Error	df Error	F-value	p-value
E. grandis	Individual within-family	16	1648E6	400E6	32	4.11	0.000
	Replicate	2	1203E6	400E6	32	3.01	0.064
Hybrids	Individual within-family	52	3447E6	1153E6	104	2.99	0.000
	Replicate	2	1713E6	1153E6	104	1.49	0.231

Table 3. Analysis of variance of LAC for family and individual within family effects in bio-assay on four second-generation hybrid-families.

Foliage Class	Source	df	SS	MS	F-value	p-value	Error line
Adult	Family	3	64378E6	21459E6	9.79	0.000	G w/n F
	Genotype w/n Family	104	227945E6	2191E6	3.20	0.000	error
	Block	2	817E6	408E6	0.60	0.552	error
	Regression insect No.[1]	1	15139E6	15139E6	22.10	0.000	error
	Error	206	141135E6	685E6			
Juvenile	Family	3	53106E6	17702E6	4.14	0.014	G w/n F
	Genotype w/n Family	32	136782E6	4274E6	7.17	0.000	error
	Block	2	4170E6	2085E6	3.50	0.036	error
	Regression insect No.[1]	1	654E6	654E6	1.10	0.299	error
	Error	65	38775E6	596E6			

[1] Count of surviving insects used as a covariate

Table 4. Ranked means and least significant difference test (LSD) for LAC of adults and juvenile foliage from four second generation families.

Foliage type	Population	n[1]	LAC[2] (pixels)	LSD[3]			
Adult	Backcross to *E. grandis* Family 10	21	65786	a			
	Backcross to *E. grandis* Family 9	37	68665	a			
	Backcross to *E. urophylla* Family 7	33	94939	b			
	Selfed F2 Family 68	17	99586	b			
Juvenile	Backcross to *E. grandis* Family 10	11	84484	a	b		
	Backcross to *E. grandis* Family 9	5	87984	a	b	c	
	Selfed F2 Family 68	12	118300		b	c	d
	Backcross to *E. urophylla* Family 7	8	142000			c	d

[1] n = number of genotypes.
[2] LAC for adult foliage adjusting for surviving beetle numbers.
[3] Treatments sharing the same letter where not significantly different at p-value = 0.5.

Bio-assays to evaluate family and individual within-family effects upon defoliation in second generation families

Four second generation families were assayed in a single bio-assay to assess the effect of family and individual within-family on consumption. As some trees in each family had been harvested, only foliage from basal coppice was available for analysis. The foliage on coppice is juvenile as coppice originates from quiescent buds of a juvenile physiological age (JACOBS 1955). To test whether beetles found juvenile foliage more palatable than the adult foliage the means for the two ontogenetic groups were compared within each family (data not shown). For all four families, a significantly greater amount of juvenile foliage was consumed (Figure 2). Hence for further testing, data for different ontogenetic stages were analysed separately.

Family and individual within-family effects for each ontogenetic class of foliage were examined in an ANOVA (Table 3). Both family and individual within-family effects were significant for both classes of foliage. A covariate, number of surviving beetles, was also significant in the analysis of adult but not the juvenile foliage. Multiple comparison testing of family means for adult foliage indicated that the backcross to *E. grandis* families (Families 9 and 10) were not different to each other (Table 4). The backcross to *E. urophylla* family (Family 7) was also not different to the self family (Family 68), however, more foliage was consumed in both of these families compared with the backcross to *E. grandis* families. Least significant difference tests upon the juvenile foliage showed less differentiation into distinct groups of families. This may have been a function of smaller sample sizes available for testing in this foliage class (Table 4).

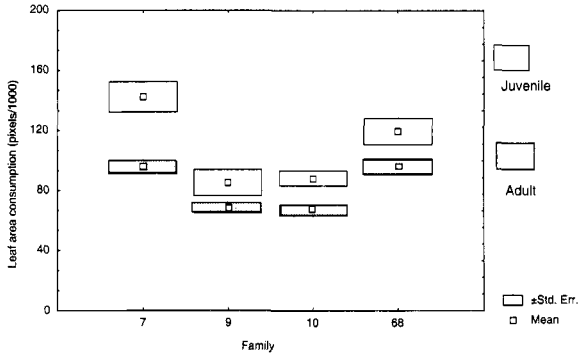

Figure 2. Comparison of LAC for adult and juvenile foliage from four second generation controlled cross hybrid eucalypt families. Families 9 and 10 are backcrosses of a *E. grandis* × *E. urophylla* hybrid (GU11) to *E. grandis* individuals. Family 68 was a self family of GU11 and family 7 was a GU 11 backcrossed to a *E. urophylla*.

DISCUSSION

Genetic control of resistance to Christmas beetle defoliation

We report on a field study and laboratory feeding trials to investigate genetic control of resistance to Christmas beetle herbivory in two generations of eucalypt families. Genetic marker analysis was used to determine sub-family structure in the first generation OP family. Analysis of RAPD markers indicated the family largely consisted of *E. grandis* × *E. urophylla* hybrids which was expected as the material originated from a seed orchard designed to promote interspecific hybridisation between these two species. However, the family also included a smaller proportion (22%) of pure *E. grandis*

progeny, probably selfs of the seed parent. *Eucalyptus grandis* has a mixed mating system and the level of selfing detected in this family was consistent with the level previously reported for this species (MORAN & BELL 1983).

The identification of family sub-structure provided a possible explanation for the observation in previous field studies where several groups of clones were uniformly resistant whereas others were variable and susceptible (SHEPHERD 1998). In the present study, we re-examined this field data in light of the new information on sub-family population structure and show that a strong relationship exists between sub-family structure and field resistance ratings. It was found that clones that were resistant in the field largely corresponded with clones that where identifiable as pure *E. grandis*. Hence, from field studies it appeared that susceptibility to Christmas beetle defoliation in hybrids originated from *E. urophylla*.

The relationship between interspecific hybridity and resistance to Christmas beetle defoliation was confirmed in a bio-assay on a large independent sample of OP individuals. In this bio-assay, individuals classified as interspecific hybrids on the basis of genetic markers, were more susceptible than *E. grandis* individuals. This was consistent with the field study where *E. grandis* clones were on the whole repeatably scored as resistant. Furthermore, the bio-assay demonstrated that resistance assessed in the field could be correlated with resistance detectable under laboratory conditions as sub-family populations performed relative to each other. The bio-assay also showed that material grown under different environmental conditions exhibits the same relative resistance levels.

Laboratory feeding trial on controlled-cross, second-generation families demonstrated that resistance at the family level could be varied dependant upon the parentage of a cross. Furthermore, inferences about the resistance phenotype of these parents was consistent with resistance phenotypes for sub-family populations and species level resistance observed in first generation material, demonstrating that family resistance levels were predictable. Ie. *E. grandis* paternal parents give rise to families which are significantly more resistant than families with *E. urophylla* paternal parents. An interspecific hybrid parent was equivalent to a susceptible parent, *E. urophylla*.

Field assessment of hybrid clones suggested there was more intra-clonal variability in resistance than *E. grandis* clones. This was most likely to be attributed to the difficulty in assaying the susceptible phenotype in the field. Resistant phenotypes were readily identifiable anywhere in the trial. Fortuitously, the trial was located in a relatively isolated location away from other natural food sources, and, in the years field assessment was carried out, beetle population density was high so that the likelihood of trees escaping exposure to herbivory was low. Susceptible phenotypes were more difficult to rate and appeared to be subject to more micro-site variation. Exposed trees at the edge of the trial tended to grow less vigorously and had less dense canopy cover, which may have confounded estimates of defoliation and led to greater variation in field scores.

Bio-assays allowed defoliation to be quantified and the design of experiments to specifically test for genetic effects. In the bio-assay assessing 70 OP individuals, as well as assessing sub-family population effect, the effect of individual genotype within a sub-family population was tested and found to be significant in both the *E. grandis* and hybrid populations. This indicates the potential to assess within sub-family determinants of resistance through approaches such as genetic mapping. A controlled-cross family where both parental contributions can be evaluated and a cross where a high level of within-family variation is evident, such as Family 7, (GU11 crossed to *E. urophylla*) would appear appropriate.

The greater susceptibility of juvenile compared with adult foliage types, indicated a factor additional to those determining family and individual within-family population effects, influenced resistance. As family ranks did not change significantly when assessed on adult or juvenile foliage, it appears that some factor associated with foliage maturation acted uniformly in all families to either increase the susceptibility of juvenile foliage or decrease the susceptibility of adult foliage. A gene or genes controlling the sclerophyllous nature of foliage or the production of a defensive compound, for example, may have lead to more resistant adult foliage.

Studies of resistance to Christmas beetle defoliation by other researchers has suggested a simple dominant mode of inheritance (PRYOR 1953 EDWARDS 1990). A constraint of our study has been the lack of parental resistance phenotypes for comparison with offspring populations. This has limited our ability to define a specific mode of inheritance for resistance. We can not distinguish between monogenetic and more complex control or clarify intra-locus gene action at this stage. A more detailed genetic model must await the testing of appropriate parental populations.

Variation in resistance between *E. grandis* and *E. urophylla*

Several individuals from the Mt Lewis provenance of *E. grandis* and a single tree from Coffs Harbour have now been tested for resistance to *A. porosus*. Addition-

ally, OP progeny of a third individual from the Gympie provenance were also found to be uniformly resistant in field trials (data not shown). This suggests resistance is widely dispersed in the natural range of *E. grandis*. There are important exceptions, however, as not all populations of *E. grandis* appear to be resistant nor are they uniform. Evidence from other researchers suggests that some provenances of *E. grandis* are susceptible to Christmas beetle defoliation (CARNE *et al.* 1974; STONE *et al.* 1998) and the present study has also shown that there is variation within a population of pure *E. grandis*. Natural selective forces have probably led to variation prevailing at many levels in *E. grandis* populations as Christmas beetles are likely to co-exist with some natural populations and not with others. *Eucalyptus grandis* generally occupies moderately fertile, lower slopes and sheltered valley bottoms, often adjacent to rainforest but is also found on upper slopes and ridgetopes (ELDRIDGE *et al.* 1994). Populations of *E. grandis* from tall closed forests are unlikely to be exposed to high densities of Christmas beetles, as conditions are not conducive to the buildup of larvae numbers (CARNE *et al.* 1974). *Eucalyptus grandis* from drier, open forest sites may represent a source of resistant individuals, as these habitats may support higher beetle numbers. *Eucalyptus urophylla* is not a native of Australia and does not encounter *A. porosus* in natural habitats. Genes conferring susceptibility in native populations of *E. urophylla* may persist in the gene pool as a result of a lack of selective pressure in this species.

ACKNOWLEDGMENTS

The authors wish to thank Queensland Forest Service and Dongmen State Forest farms for access to plant material. We wish to thank M. Podberscek, B. Vincent, D. Hatchman and D. Harrison for assistance in the field, H. Vogel, G. Smail, H. Duong, M. Gibbings, M. Jones and J. Drenth for assistance in the laboratory. Our thanks also to M. DeBarr and R. Wiley for identification of insect specimens, Y. Li and J. Hendrikz for assistance with data analysis, K. Mitchelson and S. Garland for commenting on a draft. We grateful acknowledge helpful communications with R. Floyd, P. Allsopp and M. Zalucki. M. Shepherd was supported by an Australian Postgraduate Award.

REFERENCES

BOUSQUET, J., SIMON, L. & LALONDE, M. 1990: DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. *Can. J. For. Res.* **20**: 254–257.

CARNE, P. B. 1957: A revision of the Ruteline genus *Anoplognathus* Leach (Coleoptera: Scarabaeidae). *Aust. J. Zool.* **5**:88–143.

CARNE, P. B., GREAVES, R. T. G. & MCINNES, R. S. 1974: Insect damage to plantation-grown eucalypts in north coastal New South Wales, with particular reference to Christmas beetles (Coleoptera: Scarabaeidae). *J. Aust. Ent. Soc.* **13**:189–206.

EDWARDS, P. B. 1990: Mosaic resistance in plants. *Nature* **347**:434.

EDWARDS, P. B., WANJURA, W. J. & BROWN, W. V. 1993: Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* **95**:551–557.

ELDRIDGE, K., DAVIDSON, J., HARWOOD, C. & VAN WYK, G. 1994. Eucalypt Domestication and Breeding. Oxford University Press, Oxford.

GRATTAPAGLIA, D. & SEDEROFF, R. 1994: Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: Mapping strategy and RAPD markers. *Genetics* **137**:1121–1137.

GRATTAPAGLIA, D., BERTOLUCCI, F. L. G., PENCHEL, R. & SEDEROFF, R. 1996: Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. *Genetics* **144**:1205–1214.

HILLIS, W. E. & BROWN, A. G. (eds). 1984: Eucalypts for Wood Production, 2nd ed. CSIRO/Academic Press, Melbourne.

JACOBS, M. R. 1955: Growth Habits of the Eucalypts. Canberra Forestry and Timber Bureau, Canberra.

KILE, G. A. 1981: An overview of eucalypt dieback in rural Australia. *In:* (Old, K.M., Kile, G.A. and Ohmart, C.P. Eds). CSIRO, Melbourne, p13–26.

MILLIKEN, G. A. & JOHNSON, D. E. 1984. Analysis of Messy Data, vol.1: Designed Experiments. Van Nortrand Reinhold, New York.

MORAN, G. F. & BELL, C. J. 1983: *Eucalyptus*. In (Tanksley, S. D. and Orton, T. J. eds). Elsevier Science, Amsterdam, The Netherlands, p. 423–441.

OHMART, C. P. & EDWARDS, P. B. 1991: Insect herbivory on *Eucalyptus*. *Ann. Rev. Entomol.* **36**:637–657.

PRYOR, L. D. 1953: Variable resistance to leaf-eating insects in some eucalypts. *Proc. Linn. Soc. New South Wales* **77**:364–368.

SHEPHERD, M. 1998: Genetic determination of insect resistance and foliar oil composition in a *Eucalyptus* hybrid. Ph.D. Dissertation. University of Queensland, Australia.

SHEPHERD, M., CHAPARRO, J. X. & TEASDALE, R. 1999: Genetic mapping of monoterpene composition in an interspecific eucalypt hybrid. *Theor. Appl. Genet.* **99**: 1207–1215.

SNEATH, P. H. A. & SOKAL, R. R. 1973. Numerical Taxonomy. Freeman, San Francisco.

STONE, C. SIMPSON, J. A. & ELDRIDGE, R. H. 1998: Insect and fungal damage to young eucalypt trial plantings in northern New South Wales. *Aust. For.* **61**:7–20.