Agricultural and Forest Entomology (2009), **11**, 107–114 DOI: 10.1111/j.1461-9563.2008.00423.x

Differential defoliation of *Eucalyptus grandis* arises from indiscriminant oviposition and differential larval survival

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Abstract

1 The influence of six open-pollinated families (OPFs) of *Eucalyptus grandis* on both the growth and development of larvae and the oviposition preference of a paropsine chrysomelid (*Paropsis atomaria*) was investigated. The OPFs had previously been identified as differing in their susceptibility to defoliation by *P. atomaria* in forestry progeny trials.

2 Oviposition preference for resistant and susceptible foliage was tested using binary choice tests. These tests did not demonstrate any significant preference for either resistant or susceptible open-pollinated material indicating that adult host preference for susceptible trees was not a likely cause of differential defoliation.

3 Quantification and analysis of growth and development parameters for all larval stages of *P. atomaria* showed that feeding on genetic material identified as resistant resulted in a significant reduction of relative growth rate of first instar larvae and an alteration to normal feeding behaviour. There was also a trend towards increased larval mortality on resistant *E. grandis*.

4 We argue that although the magnitude of these effects was minor, interactions with additional biotic and abiotic sources of mortality in the field have the potential, when magnified over successive generations, to result in significant variation in defoliation of host genotypes in the field.

Keywords Defence, herbivory, host variation, oviposition, plant-insect interactions, plant resistance.

Introduction

Specialist insect herbivores are assumed to be well adapted to deal with any defences the host plant has evolved. Consequently, research frequently demonstrates that varying concentrations of either a single chemical defence or group of related compounds in a host plant has minimal effects on the performance of specialist insect herbivores (Donaldson & Lindroth, 2004; Leimu *et al.*, 2005). It is increasingly clear, however, that many specialists are deleteriously affected by toxins of their host plant, albeit in subtle ways that may not be apparent under some experimental conditions (Agrawal & Kurashige, 2003). Furthermore, connecting variation in secondary metabolite concentrations to insect performance is made more difficult because most plants rely on a diverse array of defensive compounds that deter or kill by acting in a complementary manner or acting synergistically (Pennings & Paul, 1992; Berenbaum & Zangerl, 1993; Hay *et al.*, 1994; Stamp & Osier, 1998; Guillet *et al.*, 2000; Hummelbrunner & Isman, 2001; Calcagno *et al.*, 2002; Dyer *et al.*, 2003; Macel *et al.*, 2005). Apart from a few exceptional well-studied plant–insect herbivore interactions (Zangerl & Berenbaum, 1993; Osier & Lindroth, 2001; Haukioja, 2003), the extent to which any one compound or group of compounds in a host plant dictates the performance of co-evolved herbivorous insects is unknown.

Paropsine leaf beetles (Coleoptera: Chrysomelidae) are primarily specialist herbivores on the genus *Eucalyptus* with both larvae and adults feeding on eucalypt foliage. Over the past 30 years, investigation of the interaction between paropsine chyrsomelids and *Eucalyptus* species has become more economically important as a result of the detrimental effect of paropsines on the expanding eucalypt plantation forestry in Australia. Initial work in this area was focused on determining the basis of differential defoliation of eucalypt species by investigating both rates of oviposition and larval performance on various host species. These studies showed that oviposition preference for a host is not necessarily correlated with larval performance (and thus host plant quality) (Carne, 1966; de Little & Madden, 1975; Steinbauer *et al.*, 1998), suggesting greater defoliation of non-preferred host

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species may be largely derived from increased larval survivorship and subsequent consumption of foliage (Baker *et al.*, 2002). Mortality of paropsine larvae does appear to differ consistently between host species and, in common with other insect larvae (Zalucki *et al.*, 2001), can be quite high (e.g. >70% for *Chrysophtharta bimaculata* on *Eucalyptus regnans*), particularly in the first instar (de Little *et al.*, 1990; Baker *et al.*, 2002). This indicates that larval mortality may be critical in determining the severity of canopy defoliation.

In contrast, less is known about the basis of intraspecific variation in defoliation by paropsines. Patterson *et al.* (1996) did not identify differential larval mortality as a causal factor in explaining variation in defoliation of *E. regans* genotypes. In addition, this study was unable to demonstrate that larval growth and development are affected by feeding on genotypes identified as 'resistant' on the basis of consistently lower levels of defoliation in field trials. Similarly, studies on paropsine larval feeding across both multiple host eucalypts and within species have been unable to demonstrate that larval growth and development are correlated with any host plant secondary compounds (Fox & Macauley, 1977; Morrow & Fox, 1980).

Despite the difficulties in identifying causal factors that explain a eucalypt's resistance to defoliation by paropsine chrysomelids, it is likely that intraspecific variation in the resistance to paropsines is as a result of heritable plant traits (Raymond, 1995; Rapley *et al.*, 2004; Henery *et al.*, 2008a). To determine what aspect of the insect's life history is influenced by the interaction with host genotypes resistant to defoliation requires ecologically relevant and comprehensive studies. In this study, we sought to compare larval growth and development of *Paropsis atomaria* (Olivier) on foliage from open-pollinated families (OPFs) of *Eucalyptus grandis* (Hill ex Maiden). These families had previously been identified as being less or more susceptible to defoliation by this specialist herbivore in trial plantations (Henery *et al.*, 2008a). We had no *a priori* knowledge of how heritable traits of less susceptible trees affected the life cycle of *P. atomaria* and consequently gave rise to differential defoliation. Therefore, we chose to carry out our experiments in a controlled environment using leaf material from trees grown in pots in a greenhouse. By ensuring experimental conditions were as homogeneous as possible, we hoped to maximize our ability to detect subtle effects of host plant differences on all life history stages tested. In addition to a larval growth study, we undertook a binary choice experiment to detect whether adult *P. atomaria* females differentiated between OPFs less or more susceptible to defoliation when selecting sites for oviposition.

Materials and methods

Plant material

We measured growth and development of cohorts of *P. atomaria* larvae fed OPFs of *E. grandis* identified in field trials as varying in resistance to defoliation. The three most 'resistant' (least crown damage) and three most 'susceptible' (most crown damage) OPFs identified from matched progeny trials (Henery *et al.*, 2008a) were selected for the bioassay experiments. Approximately 40 seedlings from each of the selected OPFs were established outdoors in pots at the plant culture facility, School of Botany and Zoology, ANU, Australian Capital Territory, Australia in March 2003. The seedlings were well watered and fertilized and by January 2004 had reached sufficient canopy size (>2 m tall) to carry out bioassays.

Insect cultures

Clutches of *P. atomaria* eggs were collected in the field on *E. grandis* in northern New South Wales, Australia and allowed to develop through to adulthood. Adults were housed in cages 0.4 × 0.4 × 0.5 m and supplied regularly with fresh *E. grandis* foliage in containers of water. The cultures were checked every 1–2 days, eggs removed, and the foliage replaced as required. Egg batches were placed together in glass containers and when several hatched simultaneously (generally >5) they were combined in containers until the neonates had fed on the chorion and started moving in search of food. The inherent mixing of wandering neonates within the hatching container effectively randomized larvae between each bioassay (which was initiated using a pair of trees) and between replicates within each tree.

Experimental design

Five trees (replicates) per OPF were selected at random. Assays were conducted in pairs each using a tree from a resistant and a susceptible OPF. *Paropsis atomaria* larvae are gregarious and larvae generally feed down the sequence of expanding leaves from the tip of a branch, consuming each leaf and moving onto more mature leaves as the cohort changes instars. In order to replicate natural conditions as fully as possible, an assay for each tree consisted of five replicated cohorts of 20 newly hatched first instar *P. atomaria* larvae placed on five separate cut branchlets from each tree, suspended in water. The branchlets were selected so that a full array of leaves at different stages of expansion were available for the larvae to consume. Branchlets were replaced and the larvae transferred every few days at first but increasingly frequently as the third and fourth instars consumed foliage at an increasing rate. Each replicate was placed in a 350 × 250 × 125 mm plastic tub in which frass was collected. Assays were carried out in a controlled temperature room at 25°C with a 14:10 h light/dark cycle over a period of 3 months.

Measurement of leaf consumption

Area of leaf eaten was determined using digital image analysis (ImageJ v1.35s, U. S. National Institutes of Health, Bethesda, Maryland; http://rsb.info.nih.gov/ij/) of binary images of entire branchlets taken before and after larval feeding. We tracked individual leaves between images to obtain the difference between initial and final leaf areas for each instar. To measure leaf consumption, a regression model of dry weight of frass produced versus leaf dry weight consumed was generated from a subset of 12 trees (two from each OPF). For the 12 sample trees, the average dry weight of leaf eaten was calculated using data on leaf mass per unit area (LMA). Mean

LMA and moisture content of leaves from each tree were calculated from punches of lamina (excluding the leaf midrib) taken from juvenile, fully expanded, and mature leaves from each initial branchlet. A restricted maximum likelihood model (REML) was used to assess the variation of these leaf parameters between OPFs. Using 'OPF' as a fixed factor did not account for a significant amount of variation in either LMA or moisture content (within each leaf age class) in REML models. As the estimated variance component for 'Tree' as a random factor was large (Table 1), data for leaf dry weight eaten used in the regression model was calculated for each instar by multiplying leaf area eaten by the specific mean LMA for each tree and age class of leaf most relevant to that instar.

The output of frass corresponding to estimated leaf dry weight eaten (for each instar per cohort) was weighed after drying for 48 h at 50°C. Predicted values for dry weight of leaf eaten were generated for each instar per larval cohort on the remaining trees using frass dry weight as a predictor in the regression model. This model was also used to examine if resistance class could account for any variation in the relationship between frass output and leaf dry weight eaten and thus indicate whether approximate digestibility (*sensu* Waldbauer (1968)) differed between resistant and susceptible foliage.

Insect growth and development

The effect of diet on each of the four instars was quantified by measuring development time, larval fresh weight, frass dry weight, and mortality. Final adult dry weights were then measured after sexing pupae according to the method of Reid and Ohmart (1989). As all parameters are expressed per larva, values for each instar were estimated by dividing parameters by the mean number of larvae alive at the start and end of each instar. Change of instar was determined when 50% of the surviving larvae had moulted. Relative consumption rate (RCR) and relative growth rate (RGR) were calculated according to Baker *et al.* (2002).

Oviposition experiments

Groups of three female and two male *P. atomaria* adults were randomly selected from the captive population and placed in one of 12 cages, 290 × 300 × 260 mm. For each experiment, three trees from a resistant OPF were paired with three trees from a susceptible OPF. Each pair of trees had four replicates in the 12 cages. A cut branchlet approximately 30 cm long with equal numbers and distributions of expanding and fully expanded leaves from each tree was inserted through holes in the floor of each cage with their stems suspended in water. The experiment was then left undisturbed for 3 days. To renew the supply of foliage and allow sufficient time for adults to deposit egg batches, a second set of branchlets from the same three pairs of trees was offered for a further 3 days; the pairs of trees being tested were rearranged between the 12 cages so that the beetles in each cage were not exposed to the same choice of trees twice. If few egg batches were laid the branches were replaced a third time. This protocol was adopted to minimize the familiarity of ovipositing females with foliage being tested. A total of nine paired resistant versus susceptible tree comparisons were tested. All experiments were carried out in the same climate controlled conditions described previously with overhead artificial lighting to encourage breeding activity.

Statistical analysis

The effect of OPF on developmental parameters for each instar and final adult and pupal weights were analysed separately using REML models with OPF and Resistance category as fixed effects and Tree and Replicate as random effects. Total larval development time was also analysed using these random and fixed effects. This approach was deemed appropriate owing to the presence of missing values and some uneven sample sizes of pupal and adult weights due to mortality in some cohorts. Where OPF was not found to account for variation in dependent variables, we introduced a 'Resistance code' term which divided the trees into either resistant or susceptible OPFs and used this as a fixed effect in subsequent analyses.

Proportional mortality was analysed using a generalized linear mixed model for a binary distribution with a logit link with Development time for each instar and Resistance category as fixed effects. Total larval mortality was also tested with Resistance category and OPF as fixed effects and Tree as a random effect. Difference in oviposition preference in choice tests was assessed using a paired *t*-test.

Table 1 Mean leaf mass per area and moisture content values for the six open-pollinated families used in the bioassays separated by leaf age class with restricted maximum likelihood model (REML) variance components and significance tests for family differences. Each age class was considered in a separate REML analyses with Tree as a random effect and Family as a fixed effect. For each mean $n = 25$ (five trees with five replicates per tree)

		Resistant families			Susceptible families			Tree	Tree replicate	Family		
Leaf attribute	Leaf age class	1	2	3	4	5	6	Estimated component of variance (s.e.)	Residual variance (s.e.)	Wald statistic	d.f	*P*
LMA	Juvenile	25.9	26.3	35.0	27.1	30.1	32.8	39.8 (13.5)	36.2 (4.6)	7.51	6	0.276
(gmm^{-2}	Expanded	29.8	28.2	35.7	29.2	33.1	33.0	24.3 (8.3)	24.5 (3.1)	8.55	6	0.200
$*10^{-6}$)	Mature	35.0	30.9	35.9	33.3	35.7	34.7	26.0 (9.2)	28.7 (3.8)	4.37	6	0.626
% H_2O	Juvenile	0.77	0.76	0.71	0.75	0.71	0.74	0.0025 (0.0009)	0.0029 (0.0004)	4.29	6	0.637
	Expanded	0.72	0.73	0.69	0.72	0.70	0.71	0.0007 (0.0003)	0.0021 (0.0003)	7.42	6	0.283
	Mature	0.70	0.72	0.68	0.70	0.69	0.71	0.0056 (0.0003)	0.0027 (0.0003)	4.37	6	0.627

Results

Larval growth

Using OPF as a fixed effect in linear mixed models proved to be of little value for analysing *P. atomaria* larval performance on resistant and susceptible families. This was as a result of the relatively large variation in resistance between trees within each family as indicated by the negative estimated component of variance when OPF was introduced as a random effect (in addition to a random Tree effect) in the analyses. This variation within families made it difficult to detect consistent differences in larval growth between resistant and susceptible families.

The significant effects of feeding on resistant foliage on the growth and development of larvae were restricted to the first instar, which grew significantly more slowly on trees from resistant OPFs (Table 1). Lower RGR was primarily dependent on a significant lengthening of first instar development times in two of the three resistant families (Fig. 1). Development times were also more variable in resistant OPFs as indicated by larger mean standard errors (Table 2) which resulted in a broadening of the range of developmental stages within a cohort by delaying development of individual larvae to different degrees. Total development time, however, was not significantly different between trees from resistant and susceptible OPFs ($F = 1.83$, d.f. = 1,24, $P = 0.189$).

Observation of the behaviour of the larvae indicated that those feeding on some trees from resistant OPFs abandoned colonial feeding in a manner identical to that described for larvae feeding on highly resistant *E. grandis* clones and for which extensive damage to the midgut was subsequently identified indicating the action of a toxin (Henery *et al.*, 2008b). Such disrupted gregarious feeding was also described by Ohmart *et al.* (1987) for *P. atomaria* larvae feeding on nitrogen-deficient foliage.

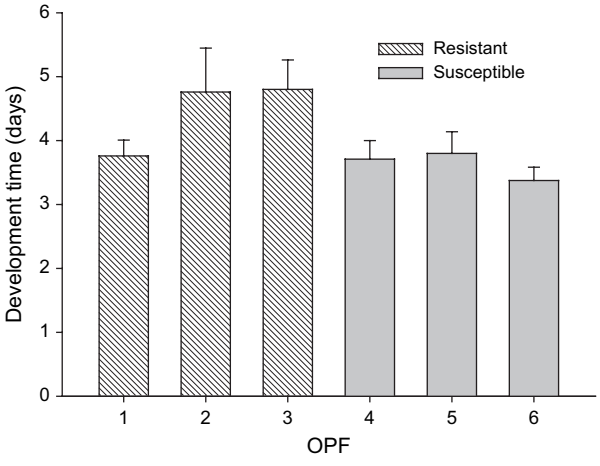

Figure 1 Mean duration of first instar *Paropsis atomaria* when fed three resistant and three susceptible open-pollinated families of *Eucalyptus grandis*. Error bars represent 95% confidence intervals.

Relative consumption rate

There were no consistent differences in relative consumption rates between either OPF families or resistance class (Table 2). The amount of leaf consumed was found to be indistinguishable between all OPFs for each instar. Similarly, we found no evidence to suggest any differences in approximate digestibility between susceptible and resistant trees. In a generalized linear model, fitting a resistance category term did not account for any significant variation in frass production ($F = 0.44$, d.f. = 1, $P = 0.507$) beyond the vast majority of variation accounted for by measured dry weight eaten ($F = 2988.7$, d.f. = 1, $P < 0.001$, $r^2 = 0.93$) (Fig. 2).

Mortality

Although there was a trend towards declining mortality with developmental time for feeding larvae, mortality was highly variable over the course of the experiment. Predicted means of proportional mortality were higher in first instar larvae developing on resistant OPF leaf material although this effect was not statistically significant (Table 2). Accurate assessment of differences in mortality between OPFs was complicated in later instars as a result of high mortality rates on some trees. There was no significant effect on overall mortality during larval development by feeding on trees from resistant OPFs ($F = 0.67$, d.f. = 1,23, $P = 0.423$).

Adult dry weights

There was a trend for lower adult dry weights of both male and female *P. atomaria* after development on resistant OPF foliage (Fig. 3) although this was not statistically significant (Wald statistic = 0.47, d.f. = 1, $P = 0.493$).

Oviposition preference

Although female *P. atomaria* showed considerable variation in oviposition preference for particular hosts, the overall direction of preference in binary choices of host plant was not consistent ($t = 1.184$, d.f. = 8, $P > 0.05$) (Fig. 4).

Discussion

This study found that, relative to the performance of *P. atomaria* larvae on *E. grandis* foliage from susceptible OPFs, neonates fed on foliage from resistant OPFs had slower growth and development. A non-significant increase in mortality was also recorded. Importantly, the larvae exhibited identical behavioural changes to those seen on other *E. grandis* clones where they accompanied toxosis (Henery *et al.*, 2008b). This overall consequence of feeding on resistant OPFs appears to be transient with later instars completing development at similar rates. There was, however, a trend towards lower mean weights of emerging adult beetles on resistant OPFs of *E. grandis*, suggesting overall host quality in this group of trees was lower than trees from susceptible families.

Table 2 Comparison of larval performance parameters from resistant and susceptible open-pollinated families of *Eucalyptus grandis*. Values are instar means (standard error) from three OPFs with significant differences (as determined by REML analysis) between the two groups indicated (*). Proportional mortalities are back-transformed from the logit scale with the logit transformed mean difference ± 95% confidence interval for differences in parentheses

Larval parameter	Instar/phase	Resistant	Susceptible	Wald statistic	d.f.	*P*
Development time (days)	L1	4.44 (0.15)	3.63 (0.08)	4.94	1	0.090
	L2	3.05 (0.11)	2.76 (0.07)	2.51	1	0.188
	L3	3.52 (0.10)	3.33 (0.08)	0.96	1	0.384
	L4	4.86 (0.15)	5.03 (0.17)	0.51	1	0.513
	Pre-pupal	6.36 (0.13)	6.11 (0.23)	3.17	1	0.147
	Pupae	7.71 (0.12)	7.49 (0.28)	0.55	1	0.509
Relative growth rate (g/g/d)	L1	1.44 (0.06)	1.73 (0.05)	4.16	1	0.041*
	L2	1.01 (0.05)	1.07 (0.04)	0.48	1	0.489
	L3	0.93 (0.03)	0.91 (0.03)	0.28	1	0.597
	L4	0.32 (0.03)	0.33 (0.02)	0.16	1	0.692
Relative consumption rate (g/g/d)	L1	1.52 (0.06)	1.58 (0.05)	0.20	1	0.680
	L2	0.96 (0.04)	0.87 (0.03)	1.16	1	0.342
	L3	0.79 (0.03)	0.75 (0.02)	0.28	1	0.627
	L4	0.60 (0.05)	0.55 (0.03)	0.64	1	0.467
Proportional mortality	L1	0.19 (0.42 ± 0.69)	0.14	1.41	1	0.235
	L2	0.11 (0.18 ± 0.51)	0.09	0.46	1	0.498
	L3	0.11 (0.28 ± 0.66)	0.14	0.71	1	0.401
	L4	0.13 (0.43 ± 0.95)	0.09	0.81	1	0.369
	Pre-pupal	0.13 (0.17 ± 1.01)	0.16	0.11	1	0.745
	Pupae	0.12 (0.35 ± 0.65)	0.16	1.09	1	0.296

Like many other eucalypt species from the subgenus *Symphyomyrtus* selected for hardwood plantation forestry, *E. grandis* is fast growing, carries a high leaf area index during its early growth, and has high rates of leaf development and turnover (Beadle & Inions, 1990). Fast-growing species commit fewer physiological resources to defence in comparison to slower growing species from resource-poor habitats (Coley *et al.*, 1985; Herms & Mattson, 1992; Tuomi, 1992; de Jong, 1995). It is therefore unsurprising that even when using *E. grandis* material that had been previously identified as less defoliated by *P. atomaria*, we could only detect minor negative effects on this eucalypt herbivore. The variability in genetic background within OPFs (half siblings only) means that the trees assayed within resistant OPFs exhibited highly variable insect responses. The labour-intensive methods required to cover the entire developmental sequence of *P. atomaria* larva through to adult emergence severely limited the number of host trees assayed per OPF. Consequently, against the same genetic background of open-pollinated plant material, the limited replication possible in an intensive bioassay experiment may have limited our ability to detect statistically significant changes in larval growth parameters between resistant and susceptible OPFs.

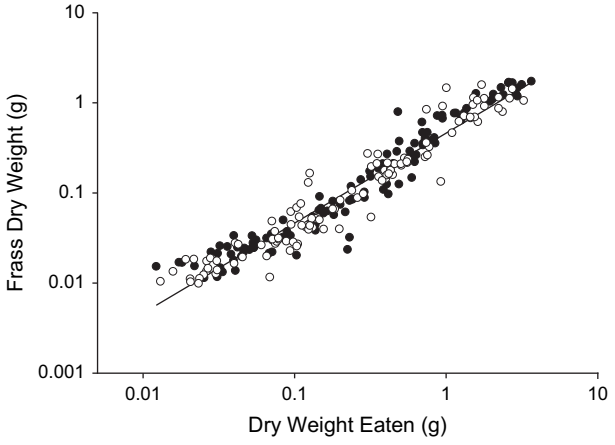

Figure 2 Frass production versus dry weight of leaf eaten for all *Paropsis atomaria* instars from a subset (n = 12) of *Eucalyptus grandis* from resistant (●) and susceptible (○) open-pollinated families. Regression line is for all data points ($\log_{10}$ dry weight eaten = 0.932*$\log_{10}$ frass dry weight + 0.269).

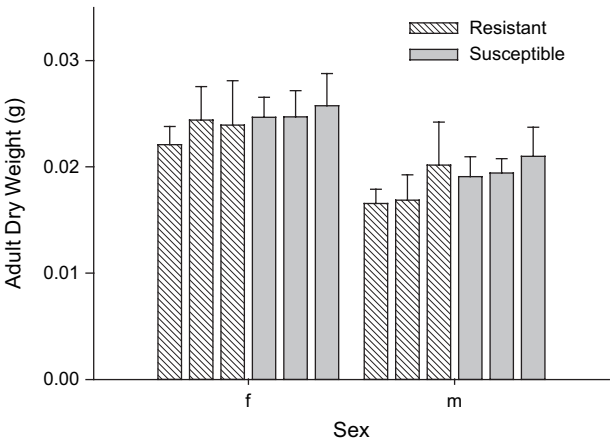

Figure 3 Mean adult dry weights of *Paropsis atomaria* females (f) and males (m) after development on three resistant and three susceptible open-pollinated families of *Eucalyptus grandis*. Error bars are 95% confidence intervals.

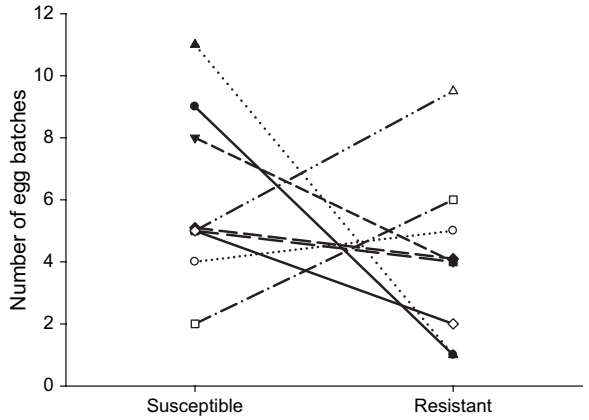

Figure 4 Egg batches oviposited by *Paropsis atomaria* on foliage from resistant and susceptible *Eucalyptus grandis* open-pollinated families in binary choice tests. Each symbol represents the summed responses of different groups of beetles exposed to foliage from a pair of test trees.

Leaf consumption data from our bioassays showed that survival of an individual larva beyond the first instar through to pupation results in a multiplication of defoliation (in terms of dry weight consumed) by a factor of approximately 80. Thus, because the later instars consume vastly more foliage, relatively minor differences in survivorship (i.e. <5%) at the first instar, when multiplied over several successive generations of insects, could explain mean differences of 20% leaf area removal in OPF crowns observed in *E. grandis* provenance trials (Henery *et al.*, 2008a).

One of the features of the response of *P. atomaria* to the foliage of resistant trees was an increase in wandering behaviour characterized by disruption and dispersal of the feeding group across the leaves on a shoot. Larvae of *P. atomaria*, like many paropsine chrysomelids, feed gregariously through all instars and have well-developed defence glands which secrete a fluid containing hydrogen cyanide (Moore, 1967) to repel attackers. Larvae respond as a group to disturbance by predators by simultaneously raising their abdomens and everting their defensive abdominal glands (Carne, 1966). A suggested benefit for gregarious larvae is that groups may be better able to defend themselves because they are able to respond in a coordinated manner to repel predators, especially when repellent defensive behaviours are involved (Tullberg & Hunter, 1996). In addition, studies have shown that gregarious feeding by a related paropsine chrysomelid *C. agricola* larvae facilitates feeding on tough leaves (Howlett *et al.*, 2001; Nahrung *et al.*, 2001). If feeding on foliage from resistant OPFs induces behaviours that reduce larval feeding efficiency and/or make larvae more vulnerable to predators it is feasible that differences in mortality rates of *P. atomaria* larvae between resistant and susceptible *E. grandis* in the field may exceed those recorded in this study.

It has also been proposed that slower growing larvae should suffer higher rates of mortality by extending the period that they are exposed to natural enemies (Feeny, 1976; Moran & Hamilton, 1980). This idea was formally proposed by Clancy and Price (1987), as the slow-growth/high-mortality hypothesis. Although this hypothesis specifically refers to increased apparency to predators and parasitoids for slow-growing larvae, it could also be expanded to encompass exposure to other causes of mortality such as extreme temperatures (Carne, 1966; Fordyce & Shapiro, 2003) or dislodgement from the plant by rain and wind (Patterson *et al.*, 1996).

Experimental tests of the slow-growth/high-mortality hypothesis have shown that parasitoids may preferentially select larger, faster growing larvae over slower growing larvae, possibly because these represent greater resources for their developing offspring (Clancy & Price, 1987; Craig *et al.*, 1990; Loader & Damman, 1991). It is also reasonable to assume that parasitism of herbivore larvae will not reduce immediate damage to the plant if a parasitoid does not kill or paralyse the herbivore's larvae before it completes its development. A review by Williams (1999) found that slow-growing surface-feeding herbivores were at greater risk of predation but at less risk of parasitism than fast-growing conspecifics. We determined that *P. atomaria* (a surface-feeding herbivore) feeding on *E. grandis* foliage from OPFs suffering less defoliation in the field experience slower growth relative to larvae on foliage from more defoliated OPFs and propose that this, in conjunction with the aforementioned disruption of group feeding behaviours, may lower feeding efficiency and make early instar larvae more vulnerable to predators and at increased risk of experiencing adverse weather conditions. Such an interaction between slow developmental rates derived from host plant effects and increased larval predation rates in early instars has been demonstrated in another chrysomelid, the Colorado potato beetle (Kaplan *et al.*, 2007).

In a study comparable to ours, Patterson *et al.* (1996) sought to determine if differential defoliation of *Eucalyptus regnans* OPFs by another species of chrysomelid *Chrysophtharta bimaculata* was explained by adverse effects of resistant genotypes on performance of neonate larvae in the field. In findings mirroring those in this study, Patterson *et al.* (1996) found that neonate survival was greater on families more prone to defoliation although, because of high variability between host families, the result was not statistically significant. Patterson *et al.* (1996) did not detect any differences in RGR on resistant and susceptible hosts and the experiment ended before the larvae moulted to the second instar. Consequently, differences in mortality on alternate hosts may have been greater if larvae had been monitored over a longer developmental time. However, on the same OPFs mean egg density per family was closely correlated with the subsequent level of canopy defoliation (Raymond, 1998). Patterson *et al.* (1996) therefore concluded that different levels of damage amongst *E. regnans* families observed in the field are more likely a result of adult host selection than differential larval mortality. Subsequent investigation by Howlett (2000), however, indicated that variation in defoliation was less dependent on differences in rate of egg deposition amongst the same *E. regnans* families but that rate of leaf deployment was lower in susceptible families resulting in less available leaf area for developing larvae per egg batch.

Despite the above example, paropsine adult host choice undoubtedly plays a role in determining levels of defoliation of *Eucalyptus* trees in the field (Rapley *et al.*, 2004). The limited oviposition assays we conducted in the laboratory suggest that ovipositing *P. atomaria* females cannot readily differentiate between trees from resistant and susceptible *E. grandis* OPFs. It has been shown that for paropsines, which rely on the presence of soft expanding foliage for the growth and survival of larvae, that choice of host for oviposition may be more influenced by the abundance of suitable expanding foliage (Strauss & Morrow, 1988; Steinbauer *et al.*, 1998) than by other leaf chemical factors (Howlett & Clarke, 2003).

A study by Rapley *et al.* (2004) demonstrated that oviposition preference for subraces and families within subraces of *E. globulus* by the paropsine *Crysophtharta agricola* was significantly positively correlated with subsequent level of damage in the field. This suggests adults were sensitive to variation between provenances, although the authors reported a significant trend for trees with an abundance of young flush foliage to suffer greater rates of infestation. As insect densities in a plantation increase, these oviposition preferences are likely to be reversed once initially preferred hosts suffer more extensive defoliation and become less attractive to ovipositing females relative to their neighbours (Carne, 1966; Clarke *et al.*, 1997; Howlett, 2000).

If even rates of oviposition on all hosts in the field are assumed, then, the findings of this study suggest over several generations of insects, mortality derived directly from plant characteristics together with the contributions of indirect causes of mortality, have the potential to produce the differences in defoliation we observed between OPFs in the *E. grandis* experimental plantations. Further investigation to identify how resistant and susceptible host plants differ chemically will be difficult because host plants factors subtly affect neonate paropsine larvae and thus any chemical differences between host trees may be restricted to very young expanding foliage where these larvae feed. Leaf chemistry continually changes during leaf development (Wait *et al.*, 1998; Laitinen *et al.*, 2002) and thus it is likely to be more difficult to isolate compounds that are deleterious to young larvae.

Acknowledgements

We thank Martin Steinbauer for advice on experimental methods, Polly Hemmings, James Woodman and Melissa Starling for assistance with the bioassays, Greg Joss for providing the image analysis macros, Helen Smith at Forests NSW for supplying the eucalypts and Jeff Wood for assistance with statistical analyses. M.H. was financially supported by the Australian Research Council and NSW Department of Primary Industries.

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Accepted 5 August 2008
First published online 20 November 2008