

# Genetic variation in susceptibility of *Eucalyptus globulus* coppice regrowth to shoot-feeding weevils (*Myllorhinus* spp.)

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## Abstract

*Genetic variation in damage caused by shoot-boring weevils Myllorhinus dentiferus (Boheman) (Coleoptera: Curculionidae) and M. bicordata (Boisduval) to juvenile coppice regrowth in a Eucalyptus globulus subsp. globulus base population in north-western Tasmania is reported. The trial contained one-year-old coppice regrowth from trees grown from 554 open-pollinated seedlots collected from 13 races and 48 localities in the native stands throughout Tasmania and Victoria. Significant variation in susceptibility is reported between races, with the Mount Dromedary race appearing highly susceptible and the King Island race least susceptible to attack. No significant variation in damage was found between localities within races or families within localities.*

## Introduction

*Eucalyptus globulus* subsp. *globulus* Labill., a native of Tasmania and south-eastern Australia, is the premier eucalypt for temperate pulpwood forestry world-wide (Eldridge *et al.* 1993). Within Australia, major plantation estates have been

established in Western Australia, Victoria, Tasmania and South Australia.

As the *E. globulus* estate continues to expand and mature, the risk of damage due to herbivorous pests and diseases is becoming of increasing concern (Davy *et al.* 1998). This risk has been realized in Tasmanian plantations of *E. globulus* subsp. *globulus*, with a number of insect species, including the autumn gum moth (*Mnesampela privata* (Guenée)), the eucalypt leaf beetles (*Chrysophtharta bimaculata* (Olivier) and *C. agricola* (Chapuis)) and sawfly (*Perga affinis* Kirby), causing major defoliation to plantations (Elliott and de Little 1984) and loss of productivity (e.g. Candy *et al.* 1992). Current control of these pests is centred around the use of chemical insecticides which may provide effective control (Elliott *et al.* 1990; Neumann and Collett 1997), with integrated pest management procedures deployed to optimise their use (Elliott *et al.* 1992; Stone and Clarke 1998). However, there is considerable interest in using further environmentally friendly and cost-effective IPM methods such as microbial insecticides (e.g. *Bacillus thuringiensis*, Elek and Beveridge 1998), enhancing natural enemies such as ladybirds (Bashford 1999) and deploying genetically more-resistant trees into the plantation (Farrow *et al.* 1994; Floyd and Raymond 1999).

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The use of natural tree resistance, in this instance, is dependent on the presence of genetic variation. Genetic variation in insect resistance within *Eucalyptus* occurs between subgenera (e.g. Stone *et al.* 1998), species (Morrow and Fox 1980), provenances (Farrow *et al.* 1994; Dutkowski and Potts 1999), families (Floyd and Raymond 1999; Raymond 1995) and even within individuals (i.e. somatic mutation, Edwards *et al.* 1990). Genetic variation in resistance of *E. globulus* subsp. *globulus* has been reported for autumn gum moth (*Mnesampela privata*), leaf blister sawfly (*Phylacteophaga froggatti* Riek), sawfly (*Perga affinis*) and the stem borer *Phoracantha semipunctata* (Fabricius) (Farrow *et al.* 1994; Dutkowski and Potts 1999; Soria and Borralho 1998). The *E. globulus* subsp. *globulus* gene pool is highly variable (Dutkowski and Potts 1999; Potts *et al.* 1999) and an understanding of how this genetic variation impacts on dependent pests and pest communities may allow the eventual selection and deployment of genetically resistant trees.

During April 1999, a noticeable amount of the young terminal coppice shoots of *E. globulus* subsp. *globulus* was being damaged at the North Forest Product's Massy Greene field trial near Burnie, north-western Tasmania. The damage was being caused predominantly by *Myllorhinus dentiferus* (Boheman) (Coleoptera: Curculionidae), although a larger weevil, *M. bicordata* (Boisduval), also contributed to the damage. *Myllorhinus dentiferus*, previously known as *Rhachiodes dentifer* (R. Bashford, pers. comm.) is a small weevil, 5–6 mm in length. This scaly, fawn to grey-brown weevil bores into young shoot tips, rasping living shoot tissue (Elliott and de Little 1984). The shoot usually dies as a result of turgor loss and lack of water. Shoot death is also commonly caused by infection from fungal or bacterial pathogens (R. Bashford, pers. comm.) introduced through the feeding scar. Females deposit single eggs in the feeding hole on larger branchlets where the larvae subsequently hatch and feed on tissue. Although this pest is not presently regarded as a serious threat

to forestry, shoot-tip death caused by adult feeding, and weakened branchlets caused by larval feeding may result in multiple forking and poor form in young trees (Elliott and de Little 1984).

In the present study, we report on genetic variation in damage to *E. globulus* subsp. *globulus* coppice regrowth in the juvenile phase caused by the shoot-boring weevils, *Myllorhinus dentiferus* and *M. bicordata*. We focus on variation between races, localities and families.

## Methods

### *Trial establishment and genetic material*

Open-pollinated seed from 596 native-stand trees was collected from 49 localities throughout Tasmania and southern Victoria, representing all 13 races classified by Dutkowski and Potts (1999). A field trial was established with families derived from this seed by North Forest Products at Massy Greene. This site is on basalt-derived krasnozem soil, 2.4 km from the coast. It is at an altitude of 115 m and has a mean daily maximum and minimum temperature of 17.1°C and 9.0°C respectively. The trial comprised five replicates, each with 25 incomplete blocks of 25 families. Each family was represented in each replicate by a two-tree plot, giving a total replication of 10 individuals. This design allows genetic differences to be diagnosed without the confounding effects of environmental variation. The trial was established in 1989 and was thinned in late 1998 for conversion to a seed orchard. The remaining stumps were allowed to coppice and this resulted in the regeneration of 1984 individuals of juvenile foliage that could be analysed for insect damage. Five hundred and fifty-four families from 48 localities, representing all of the 13 races, were included in the coppice material (Table 1). After coppicing, the number of individuals within an incomplete block averaged 17.9 and ranged from five to 30. The number of families in each

Table 1. Number of individuals, families and localities represented in each *E. globulus* subsp. *globulus* race in the Massy Greene trial after coppicing.

Race	Localities	Families	Individuals
Western Otways	4	120	409
Eastern Otways	3	39	148
Strzelecki Ranges	4	56	167
Southern Gippsland	2	14	39
Wilson's Promontory Lighthouse	1	7	9
Furneaux	7	106	403
North-eastern Tasmania	6	49	160
Dromedary	1	4	10
South-eastern Tasmania	6	61	239
Southern Tasmania	7	30	121
Recherche Bay	1	4	21
Western Tasmania	4	33	159
King Island	2	31	99

incomplete block averaged 13.7 and ranged from five to 20. This study was based on individuals that, firstly, were thinned out during conversion of the trial to a seed orchard and secondly, produced enough coppice for accurate assessment.

#### Field assessment

In late April 1999, the Massy Greene trial was scored for damage to young terminal shoots caused by *Myllorhinus* spp. The coppice was scored for the number of damaged shoots counted over 15 seconds of constant, visual scanning of the foliage. Damaged shoots were characterised by wilted, dead or missing shoots that exhibited the diagnostic bore hole below the site of damage. This eliminated confusion with shoot damage caused by other insects such as *Amorbus* sp., vertebrate herbivory and wind. This scoring technique was chosen because it was fast, it reduced bias by variation in coppice size and did not depend on any form of visual estimate of damage. In the low percentage of cases where insufficient coppice was available to complete the 15-second scan, the individuals were excluded from the analysis, as were individuals that were extremely damaged by other factors such as vertebrate damage. Previous to the scoring of insect damage, a number of other traits

related to coppice vigour had been scored, including coppice height and the number of coppice shoots.

#### Data analysis

Examination of residuals indicated that the data were best modelled using a Poisson distribution. Due to the unbalanced nature of the data, the mixed model package ASREML (Gilmour *et al.* 1999) was used with a log link function to fit the Poisson model:

$$y = \text{mean} + \text{replicate} + \text{incomplete block} + \text{stem number} + \text{coppice height} + \text{race} + \text{locality (race)} + \text{family (locality)} + \text{plot} + \text{error},$$

where:  $y$  = the number of damaged shoot tips counted; replicate is the fixed effect of field replicate number; *incomplete block* is the random effect of incomplete block within replicate; stem number is the number of coppice stems fitted as a covariate; coppice height is the mean coppice height (cm) fitted as a covariate; race is the fixed effect of geographical races, *locality (race)* is the random effect of locality within race; *family (locality)* is the random effect of the open pollinated family within locality; and *plot* is the random effect of the two tree plots. Random effects were considered to be significant when the ratio of the variance component to standard error exceeded 2

Table 2. Test for the significance of fixed and random effects on the number of coppice shoots damaged from each individual. The model was fitted with ASREML assuming a Poisson distribution. Random effects are italicised and F and Z values are presented for fixed effects and random effects respectively. n.s. = not significant, \*\*\* = P < 0.001. Variance components ( $\sigma^2$ ) are given for random effects using a model where the Race effect was treated as random to provide an indication of the relative importance of this effect.

Effect	$\sigma^2$	DF	F or Z	Probability
Replicate	–	4	5.65	***
<i>Incomplete block</i>	0.247	124	4.01	***
Stem number	–	1	28.34	***
Coppice height	–	1	90.17	***
Race	0.528	12	5.05	***
<i>Locality within race</i>	0.029	34	1.02	n.s.
<i>Family within locality</i>	0.000	504	0.00	n.s.
Plot	1.594	983	17.38	***
Error (standardised)	1.000	320	–	–

(Gilmour *et al.* 1999). Least squares means were calculated and back transformed using ASREML. The mean damage levels of each race were compared in pair-wise contrasts using family mean scores with the GENMOD procedure (that fits generalised linear models, SAS V6.12) using the model:

$$y = \text{mean} + \text{coppice height} + \text{stem number} + \text{race} + \text{error},$$

where the terms used have been described above. Bonferroni adjustment for a *posteriori* comparisons (Snedecor and Cochran 1980) was applied to the significance level to account for multiple comparisons between races.

## Results and discussion

Spatial patchiness (illustrated by significant replicate and incomplete block effects) and coppice vigour played highly significant roles in determining the distribution of damage caused by *Mylorhinus* spp. (Table 2). Such patchiness is commonly exhibited by insect communities (Linhart 1989; Farrall *et al.* 1991) and may be due to meso-scale environmental variation (within tens of metres) (Linhart *et al.* 1981) or insect dispersal patterns (Clarke *et al.* 1997). However, no obvious correlation between

environmental factors such as light and vegetation type and abundance were observed in the field.

It appears that *Mylorhinus* spp. were targeting vigorous trees with dense coppice. This effect is not uncommon (e.g. Raymond 1995) and is believed to be due to a combination of factors, including increased nutrient availability in young vigorous foliage and the protection from weather, predation and parasitism provided by dense foliage (Strauss and Morrow 1988).

Significant genetic variation in shoot tip damage by *Mylorhinus* spp. was identified among the 13 races of *E. globulus* when the effects of coppice vigour and environmental factors were taken into account (Table 2). However, no genetic variation was detected between localities within races nor families within localities (Table 2). The most susceptible race, Mount Dromedary, stood out in the field, with up to 30 damaged shoots being counted over the 15-second scan of the coppice regrowth. Severely damaged individuals were beginning to exhibit a stunted and bushy form. However, this race appears to be atypical of the *E. globulus* species. The foliage is much greener than normal (Potts and Jordan 1994a), causing Potts and Jordan (1994b)

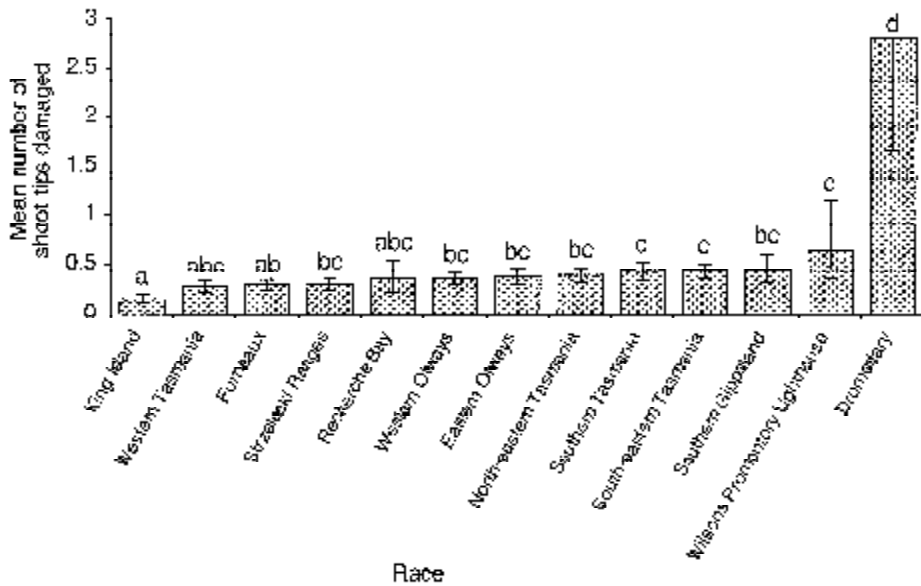


Figure 1. The least squares mean number of shoots damaged ( $\pm$  se) in each race in the Massy Greene field trial. Means and standard errors have been derived by back transformation. Races sharing the same letter displayed above the mean value column are not significantly different ( $P > 0.00064$ , after the Bonferroni adjustment).

to suggest that this race may be hybridising with *E. ovata*. The introgression of genes from *E. ovata* may be resulting in the addition of a trait that is particularly favourable to *Myllorhinus* spp. or causing a reduction in production of a resistance mechanism (Whitham 1989). The breakdown of multi-gene controlled resistance mechanisms may be the cause of reduced hybrid resistance seen in this case (Whitham *et al.* 1999).

When the Mount Dromedary race was excluded from the analysis, there was still a significant difference between the remaining races ( $F_{11,34} = 2.31$ ;  $P < 0.05$ ). The King Island race appears the most resistant race in this case, exhibiting significantly less damage than all but the Furneaux, Western Tasmania and Recherche Bay races (Figure 1). Both the Bass Strait races (King Island and Furneaux) expressed significantly less damage than the Southern Tasmania, South-eastern Tasmania, Wilsons Promontory Lighthouse and the Mount Dromedary races (Figure 1). Farrow *et al.* (1994) suggest that a King Island provenance also exhibits

increased resistance to autumn gum moth (*Mnesampela privata*) and the leaf blister sawfly (*Phylacteophaga froggatti*). Dutkowski and Potts (1999) found the King Island race to be significantly more resistant to sawfly (*Perga affinis*) than the other 12 races. Farrow *et al.* (1994) also suggest that the Flinders Island provenance (representative of the Furneaux race) exhibits a similar, but slightly less extreme, resistance to autumn gum moth (*Mnesampela privata*) and the leaf blister sawfly (*Phylacteophaga froggatti*) to that of the King Island provenance, which is also consistent with the present study.

These findings indicate that *E. globulus* originating from King Island may exhibit either a general resistance mechanism or a number of specific resistance mechanisms. However, the King Island race exhibits low resistance to the wood-boring insect, *Phorocantha semipunctata* (Soria and Borralho 1998). Three of the insects discussed are leaf defoliators that target either juvenile or mature foliage whereas *Myllorhinus* spp. are shoot feeders. Any general resistance

mechanism (e.g. presence of a chemical) would therefore have to be expressed in both juvenile and mature foliage and in both leaf and young stem tissue but not in wood and bark tissue.

In summary, while spatial patchiness and plant vigour are major determinants of susceptibility to *Myllorhinus* spp. weevil damage, once these effects are removed, significant, genetic-based differences in susceptibility have been detected between races of *E. globulus*. Such genetic variation is the basis of resistance breeding. These genetic differences are expressed in field trials where insects have a wide choice of genetic material for feeding. However, it is unclear whether deployment of resistant

genotypes from such field trials will result in decreased damage in plantations where insects are unable to choose from a range of genetic material.

## Acknowledgements

The authors acknowledge the contribution of North Forests Products (particularly Ian Ravenwood, Mike Powell and Wayne Tibbits) for establishing and maintaining the Massey Greene trial and for their permission to collect data. We thank Paul Tilyard and James Foster for their help in collection of data, Dick Bashford at Forestry Tasmania for insect identifications and Greg Jordan for his assistance and comments on the manuscript.

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