GENETIC VARIATION AND ADDITIVE INHERITANCE OF RESISTANCE OF *EUCALYPTUS GLOBULUS* TO POSSUM BROWSING

Julianne O'Reilly-Wapstra[1], Brad Potts[2] and Clare McArthur[1†]

[1] CRC for Sustainable Production Forestry, School of Zoology, University of Tasmania, Private Bag 5, Hobart, Tasmania, Australia, 7001.
[†] Present address: School of Biological Sciences, The University of Sydney, New South Wales, Australia, 2006.
[2]CRC for Sustainable Production Forestry, School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania, Australia, 7001.

ABSTRACT
Browsing of plantation seedlings and saplings by herbivores is a major problem for the successful establishment and management of plantations in Australia. The use of natural plant resistance in deployment or breeding programs may be an option for reducing such browsing. We assessed the variation in resistance of *Eucalyptus globulus* races, provenances, and families to browsing by the brushtail possum (*Trichosurus vulpecula*) in common environment field trials and captive feeding trials. There were significant genetic based differences between provenances of *E. globulus* in the extent to which they were browsed, and sideroxylonals, a group of defensive plant chemicals, were the most important determinant of coppice foliage intake by possums. Investigations of the resistance of intra- and inter-race F_1 hybrids indicated that resistance of *E. globulus* to possum browsing is additively inherited. These results suggest there is potential to deploy genotypes with greater resistance through appropriate selection and breeding.

INTRODUCTION
Browsing of seedlings by mammalian herbivores in forestry plantations is an economic problem in plantation establishment in Australia (Coleman et al 1997), through its impact on tree growth, form and survival (Wilkinson and Neilsen, 1995; Bulinski and McArthur, 1999). A strategy to minimise browsing damage to seedlings is to target the mammal species directly responsible for the damage by methods such as lethal control. Alternatively, the utilization of natural plant resistance may provide a successful and viable management strategy to reduce browsing damage in plantations. To utilize natural plant resistance there needs to be variation in resistance within the plant species of interest and this, and variation in the plant defensive trait (such as plant secondary compounds), needs to be under genetic control. Previous studies have demonstrated clear intra-specific variation in resistance of some eucalypt species to both insect and mammalian herbivores (Floyd et al. 1994; Lawler et al. 1998) and the chemical basis to herbivore resistance in the *Eucalyptus* genus has been under much investigation in recent years (e.g. McArthur & Sanson 1991; Lawler et al. 1999; Wallis et al. 2002). We aimed to determine the level of intra-specific variation in resistance of *Eucalyptus globulus* to the common brushtail possum, *Trichosurus vulpecula*, and to investigate the relationship between genetics, leaf chemistry and resistance of *E. globulus* foliage.

METHODS
We conducted two field and captive feeding trials to assess resistance of juvenile *E. globulus* foliage to browsing by *T. vulpecula*. In trial 1 we measured browsing damage on coppice foliage (<1 year old) from 2302 trees, from 13 races (after Dutkowski and Potts 1999), 48 provenances and 563 families derived from range-wide native stand seed collections of known pedigree and grown in a common environment field trial. Using a selection of 54 trees (incorporating 7 provenances) from the field trial we then conducted a no choice feeding trial with captive herbivores to assess if the genetic variation in plant resistance in the field was reflected in feeding preferences of captive animals, as measured by relative intake. In trial 2 we investigated how inter-race differences in resistance of *E. globulus* to *T. vulpecula* are inherited in their F_1 hybrids. We assessed damage to juvenile foliage on three year old trees derived from seedlings in a common environment field trial on four hybrid types of known progeny. The progeny were artificial intra-race crosses (pure parental hybrids) and reciprocal inter-race F_1 hybrids of two geographically distinct populations (races) of *E. globulus*; north-eastern Tasmania and south-eastern Tasmania. We then assessed the preferences of these trees in a series of paired feeding trials with captive animals to test the field trial results and also investigated the patterns of inheritance of plant secondary metabolites.

RESULTS AND DISCUSSION
In field trial 1 there was significant genetic based differences in browsing damage amongst provenances ($F_{47,514}$ = 2.27, P < 0.001). A large component of this variation was due to highly significant differences amongst the races of *E. globulus* ($F_{12,514}$ = 24.77, P = 0.0001). There was

In Borralho, N., *et al.* (2004). Eucalyptus in a Changing World. Proc. of IUFRO Conf., Aveiro 11-15 October 2004

a clear trend for provenances from north-eastern Tasmania to be the least resistant to browsing, particularly the St. Helens provenance, while provenances from southern Tasmania and Victoria were more resistant to browsing. There were also significant differences among families within provenances (Z = 2.65, *P* = 0.0081). These results were confirmed in the captive feeding trial where there was a significant difference in intake between provenances ($F_{6,26}$ = 4.00, *P* = 0.006), where provenances from southern Tasmanian were relatively more resistant than the St. Helens provenance from northeastern Tasmania. Chemical analysis of this coppice foliage demonstrated that a formylated phloroglucinol compound (FPC), sideroxylonal, was the dominant plant secondary metabolite that determined intake of this foliage by *T. vulpecula* and that this metabolite is also under significant genetic control. Foliage from St. Helens had significantly lower concentrations of this metabolite, while provenances from southern Tasmania had higher concentrations (*P* < 0.0038).

In field trial 2, the intra-race hybrids from the south-eastern Tasmania race were more resistant to *T. vulpecula* than those from the north-eastern Tasmania race, confirming the results of trial 1. The inter-race F_1 hybrids exhibited intermediate resistance ($F_{3,51}$ = 37.90, *P* = 0.0001). The captive feeding trial supported these results where foliage from south-eastern Tasmania was more resistant than foliage from north-eastern Tasmania. In this three year old juvenile foliage, the condensed tannins and essential oils together appeared to explain the observed patterns of resistance between the four hybrid types, while sideroxylonal was only detected in small quantities. While both oils and tannins were inherited in a dominant manner in the inter-race F_1 hybrids, the direction of dominance was opposite. Their combined concentration, however, was inherited in an additive manner, consistent with the phenotypic differences in browsing.

These two trials clearly demonstrate a strong genetic basis to variation in resistance of *E. globulus* juvenile foliage to possums, suggesting the potential to incorporate natural plant resistance into breeding programs. Alternatively, simple screening of deployment populations already selected for other economic traits may be a more pragmatic solution for reducing browsing damage
of young seedlings in plantations. Selection of these genotypes is possible not only at the broad race and provenance levels but also at the family level where families within even relatively resistant provenances exhibited variation in resistance to *T. vulpecula*. It would be advantageous to undertake such screening based on defensive chemistry. However, the chemical basis to resistance in *E. globulus* foliage appears to differ between the two
types of foliage used in this study. Sideroxylonal, an FPC, was important in determining resistance in young juvenile coppice foliage, while in three year old juvenile foliage there was no relationship between FPC concentration and relative resistance of the four hybrid types. In this foliage, two other groups of plant secondary metabolites, the condensed tannins and essential oils, appeared to confer resistance. We suggest that the production of FPCs as defensive plant metabolites may only occur in *E. globulus* when the plant is most vulnerable to mammalian herbivores such as young seedlings and coppiced foliage and further work is required to determine the most efficient developmental stage and suite of chemicals for selection.

REFERENCES

Bulinski, J. and McArthur, C. (1999). *For. Ecol.Manage.* 113: 241-249.

Coleman, J.D. Montague, T.L. Eason, C.T. and Statham, H.L. (1997) *Landcare Research Contract Report.*

Floyd, R.B. Farrow, R.A. and Neumann, F.G. (1994). *Aust. For.* 57: 45-48.

Dutkowski, G.W. and Potts, B.M. (1999). Aust . J. Bot. 47: 237-263.

Lawler, I.R. Foley, W.J. Eschler, B.M. Pass, D.M. and Handasyde, K. (1998). *Oecologia* 116: 160-169.

Lawler, I.R. Eschler, B.M. Schliebs, D.M. and Foley, W.J. (1999). *J. Chem. Ecol.* 25: 2561-2573.

McArthur, C. and Sanson, G.D. (1991). *J. Zool., London* 225: 233-251.

Wallis, I.R., Watson, M.L. and Foley, W.J. (2002). *Aust. J. Zoo.* 50: 507-519.

Wilkinson, G.R. and Nielsen, W.A. (1995). *For. Ecol. Manage.* 74: 117-124.