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INTRODUCING DURABLE SPECIES TO NEW ZEALAND DRYLANDS: GENETICS OF EARLY ADAPTATION OF *EUCALYPTUS BOSISTOANA*

1. INTRODUCTION

New Zealand plantation forestry is dominated by conifers, with radiata pine (*Pinus radiata* D. Don) and Douglas fir (*Pseudotsuga menziesii*) accounting for 95% of the 2 million ha estate. These species display considerable environmental plasticity, but they face problems when grown in increasingly dry conditions, as well as having end-uses limited by their wood properties.

Drylands – defined as areas receiving a rainfall of 500 to 1000 mm/year – cover large parts of New Zealand (Apiolaza *el al. 2011, Appendix 1*). The productive use of drylands is dominated by agriculture and sheep farming, but land owners are actively looking for alternative uses. Besides wood values, dryland plantations have the potential to bring environmental benefits influencing the carbon, water and energy exchanges (Schimel 2010).

Eucalyptus species play only a minor role in New Zealand forestry, with plantations of *E. nitens* in the South Island as the only resource of industrial-scale use. There have been a myriad attempts at introducing eucalypts in the country, but they have failed with all plantings being small scale. On one hand, small plantations have not had sufficient "critical mass" to sustain commercial use of the species. On the other, species introductions have failed to obtain a large enough collection of samples to, firstly, characterize the potential of the species and, secondly, perform selective breeding exploiting between- and within-family variation.

Eucalyptus bosistoana F. Muell. (Coast Grey Box, Gippsland Grey Box) is a medium-sized to tall forest tree of the subgenus Symphyomyrtus (Brooker and Kleinig 1999) with a reputation for highly durable wood. This species presents us with an interesting challenge, as very little is known about it from a genetics and tree-breeding point of view. Tree breeding programs rely on the existence of exploitable variability for traits of commercial importance. If traits are not variable, or heritable or we cannot assess them in a cost-effective manner there is little we can do through breeding. Therefore, a successful breeding program requires characterizing variability and estimating the degree of genetic control of each trait, as well as the association between different traits. This information is often referred to as the "genetic architecture" of the species.

This paper presents early results for growth and survival of 66 *E. bosistoana* families growing on three sites in the South Island. We present the degree of genetic control for both traits as well as the magnitude of the change in rankings for growth between sites. Finally, we discuss the prospects for selection of superior genotypes within this species.

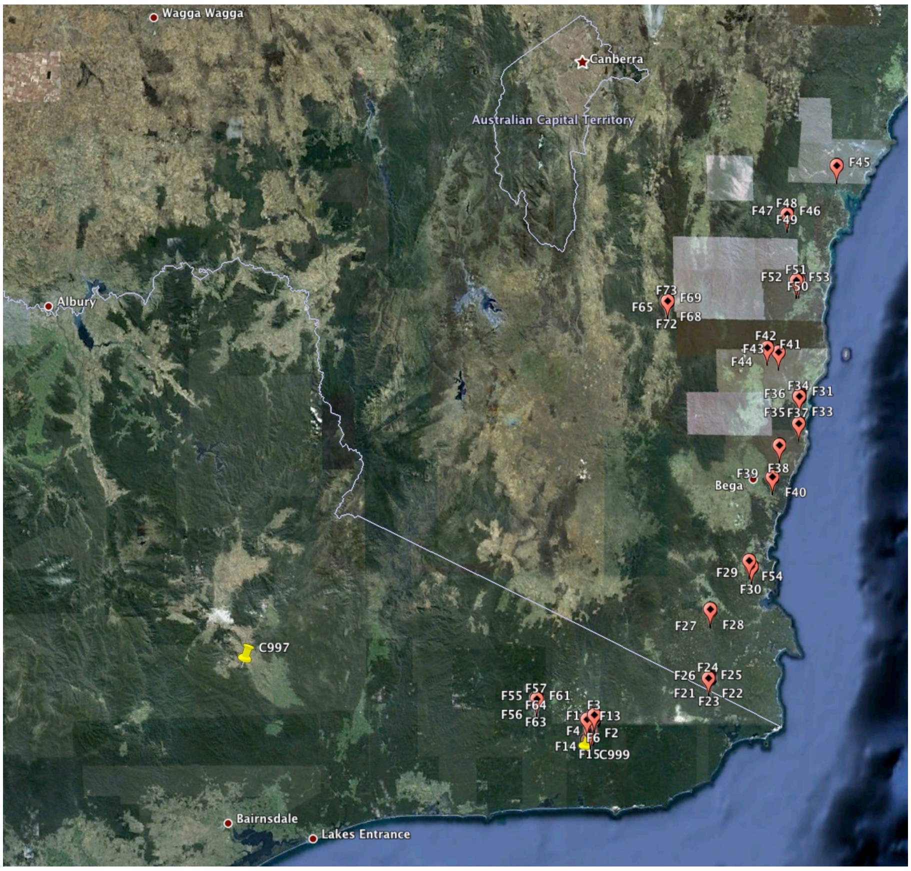

Figure 1. Location of the *E. bosistoana* plus trees represented in the progeny tests. There are a substantial number of overlapping labels in the southern part of the map.

2. MATERIALS AND METHODS

Three open-pollinated progeny tests, representing 66 plus trees from the southern coastal distribution of *E. bosistoana* (see Figure 1), were established in the South Island. The Cravens and Lawsons sites are located in the Marlborough region, while Martins is in the Canterbury region. Table 1 presents a summary of climatic data for the trials.

The trials also included three bulk-seed controls: 997 *E. globoidea* (from Swifts Creek), 998 *E. quadrangulata* and 999 *E. globoidea* (from Cann River). Control seedlots were fitted as additional fixed effects in the statistical models, as they did not have a family structure.

Table 1. Climatic data for trial sites (NIWA Cliffo database).

Site	Mean annual rainfall (mm)	Mean annual maximum temperature (C)	Mean annual minimum temperature (C)	Minimum annual temperature (C)
Cravens	668	18.3	7.6	2.3
Lawsons	590	17.1	9.3	4.2
Martins	750	17.0	5.9	1.7

Estimates of genetic parameters and predicted breeding values for tree height were obtained by fitting generalised linear mixed models. First we fitted a univariate family model to the vector of phenotypic observations $\boldsymbol{y}$:

$$\boldsymbol{y} = \boldsymbol{X m} + \boldsymbol{Z_1 b} + \boldsymbol{Z_2 f} + \boldsymbol{e} \qquad (1)$$

where $\boldsymbol{m}$ is the vector of fixed effects (overall mean, control effects), $\boldsymbol{b}$ is the vector of random block effects, $\boldsymbol{f}$ is the random effect of the open-pollinated families and $\boldsymbol{e}$ is the vector of random residuals. $\boldsymbol{X}$, $\boldsymbol{Z}_1$ and $\boldsymbol{Z}_2$ are incidence matrices linking $\boldsymbol{y}$ to the fixed and random effects. The expected values and variances were E[$\boldsymbol{y}$] = $\boldsymbol{Xm}$, Var[$\boldsymbol{b}$] = $\sigma_b^2\boldsymbol{I}$, Var[$\boldsymbol{f}$] = $\sigma_f^2\boldsymbol{I}$ and Var[$\boldsymbol{e}$] = $\sigma_e^2\boldsymbol{I}$. In addition we assumed that all covariances were zero.

The previous model was later extended to a multivariate family model by stacking up the data vectors. The variances were then Var[$\boldsymbol{b}$] = $\boldsymbol{B} = \boldsymbol{B}_0 \otimes \boldsymbol{I}$, Var[$\boldsymbol{f}$] = $\boldsymbol{F} = \boldsymbol{F}_0 \otimes \boldsymbol{I}$ and Var[$\boldsymbol{e}$] = $\boldsymbol{R} = \boldsymbol{R}_0 \otimes \boldsymbol{I}$, where $\otimes$ is the Kronecker product and

$$\boldsymbol{B}_0 = \begin{bmatrix} \sigma_{b_{11}}^2 & 0 & 0 \\ 0 & \sigma_{b_{22}}^2 & 0 \\ 0 & 0 & \sigma_{b_{33}}^2 \end{bmatrix} \quad \boldsymbol{F}_0 = \begin{bmatrix} \sigma_{f_{11}}^2 & \sigma_{f_{12}} & \sigma_{f_{13}} \\ \sigma_{f_{21}} & \sigma_{f_{22}}^2 & \sigma_{f_{23}} \\ \sigma_{f_{31}} & \sigma_{f_{32}} & \sigma_{f_{33}}^2 \end{bmatrix} \quad \boldsymbol{R}_0 = \begin{bmatrix} \sigma_{e_{11}}^2 & 0 & 0 \\ 0 & \sigma_{e_{22}}^2 & 0 \\ 0 & 0 & \sigma_{e_{33}}^2 \end{bmatrix}$$

Heavy tree mortality was due to frost in Cravens and water-logging at Martins. Therefore, survival data were analysed as separate traits with a logistic mixed model following equation (1). However, the model used a binary response variable (0 dead, 1 alive) following a binomial distribution and a logit link function.

Heritability (h^2) and genetic correlations (r_{ij}) were estimated using the standard formulas:

$$h^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_b^2 + \sigma_e^2} \qquad r_{ij} = \frac{\sigma_{f_{ij}}^2}{\sigma_{f_{ii}}\sigma_{f_{jj}}}$$

The heritability for survival used the residual variance for the logit link ($\sigma_e^2 = \pi^2/3$). Standard errors for heritabilities and genetic correlations were approximated using a Taylor series. All model fitting was done using ASReml-R, an implementation of ASReml in the R statistical system (Gilmour et al. 2002, Development Core Team 2008).

3. RESULTS

There were large among-trial differences for average survival, ranging from 38.2% for Martins to 98.5% for Lawsons (Table 2). The average differences for Cravens and Martins mask large between-family differences. In addition, there was a significant difference between the survival of the 998 (*E. quadrangulata*) and the 997 and 999 (*E. globoidea*) controls. The survival of 998 was equal to (in Cravens) or better than (in Martins) the average family performance.

Table 2. Average tree height (cm) and status (number of dead and alive) by site, percentage survival and main cause of mortality.

Site	Height	Dead	Alive	Survival	Cause of mortality
Cravens	137.0	1703	1997	54.0%	Frost
Lawsons	149.9	69	4424	98.5%	
Martins	132.8	2317	1433	38.2%	Water-logging

There were significant site-level differences for average tree height, with Lawsons — the driest site but with warmest minimum temperatures, see Table 1 — showing the best growth.

Heritabilities for height were low, ranging between 0.10 and 0.14, which is a common result for early growth of trees. This indicates that most of the observed variability potentially corresponds to within-site environmental variability and non-additive genetic effects. Interestingly, the site with the highest survival displays the lowest heritability.

The genetic control for survival presented high heritabilities for Cravens (0.50, mostly affected by Frosts) and Lawsons (0.61, high overall survival). Martins, which was plagued by water-logging problems, presented a much lower heritability (0.11), reflected the underlying environmental effects that caused patched mortality.

Table 3. Estimated genetic parameters for tree height and survival at age 2: degree of genetic control (heritability), genetic correlations between sites and their standard errors (between pathentheses).

Site	Trait	Heritability	Cravens	Lawsons	Martins
Cravens	Height	0.13 (0.04)			
Lawsons	Height	0.10 (0.02)	0.74 (0.14)		
Martins	Height	0.14 (0.05)	0.99 (0.21)	0.80 (0.19)	
Cravens	Survival	0.50 (0.10)			
Lawsons	Survival	0.61 (0.09)			
Martins	Survival	0.11 (0.03)			

Figure 2 summarizes predicted height and survival (Best Linear Unbiased Predictions—BLUP—plus the overall mean) for each site, complementing the story presented in Table 2. Lawsons displays very small between- and within-family variability for survival, while there is a large spread in survival for both Cravens and Lawsons, with a 60% difference between best and worst survival families.

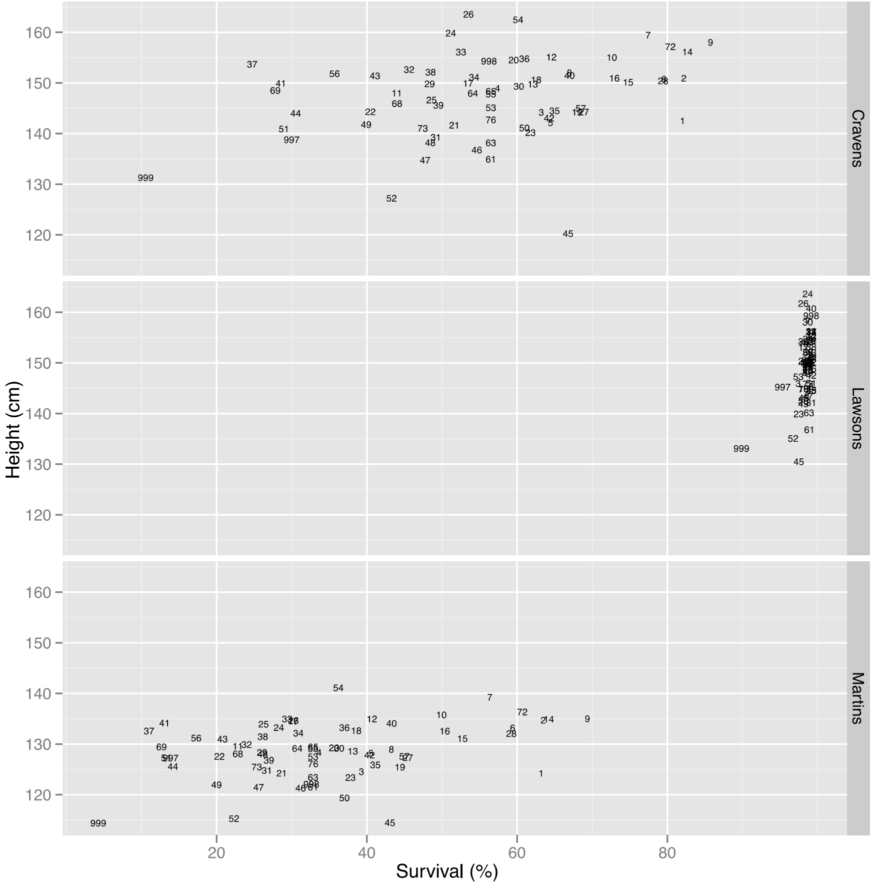

Figure 2. Breeding values for tree height and survival of *E. bosistoana* growing in three sites. Numbers correspond to family IDs, with 997, 998 and 999 referring to bulk seedlot controls. Notice the similarity of height variability for all sites, and contrast with the narrow variability of survival in Lawsons.

The situation is different for tree height, with all sites displaying similar levels of variation, a spread of roughly 30 cm between top and bottom families. However, if we consider the degree of genetic control for height (8-13%, see Table 2), much of the observed variability is of environmental rather than of genetic nature.

Another important question for breeding programs is how consistent are rankings across sites. Figure 3 displays family rankings; that is, BLUPs simply expressed as deviations from each site mean. Rankings are very consistent between Cravens and Martins (as indicated by the high genetic correlation between sites), and show some interaction in Lawsons, although the correlation is still very high (between 0.74 and 0.80).

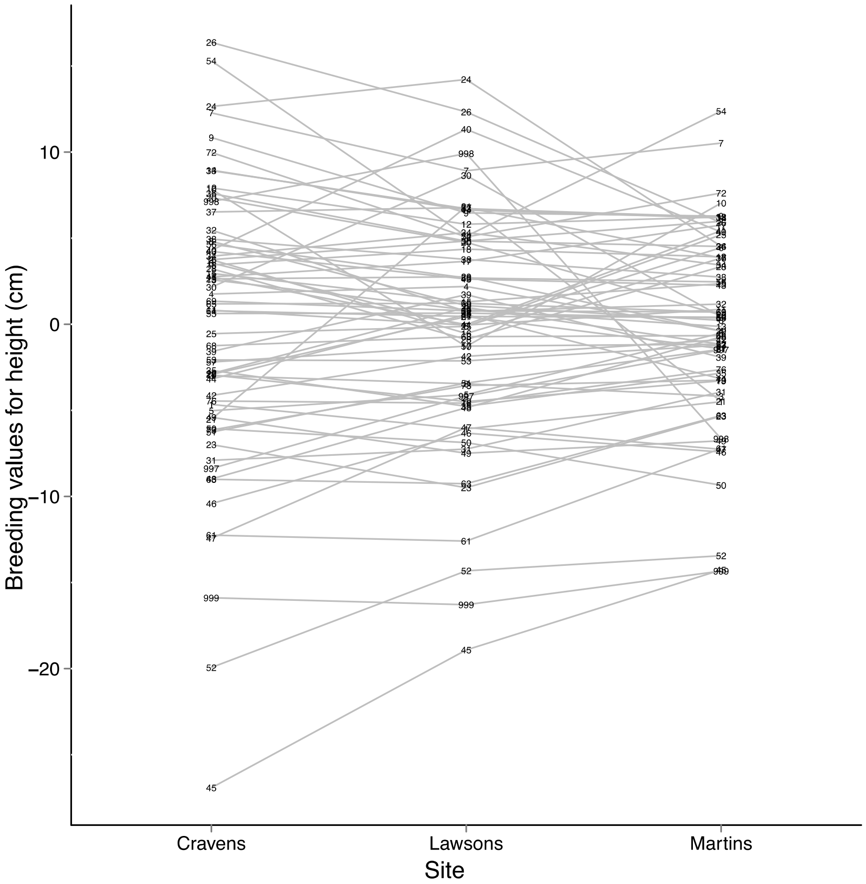

Figure 3. Family ranking for tree height (expressed as deviation from the site mean). There is high between-site correspondence, which suggests no need to create site-specific breeding and deployment populations at this stage.

4. DISCUSSION

In principle, a formal definition of breeding objectives should precede any serious work in a breeding program. In practice, when introducing new species to an environment—as is the case of *E. bosistoana* in New Zealand—the first priority is to screen for adaptation: growth rate, survival and tree health.

Our results correspond to *early* adaptation (tree height and survival) without any consideration to forest health and wood properties. A cursory view of Figure 2 reveals opportunities for selection; with the best trees in each panel (site) located in the top-right corner; that is with high growth and good survival. Except for Martins (affected by water-logging) the degree of genetic control for survival is high (~55% on average), which suggests room for selection. In addition, these results highlight the importance of working with relatively large samples, both on the number of trees under testing and on the coverage of natural populations growing in contrasting environments.

Heritability estimates assumed that, on average, we were dealing with ideal half-sib families and used a coefficient of relationship of 1/4. Nevertheless, studies in other species have used larger coefficients, e.g. 1/2.86 for *E. camaldulensis* (Apiolaza 1994) based on Squillace (1974) and between 1/3 and 1/2.5 for several species (Eldridge *et al.* 1993) to account for deviations from ideal conditions. As an example, *E. globulus* presents varying family-level selfing and small-scale spatially structured populations, almost guaranteeing deviations from ¼. Unfortunately, there is very little published information on both the reproductive biology and population structure of *E. bosistoana* as to make any realistic assumption. Nevertheless, heritabilities presented in this paper can be easily scaled to represent alternative coefficients of relationship. Estimated genetic correlations between traits will not change when assuming different coefficients of relationship among siblings.

Genotype by environment interaction is a common preoccupation in breeding programs, particularly for growth traits, (e.g. Wei and Borralho 1998; Costa e Silva *et al.* 2006, Costa e Silva *et al.* 2008). At least at this stage, there is little evidence for strong changes of ranking that would require more complex (and therefore expensive) breeding and deployment strategies. In general, wood properties tend to be less susceptible to GxE interaction, although changes of scale (as opposed to ranking) are not unusual (e.g. Raymond *et al.* 2001, Apiolaza 2011).

Genetic evaluation models did not include provenance or locality effects to account for different genetic populations, (e.g. Dutkowski and Potts 1999). At this stage, there is not enough data available to delineate these populations that would result in better fitting models. The accuracy of the predicted breeding values could also be improved by fitting within-site spatial patterns (e.g. Gilmour *et al.* 1997), as there are strong environmental gradients in some of the trials, relating to slope and aspect. Improved genetic-statistical models are under development.

One of the main objectives of the NZDFI is to generate a forest resource with naturally durable wood, i.e. that does not require preservatives. Durability in eucalypts is directly related to the presence of extractives in heartwood, which is not possible to evaluate—at least phenotypically—at this early stage.

5. FINAL REMARKS

Tree breeding is a numbers game and, as shown by these results, there is a need to screen large numbers of families and trees within families to find some winners. Domesticating a new species requires, first, finding subpopulations of good enough adaptation as to permit screening for other objective traits like growth and wood properties.

Therefore, having selected the best-adapted material from this series of trials, we can start investigating and understanding the variability of durability and tree health. The characterization of wood properties, particularly of durability, will require new tools and techniques. Developing these will require additional R&D funding.

Given that there is no much experience with *E. bosistoana* in New Zealand, the trials that we establish serve both for tree breeding and to discover the appropriate environmental conditions for optimal growth of the species. This situation carries some additional risks, as discovered at the Martins site.

The NZDFI expanded the *E. bosistoana* collection in 2010 by planting another 36 families on a further three sites, with progeny of mostly plus tree collections within the Northern natural populations. Our expectation is that Southern populations will be more successful in New Zealand drylands and on this basis, we have just completed seed collection from a further 86 plus trees that will be put to the nursery to propagate seedlings for planting in 2012. However we will also watch with interest the performance of our Northern collections as international experience with other species has delivered some surprises. These additional collected trees are not part of this series of sites, but they will be linked to this work in future trials.

6. SUMMARY

This article presents early results on the genetics of adaptation for *Eucalyptus bosistoana* growing in New Zealand drylands. A progeny trial established in 3 sites in Marlborough and Canterbury was analyzed for growth and survival at age 2 years. There was substantial variation for both height growth and survival both between- and within-family. However, only a small proportion of height variation was under genetic control (h^2 ranging from 0.10 to 0.14), while survival was under strong genetic control (h^2 from 0.50 to 0.61), except in Martins, a site damaged by water-logging. There was a low degree of genotype by environment interaction for tree height, with genetic correlations ranging between 0.74 and 0.99. This high correlation suggests that there would be no need to create breeding and deployment zones for this species.

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