An investigation of assumptions made in estimating genetic parameters and predicting genetic gain in a *Eucalyptus nitens* breeding programme in South Africa

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Abstract

It is important to have an understanding of the population genetics and validity of the pertinent underlying assumptions of a species in order to design an effective breeding strategy. In a South African breeding population of *Eucalyptus nitens*, various scenarios investigating a range of assumptions were developed and used to predict genetic gain in the F2. These were compared with realised gains achieved in a series of genetic gain trials. In the two scenarios using firstly, actual flowering for family (provenance) and, secondly, estimated flowering after 30% roguing of poor families, a coefficient of relationship of 0.33 resulted in predictions closest to realised gain on average. The statistical information suggested that outcrossing in the seed orchards was greater than 80%. Indications were that the effects were additive, and that very little or no heterosis had occurred, due to the still significant provenance effects and the lack of provenance rank changes in the F2.

The custom of assuming a degree of inbreeding (and using a coefficient of relationship of 0.33) and of including provenance effects in the models on average resulted in genetic gain predictions which were very similar to the realised genetic gains in this population of *Eucalyptus nitens*.

Introduction

A good understanding of the population genetics and underlying assumptions of a species are important for designing breeding strategies (Tibbits and Hodge 1998). *Eucalyptus nitens* is an important commercial cold tolerant eucalypt (CTE) species, grown primarily for pulp and paper production in the summer rainfall regions of South Africa. Currently there are no suitable alternative commercial eucalypt species to *E. nitens* in South Africa for sites prone to moderate frost and heavy snowfalls (Gardner and Swain 1996). There is considerable variation for several growth, reproductive and wood property traits within the species in South Africa, which is based primarily on Australian provenance origin (Swain 2013). This variation provides potential for genetic improvement, and it is important that an appropriate advanced generation breeding strategy be developed for improvement of *E. nitens*.

Several studies have been done to estimate genetic parameters in *E. nitens* (Gea et al. 1997; Johnson 1996; King and Wilcox 1988; Tibbits and Hodge 1998; Whiteman et al. 1992), with some research into the factors affecting these estimates, i.e., panmixis (Grosser et al. 2010; Tibbits 1989), outcrossing rates (Gea et al. 1997; Grosser et al. 2010; Moncur et al. 1995), self-incompatibility and inbreeding (Hardner and Tibbits 1998, Pound et al. 2003). However, there is little known about the underlying assumptions in the South African populations of *E. nitens*, and there is some concern about the impact that poor and erratic flowering, and subsequent seed production, of the species in South Africa (Gardner 2003) may have on genetic gain. In addition, the poor flowering may violate some of the basic assumptions in parameter estimation and genetic gain prediction, such as occurrence of panmixis, absence of non-additive effects, no correlation between flowering and growth, and may affect the coefficient of relationship used.

Statistical analysis of several F1 and F2 *E. nitens* trials run by the Institute for Commercial Forestry Research (ICFR) in South Africa has led to the development of estimates for genetic parameters and of

juvenile-mature and trait-trait genetic correlations, as well as an indication of the presence/absence of genotype by environment interaction (Swain 2013; Swain et al. 2013b).These have been invaluable in determining the breeding potential of the *E. nitens* population, selection of superior families and individuals, as well as informing the breeding strategy for the species. Genetic gain trials allowed for comparison of realised gain in the F2 with predicted genetic gain from the F1 (Swain 2013), and this provides an opportunity to assess the performance of the quantitative predictions using our underlying assumptions in the F1.

There are several assumptions made in the predictions of genetic gains which, if incorrect, may cause inaccuracy in predictions using deterministic methodology (Verryn et al. 2000b):

1) The assumption of the coefficient of relationship (*cr*) = 0.33 to allow for the presence of full-sibs within the open-pollinated families, and that at least some inbreeding occurs (Snedden et al. 2007; Squillace 1974).

2) The assumption of > 70% outcrossing between eucalypt seed orchard trees.
   Moncur et al. (1995) estimated that outcrossing was at a level of 75% in an *E. nitens* seed orchard, and Pound et al. (2003) found that levels of self-incompatibility in *E. nitens* ranged from 25.8 to 93.6%. The species demonstrates preferential outcrossing and appears to have a late-acting self-incompatibility system operating to reduce the production of selfed seed (Pound et al. 2003; Tibbits 1989). The assumption of > 70% outcrossing made by many authors (Butcher and Williams 2002; Grosser et al. 2010; Hodge et al. 1996; Moncur et al. 1995; Moran et al. 1989), may not be true of these F1 *E. nitens* seed orchards, due to poor flowering.

3) The assumption that flowering and mating is random, and that panmictic pollination is occurring, i.e., that all individuals have equal opportunities to mate with any other individual (Hodge and White 1993).
   i) Asynchronous flowering has been shown to exist between eucalypt provenances (Jones 2002; Moncur and Boland 2001; Tibbits 1989; Volker et al. 1990), indicating that this assumption may not be correct. However, these flowering windows do overlap (Gardner 2003; Jones 2002), which may allow for panmictic pollination some of the time. In addition to

asynchronocity, flowering in *E. nitens* is subject to seasonal influences (Gardner and Bertling 2005; Jones 2002; Moncur and Hasan 1994; Tibbits 1989) and, as the stability, or greater influence, of female effects in reproductive success in some eucalypts has been noted (Leal and Cotterill 1997; Suitor et al. 2009; Tibbits 1989), it is possible that only a few effective males may contribute to pollination of all the other trees in a seed orchard (Hodge et al. 1996; Suitor et al. 2009; Swain et al. 2013a). Conversely, pollen from a few heavy-flowering individuals may have too large a genetic influence on adjacent female trees (Moncur and Boland 2001). Grosser et al. (2010) also found that the parental contribution to progeny varied amongst clones in an *E. nitens* clonal seed orchard, suggesting that panmictic pollination was not occurring.

ii) Actual selection intensity - in reality, stratified selection based on flowering could have taken place in the ICFR seed orchards, as flowering of individual trees ranged from 15 to <50% in the year prior to seed collections (Swain et al. 2013a). This means that up to ten trees may have contributed seed from some families and as little as one tree contributed from other families, i.e., the selection intensity in the orchard could have been higher than the figures used in the predictions, resulting in higher realised genetic gains.

4) The assumption that there is no correlation between flowering and growth, i.e., flowering individuals would not be selected against due to their growth, and *vice versa*.

Varghese et al. (2009) found contrasting trends in their studies on *E. camaldulensis* and *E. tereticornis* in India. These authors found a negative genetic correlation between flowering and outstanding growth performance in unimproved provenances in *E. camaldulensis*, but no such correlation was found in an improved seed orchard of *E. teretecornis*. Although there are currently no published results on such studies on *E. nitens* in South Africa, Gardner (pers comm[1]) did not find any correlations between flowering and growth in flowering studies in *E. nitens*, nor has Jones (pers comm[2]) found any strong correlations in *E. nitens* seed orchards.

5) The assumption of the absence of non-additive effects such as inbreeding depression or heterosis, the latter as a result of mixing of provenances by outcrossing of all families in the F1. It has been

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assumed that variance is additive in this *E. nitens* breeding population, with negligible non-additive effects, as has been found in other studies on the species (Hamilton et al. 2008; Hodge et al. 1996), but if non-additive effects were playing a role, the predictions of genetic gain would be overestimated due to inflation of narrow-sense heritability estimates (Bush et al. 2011; Hodge et al. 1996).

Therefore, to determine which assumptions best fit the ICFR *E. nitens* populations for the estimation of genetic parameters in the F1, several scenarios of predicted genetic gain were developed from the F1, and compared with the gains realised from genetic gain trials (F2). Recently, similar studies have been done on stand volume in *E. globulus* (Callister et al. 2013), and on *E. cladocalyx,* incorporating molecular markers to calculate inbreeding rates and coefficients of relationship (Bush et al. 2011). This study will also indicate whether there are important digressions from the assumptions made in the F1 study (Swain et al. 2013b), and whether the *E. nitens* breeding strategy needs to be adapted to take these into account.

Material and Methods

Eight F1 provenance-progeny trials comprising *E. nitens* seedlots imported from Australia were established in the summer rainfall region of South Africa during the 1980's and 1990's. Details of these F1 trials, statistical analysis thereof, estimation of genetic parameters for the F1 and predicted gain for the F2 can be found in Swain et al. (2013b). Both the estimation of genetic parameters in the F1 and predicted gains for the F2 used a coefficient of relationship of 0.33 (Swain et al. 2013b). These trials were subsequently thinned to seed orchards, based on the results of the final measurements, and seed collected over several years to establish progeny trials (F2) of this material (Swain 2013). In addition, three genetic gain trials were established on temperate forestry sites in South Africa early in 2001 to test the improved material (Swain et al. 2013a). The genetic gain trials also allowed for comparison of realised gain in the F2 with predicted genetic gain, as well as the calculation of realised heritabilities in the F1 (Swain 2013).

The realised genetic gains in the F2 were estimated by comparing the average performance of the progeny of a range of improved bulks originating from each of four ICFR F1 seed orchards, i.e., Amsterdam,

Helvetia, Jaglust and Jessievale, with an F1 bulk (Treatment 22) in the genetic gain trials described by Swain et al. (2013a). The families within each F1 seed orchard were not related by female parent. These realised gains were then compared with the gains predicted from a range of scenarios, testing coefficients of relationship and selection intensities. The authors also investigated the effect of provenance in variance component estimation, i.e., families nested within provenance (family (provenance)) and families across provenances (Swain et al. 2013b), but as this and other studies have shown that across-provenance estimates result in overestimates of additive genetic variance (Brawner and Elizaul 2007; Johnson 1996; Tibbits and Hodge 1998), this scenario was not included in this report. The scenarios investigated were:

1) Coefficient of relationship and outcrossing -

   Following the approach of Squillace (1974), and extending his calculations, it has been shown that 30% selfing gives an average coefficient of relationship among open-pollinated (OP) progeny of 0.4 (Griffin and Cotterill 1988) and 25% selfing gives an average coefficient of relationship of 0.5 (Hodge et al. 1996). Snedden et al. (2007) assumed a coefficient of relationship of 0.33 in an *E. grandis* study where approximately 20% inbreeding was found. Extending this approach, the assumed inbreeding of 20% and the assumed coefficient of relationship of 0.33 may be too low in seed orchards where flowering is poor and pollen trees may be isolated from maternal parents. Although levels of relatedness and inbreeding are not available from ICFR seed orchards until DNA genotyping/genetic marker studies have been completed, percentage of trees flowering has ranged from 15 to 47% over orchard and year, in terms of the number of individual trees flowering (Swain et al. 2013a), which could decrease genetic gain achieved. Thus this scenario considered an additional higher level of selfing of 25% (and, by extension, outcrossing rate of ≈ 75%) for the family (provenance) parameters estimated above, using a coefficient of relationship of 0.4 as a surrogate for the selfing rate. An assumed selfing rate of 0% (surrogate $cr = 0.25$; assumed outcrossing 100%) was also included for comparative purposes. Thus the coefficient of relationship can, in effect, be used to correct for relationships closer than half-sib, which have resulted from selfing or related individuals crossing (Bush et al. 2011).

2) Selection intensity – should flowering be poorer in reality, i.e., 40% flowering, this would decrease the population size from which selections are made, given that the population size was

less than 400 (Becker 1975), resulting in lower genetic gains. The ICFR seed orchards were rogued of the poorest 30% of families and each remaining plot thinned so that only the top-ranked tree per plot remained. Thus this scenario is represented by actual flowering that occurred in the ICFR seed orchards the year before seed was collected for the ICFR genetic gain trials, and compared with an assumed 100% flowering of the remaining 70% of families after roguing and thinning. Due to the incremental selection applied to the seed orchards, incorporating roguing, thinning and bulking of selected seedlots, computation of the selection intensities was also done in an incremental or phased manner. This scenario was run for the family (provenance) model estimated above, and for the different outcrossing scenarios.

Thus, there were six different scenarios to be compared, as summarised in **Table 1**.

Genetic gains in the F2 were predicted from the following formula (Verryn et al. 2000, adapted) for the rogued scenarios:

$$\Delta G = 0.5\left(\Delta G_f\right) + 0.5\left(\Delta G_m\right), \text{ where;}$$

$$\Delta G_f = \left[\left(SI_1 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right) + \left(SI_2 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right) \right]$$

$$\text{and } \Delta G_m = \left[SI_1 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right],$$

and the predictions are the predicted genetic gains in the F2 from female and male selection in an F1 population, respectively.

(SI_1 = selection intensity between/among female or male families, respectively, i.e., number of families remaining in seed orchard after roguing of poor families out of the total original number of families,

SI_2 = selection intensity within female families, i.e., number of families used to make up bulk treatment out of those remaining in seed orchard after roguing,

cr = coefficient of relationship,

σ^2_A = additive genetic variance,

σ_{fm} = standard deviation between/among families (provenance),

SI_{wf} = selection intensity within female or male families, respectively, within plots,

t = number of trees per plot, and

σ_{wf} = within plot family standard deviation).

The selection intensity for male and females differed as, in addition to roguing and thinning, the top 8 to 16 families were selected to make up bulked seed orchard treatments. Male and female selection intensities between/among families and within families within plots were determined using the standardised selection intensity tables of Becker (1975).

The genetic gain equations used for the actual flowering scenarios are similar to those above, except that both the ΔG_f and ΔG_m equations now incorporate the actual number of families flowering, as follows:

$$\Delta G_f = \left[\left(SI_1 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right) + \left(SI_3 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right) \right]$$

and $$\Delta G_m = \left[SI_4 \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right],$$

where;

SI_3 = selection intensity within female families, i.e., proportion of families selected for the bulk
       treatment from those flowering in the seed orchard after roguing,

SI_4 = selection intensity within male families, i.e., the proportion of families remaining after roguing
       multiplied by the proportion of flowering in the seed orchard.

The remainder of the variables are defined as for ΔG_f and ΔG_m in the roguing scenario above. The same additive genetic variances were used for predicting gain from both the rouging and actual flowering (selection) scenarios, after Shelbourne (1992) and other authors (Johnson 1996; Tibbits and Hodge 1998; Volker et al. 1990), where the same variance components were used before and after selection.

The single-site estimates of heritability were used in the genetic gain calculations rather than the general

across-site heritability estimates, although these can upwardly bias the predicted genetic gain (Tibbits and Hodge 1998). However the authors felt justified in using the single-site estimates because (i) genotype-environment effects were negligible in the F1 population, with Type B genetic correlations ranging from 0.75 to 0.99 for the four seed orchards represented in the genetic gain trials (Swain et al. 2013b), with an average genetic correlation of 0.92, and (ii) the across-site heritability estimate has the disadvantage of losing accuracy with respect to individual site resolution.

Results and discussion

Comparisons of the predicted gains in dbh from the various scenarios to investigate the underlying assumptions used in estimating genetic parameters for the F1 are presented with the realised gain in **Table 1**. The data from the individual trials were erratic, and thus the gains were averaged across the trials for each scenario, this approach assumed to be more reliable, and this is summarised in **Figure 1.** The low predicted gains at Helvetia differed notably from the higher realised gains, due to a particularly low h^2 for dbh in the F1 ($h^2 = 0.14$ at 94 months). It is unclear why the h^2 was so low, relative to the gains, at this site.
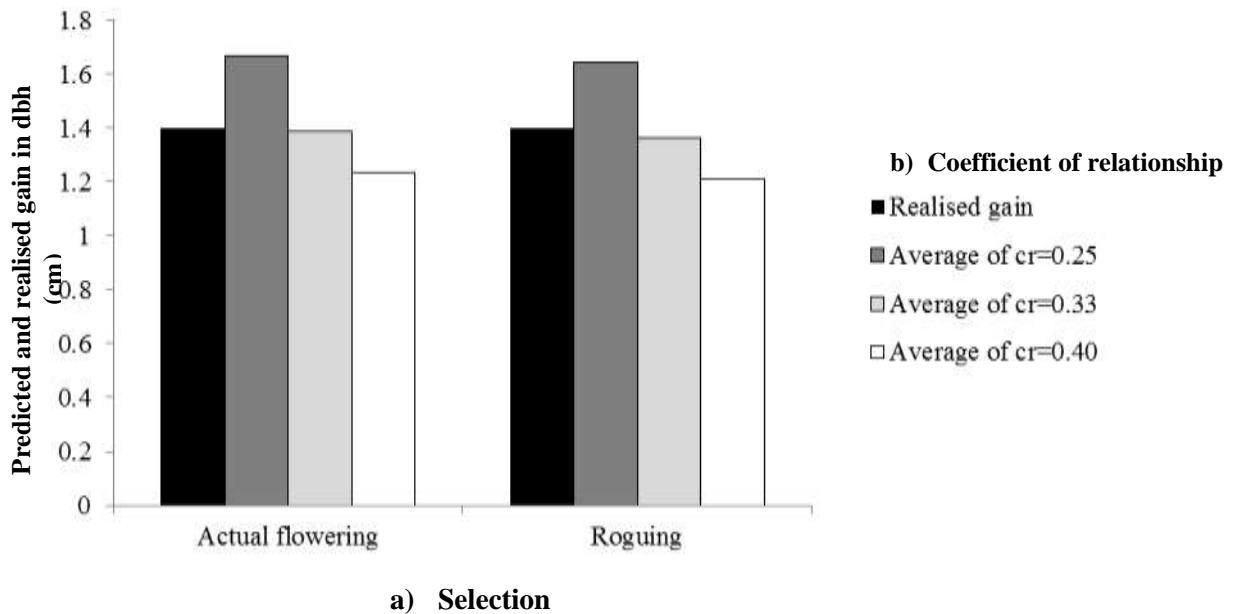

Fig. 1 Comparison of mean realised gain in dbh (cm) averaged across three *Eucalyptus nitens* genetic gain trials in South Africa with mean gains predicted from six F1 seed orchards for different scenarios, including; a) Selection Intensity, and b) Coefficient of relationship (*cr*)

Table 1   Predicted gains in dbh for various scenarios in F1, compared to realised gains of F2 *Eucalyptus nitens* bulk treatments at 87 months over unimproved F1 bulk, from six seed orchards, as estimated from genetic gain trials (Swain et al. 2013a)

		Trial series & site name (age in months at final measurement[a])					
		E88/01 Jessievale at 101 mths (cm) (%)	E88/01 Amsterdam at 101 mths (cm) (%)	E88/03 Daspoort at 113 mths (cm) (%)	E88/03 Helvetia at 94 mths (cm) (%)	E88/05 Babanango at 112 mths (cm) (%)	E88/06 Woodstock at 76 mths (cm) (%)
Realised gain in cm dbh (%)		**1.02 (7.3)**	**1.90 (13.7)**	**Helvetia bulk [b] 1.80 (12.9)**	**1.80 (12.9)**	**Jaglust bulk[c]: 1.40 (10.1)**	**Jaglust bulk[c]: 1.35 (9.7)**
Family (provenance)	**Scenarios**	Predicted gain in cm dbh (% gain)					
Roguing:	**1 – cr = 0.25**	1.17 (8.4)	1.84 (13.2)	2.47 (17.7)	0.35 (2.5)	3.00 (21.5)	1.84 (13.2)
	2 – cr = 0.33	0.99 (7.1)	1.25 (8.9)	2.05 (14.7)	0.32 (2.3)	2.52 (18.0)	1.55 (11.1)
	3 – cr = 0.40	0.89 (6.4)	0.94 (6.7)	1.82 (13.0)	0.31 (2.2)	2.26 (16.2)	1.40 (10.0)
Actual flowering:	**4 – cr = 0.25**	1.18 (8.5)	1.94 (13.9)	2.49 (17.8)	0.35 (2.5)	3.06 (21.9)	1.87 (13.4)
	5 – cr = 0.33	1.00 (7.1)	1.36 (9.7)	2.07 (14.8)	0.32 (2.3)	2.58 (18.4)	1.59 (11.3)
	6 – cr = 0.40	0.90 (6.4)	1.04 (7.4)	1.83 (13.1)	0.31 (2.2)	2.31 (16.6)	1.43 (10.2)

[a] Different ages should be noted,
[b] As no seed had been produced from this seed orchard by time of F2 establishment, parameters from the related Helvetia orchard were used,
[c] Jaglust is a seed orchard representing the material in the Babanango and Woodstock trials

Coefficient of relationship and outcrossing rate

With regards to the scenarios using actual flowering for family (provenance), which in theory, should be the most accurate predictor of gain, $cr = 0.33$ was closest to realised gain (**Table 1**), indicating the presence of some full-sibs within the open-pollinated F2 families. This was probably mainly as a result of selfing, but possibly also from biparental or neighbourhood mating (Bush et al. 2011). This coefficient of relationship was also found to produce predictions closest to realised values for the roguing scenarios. Although a coefficient of relationship of 0.4 has been used for eucalypts (Brawner and Elizaul 2007; Greaves et al. 1997; Volker et al. 1990; Whiteman et al. 1990), this coefficient of relationship supports earlier work by various authors (Griffin and Cotterill 1988; Griffin et al. 1987; Hodge et al. 1996; Verryn 1993), where it was suggested that seed orchard OP families are not true half-sib families, and may contain sufficient full-sib families to increase the coefficient of relationship from 0.25. A coefficient of relationship of 0.33 has been commonly used by many authors for OP eucalypt populations (Borralho et al. 1992), particularly in South Africa (Louw 1996; Ndlovu 2008; van den Berg and Stanger 2007; van Deventer 2009; Verryn et al. 2009). This coefficient of relationship could be used for future prediction of gains in roguing scenarios in *E. nitens*, based on our knowledge and the data presented here, and should be sufficient to correct for the amount of selfing or neighbourhood mating taking place. A further consideration however, is whether inbreeding levels are uniform within the entire population, as (i) the application of a single coefficient of relationship to the whole population may not give accurate estimates of variance components, and (ii) in cases where the levels of inbreeding are different from the single coefficient of relationship, the Best Linear Unbiased Predictor (BLUP) breeding values will be improperly regressed towards the mean, which could result in incorrect ranking of breeding values (Bush et al. 2011). Molecular marker studies will further inform the levels of selfing in this population, and whether the levels of inbreeding are variable or more uniform.

In addition, the statistical information suggests that outcrossing was >80% in the ICFR seed orchards. This is higher than expected by the authors, given the general sparse flowering in the species in South Africa (Gardner and Bertling 2005; Jones 2002). Hodge et al. (1996) found that estimates of outcrossing in *E. nitens* are higher in seed orchards than in native stands and, following microsatellite studies, Gea et al.

(2007) estimated an outcrossing rate of 0.87 in an OP *E. nitens* clonal seed orchard in New Zealand. Grosser et al. (2010) determined an average outcrossing rate of 85% in a similar orchard in Australia. Thus these suggestions of higher than expected outcrossing in the ICFR seed orchards are not unreasonable, although such indications would need to be verified with molecular marker studies in this *E. nitens* population. It is thought that outcrossing is high in *E. nitens* due to successful late-acting self-incompatibility mechanisms in the species (Hodge and White 1993; Pound et al. 2003).

As variation in the outcrossing rate between families and individuals may obscure differences in breeding values between parents when estimated with OP families (Burgess et al. 1996), it may be worth using family/individual outcrossing rates to better predict breeding values. Hodge et al. (1996), however, found that OP tests predicted breeding values well for *E. nitens,* and better than for *E. globulus*.

Selection intensity

The actual flowering scenarios had similar predicted gains to the roguing scenarios. This was initially surprising due to flowering commonly being poor in *E. nitens* in South Africa (Gardner 2003; Gardner and Bertling 2005; Jones 2002; Swain and Gardner 2003), with possible subsequent poor outcrossing and genetic gain. A possibility is that the similar predicted gains reflect the low additional selection possible in the orchards due to the low proportion of trees flowering. Further investigation, however, showed that although the proportion of individuals flowering in the ICFR orchards was relatively low in the year prior to the seed collections for the genetic gain trials (15 to 47% (Swain et al. 2013a)), the total number of families flowering in each seed orchard was generally high (59 to 91%). As genetic gains are most sensitive to the family selection intensity (as opposed to within family selection) (Shelbourne 1992), this, together with the self-incompatibility mechanisms that exist in *E. nitens*, may explain the similar predicted gains for the actual flowering and roguing scenarios.

The next logical consideration, i.e., that increased flowering in seed orchards may increase gains due to an increase in population size from which selections are made, bears further consideration. A decrease in outcrossing rates has been linked to a decrease in progeny growth in eucalypts (*E. nitens*: Hardner and

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Tibbits 1998; *E. globulus*: Hardner and Potts 1995; Patterson et al. 2004; *E. regnans*: Griffin and Cotterill 1988), and it has been shown that flowering above a certain low level may result in increased gains (Swain et al. 2013a; Varghese et al. 2009). It is unclear, however, whether additional flowering above this level will confer any further benefit. The flowering levels in the seed orchard bulk entries in the genetic gain trials represent the number of individuals flowering in the seed orchard, irrespective of the number of trees flowering per family, which may be as little as one. A future increase in flowering is most likely to result from more trees per family flowering, rather than a marked increase in the number of families flowering. This is because the number of families flowering in the orchards the year prior to selecting and making up the bulked entries for the genetic gain trials was relatively high (59 to 91%), compared to the total trees flowering (15 to 47%) (Swain et al. 2013a). An increase in the number of trees per family flowering would lead to an increase in gain if the best trees per family flowering were selected. Should an increase in the numbers of families flowering be possible in those orchards with less families flowering (e.g. 59% at Helvetia and 67% at Jaglust), this, together with selection of the best trees per family flowering, would lead to even greater gains. It is likely that outcrossing would also be improved with increased flowering, resulting in less potential inbreeding depression in the F2.

The numbers of families selected to make up the bulked entries in the genetic gain trials were small and ranged from 8 to 16 families per bulk (Swain et al. 2013a). Although these bulks could certainly be used for establishment of high productivity plantations, it is more likely that, due to the shortage of improved *E. nitens* seed in South Africa, commercial bulks would comprise at least 16 families. By contrast then, this would mean a decrease in the selection intensity in the seed orchards, with a resultant slight decrease in genetic gain.

Although superior individuals can be grafted from tree improvement trials into clonal seed orchards (CSOs) for capture of maximum genetic gain, problems associated with grafting of *E. nitens* (de Little et al. 1992; Moncur 1998) can cause delays in production from CSOs, making the South African forestry industry reliant on production of improved seed from Breeding Seed Orchards (BSOs). It is therefore important to construct BSOs with both sufficient families and individuals per family, as well as adequate numbers of

families flowering simultaneously (or with periods of overlapping flowering), to ensure sufficient outcrossing and to actually realise potential gains. Flowering and seed orchard research that results in technologies to improve and stabilise flowering (Gardner 2012; Germishuizen and Gardner 2014) will help to make this possible.

Provenance effect

Progeny trials are not generally designed to detect inter-provenance heterosis/non-additive effects (Vaillancourt et al. 1995), yet these effects are likely to be negligible in this population, due to the still significant provenance effect in the F2, the lack of provenance rank changes for dbh in the F2 (Swain et al. 2013a), and the presence of intermediate phenotypes in the F2, indicating a degree of inter-provenance crossing occurring. Although dominance effects have been found in selfed seedlots of *E. nitens* (Hardner and Tibbits 1998), Hodge et al. (1996) found negligible levels of non-additive effects in their *E. nitens* study of open-pollinated and control-pollinated progeny, and an absence of inbreeding depression (1%). These authors also found lower levels of non-additive genetic variation and deleterious abnormalities in *E. nitens* than in *E. globulus* (Hodge et al. 1996). Many authors assume negligible or zero non-additive effects in *E. nitens* (Hamilton et al. 2008).

Although the argument for negligible non-additive effects in this population is strong, the possibility exists that inbreeding depression exists in the ICFR F1 seed orchards due to biparental mating in the native stands. Thus the gains observed in the F2 genetic gain trials (Swain et al. 2013a) may have resulted from changing (decreasing) levels of inbreeding depression due to the release from biparental inbreeding. There is also the slight possibility that provenances themselves are inbred populations, although this seems unlikely, as this has not been reported in the many studies done by various authors on these *E. nitens* provenances (Gea et al. 1997; Hamilton et al. 2008; Johnson 1996; Tibbits and Hodge 1998; Tibbits and Reid 1987). Molecular studies will better inform both these scenarios.

The significant provenance effect in the F2 (Swain 2013) could be as a result of asynchronous flowering between provenances and resultant lack of panmixis. Asynchronous flowering between provenances in the

F1 BSOs would violate the assumption that pollination occurs randomly and that pollen parents are equivalent (Shelbourne et al. 2007), and would result in traits and grouping of genes by provenance in the F2. The lack of panmixis would have an effect on the variance components and heritability estimates, with a possible underestimation of family variance components and resultant heritability, if the parents were actually correlated by provenance (Squillace 1974). In addition, given the relatively small population sizes of the seed orchard bulks in the genetic gain trials (F2) and sparse flowering in some of these bulks (Swain et al. 2013a), one could be inclined to raise concerns about the lack of panmixis, which in turn, might lead to inbreeding depression and poorer performances of those bulks.

Although it is difficult to test whether panmixis occurred in these populations without a molecular study, Hodge and White (1993) argued that deviations from panmixis should have little or no effect on the average genetic value of an orchard crop. They suggested that crosses that were absent, or present in unequal frequencies, would only negatively affect overall genetic quality of a seed crop if: a) Specific Combining Ability (SCA) effects are large relative to General Combining Ability (GCA) effects; b) a specific clone crosses more with one or a few clones than with others; and c) the SCA effects of crosses are negative due to the occurrence of a rare event. However, other researchers differ on this point (Grosser et al. 2010; Moncur and Boland 2001; Squillace and Goddard 1982), and maintained that deviations from panmixis can affect the genetic worth of seed. The genetic gain evidence on hand in this study (Swain 2013; Swain et al. 2013a) showed that, barring the seed orchards that had less than 20% flowering, the observed genetic gains in the F2 population were as expected for a panmictic population.

A further consideration in the estimation of variance components for the prediction of genetic gain is that of competition. Costa e Silva and Kerr (2013) found that variances estimated for additive genetic and residual effects, using models that ignored genetic competition, tended to be biased, which could negatively impact on the total response to selection and thus genetic gain.

Simple family-based models were used to estimate genetic parameters in this study. Although these models currently account for much of the quantitative genetics forest tree literature, many of the assumptions that

these models rely on are violated in advanced generation tree improvement programmes (White et al. 2007). These assumptions are: 1) Parents must be from the same generation; 2) Parents are unrelated and not inbred; 3) Parents are an unselected random sample from the population; 4) Parents are randomly mated to form the test population; and 5) All parents are mated in a single mating design. Mixed-model methods facilitate analysis of data where different mating designs or highly selected, related and possibly inbred parents from different generations are used, provided that the models are specified correctly and all data are included. In this study, assumptions 1) and 5) were not violated; and 3) and 4) only to the degree that 30% of the poor families were rogued from the F1 orchards. With regards to assumption 2), it is unlikely that parents were inbred, although there is a possibility of parents being related, due to there being 1 to 10 trees/family remaining in the orchards, albeit at a distance (these distances are greater than 50 m and can range up to over 100m). All family-based models, however, are limited in that they do not take into account the pedigree of the trees being tested, i.e., there are no predicted breeding values for each tree measured, only of the parents (White et al. 2007), which are included in individual tree models. By applying a grandmaternal provenance grouping as a fixed effect in the family model, and using various coefficients of relationship for possible relatedness closer than half-sibs in the F2, the simple family-based modelling approach is assumed reasonable in assessing whether predicted genetic gain is in the correct range of realised gain.

The prediction of gain using various scenarios was onerous without the use of a modelling programme, and future modelling of gains in ICFR trials will be done with the assistance of such.

Conclusions

Parameter estimations using family (provenance) in the F1 *E. nitens* population resulted in predictions close to the realised gain. A coefficient of relationship of 0.33 appears to fit well in parameter estimation and gain predictions, and should be sufficient for correcting for deviations from half-sib assumptions in the population. Levels of selfing appear to be low (<20%), and indications are that levels of outcrossing may be over 80%, despite poor flowering of the species in South Africa. It is clear that molecular studies in the seed orchards and resultant progeny would provide an effective tool to monitor outcrossing rates and the

role of male and female parents in the orchards, as well as to determine whether panmixis is occurring. This information would allow for refinement of the models over time if necessary.

The stratified or phased selection which is likely taking place in these seed orchards may actually be decreasing gains and thus, in reality, the selection intensities should be revised downwards. This implies that even greater gains are possible if flowering can be increased. It is therefore important firstly, to construct seed orchards with sufficient numbers of families, sufficient individuals per family and adequate numbers of synchronously flowering families; and secondly, to apply technologies to increase flowering, not only to support outcrossing, but also to increase the population size available for selection, such that maximum potential gains can be realised.

Although the progeny trials were not designed to detect inter-provenance heterosis/non-additive effects, they are likely to be negligible in this population. It is suggested, therefore, that it is not necessary to keep provenances separate in current and future ICFR seed orchards, which will make practical management of seed orchards easier.

This study has provided an objective and quantitative assessment of the underlying assumptions used for estimating genetic parameters and predicting gain in this population of *E. nitens*. It can also be concluded that the assumptions used in the F1 study were correct and no adjustments are necessary to that step in the breeding programme.

Acknowledgments

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