A comparison of the effect of genetic improvement and seed source and seedling seed orchard variables on progeny growth in *Eucalyptus nitens* in South Africa

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ABSTRACT

Eucalyptus nitens is an important forestry species grown for pulp and paper production in the temperate, summer rainfall regions of South Africa. A tree improvement programme has been ongoing at the Institute for Commercial Forestry Research for two decades, but genetic improvement in the species has been slow due to delayed and infrequent flowering and seed production. Three trials were established to firstly, quantify the gains that have been made in the first generation of improvement in the breeding programme; and secondly, establish whether a number of seed source and orchard variables influence the performance of the progeny. These variables are: the amount of flowering trees in the seed orchard, year of seed collection, seed orchard origin and composition of seed orchard seed bulks. Diameter at breast height and tree height were measured in the trials at between 87 and 97 months after establishment and timber volumes and survival were calculated. Improved seed orchard bulks performed significantly better ($p < 0.01$) than unimproved controls in the field trials. Genetic gains ranging from 23.2 to 164.8 m^3ha^{-1} were observed over the unimproved commercial seed. There were significant differences ($p < 0.01$) in progeny growth between the levels of seed orchard flowering, with higher levels of flowering (≥ 40 %) producing substantially greater progeny growth than lower flowering levels (≤ 20 %). The seed orchard had no effect on progeny growth in this trial series. This suggests that seed collected from any of the four seed orchards tested will produce trees with significant improvement in growth.

Keywords

E. nitens, genetic gain, tree improvement, breeding, flowering levels, seed bulk composition

INTRODUCTION

Eucalyptus nitens remains one of the most important commercial cold tolerant eucalypt (CTE) species currently grown for pulp and paper production in the summer rainfall regions of South Africa. Significant variation exists among the provenances grown in South Africa for growth (Swain et al. 1998; Gardner et al. 2003) and drought (Darrow 1996; Gardner 2001), frost and cold tolerance (Gardner 2001; Swain 2001); timing and abundance of flowering (Carlson et al. 2000; Jones 2002; Gardner and Bertling 2005); seed production (Swain and Chiappero 1998; Jones 2002) and pulping properties (Clarke 2000). This makes the species ideally suited to genetic improvement.

In South Africa, *E. nitens* grows optimally where the mean annual temperature (MAT) is greater than 14^{o}C and less than 16^{o}C (Swain and Gardner 2003). The species is classified as frost tolerant, but is not as hardy as *Eucalyptus macarthurii* (Darrow 1994; 1996), and is recognised as one of the most snow hardy of the CTEs grown in South Africa (Gardner and Swain 1996; Kunz and Gardner 2001). Currently, there is no alternative commercial species to *E. nitens* for sites prone to moderate frost and heavy snows.

The *E. nitens* populations grown commercially in South Africa originate from several provenances in New South Wales (NSW) in Australia, as provenance trials have shown that the material from Victoria in Australia does not perform well in the summer rainfall regions of South Africa (Swain et al. 1998). A breeding programme for *E. nitens* has been ongoing since the early 1980s, when the Institute for Commercial Forestry Research (ICFR) took over a series of provenance/progeny trials from the South African Department of Forestry. These trials tested a range of seedlots and provenances imported from Australia, with additional trials being established by the ICFR to assess new Australian seed imports at the end of the 1980s (Swain et al. 1998).

As vegetative propagation is difficult in *E. nitens* (de Little et al. 1992; Moncur 1998), open-pollinated seed orchards have been established for the production of improved seed. The reticent or shy flowering of *E. nitens* (Gardner 2003) has hindered the breeding programme and the production of improved seed, for plantation establishment. Globally, the species is known as a light and infrequent flowerer and produces small seed crops (Pound et al. 2003). In South Africa, the species often only becomes reproductively mature at 10 to 15 years of age if grown in a plantation situation (Eldridge et al. 1993; Gardner 2003) and requires winter chilling, or hormonal treatments to replace the chilling, if flowering is to occur earlier (Gardner and Bertling 2005). The use of open-pollinated seed orchards to turn over generations in conventional breeding is therefore slow and difficult, and can result in inconsistent commercial seed production. Shy flowering may also affect realised or actual gain, in that only certain families may be contributing as pollen parents, potentially causing differences from predicted gain. On the contrary, if different or additional families start flowering with each advancing year, gain may vary significantly on an annual basis. The mixed mating system of *E. nitens*, where outcrossing is preferential but selfing is not uncommon (Griffin et al. 1987; Sedgley et al. 1989), in conjunction with the erratic flowering of the species, may result in the open-pollinated seed orchards failing to produce consistently high quality seed.

A series of genetic gain trials was established in 2001, firstly, to quantify the gain that has been made in the first generation of improvement in *E. nitens* and, secondly, to establish whether there is any relationship between level of flowering in an orchard, family composition of the seed orchard bulk, the seedling seed orchard and the genetic gain in progeny derived from the ICFR's *E. nitens* advanced generation seedling seed orchards.

MATERIAL AND METHODS

Three genetic gain trials were established on temperate sites in KwaZulu-Natal (KZN) and Mpumalanga (MPU) in South Africa early in 2001, i.e. Balgowan, Amsterdam and Lothair. Details of the trial sites and trial designs are included in **Table 1**. All trials were planted at 1667 stems per hectare stocking (2 x 3 m), with four replicates of treatments or entries in square plots of 5 x 5 trees, and only the inner 9 trees (3 x 3) being measured in order to exclude inter-treatment/entry competition effects. Twenty-five to 28 entries, details of which are in **Table 2**, were included in the trials. The improved material originated from four ICFR seedling seed orchards, i.e. Amsterdam, Helvetia, Jaglust and Jessievale. These were former provenance/progeny trials that were thinned to seed orchards using a 30 % roguing of poor families and a thinning to the best tree per plot of remaining families. After roguing, there were only three common families across all four of these seed orchards which could potentially act as pollen parents, and an additional six that were common to three of the orchards.

Table 1 Site and trial design details of three *E. nitens* genetic gain trials in South Africa

Plantation, Province	Date Planted	Latitude ^{o}(S)	Longitude ^{o}(E)	Altitude (m a.s.l.)	MAP[a] (mm)	MAT[b] (^{o}C)	Soil depth (mm)	No. of entries	Design
Balgowan, KZN[c]	05/02/01	-29.4044	30.02417	1498	1002	15.3	1000-1200	28	5x6 unbalanced lattice
Amsterdam, MPU[d]	20/02/01	-26.5728	30.72778	1478	881	14.8	700	26	5x5 unbalanced lattice
Lothair, MPU	22/02/01	-26.4833	30.63333	1600	869	14.6	800	25	5x5 triple lattice

[a] Mean Annual Precipitation [b] Mean Annual Temperature [c] KwaZulu-Natal [d] Mpumalanga

In addition to comparing improved with unimproved material, entries included seed orchard bulks comprising a mix of the same mother families originating from different seedling seed orchards, i.e. approximate half sibs, to determine if seed orchard plays a role in progeny performance. Common seed/mother trees ranged from eight to 15 families, depending on bulk composition, the low number of common pollen parents allowing for potential variation between bulks to be expressed. All bulks from a specific seedling seed orchard were also combined in another comparison, irrespective of flowering level, to further examine the relationship between seed orchard and genetic gain. In order to establish whether there was a relationship between the number of trees flowering simultaneously in a seed orchard and progeny performance (i.e. assuming increased outcrossing with increased flowering, above a certain level of flowering), entries were included that comprised bulks of the same families, but which were collected in different years to represent different levels of flowering in the orchards. Flowering assessments were made in these orchards over three years to acquire the necessary flowering figures, which were obtained

by totaling the number of flowering trees in a seed orchard and calculating these as a percentage of all trees in the orchard. Lastly, bulks comprising different family combinations were included to determine whether this played a significant role in achieved gain being commercially deployed. Flowering over the period fell between 15 and 20 % or 40 and 47 %, and was thus categorised into these two levels (≤20 % and ≥40 %, respectively) for the purposes of this study. Details of the treatment/ bulk compositions, selection intensity and grouped comparisons are included in **Tables 2** and **3**.

Table 2 Individual entry comparisons in *E. nitens* genetic gain trials at three sites

Entry no.	Origin and year seed collected (flowering percentage in previous year)	Entry/bulk composition	Level of female selection[a]
1	E88/01 Jessievale SO[b] A 1998 (15%)	27832 31332	
2	E88/01 Jessievale SO A 1999 (40%)	31328 31337 31329 32101 31331 32098	8 top families from 42 8 top families from 42
3	E88/03 Helvetia SO B 2000 (44%)	32079 32093	8 top families from 49
4	E88/01 Jessievale SO B 1998 (15%)	32087 32095	8 top families from 42
9	E88/01 Jessievale SO B 2000 (45%)	32089 32097	8 top families from 42
10	E88/05 Jaglust SO B 2000 bulk (47%)	32090 32100	8 top families from 144
5	E88/01 Amsterdam SO C 1998 (20%)	Top 70% families	25 families from 34
6	E88/05 Jaglust SO D 1998 bulk (47%)	27832 31331 31338 32084 32087 32091 32092 32094 32095 32096 32097 32099 32100 32101 32102	15 top families from 144
7	E88/01 Jessievale SO D 1998 bulk (15%)		15 top families from 42
8	E88/03 Helvetia SO E 2000 (44%)	32087 32093 32095 32096 32100 34831 34832 34833 34835 34836 34837 34838 34839 34840	16 top families from 49
11	E88/01 Jessievale SO 1998, top family	32097	
12	E88/01 Jessievale SO 2000, top family	32097	
13	E88/03 Helvetia SO 1999, top family	32097	
14	E88/03 Helvetia SO 2000, top family	32097	
16	E88/05 Jaglust SO 1998, top family	34832	
17	E88/03 Helvetia SO 2000, top family	34832	
18	E88/05 Jaglust SO 1998, top family	37232	
19	E88/05 Jaglust SO 1998, top family	37224	
20	Land race commercial bulk, ex Dorstbult SO, SA[c]	-	
21	Improved commercial bulk, ex Helvetia SO, SA	-	
22	Unimproved general bulk ex Australia	32083 32091 32092 32093 32096 32099 32101 34832 34838 37628	10 families
23	Unimproved average family ex Nelshoogte, SA	28	
24	Unimproved top family ex Badja, Australia	37232	
26	Unimproved top family ex Barren Mountain, Australia	32097	
27	Unimproved top family ex Barrington Tops, Australia	34832	
28	Unimproved local bulk *E. nitens* ex Perdestal, SA, 1989	-	
29	*E. grandis x nitens (GXN)* clone ex SA	-	
30	Controlled pollination seed ex SA	-	

[a] See text for level of male selection [b] Seedling seed orchard [c] South Africa

Table 3 Combination of entries for group comparisons in *E. nitens* genetic gain trials at three sites

Group comparison	Entries included	Group comparison	Entries included
i) Level of improvement		iv) Seed Orchard	
Unimproved	20, 21, 22, 28	Amsterdam	5
Improved	1 - 14	Helvetia	3, 8, 13, 14
ii) Flowering level		Jaglust	6, 10
≤ 20 %	1, 4, 5, 7	Jessievale	1, 2, 4, 7, 9, 11, 12
≥ 40 %	2, 3, 6, 8, 9, 10		
iii) Year of seed collection		v) Composition of Seed Orchard Bulk	
1998	1, 4, 5, 6, 7, 11	A	1, 2
1999	2, 13	B	3, 4, 9, 10
2000	3, 8, 9, 10, 12, 14	C	5
		D	6, 7

Measurements

Diameter at breast height (dbh) and tree height measurements were carried out at Lothair and Amsterdam at 87 months after establishment and at Balgowan at 97 months, which is just prior to full rotation for eucalypts grown on a pulp rotation in the temperate areas of South Africa. Formal stem form and disease assessments were not carried out because these traits were bred to the desired level in the first-generation trials (Swain et al. 1998). Individual-tree volume was calculated from these measurements using the equation developed by Schnöau (1982):

$\text{Log } V = b_0 + b_1 \log (D + \text{vald}) + b_2 \log H$

where V = total volume to 5 cm tip diameter in cubic decimetre, D = dbh in centimetre, H = total height in metre, b_0 = -2.17055, b_1 = 2.07516, vald = constant tree form value = 0, and b_2 = 1.42792. The assumption of constant tree form value throughout is satisfactory (Bredenkamp 2000). Total treatment/entry volumes per plot were calculated and then estimated per hectare, taking survival into account.

Statistical analysis

Statistical analysis was conducted using SAS® Institute, Inc., Software 9.2 (SAS 2002-2008). Dead or missing trees were removed from the dataset before analysis. To test for normality for dbh, height and volume, residuals were plotted against fitted values. None showed any detectable trends or patterns and it can therefore be said that the condition $\varepsilon_{ijk} \sim$ iid $(0,\sigma^2)$ were met for these data, and the standard ANOVA assumptions are valid. Analyses of variance for dbh, height, survival, individual-tree and total volumes were carried out for each site, as well as across sites, and F statistics were calculated to test for significant differences among entries. Proc GLM was used to calculate least squares means for dbh, height, survival, individual-tree and total volumes of each entry, as this procedure is recommended for unbalanced designs (Hettasch et al. 2007). Comparisons were made for differences between individual entries using Fisher's test for Least Significance Differences (LSD) at the 1 % significance level. Simple and partial phenotypic correlation statistics were estimated between traits using the combined site entry means.

In addition, individual entries were grouped, and statistical comparisons were made for levels of improvement, flowering levels, year of seed collection, seedling seed orchard origin and composition of seedling seed orchard bulk for all traits, across sites. Comparisons within the entry groups were made using pairwise t tests. The following model was used for the individual site, nine tree square plot analysis:

$y_{ijkl} = \mu + \text{rep}_i + \text{block}_j\,(\text{rep}_i) + \text{tmt}_k + \text{rep}_i{*}\text{tmt}_k + (\text{rep}_i{*}\text{tmt}_k) + \varepsilon_{ijkl}$

where y_{ijkl} = mean for the trait of the l^{th} tree in the i^{th} rep and k^{th} entry, μ = overall mean, rep = i^{th} rep effect (fixed), i = 1, …, 4; block = j^{th} block within i^{th} rep effect (fixed), j = 1,…, 5; tmt = k^{th} entry effect (random), k = 1,…, 14, 16,…, 25, 26 or 29; rep*tmt = interaction between the i^{th} rep and k^{th} entry (random plot effect); ε_{ijkl} = random error associated with i^{th} rep, j^{th} block within i^{th} rep, k^{th} entry and l^{th} tree where $\varepsilon_{ijkl} \sim$ iid $(0,\sigma^2)$.

The following model was used for the combined site analysis:

$y_{ijkl} = \mu + \text{site}_i + \text{rep}_j(\text{site}_i) + \text{tmt}_k + (\text{site}_i{*}\text{tmt}_k) + \varepsilon_{ijkl}$

where y_{ijkl} = mean for the trait of the l^{th} tree in the j^{th} rep and k^{th} entry at the i^{th} site; μ = overall mean; site = site effect (fixed), i = 1,…, 3; $\text{rep}_j(\text{site}_j)$ = j^{th} rep effect (fixed) within i^{th} site, j = 1,…, 4; tmt = k^{th} entry effect (random), k = 1,…, 14, 16,…, 25, 26 or 29; site*tmt = interaction between the i^{th} site and k^{th} entry (random); ε_{ijkl} = random error associated with i^{th} site, j^{th} rep within i^{th} site, k^{th} entry and l^{th} tree where $\varepsilon_{ijkl} \sim$ iid $(0,\sigma^2)$. Interactions between the grouped entries (i.e. level of improvement, flowering level, year of seed collection, seed orchard and bulk composition) were tested, and a regression analysis was performed on flowering levels with growth traits.

RESULTS AND DISCUSSION

Comparison of individual entries

Growth

Table 4 presents the final survival, individual-tree and total volume entry means for the three genetic gain trials. There were significant differences ($p < 0.01$) between entries for all traits at all three sites, and within-site replicate effects were significant for all traits except total volume. Block-within-replicate effects were not significant ($p > 0.01$) and replicate x entry interaction effects were only significant ($p < 0.01$) at Amsterdam for individual volume (details not shown). With regard to the combined site analysis, site effects were significant ($p < 0.001$) for all traits, but site x entry effects were only significant for total volume ($p < 0.001$) and survival ($p < 0.05$) (**Table 5**). **Table 4** also presents the across or combined site survival, individual and total volume entry means. Across all sites, top performing entries 11 (top improved family 32097 from Jessievale seed orchard) and 17 (top improved family 34832 from Helvetia seed orchard) significantly outperformed ($p < 0.05$) the majority of unimproved entries, the land-race commercial bulk (entry 20), the *GXN* hybrid clone (entry 29) and the two first-generation top families from Jaglust (entries 18 and 19), for total volume. Entry 27 (unimproved top family 34832 ex Barrington Tops, Australia) was only present at two sites, but performed well overall, being the top entry in the combined site analysis for total volume. At first glance, this would seem to indicate that the more northernmost Australian provenance of Barrington Tops should be widely used in future breeding. Although this is supported by results of first-generation trials in South Africa (Swain et al. 2013), the northern provenance of Barren Mountain performed as well as Barrington Tops in the first-generation trials.

With regards to poor performance, unimproved control entry 28 (unimproved South African *E. nitens* ex Perdestal) performed significantly worse ($p < 0.01$) than the majority of improved entries for most traits.

Table 4 Final percentage survival, total volume and individual-tree volume entry means, ranked for decreasing total volume, in three *E. nitens* genetic gain trials, and a combined site analysis.

Balgowan (97 months)				**Amsterdam (87 months)**				**Lothair (87 months)**				**Combined site analysis**			
Entry	Survival (%)	Total volume (m^3ha^{-1}) (p <0.05)	Indiv. tree volume[a] (m^3)	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1}) (p <0.05)**	**Indiv. tree volume[a] (m^3)**	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1})**	**Indiv. tree volume[a] (m^3)**	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1}) (p <0.001)**	**Indiv. tree volume[a] (m^3)**
17	93 a	404.8 a	0.262 a	17	63 bc	189.3 a	0.183 b	10	85 ab	240.2 a	0.164 ab	27	89 a	257.6 a	0.174 ab
11	83 ab	366.4 ab	0.264 a	13	75 a	189.2 a	0.151 b	5	81 ab	236.5 a	0.082 b	11	79 ab	252.5 a	0.195 ab
2	86 ab	331.2 ab	0.231 ab	8	72 ab	182.5 a	0.152 b	27	83 a	230.9 a	0.080 b	8	79 ab	249.7 a	0.195 ab
6	81 ab	322.7 ab	0.240 ab	1	78 a	179.1 ab	0.136 b	2	86 a	226.2 a	0.157 abc	17	70 abcde	241.7 ab	0.206 a
8	78 ab	321.9 ab	0.241 ab	11	67 b	173.0 abc	0.156 b	11	83 a	218.1 a	0.157 abc	2	76 abc	241.0 ab	0.186 ab
14	72 ab	308.7 ab	0.256 a	14	69 ab	169.8 abc	0.142 b	8	78 bc	209.9 ab	0.158 abc	5	73 abcd	226.6 ab	0.191 ab
9	89 ab	307.1 ab	0.207 ab	2	61 bc	165.6 abc	0.160 b	1	86 a	206.0 ab	0.144 bc	3	67 abcde	222.2 ab	0.178 ab
5	72 ab	296.0 ab	0.246 ab	3	75 a	160.2 abcd	0.127 b	12	81 ab	199.8 ab	0.149 bc	14	69 abcde	219.7 ab	0.185 ab
4	75 ab	290.6 ab	0.233 ab	9	58 bc	155.5 abcd	0.159 b	9	72 bc	191.0 ab	0.156 abc	6	74 abcd	218.8 ab	0.175 ab
1	82 ab	285.5 ab	0.211 ab	6	64 bc	153.7 abcd	0.144 b	17	64 bc	184.8 ab	0.172 ab	1	81 a	217.9 ab	0.160 abc
27	95 a	284.3 ab	0.181 ab	29	31 bc	148.1 abcde	0.290 a	14	72 bc	180.5 ab	0.149 bc	9	73 abcd	217.9 ab	0.179 ab
3	75 ab	284.1 ab	0.227 ab	5	61 bc	147.2 abcde	0.144 b	6	81 ab	180.0 ab	0.133 bc	13	68 abcde	216.4 ab	0.183 ab
16	64 ab	281.5 ab	0.264 a	10	61 bc	131.7 abcde	0.128 b	21	69 bc	178.2 ab	0.154 bc	10	72 abcde	213.2 ab	0.181 ab
12	78 ab	280.3 ab	0.216 ab	26	47 bc	122.8 abcde	0.154 b	7	75 bc	177.6 ab	0.145 bc	30	78 abc	200.9 abc	0.155 abc
10	70 ab	274.4 ab	0.237 ab	12	44 bc	112.3 abcde	0.159 b	20	78 bc	172.8 ab	0.142 bc	12	70 abcde	197.5 abc	0.175 ab
30	78 ab	262.8 ab	0.203 ab	21	50 bc	109.6 abcde	0.133 b	30	78 bc	169.9 ab	0.130 bc	7	73 abcde	181.9 abc	0.144 abc
7	76 ab	248.7 ab	0.182 ab	24	44 bc	105.3 abcde	0.150 b	29	42 c	155.9 ab	0.222 a	4	61 abcde	179.8 abc	0.166 abc
13	67 ab	243.5 ab	0.219 ab	4	67 b	103.2 abcde	0.092 b	28	75 bc	155.2 ab	0.121 bc	21	65 abcde	174.8 abc	0.163 abc
21	70 ab	215.9 ab	0.184 ab	7	67 b	102.6 abcde	0.094 b	22	75 bc	149.1 ab	0.118 bc	16	58 bcde	164.5 abc	0.178 ab
20	58 ab	210.2 ab	0.246 ab	20	56 bc	95.7 abcde	0.106 b	4	53 bc	145.6 ab	0.171 ab	20	57 cde	158.7 abc	0.154 abc
18	75 ab	209.3 ab	0.168 ab	22	36 cd	93.9 abcde	0.155 b	16	58 bc	129.5 ab	0.132 bc	22	62 abcde	144.3 abc	0.141 abc
23	67 ab	189.8 ab	0.171 ab	18	51 bc	93.6 abcde	0.106 b	24	50 bc	129.2 ab	0.162 abc	24	49 e	132.6 abc	0.151 abc
19	70 ab	188.8 ab	0.163 ab	16	44 bc	82.4 bcde	0.107 b	26	72 bc	118.7 ab	0.107 bc	29	51 e	130.8 abc	0.157 abc
22	67 ab	177.3 ab	0.160 ab	19	33 d	71.6 cde	0.129 b	23	50 bc	106.5 ab	0.120 bc	19	58 bcde	130.2 abc	0.152 abc
24	64 ab	163.4 ab	0.153 ab	23	39 cd	61.8 de	0.103 b	18	39 c	61.7 b	0.094 c	18	54 de	121.5 bc	0.133 bc
26	47 c	108.4 ab	0.138 ab	28	36 cd	49.5 e	0.083 b					26	54 de	116.2 bc	0.134 bc
29	72 ab	73.8 ab	0.061 b									23	50 e	114.9 bc	0.137 abc
28	30 c	38.4 b	0.078 ab									28	47 e	84.9 c	0.105 c
Trial mean	72.1	248.1	0.205		55.7	128.6	0.139		69.8	173.5	0.149		65.7	185.2	0.168
SD[b]	17.43	188.74	0.153		18.88	54.19	0.090		21.49	53.25	0.091		19.64	74.07	0.121

Values followed by the same letter of the alphabet within a column are not significantly different from each other (p > 0.01, unless indicated otherwise)

[a] Individual-tree volume [b] Standard deviation of entry means

Table 5 Analysis of variance for combined site percentage survival and growth, as well as growth within entry groups for the three genetic gain trials

Trait		Source of variation	*df*	Mean Square	*F* value	*p* value	
Percentage survival	**Entry**	Site	2	8196.743	21.27	<0.0001	***
		Rep (site)	9	611.339	1.59	0.12	
		Entry	27	1165.615	3.02	<0.0001	***
		Site*entry	49	390.207	1.01	0.46	
		Error	213	385.403			
	Level of improvement	Site	2	4751.905	16.07	<0.0001	***
		Rep (site)	9	283.383	0.96	0.48	
		Improvement	1	7330.620	24.78	<0.0001	***
		Site*improvement	2	1445.573	4.89	0.01	**
		Error	166	295.771			
Total volume	**Entry**	Site	2	354315.929	69.96	<0.0001	***
		Rep (site)	9	5559.452	1.10	0.37	
		Entry	27	25933.600	5.12	<0.0001	***
		Site*entry	49	7318.113	1.44	0.04	*
		Error	213	5064.653			
	Level of improvement	Site	2	62611.3621	12.44	<.0001	***
		Rep (site)	9	5708.6565	1.13	0.34	
		Improvement	1	207367.1799	41.19	<.0001	***
		Site*improvement	2	29193.6384	5.80	0.004	**
		Error	166	5034.029			
	Flowering level (grouped ≤ 20 and ≥ 40 %)	Site	2	225973.664	56.22	<0.0001	***
		Rep (site)	9	6466.717	1.61	0.13	
		Flower	1	16351.056	4.07	0.05	*
		Error	102	4019.636			
	Year of seed collection	Site	2	305192.646	62.28	<0.0001	***
		Rep (site)	9	4718.353	0.96	0.48	
		Year	2	1929.537	0.39	0.68	
		Error	145	4900.134			
	Seed orchard	Site	2	304054.905	61.64	<0.0001	***
		Rep (site)	9	4735.810	0.96	0.48	
		Seed orchard	3	1350.865	0.27	0.84	
		Error	144	4932.819			
	Composition of bulk	Site	2	227622.635	57.42	<0.0001	***
		Rep (site)	9	6999.527	1.77	0.08	
		Bulk	4	8479.672	2.14	0.08	
		Error	99	3963.992			
Individual tree volume	**Entry**	Site	2	0.114	8.05	0.0006	***
		Rep (site)	9	0.010	0.74	0.67	
		Entry	27	0.015	1.10	0.36	
		Site*entry	45	0.016	1.10	0.35	
		Error	102	0.014			
	Level of improvement	Site	2	0.823	56.85	<0.0001	***
		Rep (site)	9	0.051	3.57	0.0002	***
		Improvement	1	0.240	16.58	<.0001	***
		Error	1145	0.014			
	Flowering level (grouped ≤ 20 and ≥ 40 %)	Site	2	0.596	42.90	<0.0001	***
		Rep (site)	9	0.033	2.38	0.01	*
		Flower	1	0.030	2.14	0.14	
		Error	751	0.014			
	Year of seed collection	Site	2	0.844	57.55	<0.0001	***
		Rep (site)	9	0.043	2.96	0.0017	**
		Year	2	0.008	0.51	0.60	
		Error	1035	0.015			

Seed orchard	Site	2	0.091	7.31	0.0012	**
	Rep (site)	9	0.018	1.48	0.17	
	Seed orchard	3	0.012	0.97	0.41	
	Site*seed orch.	6	0.033	2.68	0.02	*
	Error	88	0.012			
Composition of bulk	Site	2	0.604	43.60	<0.0001	***
	Rep (site)	9	0.035	2.49	0.0082	*
	Bulk	4	0.024	1.73	0.14	
	Error	748	0.014			

df degrees of freedom

The control-pollinated seed (entry 30) performed at or below the trial mean at the two sites where it was established. This performance may have been relatively poor, either because the seed was produced from early control-pollinated crosses, where the technique was still being established and the levels of contamination may have been high, or due to poor specific combining ability of the genotypes.

Survival

It is notable that the ranking of many of the entries changed markedly once survival was taken into account, i.e. total volume per hectare was calculated with dead trees having a volume of zero. Survival differences were significant at varying levels at the individual sites, i.e. Balgowan ($p < 0.05$), Amsterdam ($p < 0.005$) and Lothair ($p < 0.1$), with no significant entry x replicate effects ($p > 0.1$) for the three sites (details not shown). In the combined site analysis, site effect was significant for survival ($p < 0.001$); yet the site x entry effect was non-significant ($p > 0.05$) **(Table 5)**. With the exception of entry 27 (unimproved top family 34832 ex Barrington Tops, Australia), survival or stocking of the improved entries was generally better than that of the unimproved and land-race material (**Table 4**). The *GXN* hybrid clone performed well below the trial average at Balgowan yet had good survival (72 %), and at Amsterdam, although survival was poor (31 %), individual-tree growth of surviving trees was good as trees captured the open space around them. In contrast, entry 27 performed well at the two sites where it was planted, this performance being due in part to final survival of 95 and 83 %, respectively. All other unimproved entries (22, 23, 24, 26 and 28) had lower survival than most improved entries and low total volume, as if survival itself was behaving as a genetic trait. The positive impact of survival was expected after selection, as the previous generation of improvement focused on selection of trees that (1) had improved pest and/or disease tolerance, (2) were able to capture the site better, as measured by superior growth, and (3) had good survival. Selection for these traits has apparently resulted in increased stocking contributing significantly to the gain achieved through tree improvement.

Simple correlations (r) between survival, dbh and height, as well as partial correlations between survival and total volume, are presented in **Table 6**. These indicate that the correlation between survival and height ($r = 0.77$) is greater than that of survival with dbh ($r = 0.65$), and that there is a positive correlation between dbh and height for this trial series ($r = 0.74$). This latter correlation is lower than that obtained in first-generation trials of *E. nitens* ($r \geq 0.82$), the material being related to

that included in this genetic gain trial series (Swain et al. 2013). With regard to the partial correlations of total volume with dbh and height (total volume being dependent on both dbh and height), the higher *r* of 0.90 for total volume with height for constant dbh supported the stronger correlation between survival and height. Swain et al. (2013) present further trait and juvenile-mature correlations for the related first-generation material over a range of sites and trial series, as well as genetic parameters for the *E. nitens* population.

Table 6 Selected simple phenotypic correlations (below the diagonal) and partial correlations (above the diagonal) between traits for final measurements of the three genetic gain trials

	Pearson's phenotypic trait correlations ($p \leq 0.0002$)			
Trait	**dbh**	**Height**	**Survival**	**Total volume**
dbh	-	-		
Height	0.74	-		
Survival	0.65	0.77	-	0.78 (For constant height) 0.90 (For constant dbh)
Total volume	-	-	0.91	-

Gains

These results indicate that improvement has been made through the first generation of selection in the ICFR breeding programme, with the average increase in total volume of improved over unimproved material being 62.3 % (**Tables 4** and **Table 7**). Gains that can be made by using seed orchard bulks originating from any of the four ICFR seedling seed orchards included in these trials range from 9.3 to 94.4 % in total volume depending on site and bulk used, and expressed as a percentage of the unimproved and land-race bulk means, respectively (Shelbourne 1970). There were no significant differences ($p > 0.01$) between the improved bulks, although the bulk D from Jessievale (entry 7 (15 % flowering)) performed below the mean for all traits for the combined site analysis, and similarly, bulk B from Jessievale (entry 4 (15 % flowering)) performed just below the mean for total volume and individual-tree volume (**Table 4**). Both commercial bulks i.e. the improved commercial bulk from Helvetia (entry 21) and the land-race commercial bulk from Dorstbult (entry 20), were average performers for dbh but were below the mean for volumes and height in the combined site analysis (**Table 4**). As there were no significant differences between the different *E. nitens* seed orchard bulks in this study, nor the individual top-performing families, the homogeneity of the various entries was investigated. This showed that the range of dbh was similar for all entries across all sites, in the range of 15 to 20 cm, with only two exception: *GXN* (entry 29) had a narrower range of variation of 9 cm, as would be expected from a clone, and the unimproved South African bulk from Perdestal (entry 28) had a narrow range in the lower dbh range. Although the literature provides many comparisons between unimproved and improved eucalypt seedlots, most of which show significant improvements of the bred material over the unimproved material, very few comparisons have been found that displayed significant differences between improved eucalypt open-pollinated seed orchard bulks of the same species and nominal level of improvement. This is supported by findings in previous *E. macarthurii*

(Swain et al. 1999) and *E. nitens* genetic gain trials (Jones, pers. comm[1]) in South Africa and in *E. camaldulensis* genetic gain trials in India (Varghese et al. 2009), where bulks of the same nominal level of improvement did not differ significantly from each other.

Although there were no significant differences ($p > 0.01$) between the improved seed orchard bulks, the yield improvement of these bulks over the unimproved controls varied markedly according to which bulk was used in the comparison. The improved commercial bulk E from Helvetia (entry 8, 44 % flowering) produced an average of 91.0 and 164.8 m^3ha^{-1} more than the land-race commercial bulk from Dorstbult (entry 20) and the unimproved South African bulk from Perdestal (entry 28), respectively. By contrast, the bulk D from Jessievale (entry 7, 15 % flowering) produced only 23.2 and 97.0 m^3ha^{-1} more than the two controls, respectively.

Comparison of grouped entries

Tables 5 and **7** present comparisons of grouped entries for different levels of improvement, flowering, year of seed collection, seedling seed orchard and seed orchard bulk composition.

Table 7 Comparison of growth within entry groups in *E. nitens* genetic gain trials across all sites

	Grouping	Total volume (m^3ha^{-1})	dbh (cm)	Height (m)	Individual-tree volume (m^3tree^{-1})
Level of improvement	Improved	217.95 a	15.26 a	20.59 a	0.178 a
	Unimproved	114.61 b	13.60 b	18.33 b	0.125 b
Flowering percentage	≥ 40 %	227.62 a	15.50 a	20.77 a	0.183 a
	≤ 20 %	200.79 b	14.81 b	20.20 b	0.115 b
Year of seed collection[a]	1999	231.14 a	15.51 a	20.83 a	0.185 a
	2000	219.98 a	15.40 a	20.56 a	0.183 a
	1998	212.41 a	15.06 a	20.55 a	0.172 a
Seed orchard	Helvetia	228.51 a	15.60 a	20.59 a	0.187 a
	Amsterdam	226.57 a	15.77 a	20.60 a	0.190 a
	Jaglust	216.11 a	15.25 a	20.74 a	0.178 a
	Jessievale	212.20 a	15.03 a	20.55 a	0.172 a
Composition of seed orchard bulk[b]	E	249.69 a	15.78 a	20.48 a	0.195 a
	C	226.57 ab	15.78 a	20.61 a	0.191 ab
	A	229.95 ab	15.19 a	20.63 a	0.173 ab
	B	206.84 bc	15.23 a	20.60 a	0.176 ab
	D	199.60 bc	14.66 a	20.33 a	0.159 b

Values within an entry grouping within a column followed by the same letter of the alphabet are not significantly different from each other ($p > 0.05$)

[a] Refer to Table 2 for details of flowering in these years

[b] Refer to Table 2 for details of bulk composition

[1] Jones W (2010) Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA.

Levels of improvement

There were significant differences ($p < 0.01$) between the level of improvement for all traits (supporting the findings in **Table 4**).

Flowering level

Significant differences ($p < 0.01$) were found between flowering levels for all traits, with seed collected from seed orchards that had ≥ 40 % flowering producing progeny with significantly greater volume than seed that was collected from seed orchards with ≤ 20 % flowering (**Table 7**). It is unlikely that survival in the parent seed orchards would have affected flowering percentage, as there was good representation of the top 70 % of families in the seed orchards, despite subsequent poor flowering in a few of the orchards some years. Mining of the survival data of the progeny for the different flowering levels did not show any consistent resultant high or low survival for the ≥ 40 % or ≤ 20 % flowering entries, respectively, as these seemed to differ across site and with flowering level (**Table 4**).

There is little research on the breeding system of *E. nitens*, but Moncur et al. (1995) estimated a 75% outcrossing rate in this species, and Pound et al. (2003) found that levels of self-incompatibility in *E. nitens* ranged from 25.8 to 93.6%. Self-pollination is definitely possible in *E. nitens* (Griffin et al. 1987; Tibbits 1988), particularly in areas where the presence of natural pollinators is low, and pollen load is poor. This is despite selfing being controlled by a late–acting self-incompatibility system where ovule abortions occur after self-pollination (Pound et al. 2003), and resultant inbreeding depression has been reported in nine-year-old trees originating from controlled self-pollinations of *E. nitens* (Hardner and Tibbits 1998).

A regression analysis performed on the complete range of flowering levels for the four different growth traits indicated a slight significant positive trend ($p < 0.1$) between increasing levels of flowering and progeny tree growth for all traits except total volume. However, the R^2 values were very low for all traits, indicating a poor fit of the model, and no conclusions can be drawn from this analysis. A comparison of percentage improvement, as determined by flowering level, showed the following improvements in total volume over the 15 % flowering level: 40 % flowering (25.4 %), 45 % flowering (20.1 %), 20 % flowering (17.9 %) and 47 % flowering (12.0 %). The flowering levels happened to be specific to the design of each of the seed orchards that seed was collected from both in terms of family and final spacial distribution of parent trees; i.e. these were trials thinned to seed orchards based on family and individual performance and were not originally planted as seed orchards. This may partly explain the inconsistency of gain related to flowering level. Although a decrease in outcrossing rates has been linked to a decrease in progeny growth in forestry species (*E. nitens*, Hardner and Tibbits 1998; *Eucalyptus globulus*, Hardner and Potts 1995; Patterson et al. 2004; *Acacia mangium*, Butcher et al. 2004; Harwood et al. 2004), the flowering levels in this study do not necessarily represent the rate of outcrossing in the seed orchards, although the trends appear to be similar. Consequently, it could be assumed that an increase in flowering above a certain low level may result in increased gains in a

population due to an increase in outcrossing rate, a decrease in selfing and subsequent inbreeding depression, but that additional flowering above this level may confer very little, if any benefit.

Year of seed collection

The year of seed collection did not differ significantly ($p > 0.1$) (**Tables 5** and **7**).

Bulk composition

The composition of the seed orchard bulks differed in that bulk E performed significantly better ($p < 0.1$) than bulks D and B for total volume, and better than bulk D for individual-tree volume (**Tables 5** and **7**). Bulk E comprised a mix of 14 top-performing families where seed was collected in a year following ≥ 40 % flowering. By contrast, the poorer performing bulk D comprised two entries of 15 families representing the top 40 % of families during years of ≤ 20 % and ≥ 40 % flowering in two different seed orchards, respectively. Bulk B comprised seven top and one average family in years following ≤ 20 % and ≥ 40 % flowering in three different seed orchards. Although this might imply that flowering level was influencing the bulk performance, bulk E did not perform significantly better than other bulks with low flowering levels, i.e. bulk A (15 and 40 %, bulk D 20 %).

Seed orchard

There were no significant differences ($p > 0.1$) in progeny growth based on seedling seed orchard. As not all seed orchards were represented by both high and low levels of flowering, which may have been biasing the data, the ≤ 20 % flowering levels were removed from a subsequent analysis so that only the higher flowering levels were represented in all seed orchards. This had no effect on significance, with seed orchard still showing no impact on progeny growth.

This could imply that, irrespective of flowering levels in these seed orchards, seed can be utilised from any of these four seed orchards to achieve the same appreciable level of gain and production in commercial plantations. This is similar to what was found in *Pinus taeda* (Sluder 1988). Unfortunately there were insufficient degrees of freedom for the seed orchard x flowering level interaction to be tested in the current study, which may have further informed this. Although the seed orchard x bulk interaction was not significant ($p > 0.05$) for dbh or volume, certain combinations of flowering level, bulk composition and seedling seed orchard resulted in marked differences in progeny growth, as discussed earlier (**Table 4**). Caution should thus be exercised when compiling bulks from seedling seed orchards with low flowering levels in any given year. It may be necessary to ensure that certain maternal families that produce high-yielding progeny are included in these, or all, seedling seed orchard bulks.

To this end, a study on the mating system of this population of *E. nitens* should be carried out to determine how many individuals or families are involved in pollination in these *E. nitens* seed orchards, the levels of outcrossing and how much self-incompatibility varies with genotype. This will add to an understanding of the degree of selfing and outcrossing which is occurring in the seed

orchards and the effect on the genetic quality of the seed.

CONCLUSIONS

Significant improvements have been made over the first generation of selection in the ICFR *E. nitens* breeding population. It is therefore recommended that seed from any of the ICFR improved bulks be accessed for commercial deployment when available, rather than using unimproved or land-race material from Australia and South Africa, respectively.

Improvement in survival of the advanced-generation material plays an important role in the gains in total volume per hectare achieved. In addition, indications are that levels of flowering have an impact on progeny growth. These results suggest that seed orchards with 15 % flowering result in poorer progeny growth than those with ≥ 40 % flowering, although this is not consistent and it is thus difficult to draw any definite conclusions in this regard. Indications are that flowering above a certain low level may result in increased gains in a population due to a decrease in selfing or related crosses, but that additional flowering above this level may confer very little, if any benefit. Further investigation of flowering levels should be carried out with larger numbers of observations per flowering level. Until then, it is recommended that seed should be collected, where possible, from seed orchards where 40 % or more flowering was observed in the previous year. This is supported by substantial percentage improvement in total volume of the progeny, generally being more than 20 % (and $p < 0.05$) at these higher levels of flowering.

The orchard from which the seed is collected appears to have no effect on progeny growth in this trial series, irrespective of flowering levels. This suggests that seed collected from any of the four ICFR seedling seed orchards tested in the trial series will produce trees with significant improvement in growth over the unimproved and commercial material. It should however be noted that certain combinations of seedling seed orchard and bulk composition, particularly at the lower levels of flowering, produced much better progeny growth than others, even if this difference is not statistically significant. It is thus recommended that such higher yielding bulk and seedling seed orchard combinations be used for commercial deployment. This will impact on management of ICFR seed orchards and future seed bulk composition.

Molecular studies in the *E. nitens* seed orchards will provide a better understanding of selfing and outcrossing in this breeding population. This, in turn, will allow for manipulation of current and future seed orchards to ensure that maximum gains are captured in the seed for commercial deployment.

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Conflict of interest

The authors declare that the experiments described in this research paper comply with the current laws of South Africa and that there is no conflict of interest between authors.

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