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Effect of seed size on field survival and growth of *Eucalyptus* in KwaZulu-Natal, South Africa

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This study was established to determine the effect of seed size on nursery performance, field survival and growth. Results indicated that germination was greater for larger seed whereas the smallest seeds germinated poorly. In addition, seedling survival in the nursery was also lower for Grade 4 *Eucalyptus grandis* but not for *E. smithii*. Grade 4 seeds produced the shortest seedlings. This was attributed to a slower rate of germination within a tray of seedlings produced from Grade 4 seed. The Grade 4 seeds, however, produced seedlings with larger root collar diameters due to a greater amount of empty spaces within a tray (a function of poor germination). Field results indicated good survival irrespective of species or seed grade. At 12 months, only seed orchard source was significant for all response variates for *E. grandis*, whereas there were no significant inter-treatment differences for *E. smithii*. This study showed that *Eucalyptus* seed size effects were transitory, only apparent in the nursery and not perceptible one year after planting in the field. Smaller seeds can therefore be used in a commercial nursery as long as they are sown with similar-sized seeds to allow for management of poor germination and crop uniformity. Seed orchard source proved to be very important in predicting field growth.

Keywords: germination, nursery, seedling

Introduction

Eucalyptus seeds are small, irregularly shaped and must be graded according to size (Table 1). Grading of *Eucalyptus* seeds aids precision sowing and increases crop uniformity in the nursery. The latter is attributed to the varying germination rates of the different seed grades with the largest seeds germinating the fastest and producing the highest final germination (McRae, 2005). The superior germination of larger seeds is ascribed to a greater amount of endosperm reserves that are available during the germination process (Schmidt, 2000). *Eucalyptus* seeds can be classified into four size classes, with the top three grades being suited to commercial sowing. Seed availability is often a constraint in nurseries, especially for high value seeds and recalcitrant flowering species. With this in mind, utilisation of the current non-commercial Grade 4 seeds could alleviate some of the demand constraints. The aim of this study was to determine whether seedlings produced from different grades of *Eucalyptus* seeds perform differently in the field.

The benefits of using Grade 4 seeds include (1) better usage of high-value seeds in the nursery and (2) increased realisation of gain in the field as a result of enhanced deployment of high-value material. However, the disadvantage of using Grade 4 seeds is that (1) smaller seeds have a lower rate of emergence and lower final germination, which results in more nursery space and resource utilisation and increased labour time to blank empty cavities and (2) smaller seeds also have a high amount of debris that is of similar size, shape and density to seeds, which makes cleaning, handling and sowing difficult.

Materials and methods

Four seed grades of two species were used to generate 16 treatments (Table 1). *Eucalyptus grandis* was sourced from three seed orchards of different genetic value (Clan, Salpine and Venus) and *E. smithii* was sourced from a single orchard (Seven Fountains). Seeds were pre-treated by imbibing overnight in aerated distilled water at 25°C. Seeds were sown into moistened composted pine bark and covered with a capping of 5mm sieved bark. Trays were stacked and covered with plastic (to reduce water loss and temperature fluctuations) to promote uniform germination. At the first sign of radicle emergence, trays were moved out to the nursery. Trays in the nursery were arranged in a randomised complete block design with six replications per treatment. Plants were watered according to prevailing weather conditions, i.e. more frequently on hot days. Germination was monitored biweekly for one month. Seedling survival, growth (height and root collar diameter [RCD]) and sturdiness ratio (height:RCD) were measured prior to planting. All the plants were measured for germination, survival and growth whilst in the nursery. Seedlings were planted out to assess whether nursery differences would manifest in the field.

Location of study and site description

The study site was located in the KwaZulu-Natal (KZN) Midlands region near Howick, South Africa, at Sappi's Shafton plantation. Site characteristics and climatic data are presented in Table 2.

Re-establishment, fertilisation and weed control

Coppice shoots were removed from stumps remaining from the previous crop. The site was burnt to reduce the slash load and remove weeds. Five days after burning and 2d after pitting (Table 3), five-month-old seedlings of *E. grandis* and *E. smithii* were planted. The spacing used was 2.4m by 2.4m. Two border rows of *E. grandis* seedlings were also planted to buffer the trial from any edge effects. A 1l drench consisting of an aqueous Pyrinex® (active ingredient, Chlorpyrifos) solution was applied to the seedlings at planting to reduce problems with insects at establishment. In addition, 60g of granular fertiliser (diammonium phosphate) was applied as four spot applications around each seedling (15g per spot). Each spot application was buried in shallow holes, 5cm deep, approximately 15cm from the seedling. During winter (from early June until late August 2004), the seedlings were covered with porous maize bags to provide frost protection. In November 2004, an additional manual weeding was performed to control coppice and weed growth around the seedlings.

Experimental design

The field study was designed as a randomised complete block with 16 treatments and five replications. Each plot consisted of 16 trees in a 4 x 4 configuration. The total area used for this study was 0.74ha.

Table 1: *Eucalyptus* species, orchard and size of seeds used in this study

Species	Orchard	Grade	Size (μm)
E. grandis	Clan	1	> 1 000
		2	> 850 < 1 000
		3	> 710 < 850
		4	> 500 < 710
E. grandis	Salpine	1	> 1 000
		2	> 850 < 1 000
		3	> 710 < 850
		4	> 500 < 710
E. grandis	Venus	1	> 1 000
		2	> 850 < 1 000
		3	> 710 < 850
		4	> 500 < 710
E. smithii	Seven Fountains	1	> 1 000
		2	> 850 < 1 000
		3	> 710 < 850
		4	> 500 < 710

Data analyses

All statistical analyses were performed using GenStat® for Windows 7 (Lane and Payne, 1996). Residuals were plotted against fitted values to test whether the assumptions of homogeneity and normality were valid. Percentage data for nursery germination and field survival were angular transformed prior to analyses to fulfil the assumption of normality. The assumptions were found to be valid for the remaining variates that were tested. Nursery germination data were submitted to REML analysis while field data were submitted to an ANOVA with replication as the blocking term and orchard source by seed grade as the fixed factors, as per the following linear model:

$$Y_{ijkl} = \mu + rep_i + orchard_j + grade_k + (orchard \times grade)_{jk} + \varepsilon_{ijkl} \quad (1)$$

Where y_{ijkl} is the parameter of interest.

μ = overall mean

Rep_i = i^{th} rep effect, $i = 1,...5$.

$orchard_j$ = j^{th} fixed orchard effect, $j = 1,...3$ (for *E. grandis*) and $j = 1$ (for *E. smithii*).

$grade_k$ = k^{th} fixed seed grade effect, $k = 1,...4$.

$orchard.grade_{jk}$ = interaction between the j^{th} orchard and the k^{th} grade.

ε_{ijkl} = random error associated with the i^{th} *rep*, j^{th} orchard, k^{th} *grade* and the l^{th} tree.

Where $\varepsilon_{ijkl} \sim$ iid $(0, \sigma^2)$.

Field response variates included survival, height, ground line diameter (GLD) and volume index (VI). Volume index was calculated as follows:

$$VI\ (dm^3) = GLD^2 \times height \quad (2)$$

In addition, seedling measurements at planting were used as covariates in the analyses. Correlations and multiple linear regressions were also performed to determine whether any relationships existed between nursery and field measurements. Survival analyses were performed using whole plot data but only data for the inner four trees were used when analysing other response variates to minimise interspecific competition effects. Least significant differences (LSD) were used to determine differences between treatment means.

Table 2: Site characteristics and climatic data

Site parameters	Description
Latitude	29°24'S
Longitude	30°12'E
Altitude	1 260m asl
Frost risk	Week of 1st frost is 5th week in May; frost period continues for 8 weeks with a probability rating of 14% for at least one frost
MAP	c. 950mm
MAT	16.2°C (southern end of warm temperate zone)

MAP = mean annual precipitation; MAT = mean annual temperature; asl = above sea level

Table 3: Measurements and tasks conducted

Measurement and tasks	Period
Site preparation	12 February 2004
Burning	20 February 2004
Pitting	23 February 2004
Planting	25 February 2004
Survival at 2 weeks	10 March 2004
Blanking (no fertiliser used at blanking)	12 March 2004
Survival, height and GLD at 6 months	1 September 2004
Manual weeding	22 November 2004
Survival height and GLD at 12 months	25 February 2005

GLD = ground line diameter

Results

Nursery data

For both species, the rate of germination and final germination was higher for the larger grade seeds whereas the smallest, Grade 4 seeds germinated poorly (Table 4). Depending on the species, a 40–50% difference was observed between the germination of Grade 1 and Grade 4 seeds.

Seedling survival was poorer for Grade 4 *E. grandis* but not for *E. smithii*. To illustrate the variation among treatments at planting, seedling nursery height and RCD were plotted (Figures 1 and 2). The trend observed in the nursery was for the smallest Grade 4 seeds to produce the shortest seedlings (Figure 1).

There was no consistent trend observed in the nursery for RCD (Figure 2), except for *E. smithii*, where Grade 4 material had the highest RCD.

Field survival

None of the covariates (i.e. seedling measurements at planting) were significant for field survival or growth. Survival for both species (Tables 5 and 6) was good in this study due to rainfall prior to planting (168mm from January 2004 until planting with a mean maximum temperature of 27.7°C) and subsequent to planting (77mm from planting until the end of March 2004 with a mean maximum temperature of 26.6°C). The weather data was obtained from Sappi's Shaw Research Centre, which is located about 8km from the site.

Table 4: Nursery germination percentage over time for *Eucalyptus grandis* and *E. smithii*

Treatments	Germination* (%)					
	Week 1		Week 3		Week 5	
E. grandis						
Orchard						
Clan	22.2	*23.8*	61.7	*51.9*	73.6	*60.5*
Salpine	4.7	*9.0*	52.6	*44.3*	74.6	*61.1*
Venus	11.5	*16.5*	60.1	*51.1*	74.2	*61.0*
LSD		*3.71*		*4.60*		*5.00*
p-value		*<0.001*		*<0.001*		*0.521*
Grade						
1	24.8	*26.3*	81.6	*65.9*	88.8	*71.3*
2	15.4	*21.4*	75.7	*61.0*	85.6	*68.4*
3	9.8	*14.8*	62.9	*52.7*	78.6	*62.9*
4	1.2	*3.32*	12.1	*16.9*	43.5	*40.9*
LSD		*4.26*		*5.27*		*5.74*
p-value		*<0.001*		*<0.001*		*<0.001*
Orchard x *Grade Interaction*						
Clan						
1	49.7	*44.8*	84.4	*67.1*	90.1	*72.1*
2	23.1	*28.1*	76.5	*61.4*	86.1	*68.9*
3	15.0	*19.8*	65.7	*54.4*	78.9	*63.1*
4	0.9	*2.7*	20.1	*24.8*	39.1	*38.0*
Salpine						
1	5.8	*9.8*	74.2	*60.3*	86.4	*68.4*
2	8.5	*14.1*	78.6	*62.9*	90.5	*72.1*
3	2.0	*6.0*	55.1	*48.0*	76.2	*61.5*
4	2.6	*6.2*	2.6	*6.2*	45.2	*42.2*
Venus						
1	19.1	*24.4*	86.4	*70.3*	89.8	*73.3*
2	14.6	*21.9*	72.1	*58.8*	80.3	*64.1*
3	12.3	*18.6*	68.0	*55.7*	80.6	*64.0*
4	0.3	*1.1*	13.7	*19.5*	46.2	*42.5*
LSD		*7.34*		*9.10*		*9.89*
p-value		*<0.001*		*0.006*		*0.572*
E. smithii						
Grade						
1	36.2	*36.6*	78.6	*63.3*	87.9	*70.1*
2	24.0	*28.7*	78.1	*62.4*	85.5	*67.9*
3	13.6	*19.2*	60.9	*51.6*	76.7	*61.5*
4	2.2	*6.6*	26.3	*29.1*	36.5	*35.6*
LSD		*6.38*		*7.89*		*7.12*
p-value		*<0.001*		*<0.001*		*<0.001*

* Percentage germination angular-transformed data given in italics; LSD = least significant difference calculated from REML at 95% confidence level

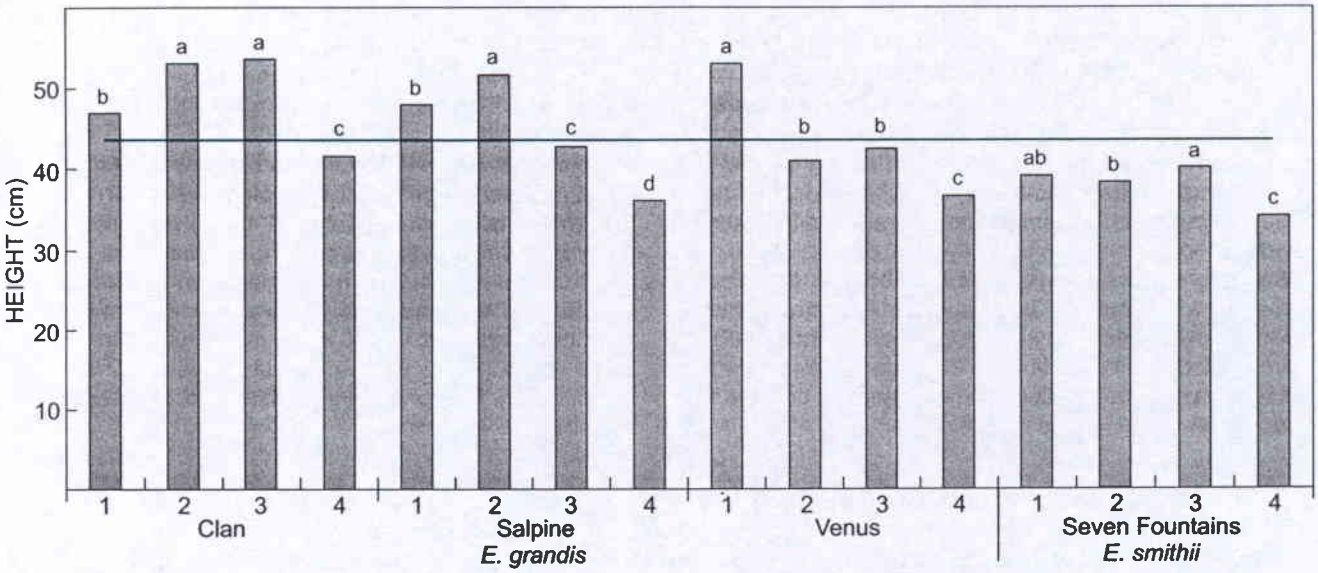

Figure 1: Seedling nursery height prior to planting. The numbers on the x-axis refer to seed grade. The x-axis is grouped according to orchard source and species. The horizontal line represents the trial mean. Common letters above the bars indicate no significant difference at the 95% confidence level (derived using LSD values) and are only comparable within an orchard source

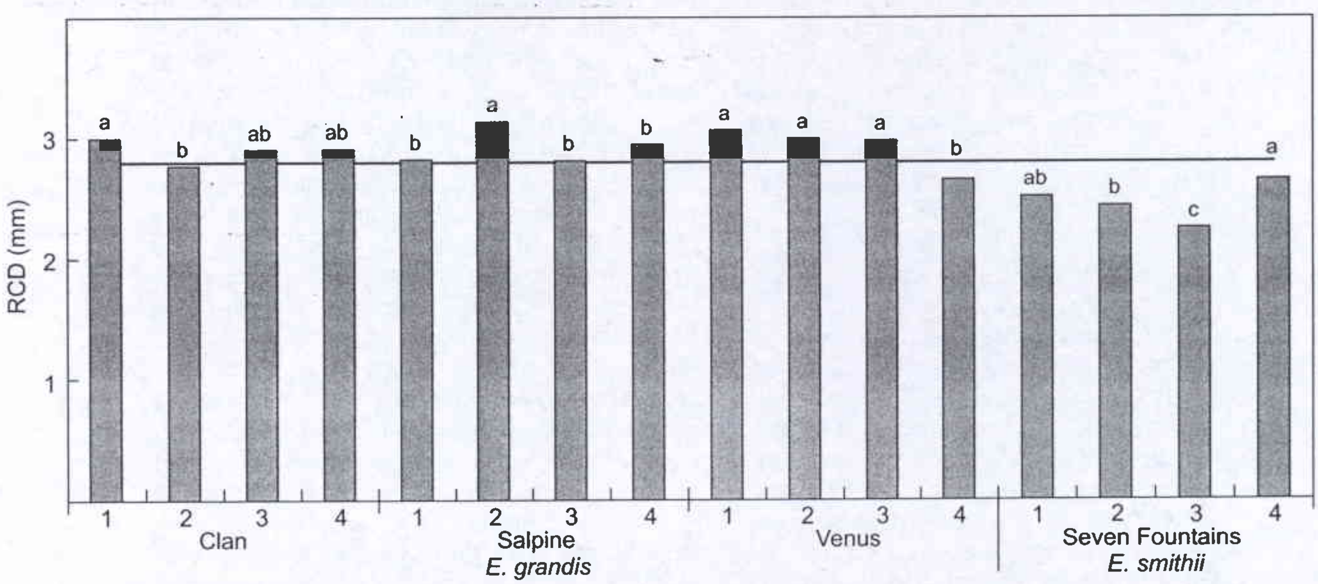

Figure 2: Seedling nursery root collar diameter (RCD) prior to planting. The numbers on the x-axis refer to seed grade. The x-axis is grouped according to orchard source and species. The horizontal line represents the trial mean. Common letters above the bars indicate no significant difference at the 95% confidence level (derived using LSD values) and are only applicable within an orchard source

For *E. grandis*, survival at six months was significantly lower for seedlings from Grade 1 seeds compared to Grades 3 and 4. This was due primarily to the lower survival for Grade 1 material from the Venus orchard (Table 5). However, by 12 months, the difference was only significant between Grade 1 and Grade 3 seeds. There were no other major significant differences.

There were no significant inter-treatment differences in survival for *E. smithii* (Table 6). Survival in this study was still good even after a snow event that occurred a week after the six-month measurements.

Growth

Eucalyptus grandis

Only orchard source was significant for all response variates, with significant interactions observed for height and growth rate. There was also a significant interaction for volume index and its growth rate but only at the 10% level (Table 7). The lower-value orchard, Salpine, produced lower values for the response variates and was responsible for the significant differences observed for orchard source (Table 7). Grade was not significant for any of the response

Table 5: Field survival for *Eucalyptus grandis* from three orchards. Whole plot data used

Treatment	Survival (%)			
	6 months		12 months	
Orchard				
Clan	99.1	*89.1*	97.8	*88.0*
Salpine	98.8	*88.8*	98.1	*88.2*
Venus	98.4	*88.5*	97.5	*87.6*
LSD		*1.6*		*2.0*
p-value		*0.745*		*0.945*
Grade				
1	97.0	*87.2*	97.0	*87.2*
2	98.8	*88.8*	97.9	*88.1*
3	99.2	*89.2*	98.8	*88.8*
4	100.0	*89.9*	97.5	*87.7*
LSD		*1.8*		*2.4*
p-value		*0.027*		*0.737*
Orchard x *Grade Interaction*				
Clan				
1	98.8	*88.8*	98.8	*88.8*
2	98.8	*88.8*	98.8	*88.8*
3	98.8	*88.8*	97.5	*87.7*
4	100.0	*89.9*	96.3	*86.6*
Salpine				
1	97.5	*87.7*	97.5	*87.7*
2	98.8	*88.8*	96.3	*86.6*
3	98.8	*88.8*	98.8	*88.8*
4	100.0	*89.9*	100.0	*89.9*
Venus				
1	94.8	*85.3*	94.8	*85.3*
2	98.8	*88.8*	98.8	*88.8*
3	100.0	*89.9*	100.0	*89.9*
4	100.0	*89.9*	96.3	*86.6*
LSD		*3.1*		*4.1*
p-value		*0.500*		*0.259*

Angular transformed data in italics; LSD from ANOVA at 95% confidence level; snow event on 6 September 2004, a week after the six month measurement

variates tested. The significant interactions showed greater growth for Grade 4 compared to Grade 1 material from Salpine and Venus orchards but a reversal of this trend was observed for Clan (Table 7).

Eucalyptus smithii

Only volume index and growth rate was significant at the 10% level with Grade 1 material producing a lower volume index (and growth rate) than Grade 2 and 3 material but a statistically comparable volume index (and growth rate) with Grade 4 material (Table 8).

Correlations and regressions

Simple linear correlations were performed on the inner tree data set to observe whether relationships existed between nursery measurements (prior to planting) and field measurements (Table 9). Nursery parameters were not highly correlated to field parameters (Table 9).

Regression analyses of field response variates with nursery explanatory variates did not yield any major significant relationships. As expected, due to the good survival in this study, no significant regressions were noted for this response variate (data not shown). A significant ($p = 0.033$) simple linear regression was observed for *E. grandis* field height against planting height with planting height only accounting for 1.5% of the variance in field height. The following equation was derived from the regression model:

$$y = 0.0691\chi + 309.4$$

Table 6: Field survival for *Eucalyptus smithii* from a single orchard source (Seven Fountains). Whole plot data used

Treatment	Survival (%)			
	6 months		12 months	
Grade				
1	97.5	*87.7*	92.5	*83.2*
2	100.0	*89.9*	92.5	*83.2*
3	100.0	*89.9*	97.5	*87.7*
4	98.8	*88.8*	92.5	*83.2*
LSD		*2.7*		*6.8*
p-value		*0.296*		*0.469*

Angular transformed data in italics; LSD from ANOVA at 95% confidence level; snow event on 6 September 2004, a week after the six-month measurement

Table 7: Field growth for *Eucalyptus grandis* from three orchards. Inner tree data used

Treatment	Growth at 12 months					
	Height (cm)	GLD (mm)	VI (dm^3)	Height GR (cm d^{-1})	GLD GR (mm d^{-1})	VI GR (dm^3 d^{-1})
*Orchard**						
Clan	354.1	57.5	12.1	0.83	0.149	0.033
Salpine	324.4	55.0	10.1	0.77	0.142	0.028
Venus	345.4	58.3	12.0	0.82	0.151	0.033
LSD	*17.1*	*2.1*	*1.1*	*0.05*	*0.006*	*0.003*
p-value	*0.002*	*0.01*	*<0.001*	*0.01*	*0.01*	*<0.001*
Grade						
1	340.8	56.7	11.4	0.80	0.147	0.031
2	343.9	57.5	11.8	0.81	0.149	0.032
3	334.9	55.6	10.6	0.79	0.144	0.029
4	345.7	57.8	11.9	0.84	0.150	0.032
LSD	*19.7*	*2.4*	*1.3*	*0.05*	*0.007*	*0.004*
p-value	*0.72*	*0.28*	*0.18*	*0.24*	*0.23*	*0.18*
Orchard x *Grade Interaction*						
Clan						
1	354.3	58.6	12.7	0.84	0.152	0.035
2	369.1	57.7	12.8	0.86	0.150	0.035
3	365.8	57.0	12.2	0.85	0.148	0.033
4	327.2	56.5	10.8	0.78	0.146	0.030
Salpine						
1	320.6	54.3	9.9	0.75	0.141	0.027
2	314.9	55.1	9.9	0.72	0.142	0.027
3	320.4	52.9	9.1	0.76	0.137	0.025
4	341.7	57.6	11.7	0.84	0.150	0.032
Venus						
1	347.4	57.2	11.6	0.80	0.148	0.032
2	347.7	59.7	12.8	0.84	0.156	0.035
3	318.4	56.9	10.4	0.75	0.147	0.028
4	368.2	59.4	13.1	0.90	0.155	0.036
LSD	*34.1*	*4.2*	*2.3*	*0.09*	*0.012*	*0.006*
p-value	*0.01*	*0.52*	*0.09*	*0.01*	*0.46*	*0.09*

LSD from ANOVA at 95% confidence level; GLD = ground line diameter; VI = volume index; GR = growth rate
* Orchard source, Salpine produces lower yield material compared to the other orchards in this study

Table 8: Field growth for *Eucalyptus smithii* from a single orchard source (Seven Fountains). Inner tree data used

Treatment	Growth at 12 months					
	Height (cm)	GLD (mm)	VI (dm^3)	Height GR (cm d^{-1})	GLD GR (mm d^{-1})	VI GR (dm^3 d^{-1})
Grade						
1	326.8	55.8	10.4	0.79	0.146	0.028
2	363.6	60.6	13.6	0.89	0.160	0.037
3	369.9	58.7	13.7	0.90	0.154	0.037
4	364.3	57.8	12.6	0.91	0.151	0.034
LSD	*44.4*	*5.1*	*2.8*	*0.12*	*0.014*	*0.008*
p-value	*0.20*	*0.30*	*0.08*	*0.22*	*0.24*	*0.08*

LSD from ANOVA at 95% confidence level; GLD = ground line diameter; VI = volume index; GR = growth rate

Discussion

This study showed that the small Grade 4 seeds germinated poorly. A similar trend was also recorded for *E. globulus*, where larger seeds germinated better than smaller seeds (López *et al.*, 2000). This is of operational importance in a nursery as seeds can be sown according to size to enhance homogeneity within the crop.

In addition, the small Grade 4 seeds produced the shortest seedlings. This trend was also observed in a study conducted by Khurana and Singh (2004) on five tropical tree species in India. The lower height for the Grade 4 material may be a function of delayed germination, which indicates that seedling growth, although indirectly an effect of seed size, may be directly linked to rate of germination (or germination date). A similar result was obtained by Dunlap

Table 9: Correlation matrix of nursery and one-year field data. Inner tree data used

Parameter		Nursery				Field			
		$VI_{planting}$	Height	RCD	SR	Height	GLD	VI	Survival
Nursery	$VI_{planting}$	1							
	Height	**0.73**	1						
	RCD	**0.88**	**0.44**	1					
	SR	**–0.21**	**0.38**	**–0.59**	1				
Field	*Height*	0.00	0.05	–0.04	0.06	1			
	GLD	–0.03	–0.04	–0.01	–0.05	**0.46**	1		
	VI	–0.03	–0.01	–0.03	–0.01	**0.79**	**0.88**	1	
	Survival	0.06	0.05	0.03	0.03	0.00	0.00	0.00	1

GLD = ground line diameter; RCD = root collar diameter; SR = sturdiness ratio (a function of height divided by RCD); VI = volume index; Significant correlations are in bold, calculated at 95% level

and Barnett (1983), who found that large loblolly pine seeds germinated faster and produced larger seedlings compared to small seeds. Delayed germination may be overcome by sowing germinants (seeds that have been imbibed in water until the start of radicle emergence from the seed coat). Sappi has previously explored germinant technology to sow *Eucalyptus* seeds, i.e. sowing suspended germinants in water using a fluid drilling machine (South and Young, 1995) but this technique is no longer practised due to (1) the cessation of using fluid drilling technology at Sappi in a move to pursue faster, more accurate precision seeders that can sow seeds dry; (2) germinant separation has proved to be difficult on a commercial scale and often results in the loss of viable seeds due to inefficient separation — the key to the success of germinant technology is efficient separation of dead from live seeds (South and Young, 1995); (3) germinant technology was mainly used for species that produced a high number of non-viable seeds and for species that have similar-sized chaff and seeds (current commercial species at Sappi have a high percentage of viable seeds and commercial cleaning methods can efficiently separate chaff from seeds); and (4) easier techniques of improving seed-use efficiency are now being explored with Sappi opting to pursue alternative methods of single-sowing (one seed per cell) in order to maximise the use of valuable, genetically improved seeds.

Although no consistent trend was observed in the nursery for RCD in *E. grandis*, Grade 4 material had the highest RCD for *E. smithii*. This is possibly a function of poor germination leading to a lack of competition effects and thus allowing partitioning of resources to RCD growth rather than height growth. A study on *Pinus palustris* reported that seedlings grown at a lower density within a container have a greater diameter and this increase in diameter growth was attributed to more light availability to the seedlings (South *et al*., 2005).

Contrary to this study, observations on pine seedling morphology have shown that both seedling height and diameter are good predictors of field survival and growth (Iverson, 1984; Thompson, 1985; South, 2002; South *et al*., 2005).

Thus, these data showed that seed size effects for *Eucalyptus* were transitory, only apparent in the nursery and not perceptible after one year in-field. Similar findings were recorded for *P. elliottii* (slash pine), which showed that seed size had no effects on tree height after one year in-field (Sluder, 1991). A study on three Costa Rican hardwoods also found that differences in nursery planting stock did not translate to significant differences in plant size after one year in-field (Wightman *et al*., 2001). A study on *E. globulus* seedling specifications in Australia found that mortality was not related to seedling size at six months after planting (Close *et al*., 2006). In addition, this study also showed that seedling size had no effect on growth at six months after planting (Close *et al*., 2006).

Conclusions

This study investigated whether seed grade differences recorded in the nursery would translate to differences in the field. Results indicated poor nursery germination for the small Grade 4 seeds but good field survival was recorded irrespective of species or seed grade. At 12 months, only orchard source was significant for all response variates for *E. grandis*, with the lower-value orchard, Salpine, producing lower values for all response variables. For *E. smithii* at 12 months, there were no significant inter-treatment differences recorded at the 5% level. Nursery parameters were not highly correlated to field parameters. Regression analyses of field response variates with nursery explanatory variates did not yield any major significant relationships. A significant ($p = 0.033$) simple linear regression was observed for *E. grandis* field height against planting height, but planting height only accounted for 1.5% of the variance in field height. Thus, this study showed that seed size effects for *Eucalyptus* were transient, only apparent in the nursery and not noticeable after one year in-field. Therefore smaller seeds can be used in a commercial nursery as long as they are sown with similar-sized seeds to allow for management of poor germination and crop uniformity. Seed orchard source proved to be very important in predicting field growth.

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