

# FINAL RESULTS FROM A TRIAL TO TEST THE EFFECT OF PLOT SIZE ON *EUCALYPTUS* HYBRID CLONAL RANKING IN COASTAL ZULULAND, SOUTH AFRICA.

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Hybrid clones with *Eucalyptus grandis* as one parent and generally, *E. urophylla* as the other are deployed commercially as mono-clonal blocks by Sappi in the sub-tropical region of coastal Zululand, South Africa. Large numbers of clones selected in full-sib hybrid progeny trials are screened in field trials with six replications of three tree row plots to rapidly identify superior clones. Prior to commercialisation, these are then planted in more comprehensive clone by site interaction trials, using three to four replications of 6 x 6 tree square plots. Only the inner 4 x 4 tree plot, that simulates the competition that would occur between individuals of the same clone under plantation conditions, are measured in each plot. In mono-clonal blocks unique genotypes are grown in self-competition. Performance under this scenario may not necessarily correlate well with performance where genotypes are competing for resources with a number of genetically dissimilar genotypes.

There is an uncertainty whether plot configuration or size influences clonal ranking and therefore selection of clones for commercialisation. Sappi's Hardwood Breeding Programme planted a trial testing eight selected *Eucalyptus* hybrid clones in single tree, eight tree line and 8 x 8 tree square plots, in coastal Zululand, South Africa. Final results at eight years of age are reported on in this paper. There were clonal rank changes between the single tree, line and square plots. This raises the question: Are we selecting the best clones using statistically more efficient field designs that do not take into account the possibility that inter-genotypic competition effects clonal performance? Gain predictions using data from the single tree plots are also compared with realised gains measured in the large square plots.

## Key Words

Clones, clonal ranking, hybrid, plot size, inter-genotypic competition

## INTRODUCTION

Due to its rapid growth and desirable wood properties *E. grandis* has historically been an important tree species for pulp production in South Africa. However the species suffers from poor survival in coastal Zululand, which is a sub-tropical area, because of fungal diseases. *E. urophylla* has been found to be more disease tolerant and hybrids between the two species have been produced that combine the good survival and rapid early growth of the parent species. These hybrid clones have been deployed commercially as mono-clonal blocks of average size 20ha since 1992 by Sappi in Zululand, South Africa.

As part of the Sappi breeding programme large numbers of clones selected in full-sib hybrid seedling progeny trials are initially screened in field trials with six replications of three tree row plots to identify superior clones. These are then evaluated more comprehensively in clone by site interaction trials (CSI's) using four replications of 6 x 6 tree plots in a randomised complete block field design. Trials are planted at the commercial espacement and receive standard silvicultural treatment. Only the inner 4 x 4 trees in each plot are measured to simulate the competition that would occur between individuals of the same clone under plantation conditions. Each trial series are tested across a range of different sites.

Fasoula and Fasoula (1997) argue that two of the ecosystems in which genotypes are tested by plant breeders are a competition environment and a crop environment. They define a competition environment as being composed of interactions between genetically dissimilar genotypes, while a crop environment is composed of interactions between genetically identical genotypes. Fasoula and Fasoula (1997) report that for crop plants there is a poor negative correlation between the best yield producers in a competition environment and the best yield producers in a crop environment. In forestry all plantings of seed orchard bulks, half-sib or full-sib families can be classified as growing in a competition environment, while mono-clonal plantings can be classified as a crop environment. The vast majority of selections made in tree breeding trials are made in trials with different genotypes growing adjacent to each other (competition environment). Offspring from these selections are then deployed in plantations in a competition environment. Since Sappi deploy their clones in the coastal Zululand region as mono-clonal blocks (crop environment), the question arises whether clonal selections should be done in a competition environment or crop environment to maximise the probability of identifying the best clones for commercial deployment.

The cost of having large multiple tree plots to simulate a crop environment in a clonal screening programme is high and thus the number of clones that can be included is restricted. There is uncertainty whether plot type influences clonal ranking and therefore selection of clones for commercialisation. In order to overcome this, the breeding programme planted a trial to compare the precision with which clones are ranked by single tree, line and square plots.

Trial designs with small plots and large numbers of replicates give the highest statistical precision (Eldridge *et al.*, 1993). The advantages of single tree plots are that they maximise the variety of micro-sites sampled by a given clone (Loo-Dinkins and Tauer, 1987). The disadvantage of single tree plots is that they are more difficult to establish than line or block plots, because the identity of each seedling must be maintained (Eldridge *et al.*, 1993). As trees grow and competition sets in, single tree plots do not always resemble actual stand conditions in plantations. Early results from this trial have shown that there was a weak rank correlation between the single, line and square plots and that it may not be possible to extrapolate conclusions derived from single tree plots to plantation conditions (Retief *et al.*, 2001).

To evaluate characteristics influenced by stand conditions, multiple tree plots with fairly large numbers of trees per plot are preferred (Shiue and Pauley, 1961). To test the same number of entries in multiple tree block plots, field trials can become very large and therefore require extensive resources (Janson *et al.*, 1998). Many forestry test sites have a large amount of environmental variability and trials with large block sizes may result in poor statistical precision (Franklin, 1971), for these reasons they are often not the preferred design type.

## **MATERIALS AND METHODS**

The trial reported on in this paper was established in the Zululand coastal region of South Africa. The Zululand coastal region is a flat landscape, with deep, sandy soils and altitudes ranging from 50m to 100m above sea level. This area has a mean annual temperature of 21°C and a rainfall gradient that decreases from south to north and with distance inland from the coast (Pallett & Mitchell, 1993). Mean annual rainfall at the trial site is predicted to be 1149mm.

The trial design is a randomised block design with three “main” replications (See Figure 1). In each replication there is one 8 x 8 tree square plot for each of the eight clones, four sets of eight tree line plots of each clone and four sets of 64 single tree plots with each clone appearing eight times per set.

There are three replications of the square plots, 12 replications of the line plots and 96 replications of the single tree plots for each clone. In the 8 x 8 tree square plots, the outside row serves as a buffer row and only the measurements from the inner 6 x 6 trees were used in these analyses. There were seven *E. grandis* x *E. urophylla* (GU) clones and one *E. grandis* x *E. camaldulensis* (GC) clone included in the trial. Six of the eight clones have been planted operationally by Sappi and are known to be good performers.

Rep 1 Plot 1 Square GU 5	Rep 1 Plot 5 Line	Rep 1 Plot 9 Square GU 7	Rep 1 Plot 13 Square GU 1	Rep 2 Plot 1 Square GU 2	Rep 2 Plot 5 Square GU 7	Rep 2 Plot 9 Square GC 1	Rep 2 Plot 13 Square GU 6	Rep 3 Plot 1 STP	Rep 3 Plot 5 Line	Rep 3 Plot 9 Square GU 4	Rep 3 Plot 13 Line
Rep 1 Plot 2 STP	Rep 1 Plot 6 Square GU 2	Rep 1 Plot 10 Square GU 3	Rep 1 Plot 14 Square GU 6	Rep 2 Plot 2 Square GU 1	Rep 2 Plot 6 Square GU 4	Rep 2 Plot 10 STP	Rep 2 Plot 14 STP	Rep 3 Plot 2 Square GU 5	Rep 3 Plot 6 Square GU 6	Rep 3 Plot 10 Line	Rep 3 Plot 14 Square GU 1
Rep 1 Plot 3 STP	Rep 1 Plot 7 Square GC 1	Rep 1 Plot 11 Line	Rep 1 Plot 15 Line	Rep 2 Plot 3 Line	Rep 2 Plot 7 STP	Rep 2 Plot 11 Line	Rep 2 Plot 15 Line	Rep 3 Plot 3 Line	Rep 3 Plot 7 Square GU 7	Rep 3 Plot 11 Square GC 1	Rep 3 Plot 15 STP
Rep 1 Plot 4 STP	Rep 1 Plot 8 STP	Rep 1 Plot 12 Square GU 4	Rep 1 Plot 16 Line	Rep 2 Plot 4 Square GU 5	Rep 2 Plot 8 Square GU 3	Rep 2 Plot 12 Line	Rep 2 Plot 16 STP	Rep 3 Plot 4 Square GU 2	Rep 3 Plot 8 Square GU 3	Rep 3 Plot 12 STP	Rep 3 Plot 16 STP

FIG. 1- Field layout of the trial (STP = single tree plot)

Results from an analysis of the 44 (half rotation) and 94 month (full rotation) measurements of the standing trees are reported on in this paper. All statistical analysis was conducted using SAS® ver 9.1 (SAS Institute Inc. 2004).

The models used for the single tree plot analysis [1] and the line plot and square plot analysis [2] were:

$$y_{ij} = \mu + \beta_i + \alpha_j + \varepsilon_{ij} \dots \dots \dots [1]$$

$$y_{ijk} = \mu + \beta_i + \alpha_j + (\beta\alpha)_{ij} + \varepsilon_{ijk} \dots \dots \dots [2]$$

where  $\mu$  = overall mean

$\beta_i$  =  $i^{\text{th}}$  block effect,  $i = 1, \dots, 96$  for single tree plots,  $i = 1, \dots, 12$  for line plots and  $i = 1, \dots, 3$  for square plots

$\alpha_j$  =  $j^{\text{th}}$  clone effect,  $j = 1, \dots, 8$

$\varepsilon_{ij}$  = random error associated with  $i^{\text{th}}$  block,  $j^{\text{th}}$  clone and  $k^{\text{th}}$  tree where  $\varepsilon_{ij} \sim \text{iid}(0, \sigma^2)$

PROC VARCOMP (SAS Institute Inc. 2004) was used to estimate variance components using a completely random model, and PROC GLM (SAS Institute Inc. 2004) with fixed clonal and replication effects to estimate clonal means. Residuals were plotted against fitted values to test whether the assumptions of homogeneity and normality were valid.

The correlations between clonal means were calculated using Spearman's rank order correlation coefficient  $r_s$ , as follows:

$$r_s = 1 - 6\sum d_i^2 / n(n^2 - 1)$$

where  $d_i$  = the difference between the y rank and the x rank on observation  $i$

$n$  = is the number of  $x_i, y_i$  observations

(Ott, 1993)

The variance among clonal means,  $\sigma_{c\text{ mean}}^2$ , was calculated using the following expression:

$$\sigma_{c\text{ mean}}^2 = \sigma_c^2 + \sigma_p^2/b + \sigma_w^2/nb$$

where  $\sigma_c^2$  = clonal variance component

$\sigma_p^2$  = between plot variance component

$\sigma_w^2$  = within plot variance component

$b$  = number of blocks or replications

$n$  = number of trees per plot

Clonal repeatability's (clonal heritability,  $H^2$ ), using the variance components defined above, were calculated for each design using the following expression:

$$H^2 = \sigma_c^2 / (\sigma_c^2 + \sigma_p^2/b + \sigma_w^2/nb)$$

## RESULTS

For each design the plots of residuals against fitted values showed no detectable trends or patterns. It can therefore be said that the conditions that  $\varepsilon_{ijk} \sim \text{iid}(0, \sigma^2)$  have been met for this data and the standard ANOVA assumptions are valid. Overall survival (96.4%) in the trial at 44 months was good. Half rotation (44 month) and full rotation (94 month) data were included in this analysis. Inter-genotypic competition dynamics may change over time. Clonal ranking was compared using the 44 and 94 month data. The phenotypic correlations between mean tree volume at 44 and 94 months were 0.91, 0.94 and 0.92 for the single tree, line and square plots respectively. All were statistically significant ( $P < 0.0001$ ). Similarly the Spearman rank correlations were 0.98, 0.98 and 0.40 for the single tree, line and square plots respectively, with the first two being statistically significant ( $P < 0.0001$ ). Ranking between these two ages of assessment have remained stable in the single tree and line plots, but there have been some changes in the square plots (Figure 2).

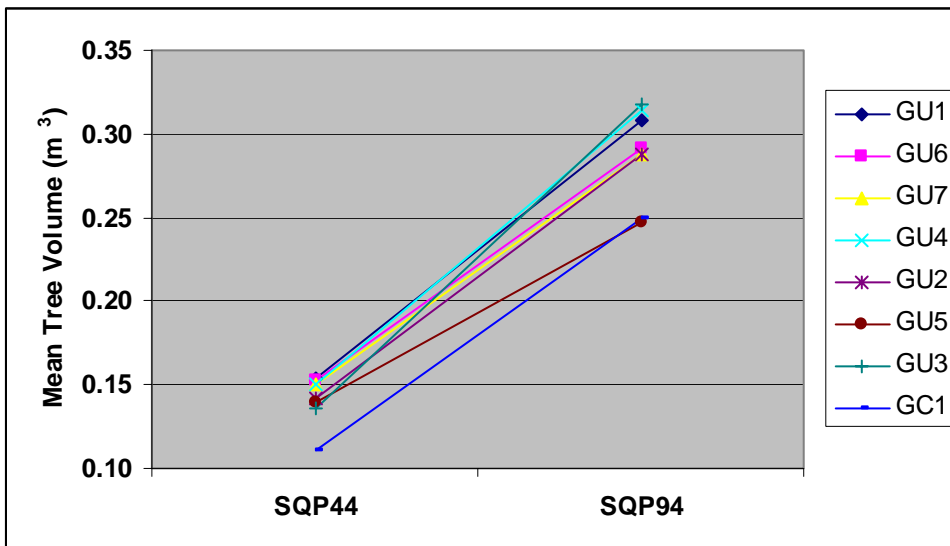


FIG. 2 - Comparison of clonal ranking using mean tree volume ( $\text{m}^3$ ) from square plots (SQP) at 44 and 94 months.

Further analyses focussed on the 94 month data and the changes in clonal ranking between the single tree, line and square plots. Clonal ranking between the single tree and line plots was consistent (Spearman's rank correlation = 0.98,  $P < 0.0001$ ) with only two clones, GU5 and GU6 swapping ranks (Table 1). If a breeder wanted to select the top two clones from the eight in these tests, the same two would have been chosen from using either a single tree or line plot configuration (Table 1). These two

clones, GU1 and GU2 were ranked third and fifth using the data from the large square plots. When comparing the single tree to the large square plots rank changes have been significant (Spearman's rank correlation = 0.57, P = 0.1390). Clone GU2 has changed rank the most. While this clone would likely have been selected, from this group of eight clones, as a commercial clone using single and line plot data, it only ranked fifth overall when ranked using square plot data (Table 1).

TABLE 1 - Clonal mean tree volume (m<sup>3</sup>) at 94 months and clonal rank for the three different plot designs. (LSD = least significant difference at the 5% level).

Clone	Single Tree Plot		Line Plot		Square Plot	
	Vol94	Rank	Vol94	Rank	Vol94	Rank
GU1	0.5245	1	0.4380	1	0.3081	3
GU2	0.4180	2	0.3943	2	0.2878	5
GU3	0.3919	3	0.3777	3	0.3178	1
GU4	0.3180	4	0.3017	4	0.3145	2
GU5	0.2371	5	0.2318	6	0.2469	8
GU6	0.2250	6	0.2723	5	0.2910	4
GU7	0.1599	7	0.1474	7	0.2874	6
GC1	0.1203	8	0.1366	8	0.2499	7
Mean	0.3043		0.2915		0.2877	
LSD <sub>0.05</sub>	0.05371		0.04926		0.04692	

The range of the clonal means for the single tree plots (0.4042 m<sup>3</sup>) is more than five times the range for the square plots (0.0709 m<sup>3</sup>)(See Figure 3). This is more dramatic than that reported on for the 31 month diameter by Retief *et. al.* (2001). The mean tree volume for the best performing clone, GU1, drops from 0.5245 m<sup>3</sup> in the single tree plots to 0.3081 m<sup>3</sup> in the square plots. GU1 is known to be a “fast starter” and it appears that in the single tree and line plot designs that GU1 out-competes its neighbours and gains a competitive advantage. In the square plots of GU1, the environmental resources are distributed more evenly among the individuals resulting in a smaller tree volume and lower within plot variance. GU7 and GC1 appear to be suppressed in the single tree and line plot designs (Figure 3). This might be a function of competition due to root or canopy architecture. The overall means for the three different plot types are very similar (Table 1).

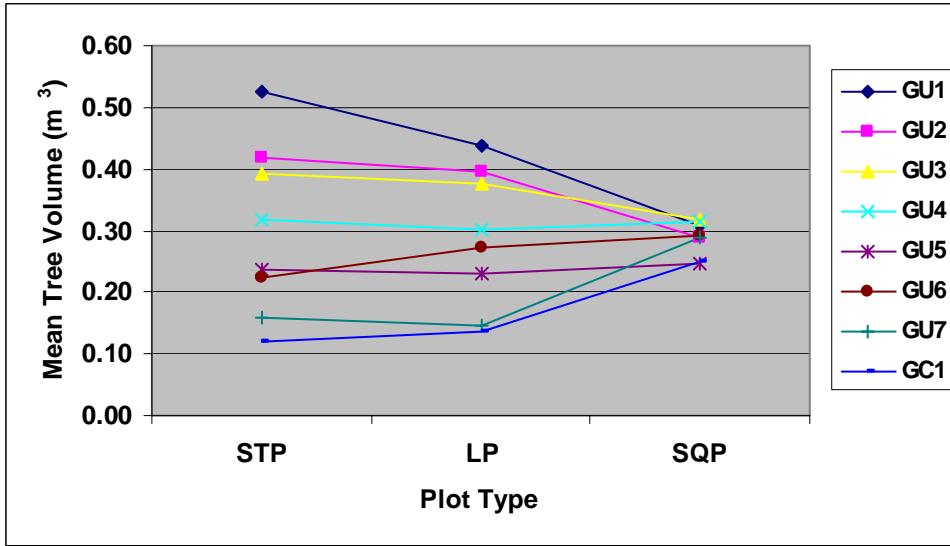


FIG. 3 - Mean tree volume (m<sup>3</sup>) at 94 months for each clone for the three different plot designs. Single tree plot (STP), line plot (LP) and square plot (SQP)

The clonal repeatability's were high for the single tree and line plot designs, and substantially lower for the square plots (Table 2).

TABLE 2 - Summary of the variance components, environmental portion of the variance of a clonal mean ( $E_{c \text{ mean}}$ ) and relative efficiencies (RE) for the three different plot designs based on individual tree volume at 94 months.

Design	Single tree	Line	Square
No. of blocks	96	12	3
Ramets per block	1	8	36
$\sigma_c^2$	0.01841	0.01208	0.0004478
$\sigma_{\text{block}}^2$	0.00064	0	0
$\sigma_p^2$	-	0.0015676	0
$\sigma_w^2$	0.0278	0.02816	0.02876
$\sigma_{c \text{ mean}}^2$	0.019	0.013	0.001
$H^2$	0.98	0.97	0.63
$E_{c \text{ mean}}$	0.028	0.030	0.029
RE	1.00	0.94	0.97



In progeny trials, the terms  $\sigma_p^2/b$  and  $\sigma_w^2/nb$  are largely attributable to the environment (Loo-Dinkins and Tauer, 1987). In this trial, where clones were used, these two terms are totally determined by environmental effects. The environmental portion of the treatment mean variance can be influenced by changing the number of trees per plot (n) or the number of blocks (b) (Loo-Dinkins and Tauer, 1987). Loo-Dinkins and Tauer (1987) defined the sum of  $\sigma_p^2/b$  and  $\sigma_w^2/nb$  as the environmental portion of the variance of a treatment mean, denoted by  $E_{c \text{ mean}}$ . Loo-Dinkins and Tauer (1987) defined the relative efficiency (RE) of a design as: The inverse of the  $E_{c \text{ mean}}$  for a design relative to the  $E_{c \text{ mean}}$  for the single plot design. The results for the three different plot designs are summarised in Table 2.

The portion of the clonal mean that is influenced by the environment  $E_{c \text{ mean}}$ , was very similar for all three plot types at 94 months. It has been reported that this portion was larger for the single tree plots than for the line plot design at 31 months (Retief *et al.* 2001). At 31 months the three replications of the 8 x 8 square plots were the most efficient design and the line plot design was marginally more efficient than the single tree plot design (Retief *et al.* 2001). This seems to have stabilised and is more in line with expectations with the single tree plots being statistically the most efficient.

It is common practice in clonal selection programmes to screen large numbers of clones in trials using single tree or small line plots. Breeders face constant pressure from operational management to provide estimates of gain that can be expected from new clones about to be introduced for operational planting. A classical formula;  $R=h^2S$  to estimate genetic gain, uses the selection differential (S) and heritability ( $h^2$ ) to calculate a response to selection (Falconer, 1981). Gains relative to the trial mean were predicted for all eight clones using this formula and compared to realized gains using the data from the large square plots (Table 3). The gains predicted using the single tree plot data were gross overestimates compared to the realized gains measured on the square plots (Table 3). Clone GU1 had a predicted gain of 74% over the trial mean, but in reality only yielded 7% more in the square plots. Similarly clone GC1 did not perform as poorly as what was predicted and produced 13% less than the trial mean on the square plots (Table 3). It is well known that small single tree or line plots cannot be used to predict yield on a volume per hectare basis, but it is common for breeders to predict mean tree volumes from these plots and to extrapolate this to a future yield scenario. The results from this trial clearly demonstrate that this approach can lead to gross over estimations of yield.

TABLE 3 - Predicted gains (%) relative to the trial mean using single tree plot (STP) data and actual yields (m<sup>3</sup>) and realised gains (%) from square plot data.

Clone	STP	S	H <sup>2</sup>	Gain	Predicted Yield	Predicted Gain	Actual Yield	Realized Gain
GU1	0.5245	0.220	0.98	0.216	0.5201	74	0.3081	7
GU2	0.4180	0.114	0.98	0.111	0.4157	39	0.2878	0
GU3	0.3919	0.088	0.98	0.086	0.3901	30	0.3178	10
GU4	0.3180	0.014	0.98	0.013	0.3177	6	0.3145	9
GU5	0.2371	-0.067	0.98	-0.066	0.2384	-20	0.2469	-14
GU6	0.2250	-0.079	0.98	-0.078	0.2266	-24	0.2910	1
GU7	0.1599	-0.144	0.98	-0.142	0.1628	-46	0.2874	0
GC1	0.1203	-0.184	0.98	-0.180	0.1240	-59	0.2499	-13
Mean	0.3043				0.2994		0.2877	

## DISCUSSION

In a single tree or line plot, an individual is totally or partly surrounded by dissimilar genotypes, while in square plots, the inner 6 x 6 trees (the measured plot) are totally surrounded by genetically identical trees. Although the means for tree volume differed slightly between the single and line plot designs, the clonal rankings were very similar with only one rank change. There were however significant differences in clonal ranking between the first two plot types and the square plots.

The results show that to select clones that will perform best when growing in competition with themselves, an experimental design where trees are surrounded by identical genotypes, should be used. A competition environment as defined by Fasoula and Fasoula (1997) is composed of interactions between genetically dissimilar genotypes, which is comparable to the single tree and the line plots in this study. A crop environment is composed of the interactions between genetically identical genotypes, which is simulated by the square plots. In a crop environment (identical genotypes) resources are shared equally by all plants and consequently the yield of all plants is evenly suppressed. In the competition environment (dissimilar genotypes) the sharing of resources can never be equal, since the interacting plants are genetically different. The best performers in a competition environment are the strongest competitors. Results have shown that successful competitors in a competition environment can be poorer yield producers in pure stand. The genotypes that perform best when grown in a monoculture are the ones with high individual buffering. Due to the negative correlation

between yielding and competitive ability, Fasoula and Fasoula (1997) regard the competition environment as unfit for direct selection based on yield. This might explain the poor correlation between the clonal means in the single tree and line plots when compared to the square plots. Square plots simulate mono-clonal plantations and are more reliable for selecting commercial clones. Although still very young a follow up trial with 25 clones is showing similar trends to the ones reported on in this paper.

## **CONCLUSIONS**

Based on our field experience, the arguments of Fasoula and Fasoula (1997) derived from crop breeding, warrant further investigation in forestry. Clonal forestry with eucalypts offers an opportunity to further understand some of the mechanisms that may be involved in "between genotype" competition. The results reported in this paper raise some interesting questions that can perhaps only be answered by further investigation. Although testing genotypes in square or rectangular plots is more expensive than single tree or line plots, this study indicates that for clones in coastal Zululand, it may be the most reliable and efficient way of identifying the best *Eucalyptus* hybrid clones for commercial deployment in mono-clonal blocks.

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