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Experimental strategies for clonal eucalyptus

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Abstract

The success of any forestry operation depends on the careful choice of the clones to be planted. At existing yield levels, the differences among clones are becoming smaller, leading to the need to use experimental strategies for a more successful choice. To obtain information in regard to better experimental strategies, data from two clonal tests of eucalyptus were used. One of the tests consisted of 35 clones and another of 48 clones. In both tests, the experimental plot consisted of 100 plants at commercial spacing (12 m² per plant). The trait wood volume was evaluated, taking the relative position of the plant in the plot as a reference. The data were evaluated by different means, and it was observed that the use of border plot in clonal tests in eucalyptus is not necessary. Experimental accuracy with 15 to 20 plants is similar in comparison to the plot with 100 plants.

Key words: *Eucalyptus*, experimental precision, accuracy, plot size.

Introduction

In a eucalyptus genetic breeding program, the clonal evaluation stage, especially that which precedes planting recommendation on a commercial scale, is one of the most expensive because it requires that the experiments be set up in representative environments of the operational area of the company. The clonal tests are used for this purpose because they simulate the conditions to which the clone will be subjected on a small scale. For selection to be successful in this phase of the program, evaluating the greatest number of clones possible is indispensable and the test plots must be highly representative. When all this information is analyzed together, it may be clearly perceived how difficult and expensive operationalization of a breeding program is. Therefore, alternatives to facilitate and decrease the cost of this phase of the program should be considered, and one of them is in regard to carefully defining the size of the plots and/or number of plants to be evaluated in the final stages of the program.

Theoretically, the larger the plot, the greater the accuracy. However, the cost of each plot increases with an increase in the number of plants. Thus, identifying this size so as to have efficiency in the recommendation of new clones at the lowest possible cost is fundamental. Normally, to obtain information in respect to the form and the size of the experimental plots, an experimental area is planted to a single clone such that at the time of data collection, the area is split into basic units of minimum size (PARANAÍBA et al., 2009; SILVA, 2010). This pro-

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cedure allows one to obtain, among other data, the heterogeneity of the experimental area. In the forestry sector, however, experiments are set up together with the commercial planting areas and, therefore, there is not a defined experimental area. Under these conditions, the information obtained in the blank test is of little use.

For many planted species, there are studies seeking to identify the best size of the experimental plot and whether there is need for a border. In eucalyptus, this information was already obtained in some studies (ANDRADE et al., 2006; STORCK et al., 2011). ANDRADE et al. (2006), for the purpose of charcoal production, sought to identify the best plot size based on evaluation of seven clones in separate experiments with four plot sizes. This strategy, although viable, has some restrictions. First, the number of clones evaluated was small and generalizing the information is difficult, due to the small number of sizes evaluated. Another option is to use a single large plot size and simulate different plot sizes from pre-existing data in the larger plot. This procedure has been used in other species (TOLEDO et al., 2013). Nevertheless, references to its use in the forestry sector were not found. In light of this, the present study was carried out for the purpose of estimating the need for a border area in the clonal tests and the ideal size of the experimental area in eucalyptus.

Materials and Methods

To carry out this study, we evaluated two clonal tests of hybrids of *Eucalyptus grandis* with *Eucalyptus urophylla* belonging to the genetic breeding program of Veracel Celulose SA, located in Eunápolis, in the extreme south of the Brazilian state of Bahia. The information from the experiments is shown in Table 1.

One of the tests consisted of 35 and the other of 48 clones. A randomized block experimental design was used with three replications. The plots consisted of 100 plants, each one at a spacing of 3 m x 4 m (12 m² per plant). Taking the plant relative position in the plot as a reference, the wood volume was obtained, as showed in the Figure 1.

To verify the need for a border, analysis of variance was carried out, grouping the data in three different ways: 1) it was evaluated the following five alternatives: a) considering all the 100 plants in the plot (B0), b) considering 64 plants in the useful area, with only one row and one column as border area (B1), c) considering 36 plants in the useful area, with two rows and two columns as the border area (B2), d) considering 16 plants in the useful area, with three rows and three columns as the border area (B3) and e) considering 4 plants in the useful area, with four rows and four

columns as the border area (B4) (Figure 1); 2) by considering the split plot rows, and 3) by considering the split plot columns.

According to STEEL et al. (1997), the suitable model for such analysis is:

$$Y_{ijk} = m + c_i + r_j + (cr)_{ij} + p_k + (cp)_{ik} + (rp)_{jk} + e_{ijk}$$

where:

Y_{ijk} : observation in regard to clone i, in replication j, in position k;

Row/ Column	1	2	3	4	5	6	7	8	9	10
1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	1.10
2	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	2.10
3	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	3.10
4	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	4.10
5	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	5.10
6	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	6.10
7	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	7.10
8	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	8.10
9	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8	9.9	9.10
10	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.9	10.10

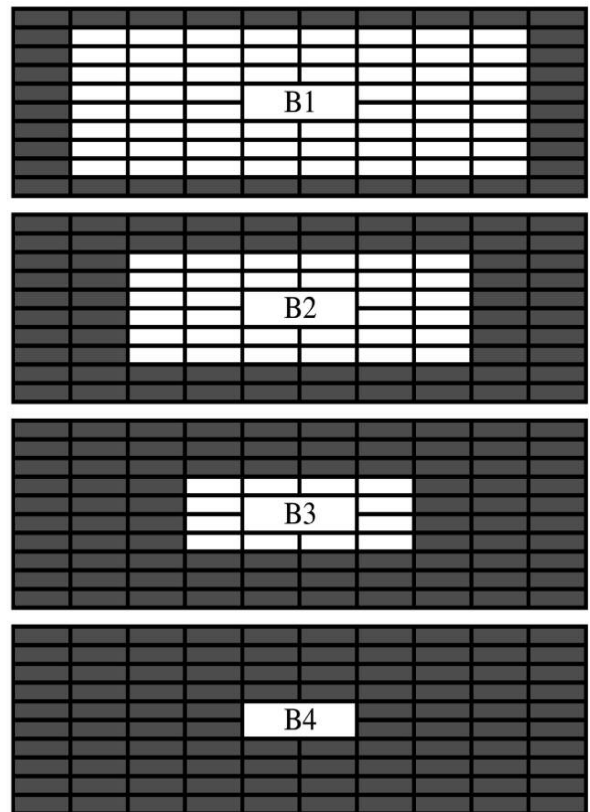


Figure 1. – Design of the relative position of the plants in the plot (B0) and the simulated border areas of one (B1), two (B2), three (B3) and four (B4) rows and columns.

Table 1. – Planting date of the tests and edaphic and climatic information of the region where the clonal tests were set up.

Test	Planting date	Rainfall (mm)	Temperature (°C)	Soil Type
Clonal test 1	09/03/2008	1050	23	LAd1.1
Clonal test 2	07/27/2007	1310	23	PAdx5.1

- m : general mean of the experiment;
 c_i : effect of clone i ;
 r_j : effect of replicate j ;
 $(cr)_{ij}$: interaction between the effect of clone i and the effect of replicate j , which corresponds to the error among plots;
 p_k : effect of position k ;
 $(cp)_{ik}$: interaction between the effect of clone i and the effect of position k ;
 $(rp)_{jk}$: interaction between the effect of replicate j and the effect of position k , because there was no randomization of the subplots within the plot;
 e_{ijk} : experimental error, presupposing $e_{ijk} \sim N(0, \sigma_e^2)$.

To estimate the ideal size of the experimental plot, the resampling method was used. This methodology was applied with the aid of an algorithm that sorted out the plants (without replacement) in each plot of the experiment, to then perform analysis of variance of each simulated experiment. This algorithm was done in the software R and it was already used in another work with the same objective (TOLEDO et al., 2013). The simulated sizes began as of five plants. One thousand experiments were simulated for each size n , with the estimates of the

following parameters being stored in each simulation: mean square error among plots (V_e), within-group variance within the plot (V_w) and accuracy (r_{gg}). In the forestry sector, experimental precision has been evaluated by means of the experimental coefficient of variation (CV_e) (RODRIGUES et al., 2008; MASSARO et al., 2010; LIMA et al., 2011; SOUZA et al., 2011). Nevertheless, RESENDE and DUARTE (2007) emphasize that the use of the CV_e , as a measure of precision has the limitation of being highly affected by the mean. They then proposed use of the accuracy estimate (r_{gg}), i.e., a measure of the repeatability of the clone performance in the different replicates. According to them, accuracy above 0.7 is an indication of acceptable precision. In other words, r_{gg} refers to the correlation between the estimated genetic values and the true ones, i.e., it corresponds to the square root of heritability.

It was estimated the probability of identifying the four, three, two and one best clones, and also the probability of identifying the best clone between the top four and as the first best one classified. The estimates obtained in each plot size were compared with the analysis of the plot with 100 plants. All analyses were made on the software R (R Development Core Team 2013).

Table 2. – Summary of analysis of variance of clonal test 1 for wood volume (m^3) considering the border of one (B1), two (B2), three (B3) and four (B4) rows.

Effect	DF	Probability(>F)				
		B0	B1	B2	B3	B4
Clone	34	<0.01	<0.01	<0.01	<0.01	<0.01
Replicate	2	<0.01	<0.01	<0.01	<0.01	<0.01
Clone*Replicate	68					
Border	1		0.764	0.428	0.55	0.844
Clone*Border	34		0.061	0.104	0.315	0.932
Error	70					
Accuracy		95.24	95.32	95.32	95.14	94.56

Table 3. – Summary of analysis of variance of clonal test 2 for wood volume (m^3) considering the border of one (B1), two (B2), three (B3) and four (B4) rows.

Effect	DF	Probability (>F)				
		B0	B1	B2	B3	B4
Clone	47	<0.01	<0.01	<0.01	<0.01	<0.01
Replicate	2	<0.01	<0.01	<0.01	<0.01	<0.01
Clone*Replicate	94					
Border	1		0.303	0.246	0.427	0.337
Clone*Border	47		0.359	0.405	0.603	0.887
Error	96					
Accuracy		93.61	93.39	93.61	93.37	92.88

Table 4. – Summary of analysis of variance of the clonal test 1 and 2 for wood volume (m^3), considering the effect of the rows and columns of the experimental plot.

Effect	DF	Clonal test 1		DF	Clonal test 2	
		Probability (>F)			Probability (>F)	
Clone	34	<0.01	<0.01	47	<0.01	<0.01
Replicate	2	<0.01	<0.01	2	<0.01	<0.01
Clone*Replicate	68			94		
Row/Column	9	0.114	<0.01	9	0.145	0.927
Clone*(Row/Column)	306	<0.05	0.949	423	0.946	0.506
Error	630			861		

Results and Discussion

To make good inferences in regard to the information to be obtained in studies of this nature, it is necessary for the clones evaluated to be different. It may be observed in *Tables 2* and *3*, for clonal tests 1 and 2, that this requirement was met, i.e., the clone source of variation was significant ($P \leq 0.01$). Another important point is checking if the evaluation using the original plot had good precision. It may be observed in the two experi-

ments that when analysis was made involving all 100 plants of the plot, accuracy was greater than 93% (*Tables 2* and *3*), indicating high precision in clonal evaluation.

Another important question is if the experimental area used was uniform or not. Observe that the replicate source of variation was significant ($P \leq 0.01$), showing that the experimental area and/or management system was not uniform (*Tables 2* and *3*). As has already been

Table 5. – Mean value and confidence interval (CI) for the parameters of: accuracy (r), mean square error (V_e), within-group variance (V_w) for 1000 simulated experiments, for each size n , considering the wood volume variable of clonal test 1.

n	Accuracy		V_e		V_w	
5	0.88		0.006		0.063	
CI	0.879	0.882	0.006	0.006	0.063	0.064
10	0.917		0.006		0.064	
CI	0.916	0.918	0.006	0.006	0.063	0.064
15	0.93		0.006		0.063	
CI	0.929	0.931	0.006	0.006	0.063	0.063
20	0.936		0.006		0.063	
CI	0.936	0.937	0.006	0.006	0.063	0.064
25	0.94		0.006		0.063	
CI	0.94	0.941	0.006	0.006	0.063	0.064
30	0.93		0.006		0.064	
CI	0.942	0.943	0.006	0.006	0.063	0.064
40	0.946		0.006		0.063	
CI	0.946	0.947	0.006	0.006	0.063	0.063
60	0.95		0.006		0.063	
CI	0.95	0.95	0.006	0.006	0.063	0.064
80	0.951		0.006		0.063	
CI	0.951	0.952	0.006	0.006	0.063	0.063
100	0.953		0.006		0.063	

mentioned, in the forestry sector there is rarely a defined experimental area; experiments are normally carried out within the commercial planting areas. That way, environmental variation is very similar to the condition of the commercial planting area.

Initially analyses were performed to verify the need for border in the plots. Summaries of analysis of variance considering simulation of the different types of border are shown in *Tables 2* and *3*. Regardless of the number of rows used in the border – one, two, three or four – the accuracy estimate of the experiments practically did not change. That way, from the perspective of experimental precision, the use of the border did not contribute to better precision. It may also be observed that there was no difference between the development of the clones in the useful area or in the border area since the border source of variation was not significant. Even more important is the fact that the clone x border interaction was not significant in almost all situations, except for the B1 border in the clonal test 1, which this effect was almost significant. From this information, it may be deduced that behavior of the clones was similar in the presence of the border or without it. These results are consistent with some others already reported in the literature with other species in Brazil (ANDRADE et al., 2006).

Another way of checking if there is variation in clone behavior in relation to position is to proceed to analysis of variance considering the effect of rows and columns of the experimental plot. Taking clonal test 2 as a reference, it was observed that both the effect of rows and columns and the clones x rows and clones x columns interactions were not significant. For clonal test 1, mean performance of the clones varied among columns, which indicates that, in this case, there was heterogeneity under the environmental conditions in this regard. In general the clones x rows and clones x columns interaction were not significant, indicating coincidence in clone performance in relation to position (*Table 4*).

One of the arguments for using a border is the fact that there is intergenotypic competition among plots; i.e., the performance of one clone may be changed as a result of the clone located in the adjacent plot. From all indications, this fact did not occur in these experiments. Nevertheless, to mitigate this competition, avoiding the use of a border, the best option is the use of plots of more than one row. FEHR (1987) comments that with two rows, intergenotypic competition is cut in half. With three rows, the reduction is 66.7%.

An interesting study was performed to verify the need for a border in experiments with eucalyptus clones (ANDRADE et al., 1997). Data on diameter at breast

Table 6. – Mean value and confidence interval (CI) for the parameters of: accuracy (r), mean square error (V_e), within-group variance (V_w) for 1000 simulated experiments for each size n , considering the wood volume variable of clonal test 2.

n	Accuracy		V_e		V_w	
5	0.832		0.025		0.309	
CI	0.83	0.835	0.024	0.025	0.308	0.311
10	0.884		0.024		0.31	
CI	0.883	0.885	0.024	0.025	0.309	0.311
15	0.903		0.024		0.311	
CI	0.902	0.903	0.024	0.025	0.31	0.312
20	0.913		0.024		0.31	
CI	0.912	0.913	0.024	0.025	0.31	0.311
25	0.919		0.024		0.31	
CI	0.919	0.92	0.024	0.024	0.31	0.311
30	0.923		0.024		0.31	
CI	0.922	0.923	0.024	0.025	0.31	0.311
40	0.928		0.024		0.311	
CI	0.928	0.929	0.024	0.024	0.31	0.311
60	0.933		0.024		0.31	
CI	0.933	0.934	0.024	0.024	0.31	0.31
80	0.936		0.024		0.31	
CI	0.936	0.936	0.024	0.024	0.31	0.31
100	0.938		0.024		0.31	

Table 7. – Percentage of coincidence between the classification of the four best clones for each plot size with classification of the standard plot (100 plants) for the wood volume variable.

Clonal test 1					Clonal test 2						
n	Number of clones				C6	n	Number of clones				C20
	4	3	2	1			4	3	2	1	
5	27.8	90	99.9	100	99.9(79.5)*	5	1.9	25.4	74.5	98.4	53.1(10.9)
10	47.7	96.9	100	100	100(90.6)	10	5.8	42.5	86.5	99.6	66.9(18)
15	57.5	98.9	100	100	100(96.8)	15	8.6	53.7	91.9	100	75.9(20.8)
20	60.5	99.2	100	100	100(98.4)	20	11.1	62.2	94.9	99.9	77.5(20.6)
25	70.8	99.9	100	100	100(99.7)	25	11.4	65.1	97.3	100	82.4(25.1)
30	72.2	99.7	100	100	100(99.3)	30	15.8	73.1	98.9	100	84(25)
35	79	100	100	100	100(99.9)	35	18.5	79.8	99.1	100	88.5(30)
40	83.2	100	100	100	100(100)	40	19.9	81.8	99.4	100	91(30.2)
45	85.3	100	100	100	100(100)	45	24.3	88.8	99.7	100	93(30.5)
50	87.4	100	100	100	100(100)	50	28	89.4	99.4	100	94.1(34.2)
55	90.9	100	100	100	100(100)	55	26.7	91.1	100	100	95.1(36)
60	93.5	100	100	100	100(100)	60	38.7	95.2	100	100	97.5(38.8)
65	94.9	100	100	100	100(100)	65	40	95.1	99.9	100	98.8(41.3)
70	97.3	100	100	100	100(100)	70	43.3	97.6	100	100	98.3(42.6)
75	98.4	100	100	100	100(100)	75	49.3	98.2	100	100	99.4(43.6)
80	99.2	100	100	100	100(100)	80	53.1	99.7	100	100	99.8(47.4)
85	99.8	100	100	100	100(100)	85	56.1	99.9	100	100	100(50.9)
90	100	100	100	100	100(100)	90	63.2	100	100	100	100(56.2)
95	100	100	100	100	100(100)	95	70.6	100	100	100	100(65.2)

* Values between parentheses refer to the probability of identifying the best clone in the first place.

height (BD) in centimeters were analyzed as follow: a) using all the trees of the plot and b) only the useful area of the plot. A significant difference was observed ($P \leq 0.01$) among the clones in all the situations. Nevertheless, in all cases, the analyses that used all the plants of the plot always exhibited a lower estimate of the coefficient of variation; therefore, they exhibited greater precision than those containing only the plants of the useful area. The effect of the planting rows was also verified. In both experiments, there was no effect of rows and of clones x rows interaction.

Another question is how many plants the experimental plot should have. In other words, what is the ideal plot size, i.e., that which allows good inferences at the lowest cost. Through the results shown in *Tables 5* and *6*, it may be observed that as of 15 plants per plot, experimental precision did not change very much with an increase in the number of plants. Accuracy estimates were always greater than 90%. From the practical point of view, this information is quite interesting because it directly results in the use of smaller areas for setting up tests, less production of plantlets from the nursery,

which results in better quality plantlets and this is reflected in more uniform planting. In addition, the use of smaller plots, without the use of the border, facilitates control of the experiment and of the evaluation and selection stage, without loss of information from the visual field for selection of silviculture characteristics (trunk shape, form of the canopy, presence of diseases and pests).

Another question that may be raised is in relation to variation in experiments with eucalyptus, i.e., where greater variation occurs, among plants within the plot (V_w) or among plots that received the same treatment in the different replications (V_e). In this case, since clones were evaluated, variation among plants within the plots is all environmental (RAMALHO et al., 2012). However, V_e , in some circumstances, may also be due to deficient genetic sampling, i.e., plants in the plot might not represent the genotype, in this case, the clones. Thus, such deficiency contributes to variation among the plots that received the same treatment in the different replicates, not only due to the environment. In *Tables 5* and *6*, the mean value and the confidence interval of the 1000 sim-

ulations for each plot size are shown. It may be observed that estimates of V_w and V_e practically did not change in the different size plots in relation to the experiment with 100 plants per plot. Estimates of V_w were always greater than the estimates of V_e , i.e., most of the variation responsible for experimental error occurs within the plots. This fact is consistently observed for other cultivated species, involving other traits (SOUZA and RAMALHO, 1995; MORETO et al., 2007).

The most important thing in experiments of this nature is to identify one or more clones to be recommended for planting on a commercial scale. The implication of the number of plants in the plot on the efficiency of this recommendation was performed by means of simulation. Based on 1000 simulations, the probability of identifying the four best clones was obtained according to the ranking in the plot of 100 plants for each number of plants of the simulated plot. Observe in *Table 7*, for both experiments, that the probability of identifying one of the four superior clones is very high, even with only five plants per plot, whereas the probability of identifying the four clones simultaneously is a little less. Observe that as of 20 plants, the probability is expressive. For the clonal test 1 the best clone was the number six, and for the clonal test 2 the best clone was the number 20. The probability of identifying the best clone was high for the clonal test 1 and less for the clonal test 2 (*Table 7*).

Based on the results obtained, it may be inferred that the use of a border plot is not necessary in the clonal tests with eucalyptus. The plot does not need to have many plants. With 15 to 20 plants, experimental accuracy is similar when compared to the plot with 100 plants. It is important for the plot to have more than one row.

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