

Longitudinal analysis in the selection of *Eucalyptus globulus* clones under contrasting water availability conditionsAnálise longitudinal na seleção de clones de *Eucalyptus globulus* em condições contrastantes de disponibilidade hídricaRodolfo Schmit<sup>1</sup>, Freddy Mora<sup>2</sup>, Veronica Ingrid Emhart<sup>3</sup> e Rafael Rubilar<sup>4</sup>**Resumo**

O objetivo deste trabalho foi avaliar o crescimento de clones de *Eucalyptus globulus* em condições contrastantes de disponibilidade hídrica com base na análise multivariada de medidas repetidas no tempo. O experimento foi conduzido na região administrativa de Bío-Bío, no sul do Chile. O delineamento utilizado foi de blocos completos ao acaso com três repetições, em condições de suplementação hídrica e sem irrigação. O experimento consistiu de 30 clones de *E. globulus*, avaliados nos anos de 2008 a 2011. Ajustaram-se as seguintes estruturas de variâncias e covariâncias na matriz de resíduos: desestruturada (UN), auto regressiva de primeira ordem (AR[1]), auto regressiva heterogênea de primeira ordem (ARH[1]), simetria composta heterogênea (CHS), usando o método da Máxima Verossimilhança Restrita (REML). Conforme os critérios de informação, a melhor estrutura de variância e covariância foi a UN. Os valores de herdabilidade no sentido amplo foram de  $H^2=0.61$ ,  $H^2=0.37$  e  $H^2=0.12$  para os caracteres altura, diâmetro basal e volume da madeira, respectivamente. Interação complexa foi observada entre clone x irrigação. De acordo com o Melhor Preditor Linear Não-Viesado (BLUP), o clone com melhor desempenho no local com irrigação foi o Eg29. Nas condições sem irrigação, os clones Eg11, Eg23, e Eg18 foram promissores para seleção de maior volume. O teste clonal, avaliado utilizando uma análise multivariada via modelos mistos, mostrou suficiente variabilidade genética no experimento, podendo ser utilizada em diferentes condições de disponibilidade hídrica no sul do Chile.

**Palavras-chave:** estruturas de variância-covariância, herdabilidade, REML, BLUP, irrigação.

**Abstract**

The objective of this study was to evaluate the growth of *Eucalyptus globulus* clones under contrasting water availability conditions using a multivariate analysis with repeated measures over time. The experiment was arranged in a randomized complete block design with three replicates with and without irrigation (rainfed) in the administrative region of Bío-Bío in southern Chile. The trial consisted of 30 *E. globulus* clones assessed between 2008 and 2011. The following variance-covariance structures were fitted to the matrix of residuals using the restricted maximum likelihood (REML) method: unstructured: UN, first-order autoregressive: AR(1), heterogeneous autoregressive: ARH(1), and heterogeneous compound-symmetry: CSH. According to the information criteria, the best variance-covariance matrix was UN. The broad-sense heritability values were  $H^2=0.61$ ,  $H^2=0.37$ , and  $H^2=0.12$  for height, basal stem diameter, and wood volume, respectively. A complex clone x irrigation interaction was detected. According to the best linear unbiased predictor (BLUP), the Eg29 clone exhibited the best on-site performance under irrigation condition. The Eg11, Eg23, and Eg18 clones were more promising for selecting larger wood volume under rainfed condition. This clonal test, assessed using a multivariate analysis via mixed models, showed enough genetic variability in the experiment, which can be used for different water availability conditions in southern Chile.

**Keywords:** variance-covariance structures, heritability, REML, BLUP, irrigation.

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## INTRODUCTION

*Eucalyptus globulus* Labill ssp. *globulus* is one of the most important *Eucalyptus* species cultivated in the world and is used as raw material in the pulp and paper industry. Several breeding programs continuously improve traits of interest in *E. globulus*, such as wood growth and density, under various environmental conditions (BUNDOCK et al., 2008; MORA; SERRA, 2014). This species has been widely cultivated in temperate regions (ACUÑA et al., 2012) in which abiotic stresses such as drought and cold are the main limiting factors for the crops (COSTA E SILVA et al., 2008; MORA; SERRA, 2014).

The *Eucalyptus* genus comprises about 700 species distributed naturally over a wide range of environmental conditions and is cultivated worldwide on more than twenty million hectares (BARTHOLOMÉ et al., 2013). *E. globulus* is highly cultivated because it uses 25% less wood to produce one ton of pulp compared with other eucalypt species, requires less energy in the manufacturing process and exhibits greater fiber length and thickness (SANTELICES, 2005).

Due to the benefits of the raw material, the global production of *E. globulus* has been rising annually to meet the increasing demands for paper. However, situations such as a lack of water resources can cause slower growth rates due to inhibition of cell expansion and reduced carbon assimilation (FRITSCHÉ-NETO; BORÉM, 2011). These situations become a challenge for the breeding of eucalyptus in selecting clones with promising traits in response to water stress.

Supplemental irrigation of *Eucalyptus* promotes quality and safety. Despite some empirical controversies, water irrigation at critical stages of development may be essential for obtaining high yields. A lack of water leads to plant water stress (desirable only at the hardening phase) and reduces the absorption of nutrients necessary for plant development. Limitations to physiological acclimation can arise from species characteristics and the environmental regime in which the species evolved (CROUS et al., 2013). Several studies with *Eucalyptus* have been conducted that have evaluated clones in diverse environmental conditions of water supply and water stress, and have emphasized the advantage of drought preconditioning in *E. globulus* to improve behavior and survival after transplanting (LOPES et al., 2005; VELLINI et al., 2008; GUARNASCHELLI et al., 2006).

In Chile, *E. globulus* plantations cover over 450,000 hectares (MORA et al., 2013), which represents approximately 20% of the total area of forest plantations in the country. These values are expected to increase over time due to the progress made with studies related to tolerance capacity to different environmental conditions (MORA et al., 2013; MORA; SERRA, 2014). In this context, Navarrete-Campos et al. (2013) studied the drought effects on water use efficiency, freezing tolerance, and survival of *E. globulus* Labill. and *E. globulus* x *nitens*, exhibiting significant differences among genotypes and irrigation treatments. On the other hand, Costa e Silva et al. (2008) confirmed the hypothesis that *E. globulus* genotypes that are more resistant to dry environments might also exhibit higher cold tolerances than drought-sensitive plants.

To elucidate the effect of irrigation in the cultivation of *Eucalyptus* sp., it is necessary to perform successive assessments of the same trees. However, longitudinal data arise when trees are repeatedly evaluated over the years. Longitudinal or hierarchical data are often observed in forestry, which can pose both challenges and opportunities when performing statistical analyses. The current standard approach for analyzing these types of data is mixed-effects models under the classical statistical inference (LI et al., 2012). The traits of interest in breeding can be expressed more than once in each individual (as in the case of perennial plants) to generate longitudinal data or repeated measures over time. Therefore, data on these traits may exhibit correlated structure over time, harvest seasons, or measurements (RESENDE, 2007). Mixed linear models are one of the best methods for the analysis of longitudinal data (repeated measures) in either space or time. A major advantage of this method is that it accommodates the complexity of typical sets of longitudinal data. According to Yang et al. (2006), when a trait is measured repeatedly over time, it is referred to as a time-dependent trait or longitudinal trait. The typical repeated measures experiment in forest research consists of trees randomly assigned to treatments with response variables measured on each tree over a sequence of time points (ZAMUDIO et al., 2008). The objective of this study was to genetically evaluate *E. globulus* clones in different water availability conditions in southern Chile using a multivariate analysis of repeated measures over time.

## MATERIALS AND METHODS

The experiment was conducted in the administrative region of Bío-Bío, southern Chile (37° 45' S and 72° 18' W) with assessments performed between 2008 and 2011. The selected area corresponds to a seed orchard of *Eucalyptus* that belongs to FORESTAL MININCO S.A., a subsidiary of Paper and Cardboard Manufacturing Company (CMPC). The average annual rainfall is 1,200 mm, with greater precipitation during winter. The average annual temperature is 13.1° C, with an average of 18° C in summer and minimum of 6° C during winter. The soils used in the experiment are Collipulli soils and Luvisols with red clay highly weathered with old volcanic ash, which form deep, red clay soils on uneven terrain at the central valley of Chile.

The experiment considered the evaluation of 30 clones of *E. globulus* with and without supplemental irrigation. The clonal test was arranged in a randomized complete block design with three replicates and four ramets per plot. The selected seedlings with homogeneous sizes were obtained from rooted cuttings after seven months in the nursery. Seedlings were planted in October 2008 using a planting spade in boxes of 20x20x20 cm. Fertilization of the planting boxes for each plant was conducted with doses of 30 g Basacote Plus 9M (Compo S.A.) composed of 16% N + 8% P<sub>2</sub>O<sub>5</sub> + 12% K<sub>2</sub>O + 2% MgO + 5% SO<sub>2</sub> + microelements. Subsequent weed control was performed in each study area until the closing of the crown in plots with and without irrigation to maintain the best homogeneity conditions for expression of responses of each clone.

The irrigation treatment was applied only during summer due to the high water retention capacity of the soil and the clay texture. The amount of daily irrigation was estimated considering the replacement of the water content lost by evaporation with a maximum of 3.2 mm.day<sup>-1</sup> supplementation. Irrigation was performed in a drip system with the drip tape adjacent to the surface of each plant to ensure better water availability. The water availability in the soil was evaluated every two weeks using three TDR sensors located between 0-15 and 15-30 cm depth in each block and 0-20 cm deep moisture sensors with continuous monitoring via an ECHO-EC20 Soil Moisture Probe (DECAGON Devices).

*Eucalyptus globulus* clones were assessed from 2008 to 2011 for the following growth traits: a) total tree height (TTH) in centimeters; b) basal stem diameter (BSD) in centimeters; c) wood volume (WV) in cubic centimeters.

Consider the following mixed effects model that represents the experimental data (MORA et al., 2010; CANÉ-RETAMALES et al., 2011) in which  $y_i$  is the vector of repeated measures on the  $i$  th subject (KHATTREE; NAIK, 1999);  $X_i$ ,  $Z_i$ , and  $W_i$  are the incidence matrices (known) of the vectors  $\beta$  that represent the fixed effects of irrigation, year, and block, respectively;  $c_i$  represents the random effect due to clone and the clone\*irrigation interaction; and  $p_i$  represents the effects of the plots.

$$y_i = X_i\beta + Z_i c_i + W_i p_i + \varepsilon_i$$

The other interaction effects were not considered in the model for simplification (convergence of the restricted maximum likelihood function) and to further acquire the most appropriate variance and covariance structure for the data (GONÇALVES; FRITSCHENETO, 2012).

The estimation of variance and covariance components and broad-sense heritability and prediction of clonal effects were performed by the REML/BLUP methodology (Restricted Maximum Likelihood/Best Linear Unbiased Prediction). The broad-sense heritability for each response variable was calculated using the following expression in which  $\sigma_c^2$  corresponds to clonal variance,  $\sigma_p^2$  is the variance of the clone\*irrigation interaction, and  $\sigma_\varepsilon^2$  is the residual variance.

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_p^2 + \sigma_\varepsilon^2}$$

After the characterization of the general model, the following variance-covariance structures were fitted to the residual matrix:

a) First-order autoregressive, AR(1):

$$\sigma^2 \begin{bmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{bmatrix}$$

b) Heterogeneous autoregressive, ARH(1):

$$\begin{bmatrix} \sigma_1^2 & \sigma_1\sigma_2\rho & \sigma_1\sigma_3\rho^2 & \sigma_1\sigma_4\rho^3 \\ \sigma_2\sigma_1\rho & \sigma_2^2 & \sigma_2\sigma_3\rho & \sigma_2\sigma_4\rho^2 \\ \sigma_3\sigma_1\rho^2 & \sigma_3\sigma_2\rho & \sigma_3^2 & \sigma_3\sigma_4\rho \\ \sigma_4\sigma_1\rho^3 & \sigma_4\sigma_2\rho^2 & \sigma_4\sigma_3\rho & \sigma_4^2 \end{bmatrix}$$

c) Unstructured, UN:

$$\begin{bmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ \sigma_{21} & \sigma_2^2 & \sigma_{23} & \sigma_{24} \\ \sigma_{31} & \sigma_{32} & \sigma_3^2 & \sigma_{34} \\ \sigma_{41} & \sigma_{42} & \sigma_{43} & \sigma_4^2 \end{bmatrix}$$

d) Heterogeneous compound-symmetry, CSH:

$$\begin{bmatrix} \sigma_1^2 & \sigma_1\sigma_2\rho & \sigma_1\sigma_3\rho & \sigma_1\sigma_4\rho \\ \sigma_2\sigma_1\rho & \sigma_2^2 & \sigma_2\sigma_3\rho & \sigma_2\sigma_4\rho \\ \sigma_3\sigma_1\rho & \sigma_3\sigma_2\rho & \sigma_3^2 & \sigma_3\sigma_4\rho \\ \sigma_4\sigma_1\rho & \sigma_4\sigma_2\rho & \sigma_4\sigma_3\rho & \sigma_4^2 \end{bmatrix}$$

where  $\rho$  is the correlation coefficient between two consecutive years ( $-1 < \rho < 1$ ).  $\sigma_i^2$ ,  $\sigma_{ij}$  and  $\sigma_i$  are the variance, covariance and standard deviation, respectively.

The best variance-covariance structures for representing the dependence or independence between observations were determined. The hypothesis of correlated error arises for the continuous evaluation of the same trees over the years. The growth traits are larger in magnitude in the final year of assessment. For this reason, data were corrected by the variance and covariance matrices to better represent the error in the evaluation of longitudinal data and to assess and correct for the existence of heteroscedasticity. To test the best model (according to the variance-covariance structure), the Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used:

$$AIC = -2 \log L + 2 d$$

$$BIC = -2 \log L + 2 \log n$$

where L corresponds to the (restricted) maximum likelihood value, d is the number of model dimensions, and n is the number of observations.

Pearson's correlation coefficients were calculated among growth traits using the prediction of clonal effects (BLUP), as a measurement of the genetic association between two traits.

## RESULTS AND DISCUSSION

In the present study, the AIC and BIC obtained by the REML method indicate lower values (best model) for the UN (unstructured) variance-covariance matrix (Table 1). The information criteria are important in statistical analysis because they penalize models with excessive numbers of parameters and identify

the most parsimonious models (parsimony) that best explain the data.

**Table 1.** Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the response variables of total tree height (TTH), basal stem diameter (BSD), and wood volume (WV). Tests with the variance and covariance matrices (VCM): unstructured (UN), first-order heterogeneous autoregressive [ARH(1)], first-order autoregressive [AR(1)], and heterogeneous compound-symmetry (CHS).

**Tabela 1.** Critério de informação Akaike (AIC) e critério de informação Bayesian (BIC). Para variáveis de resposta da altura total da árvores (TTH) diâmetro basal (BSD) e Volume de Madeira (WV). Testes com matrizes de variância e covariância (VCM) desestruturada (UN), autorregressivos heterogêneos de primeira ordem [ARH(1)], autorregressivos de primeira ordem [AR(1)], e simetria composta (CHS).

Traits	VCM	-2 RLL*	AIC	BIC
TTH	UN	20998	21022	21039
	ARH(1)	21280	21294	21304
	AR(1)	24269	24277	24282
	CHS	21512	21526	21536
BSD	UN	1230	1254	1271
	ARH(1)	1569	1583	1593
	AR(1)	5270	5278	5284
	CHS	1899	1913	1923
WV	UN	38631	38655	38672
	ARH(1)	40720	40734	40744
	AR(1)	43532	43540	43545
	CHS	40884	40898	40908

\*RLL is the restricted log-likelihood

For all growth traits, the AR(1) matrix showed the highest AIC and BIC values (Table 1). In fact, the residual variance differed each year ( $p < 0.01$ ). The AR(1) matrix was the only matrix that represented homoscedasticity in the structuring of residual variances. The greatest inadequacy of this model is its exclusion of specific variances for each year of assessment (LITTELL, 2006). In general, the correlations of errors in assessments over the years follow a random behavior (i.e., unstructured), which can be explained by the UN matrix. According to Zamudio et al. (2008), the linear mixed model methodology permits the presence of heterogeneity of variance in the linear model and allows the researcher to directly address the covariance structure. Modeling the covariance structure of the data can improve the analysis of repeated measures data by providing valid standard errors and efficient statistical tests.

In general, the residual variance increased proportionally over time, and the largest covariance was detected between the years of 2010 and 2011 (Table 2). Variation values among replicates of the same clone increased due to

the sum of environmental effects assigned to the four consecutive years of assessment. The highest values are always found between two consecutive years, and higher covariance values allow more accurate prediction of statistical inferences by reducing the predicted residual variance (FORTIN, 2007).

To estimate the broad-sense heritability (genetic parameter of interest), only one residual variance must be selected. According to Zamudio et al. (2008), the residual variance that exhibits the lowest standard error must be selected. However, the best way to select the residual variance may use the proportion of variance and standard error (RESENDE, 2007). The calculation of heritability using any of the methods led to the same results. Therefore, the selected residual variances obtained by REML were 57.71 cm<sup>2</sup>, 0.003 cm<sup>2</sup>, and 3,316,054 cm<sup>6</sup> for the TTH, BSD, and WV, respectively.

Clonal effects were predicted using BLUP and the unstructured variance-covariance matrix. Pearson's correlation coefficients were found to be high and highly significant ( $p < 0.01$ ) between pair of traits predicted using BLUP:  $r = 0.97$  between TTH and BSD,  $r = 0.90$  between TTH and WV, and  $r = 0.88$  between BSD and WV. These results agree with those reported by Mora and Serra (2014), and Bundock et al. (2008) in *E. globulus*, and Vargas-Reeve et al. (2013) and Harrand et al. (2009) in other *Eucalyptus* species. Genetic correlations provide valuable information about the prospects of obtaining gains by selection and breeding of *E. globulus*

(CALLISTER et al., 2011; MORA; SERRA, 2014).

The variance of the clone x irrigation interaction contributed to 7%, 17%, and 9.5% of the phenotypic variance of the TTH, BSD, and WV traits, respectively (Table 3). The TTH trait exhibited high broad-sense heritability (0.61), which indicates effective selection of genetic gain in the height of *E. globulus* trees. The heritability for BSD and WV were moderate (0.37 and 0.12, respectively). This finding is partially consistent with a study conducted by Lopez et al. (2002), who found that growth was under moderate genetic control in a base population of *E. globulus* assessed on four sites in Argentina; in general, single site heritability estimates for growth traits ranged from 0.09 to 0.36, and the average heritability of four single trial estimates for diameter across ages was 0.25. Based on Bayesian analyses, Mora and Serra (2014) found that growth traits were weakly to moderately heritable ranging from  $h^2 = 0.04$  (tree height) to  $h^2 = 0.11$  (volume) in *E. globulus* open-pollinated families under cold conditions in southern Chile. The heritability values found in this study are important because the distribution of *E. globulus* plantations in Chile is mostly limited by cold or by low rainfall during the growing season (MORA; SERRA, 2014). Moreover, the area planted with *E. globulus* is continually increasing in southern Chile, and this search resulted in a tendency to include local weather conditions far from optimum for planting, such as frost or frequent drought conditions (COSTA E SILVA et al. 2008).

**Table 2.** Estimates of residual variances (diagonal) and residual co-variances (above the diagonal) for the four years of assessment (2008-2011) for total tree height (TTH), basal stem diameter (BSD), and wood volume (WV), which were estimated using the principles of mixed linear models (REML unstructured residual matrix).

**Tabela 2.** Estimativas de variâncias residual (diagonal) e co-variâncias residual (acima da diagonal) para os quatro anos de avaliação (2008-2011) para a altura total da árvore (TTH), Diâmetro Basal de Tora (BSD) e volume de madeira (WV) estimados usando o princípio de modelos lineares mistos (REML matriz residual não estruturada).

Traits	Year	2008	2009	2010	2011
TTH	2008	57.72	19.85	-17.56	-6.74
	2009		1160	3001	2499
	2010			10801	9197
	2011				13686
BSD	2008	0.003	0.002	-0.001	0.002
	2009		0.572	1.198	1.037
	2010			2.996	2.719
	2011				3.051
WV	2008	3910330	3492365	-3849566	-12200000
	2009		3316054	-3499778	-11300000
	2010			7916567	26951008
	2011				111000000

**Tabela 3.** Estimates of variance components: clonal variance ( $\sigma_C^2$ ), variance of the clone-irrigation interaction ( $\sigma_I^2$ ) and phenotypic variance ( $\sigma_F^2$ ), and broad-sense heritability ( $H^2$ ) for the response variables total tree height (TTH), basal stem diameter (BSD), and wood volume (WV) in a longitudinal analysis via REML (2008-2011).

**Table 3.** Estimativas de componentes de variância : variância clonal ( $\sigma_C^2$ ), variância da interação clone -irrigação ( $\sigma_I^2$ ) e variância fenotípica ( $\sigma_F^2$ ), e herdabilidade ( $H^2$ ), para variáveis de altura total da árvore (TTH), Diâmetro Basal de Tora (BSD), e Volume de Madeira (WV) em uma análise longitudinal via REML (2008-2011).

Parameter estimates	TTH	BSD	WV
$\sigma_C^2$	109.940	0.002	513364
$\sigma_I^2$	13.024	0.001	401824
$\sigma_F^2$	180.678	0.006	4231242
$H^2$	0.60	0.37	0.12

According to the values predicted via Best Linear Unbiased Prediction (BLUP) under irrigation conditions, the Eg28 (BLUP= 4.9 m), Eg29 (BLUP= 3.36 m), and Eg30 (BLUP= 3.34 m) clones exhibited the best responses for the trait tree height. In the treatment without irrigation, these clones exhibited the lowest values for height, which is not desirable for selection and confirms the existence of a significant (and complex) clone x irrigation interaction. In the cultivation without irrigation, the best clones according to BLUP were Eg3 (BLUP= 3.86 m), Eg18 (BLUP= 3.77 m), and Eg12 (BLUP= 2.83 m).

According to the values predicted via BLUP, *E. globulus* clones Eg6 (BLUP= 0.037 cm), Eg28 (BLUP= 0.019 cm), and Eg29 (BLUP= 0.011 cm) exhibited the best BSD performance under irrigation conditions. In the absence of supplemental irrigation, the promising clones for selection were Eg9, Eg3, and Eg23. The Eg29, Eg6, and Eg26 clones exhibited larger wood volume in the presence of water irrigation. The Eg29 clone displayed the most promising values for selection under supplemental irrigation for the set of evaluated traits. For treatment without irrigation, the Eg11, Eg23, and Eg18 clones were more promising for selecting larger wood volume. The results confirm the presence of a complex (clone x irrigation) interaction. For the Eg29 clone, irrigation promoted an increase of 46.55 cm<sup>3</sup> in wood volume, which was higher than the Eg11 clone without water irrigation. The complexity of genotype-environment

interaction can reduce the ability to statistically identify superior genotypes, which can limit genetic gain for complex traits (CHENU et al. 2012). In fact, to produce suitable genotypes for multiple environments, genotype-environment interaction should be accounted for and assessed in the current breeding program of *E. globulus*.

## CONCLUSIONS

The assessment of *E. globulus* clones using a multivariate analysis via mixed models showed that the growth traits were moderate to highly heritable. This genetic variability may be used for selection and breeding in different water availability conditions in southern Chile.

The difficulty of selection is given by the existence of a significant and complex clone x irrigation interaction. According to the values predicted via BLUP under water irrigation conditions, the Eg29 clone is of particular relevance given its promising performance for the three growth traits of interest. For the rainfed treatment, the Eg11, Eg23, and Eg18 clones were more promising for selecting larger wood volume.

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