DE GRUYTER
OPEN

Maintenance of genetic diversity in *Eucalyptus urophylla* S. T. Blake populations with restriction of the number of trees per family

P. H. M. Silva[1,2,3*]; A. Brune[4]; S. Pupin[5]; M. L. T. Moraes[5]; A. M. Sebbenn[5,6]; R. C. de Paula[7]

[1] Instituto de Pesquisa e Estudos Florestais (IPEF), Via Comendador Pedro Morganti, 3500, Bairro Monte Alegre, CEP 13415-000, Piracicaba, São Paulo State, Brazil.
[2] Universidade Estadual de São Paulo (UNESP/Botucatu), Rua José Barbosa de Barros, 1780, CP 237, CEP 18.603-970, Botucatu, São Paulo State, Brazil.
[3] Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Avenida Pádua Dias, 11, CP 9, CEP 13418-900, Piracicaba, São Paulo State, Brazil.
[4] APSD- Ghana, Plot 27, Block 27, Nhyiaeso, Kumasi, Ghana.
[5] Universidade Estadual de São Paulo (UNESP/Ilha Solteira), Av. Brasil Centro, 56, CP 31, CEP 15385-000, Ilha Solteira, São Paulo State, Brazil.
[6] Instituto Florestal de São Paulo (IF), CP 1322, CEP 01059-970, São Paulo State, Brazil.
[7] Universidade Estadual de São Paulo (UNESP/Jaboticabal), Via de Acesso Prof. Paulo Donato Castellane, km 5, CEP 14.884-900, Jaboticabal, São Paulo State, Brazil.

[*] Corresponding author: P.H.M. Silva, E-mail: paulohenrique@ipef.br.

Abstract

Our aim was to verify the effect on gain and genetic diversity through the restriction of the number of trees per family in selection, in order to compose an elite population of *Eucalyptus urophylla* in two trials under distinct management levels. We studied 166 open-pollinated families of *E. urophylla* in Anhembi, Sao Paulo State, Brazil under commercial practices, and the same families in Selvíria, Mato Grosso do Sul State, Brazil under lower management level (mainly no mineral fertilization). Mortality, height and diameter at breast height (DBH) were measured. DBH was analyzed by the REML/BLUP to select the best 25 trees, with four levels of tree restriction per family (no restriction; 1; 2 and 3 per family). We evaluated heritability; genetic gain and effective size of number of total and private alleles; observed and expected heterozygosity; coancestry and fixation index. A large difference in survival (48 and 83 %) and productivity (MAI of 26 and 44 $m^3ha^{-1}y^{-1}$) was observed between trials due to the different levels of management applied. The highest restriction in number of individuals per family caused a small decrease in gain, corresponding to 7 % in the more productive trial and 3 % in the less productive one. Observed and expected heterozygosity, coancestry and fixation index were not significantly (lower than 5 %) affected by the restriction in both sites. The restriction of one tree per family allowed different alleles to be kept in the selected population and higher effective population size in order to insure variation for the next generations.

Keywords: *Elite population, genetic diversity, heritability, inbreeding, tree breeding*

Introduction

Eucalyptus urophylla S. T. Blake, or Timor mountain gum, is an important economic tropical tree species around the world as a pure species or in hybrid combination (Denison and Kietzka, 1993; Harwood, 2011). In Brazil, the species is one of the most important exotic planted trees, being introduced for improving stress tolerance in the 1970's, to which the then usually planted *Eucalyptus grandis* was not adapted (Ferreira, 2015). Recently, commercial plantations in Brazil today yield a mean of 40 $m^3ha^{-1}y^{-1}$ (Gonçalves et al., 2013), based mainly on highly selected hybrid clones between the species *E. urophylla x E. grandis*.

The focus of tree improvement programs is to increase the productivity of commercial stands. In order to do that it is important to select the correct commercial genotypes, while considering environmental conditions and management practices (Gonçalves et al., 2013). However, high selection intensity to increase productivity may decrease genetic diversity and effective population size, as observed in individual selection of *E. grandis* (Oda et al., 1989) and *Eucalyptus benthamii*, which already has a narrow genetic base in its natural occurrence (Costa et al., 2016). It is important to keep a balance between gain and diversity in selection. Genetic diversity is needed in order to advance genetic gain and it is a buffer

DOI:10.2478/sg-2018-0005
edited by the Thünen Institute of Forest Genetics

against biotic and abiotic stress which strongly decreases forest productivity (Jurskis, 2005; Wingfield et al., 2008; Brawner et al., 2013; Garcia et al., 2014; Silva et al., 2016; Silva et al., 2017). Genotype is one factor influencing tree growth. Other factors of growth decrease under stress are derived from silvicultural practices and environmental conditions (Brawner et al., 2011; Silva et al., 2013; Campoe et al., 2016).

Therefore, balancing selection intensity for high productivity with the size of the population being improved is a challenge to breeders in order to compose an elite population with a minimum of inbreeding. In such situations, a quantitative approach and genetic markers can be useful to estimate genetic variation, heritability, genetic gains, and effective number of parent trees (Kumar and Richardson, 2005; Steane et al., 2006; Payn et al., 2008; Resende, 2016). Thus, the aim of this study was to evaluate the effects of selection on the number of families and trees per family. We specifically wanted to determine how the restriction in number of trees selected per family affects genetic gain, effective size and genetic diversity.

Materials and Methods

Sites and families' trials

Two trials with 166 open-pollinated families of *E. urophylla* from several Brazilian provenances were evaluated (Table 1). Both trials were established, using a randomized complete block design, with 166 treatments, four blocks and linear plots of six trees at 3 x 2 m spacing. Trial 1 was planted in Anhembi, São Paulo State (22° 28′ S, 48° 07′ W, altitude of 472m, Climate: Aw; annual mean temperature of 21.8° C and annual mean precipitation of 1300 mm) under commercial Brazilian plantation practices as described by Gonçalves et al. (2013). It was done with minimum soil cultivation, mineral fertilization (during planting and after planting), leaf-cutting ant (baits application) and weed control (herbicides). Trial 2 was established in Selvíria, Mato Grosso do Sul State (20° 21´ S, 51° 24′ W, altitude of 375 m, Climate: Aw, annual mean temperature of 24.8° C, and annual mean precipitation 1300 mm) under lower level of silvicultural practices, such as no fertilization with weed and ant control only at planting. Survival, tree height (H) and DBH were measured in order to calculate stem volume using a form factor (f) of 0.5 at half the rotation age (close to three years) and at the harvest age (between five and six years) in both trials.

$$Vol = \pi(DBH/2)^2 Hf$$

Analysis of variance and estimates of genetic parameters

Our estimates were based on the selection of 25 trees with high DBH at four different levels of selection among and within families: i) no restriction in the number of trees per family; ii) selection of 1 tree per family; iii) selection of up to 2 trees per family; iv) selection of up to 3 trees per family. Variance components and genetic parameters were estimated for DBH by the method of maximum restricted likelihood and best unbiased linear prediction (REML/BLUP), using the Selegen-REML/BLUP software (Resende, 2016). In the analysis, the open-pollinated families were assumed to be half-sib families. We used the mixed model that considers the effect of replications (r) and measurement(age)-repetition-trial (environment) (m) as fixed; and additive genetic variance (a), plot variance (p), environment (s), genotype x environment interaction (i) and error (e), as random (Resende, 2016):

Table 1
Description of provenances, origins and breeding level

	Provenance	n_f	Origin	Generation
1	Turmalina and, Timóteo, MG	44	Flores and Timor	SSO 2 to 4º generation
2	Ipatinga, MG	50	Flores and Timor	SSO 2 and 3º generation
3	Avaré, SP	14	Flores and Timor	SSO 2 and 3º generation
4	Lençóis Paulista, SP	15	Remexio -Timor	SSO 2 and 3º generation
5	Altinópolis, SP	13	Flores and Timor	SSO 3º generation
6	Itamarandiba, MG	15	Flores	SSO 2º generation
7	Teixeira de Freitas, BA	15	Timor	SPA 1º generation

n_f is the number of families; SSO is Seedling Seed Orchard; SPA is Seed Production Area

Individual analyses:

$$y = Xr + Za + Wp + e$$

Joint analyses:

$$y = Xm + Za + Wp + Ts + Qi + e$$

Where capital letters represent the incidence matrixes for replications (X), family (Z), block (W), trial (T) and GxA (Q) effects (Resende, 2007). Estimated variance components were: additive genetic variance (σ_a^2), environmental variance (σ_e^2), phenotypic variance within plots (σ_w^2) and phenotypic variance ($\sigma_p^2 = \sigma_a^2 + \sigma_e^2 + \sigma_w^2$). The estimated genetic parameters were:

Narrow sense individual heritability:

$$h_a^2 = \frac{\sigma_a^2}{\sigma_p^2},$$

Genetic gain:

$$g = b_1 Y_{ijk} + (b_2 - b_3)\overline{Y}_{i..} + (b_3 - b_1)\overline{Y}_{ij.} - b_3\overline{Y}_{.j.} + (b_3 - b_2)\overline{Y}_{...},$$

where $\overline{Y}_{ijk}$ is the individual value of the trait; $\overline{Y}_{i..}$ is the mean of the family in the trial; $\overline{Y}_{ij.}$: is the mean of family in a determined block (mean plot); $\overline{Y}_{.j.}$ is the block mean, and $\overline{Y}_{...}$ is the general mean of trait in the trials; $b_1 = h_w^2$ is the narrow sense heritability within family.

The narrow sense heritability within family:

$$h_w^2 = \frac{0.75\sigma_a^2}{\sigma_w^2},$$

$b_2 = h_f^2$ is the narrow sense mean family heritability:

$$h_f^2 = \frac{\left[(3+n_w b)/(4n_w b)\right]\sigma_a^2}{\sigma_f^2 + (\sigma_e^2/b) + (\sigma_w^2/n_w b)},$$

where n_w is the mean number of trees per family and b is the number of blocks,

$b_3 = h_p^2$ is the narrow sense heritability within plots:

$$h_p^2 = \frac{(3/4n_w)\sigma_a^2}{\sigma_e^2 + (\sigma_w^2/n_w)},$$

The effective population size is:

$$N_e = \frac{4n_f n_w}{3 + n_w + (\sigma_n^2/n_w)},$$

where n_f is the number of families, n_w is the mean number of trees per family and σ_n^2 is the variance in the number of trees per family. Genetic gain was estimated by: g(%)=100 (g/x), where x is the mean of the trait in the sites.

Analyses of microsatellite loci

DNA extraction and genotyping were carried out at Genomax/ Heréditas (Tecnology in DNA Analysis, Brasília Federal District, Brazil). DNA was extracted from 100-150 mg leaf material from 72 (40 in Anhembi + 32 in Selviria) selected trees using the method of Doyle and Doyle (1987). Nineteen microsatellite loci were amplified based on Faria et al. (2010): EMBRA2, EMBRA3, EMBRA10, EMBRA11, EMBRA12, EMBRA21, EMBRA28, EMBRA32, EMBRA38, EMBRA45, EMBRA63, EMBRA128, EMBRA157, EMBRA204, EMBRA210, EMBRA681, EMBRA915, EMBRA1144 and EMBRA1349. One locus (EMBRA333) showed some individuals that did not amplify, so the locus was not considered in the analysis. The loci EMBRA2, EMBRA3, EMBRA10, EMBRA11, EMBRA12, EMBRA28, EMBRA38, EMBRA63, EMBRA128, MBRA157, EMBRA204, EMBRA210, and EMBRA681, present regular Mendelian segregation and are not genetic linked (Pupin et al., 2017).

Analyses of genetic diversity

Genetic diversity was characterized by the total number of alleles (k); mean number of alleles per loci (A); observed (H_o) and expected (H_e) heterozygosity and fixation index (F), using FSTAT software (Gouded, 1995). The statistical significance of F values was determined through the permutation of alleles among individuals, associated to a Bonferroni correction for multiple test (95 %, α= 0.05). Estimates of gene frequencies were used to determine the number of private alleles (P_a) for the selected populations in the simulated scenarios. Group co-ancestry was estimated by:

$$\Theta = \frac{\sum_{i=1}^{n} 0.5(1+F_i) + \sum_{i=1}^{n}\sum_{i\neq j}^{n}\theta_{ij}}{n^2},$$

where n is the number of selected trees (25), F_i is the individual fixation index (F_i) and θ_{ij} is the pairwise co-ancestry coefficient (Lindgren et al., 1996). The indices F_i and θ_{ij} were estimated using the SPAGEDI 1.3 software (Hardy and Vekemans, 2002). Negative values of F_i and θ_{ij} were assumed as being zero, because Θ was derived based on the inbreeding coefficient and the true co-ancestry coefficient between pairwise individuals (both indices range from zero to 1), and estimate of F_i and θ_{ij} using a correlation coefficient (ranging from -1 to 1). The effective population size was calculated following Cockerham (1969) with base in the sample variance in gene frequencies by, $N_e = 0.5/\Theta$ (Sebbenn, 2002; Gonzaga et al., 2016).

Results

Survival and mean annual increment (MAI) were higher in Anhembi than in Selvíria . The MAI was 2.4 (36/15) times higher for the rotation age and 1.7 (44/26) times higher at harvest age in Anhembi. The individual heritability was higher in Selvíria and increased from the middle of rotation to the harvest age. Sites genotypic correlation was 0.67 and the coefficient of determination of GxE was low (Table 2).

Table 2
Survival, mean annual increment volume (MAI) and narrow sense individual heritability (h_a^2) in Anhembi, Selvíria at half the rotation age and at harvest age. Narrow sense individual heritability (h_a^2), coefficient of determination for G x E (C_s^2) and genotypic correlation (r_g x $_{site}$) in joint analysis in both sites and ages

	Anhembi		Selvíria	
	Half rotation	Harvest age	Half rotation	Harvest age
Survival (%)	86	83	53	48
MAI (44 $m^3ha^{-1}y^{-1}$)	36	44	15	26
h_a^2 (SE)	0.15 (0.04)	0.16 (0.04)	0.18 (0.05)	0.22 (0.06)
Joint analysis (ages and trials)				
h_a^2 = 0.19 (0.02)	c_i^2 = 0.024		$r_{g\,x\,site}$ = 0.67	

The number of selected families (n_f), genetic gain in percentage (g(%)) and the effective population size (N_e) with no restriction and 3, 2 and 1 selected individuals per family were higher in Selvíria than in Anhembi. In Selvíria, g(%) ranged between 78.5–91.8 % and N_e were up to 10.2 % higher than in Anhembi. The effect of restriction was stronger in Anhembi for number of families (n_f : 13–25) and for N_e (18.7–25) than for Selviria (n_f: 18–25; N_e: 20.6–25). The coincidence of families in Anhembi selection with those in Selviria ranged from 44–47 %. Coincidence in the selection of Selviria with those in Anhembi improved from 33 % without restriction to 44 % with 1 tree selected per family restriction (Table 3)

In Anhembi the total number of alleles (k = 226 to 253) and mean number of alleles per loci (A = 11.9 to 13.3) increased with the increase of the restriction level (Table 4). The observed

Table 3
Results of the number of families (n_f), family coincidence (*fc*), genetic gain (*g*), effective size (N_e), co-ancestry (Θ) for selection with and without restriction of individuals per family in Anhembi and Selvíria

	Anhembi				Selviria					Difference	
	n_f (*fc*)	*g* (%)	Θ	N_e	n_f (*fc*)	*g* (%)	Θ	N_e	*fc*	*g* (%)	N_e (%)
No restriction	13 (46%)	14.9	0.0267	18.7	18 (33%)	26.6	0.0242	20.6	6	78.5	10.2
3 trees selected	15 (46%)	14.6	0.0242	20.6	18 (39%)	26.6	0.0232	21.5	7	82.2	4.4
2 trees selected	17 (47%)	13.9	0.0225	22.2	19 (42%)	26.5	0.0224	22.4	8	90.6	0.9
1 tree selected	25 (44%)	13.4	0.0200	25.0	25 (44%)	25.7	0.0200	25.0	12	91.8	0

Percent of relative difference of *g* and N_e between Selvíria and Anhembi:

$g = 100[(g_{(Selviria)} - g_{(Anhembi)}) / g_{(Anhembi)}]$ and
$N_e = 100[(N_{e(Selviria)} - N_{e(Anhembi)}) / N_{e(Anhembi)}]$.

Discussion

The difference in survival and productivity between the sites is mainly due to differences in silvilculture practices, since both sites are suitable for *E. urophylla* (Flores et al., 2016). For the proper development of eucalypts, it is mandatory to use the correct pest and weed control and fertilizer (Gonçalves et al., 2013; Ferreira Filho et al., 2015). In Anhembi, the test was established using commercial Brazilian plantation practices, such as mineral fertilization during and after planting; leaf-cutting ant (use of bait) and weed control (herbicides). In Brazil, eucalypt plantations are very productive and one of the reasons is the adequate use of fertilizer (Silva et al., 2013a; Laclau et al., 2013; Melo et al., 2015). Mineral fertilization was not done in Selvíria.

The absence of soil fertilization probably resulted in high selection in Selviria due to worse growth conditions, so individuals and families more adapted to this condition were favored, yielding higher additive genetic variance between

Table 4
Results of genetic diversity in Eucalyptus urophylla under different restriction of the number of individuals per family in Anhembi and Selvíria.

	No restriction		3 trees selected		2 trees selected		1 tree selected	
	Anhembi	Selvíria	Anhembi	Selvíria	Anhembi	Selvíria	Anhembi	Selvíria
Total number of alleles: *k*	226	235	225	235	236	234	253	234
Mean number of alleles: *A*	11.9	12.4	11.8	12.4	12.4	12.3	13.3	12.3
95% CI: *R*	10.3–13.5	10.6–14.2	10.2–13.4	10.6–14.2	10.7–14.1	10.5–14.1	11.5–15.2	10.4–14.2
Number of private alleles: P_a	2	0	0	0	0	0	9	4
Observed heterozygosity: H_o	0.77	0.73	0.78	0.73	0.79	0.73	0.78	0.73
95% CI: H_o	0.72–0.82	0.65–0.81	0.73–0.84	0.65–0.81	0.73–0.85	0.64–0.81	0.71–0.85	0.66–0.80
Expected heterozygosity: H_e	0.84	0.85	0.85	0.85	0.86	0.85	0.87	0.85
95% CI: H_e	0.81–0.88	0.82–0.89	0.82–0.89	0.82–0.89	0.83–0.90	0.82–0.89	0.84–0.90	0.82–0.89
Fixation index: *F*	0.09*	0.15*	0.08*	0.15*	0.08*	0.15*	0.10*	0.15*
95% CI: *F*	0.03–0.14	0.06–0.24	0.02–0.14	0.06–0.24	0.02–0.15	0.06–0.24	0.03–0.18	0.07–0.22

95% CI is the 95% confidence interval; * P< 0.05.

(H_o) and expected heterozygosity (H_e) and fixation index (*F*) were weakly affected by the restriction of the number of individuals in both sites. Fixation index was significantly higher than zero in both sites, regardless of restriction; slightly higher means were observed in Selviria (0.15) when compared to Anhembi (0.10). Private alleles (P_a) were observed with the most restricted selection in both sites, nine in Anhembi and four in Selviria (Table 4). However, in Anhembi with no restriction selection, P_a were found in four trees from three distinct families (Table 5). The highest numbers of loci with P_a were found with the highest restriction in Anhembi, where a single individual presented nine P_a.

Table 5
Number of families (n_f) and trees (n_w) selected, and loci (n_l) with private alleles (P_a)

Population	n_f	n_w	n_l	Mean	min	max
				P_a		
Anhembi: No restriction	3	4	5	1.3	1	2
Anhembi: 1 tree selected	8	8	28	3.5	1	9
Selvíria-1: tree selected	6	6	14	2.3	2	3

Note: other populations (selection restriction) had no private alleles

individuals and increasing the values. As the same selection intensity was applied in both trials, the difference in genetic gain between trials was due to the lower heritability in Anhembi (0.16) than in Selvíria (0.22), resulting in largest genetic gains in Selvíria. In spite of growth discrepancy between individuals in Selvíria, some trees from several families ranked higher than the mean. Genetic gain was measured in percentage points above the mean of the population traits; the mean being low, trees with higher values for the traits resulted in high genetic gains.

Productive and stable families across sites and age in the joint analyses were found; there was only a change in the scale due to the good correlation between the trials, as already discussed about the same families of *E. urophylla* across six trials (Pupin et al., 2015).

Restriction in the number of individuals per family caused a decrease in genetic gain. It corresponded to 1.5 % (14.9-13.4 %) of total gain in Anhembi and 0.9 % (26.6-25.7 %) in Selvíria. A decrease in genetic gain was expected when applied at lower selection intensity (Borralho et al., 1992). In our study, the decrease was due to small difference among individual additive genetic values of individuals belonging to different families. On the other hand, the effective population size (N_e) in Anhembi was 25.2 % and in Selvíria was 16.8 % higher with the restriction of one individual selected per family than under no restriction. This is clearly a result of the numbers of families and individuals within family. The coincidence of families in the selection in Selvíria with no restriction had the smallest value of family coincidence, showing a specific behavior of the selected families. The estimates are based on the final selection of 25 plus trees; with no restriction, the number of families was lower, causing some individuals to be selected in the same family.

After the selection, there were some related offspring in the same family. As N_e is determined by the co-ancestry among individuals and inbreeding within individuals; and since without restriction some related individuals were selected, this resulted in a higher group of co-ancestry and lower N_e than when selecting one tree per family. Maintenance of a high N_e is important to avoid the loss of genetic diversity and allele fixation, which permits advancing genetic gain and is a buffer against stress (Lande and Barrowclough, 1987; Jurskis, 2005; Brawner et al., 2013; Bertoncini et al., 2017).

The selection of more than one tree per family may result in mating among related individuals and inbreeding in the improved seed produced. Inbreeding in eucalypt species causes negative effects due to inbreeding depression; such as decrease in survival, seed production, and growth under field conditions and in tree stem form (Hardner and Potts, 1995; Costa e Silva et al., 2011; Hedrick et al., 2016).

The indices H_o and H_e were higher than reported in *Eucalyptus pilularis* (H_o = 0.57, H_e = 0.66) in a Brazilian breeding population (Silva et al., 2015). The estimation of fixation index (*F*) indicates inbreeding for all selections in both sites, especially in Selviria, as well as non-significant differences between restrictions in the selection. This result suggests that the selected trees are inbred. However, this inbreeding may be eliminated by mating among non-related individuals. This result indicates also that some inbred individuals do not present inbreeding depression in the present population up to 3 years of age. Inbreeding has been observed in progeny trials of *E. grandis* open-pollinated seeds from two landraces in Brazil (Bertoncini et al., 2017), and in *Eucalyptus globulus* the effect of self-pollination was observed under field conditions months after planting (Hardner and Potts, 1994).

The higher restriction generated an increase in the number of private alleles in both trials that decrease the population genetic bottlenecks effect caused by changes in allelic frequencies through selection (Luikart et al., 1998). However there was one exception: the population no restriction in Anhembi (Anhembi-No restriction), in which private alleles appeared; these must have arrived with foreign pollen (open pollination).

Conclusion

The restriction of one tree per family, resulting in a slight decrease in the genetic gain, still allowed for different alleles to be kept in the selected population and higher effective population size; which insures variation for the next generations. This was more pronounced in the more productive trial.

Acknowledgement

Thanks are due to Amcel, Arborgen, Aperam, Copener, Duratex, Eldorado, Fibria, International Paper, Jari, Klabin, Lwarcel, Montes del Plata, Palmasola, Stora Enso, Vallourec, Veracel and Votoratin Siderurgia (companies which are part of the Genetic Improvement Cooperative Program - IPEF), Suzano, University of São Paulo – USP/ESALQ, São Paulo State University – UNESP, COTEC-Instituto Florestal (Forest Institute in São Paulo State), and FAPESP (15/15651-2) for providing information, seeds, land and financial support.

References

Bertoncini, G.H., Tambarussi, E.V., Sebbenn, A.M., Moraes, C.B., Moraes, M.L.T., Furtado, E.L., Mori, E.S. (2017). Rust resistance and mating system in Eucalyptus grandis Hill ex Maiden progênies. Scientia Forestalis 45(114):405–4013. Available at http://dx.doi.org/10.18671/scifor.v45n114.16.

Borralho, N.M.G., Cotterill, P.P., Kanowski, P.J. (1992). Genetic parameters and gains expected from selection for dry weight in Eucalyptus globulus ssp globulus in Portugal. Forest Sience 38: 80–94. Available at http://dx.doi.org/10.1093/forestscience/38.1.80.

Brawner, J.T., Lee, D.J., Hardner, C.N. (2011). Relationships between early growth and Quambalaria shoot blight tolerance in Corymbia citriodora progeny trials established in Queensland, Australia. Tree Genetic and Genomes 7:759–772. Available at http://dx.doi.org/10.1007/s11295-011-0372-8.

Brawner, J.T., Lee, D.J., Meder, R., Almeida, A.C., Dieters, M.J. (2013). Classifying genotype by environment interactions for targeted germplasm deployment with a focus on Eucalyptus. Euphytica 191:403–414. Available at http://dx.doi.org/10.1007/s10681-013-0892-4.

Campoe, O.C., Munhoz, J.S.B., Alvares, C.A., Carneiro, R.L., Mattos, E.M., Ferez, A.P.C., Stape, J.L. (2016). Meteorological seasonality affecting individual tree growth in forest plantations in Brazil. Forest Ecology and Management 380:149–160. Available at http://dx.doi.org/10.1016/jforeco201608048.

Cockerham, C.C. (1969). Variance of gene frequencies. Evolution 23:72–84. Available at https://doi.org/10.1111/j.1558-5646.1969.tb03496.x.

Costa RMLD, Estopa RA, Biernaski FA, Mori ES (2016) Predição de ganhos genéticos em progênies de Eucalyptus benthamii Maiden & Cambage por diferentes métodos de seleção. Scientia Forestalis 44:105–113. Available at http://dx.doi.org/10.18671/scifor.v44n109.10.

Costa e Silva, J., Hardner, C., Tilyard, P., Potts, B.M. (2011). The effects of age and environment on the expression of inbreeding depression in Eucalyptus globulus. Heredity 107:50–60. Available at http:// doi: 10.1038/hdy.2010.154

Denison, N.P., Kietzka, J.E. (1993). The use and importance of hybrid intensive forestry in South Africa. South Africa Forest Journal 165:55–60. Available at http://dx.doi.org/10.1080/0038216719939629390.

Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bulletin 9:1–15

Ferreira Filho, P.J., Wilcken, C.F., Neves, D.A., Pogetto, M.H., Carmo, J.B., Guerreiro, J.C., Zanuncio, J.C. (2015). Does diatomaceous earth control leaf-cutter ants (Hymenoptera: Formicidae) in the Eucalyptus plantations? Journal of Economic Entomology 108: 1-5. Available at http://dx.doi.org/10.1093/jee/tov066

Ferreira, M. (2015). A aventura dos Eucaliptos. In: Schumacher MV, Viera M (eds) Silvicultura do Eucalipto no Brasil. Santa Maria, Brasil, UFSM, pp 13–48. ISBN 9788573912234

Flores, T.B., Alvares, C.A., Souza, V.C., Stape, J.L. (2016). Eucalyptus no Brasil: zoneamento climático e guia para identificação Piracicaba. IPEF. 448p.

Garcia, L.G., Ferraz, S.F.B., Alvares, C.A., Ferraz, K.M.P.M.B., Higa, R.C.V. (2014). Modeling suitable climate for Eucalyptus grandis under future climates scenarios in Brazil. Scientia Forestalis 42:503–511.

Gonçalves, J.L.M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F.B., Lima, W.P., Brancalion, P.H.S., Hubner, A., Bouillet, J-P.D., Laclau, J-P., Nouvellon, Y., Epron, D. (2013). Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. Forest Ecology and Management 301: 6–27. Available at http://dx.doi.org/101016/jforeco201212030.

Gonzaga, J.M.S., Manoel, R.O., Sousa, A.C.B., Souza, A.P., Moraes, M.L.T., Freitas, M.L.M., Sebbenn, A.M. (2016). Pollen contamination and nonrandom mating in a Eucalyptus camaldulensis Dehnh seedlings seed orchard. Silvae Genetica 65(1):1–11. Available at http://dx.doi.org/10.1515/sg-2016-0001.

Goudet, J (1995). FSTAT (Version 1.2): A computer program to calculate F-statistics. Heredity 86:485–486. Available at https://doi.org/10.1093/oxfordjournals.jhered.a111627

Hardner, C.M., Potts, B.M. (1995). Inbreeding depression and changes in variation after selfing in Eucalyptus globulus ssp. globulus. Silvae Genetica 44:46–54.Hardy, O.J., Vekemans, X. (2002). SPAGeDI: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620. Available at https://doi.org/10.1046/j.1471-8286.2002.00305.x

Harwood, C. (2011). New introductions - doing it right. In: Developing a eucalypt resource Learning from Australia and elsewhere. Ed J Walker Wood Technology Research Centre, University of Canterbury, Christchurch, New Zealand, 125–136.

Hedrick, P.W., Hellsten, U., Grattapaglia, D. (2016). Examining the cause of high inbreeding depression: analysis of whole-genome sequence data in 28 selfed progeny of Eucalyptus grandis. New Phytologist 209:600–611. Available at http:// doi: 10.1111/nph.13639.

Jurskis, V. (2005). Eucalypt decline in Australia and a general concept of tree decline and dieback. Forest Ecology and Management 215:1–20. Available at http://dx.doi.org/101016/jforeco200504026.

Kumar, S., Richardson, T.E. (2005). Inferring relatedness and heritability using molecular markers in radiata pine. Molecular Breeding 15:55–64. Available at http://dx.doi.org/10.1007/s11032-004-2059-4

Laclau, J-P., Da Silva, E.A., Lambais, G.R., Bernoux, M., Le Maire, G., Stape, J.L., Jean-Pierre, B., Gonsalves, J.L.M., Jourdan, C., Nouvellon, Y. (2013). Dynamics of soil exploration by fine roots down to a depth of 10 m throughout the entire rotation in Eucalyptus grandis plantations. Frontiers in Plant Science 4:423. Available at https://doi.org/10.1017/CBO9780511623400.007.

Lande, R., Barrowclough, G.F. (1987). Effective population size, genetic variation, and their use in population management. Viable Population for Conservation. pp 87–124. Available at https://doi.org/10.1017/CBO9780511623400.007.

Lindgren, D., Gea, L., Jefferson, P. (1996). Loss of genetic diversity monitored by status number. Silvae Genetica 45:42–59.

Luikart, G., Allendorf, F.W., Cornuet, J.M., Sherwin, W.B. (1998). Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238–247. Available at http://doi:10.1093/jhered/89.3.238.

Melo, E.A.S.C.D., Gonçalves, J.L.M., Rocha, J.H.T., Hakamada, R.E., Bazani, J.H., Wenzel, A.V.A., Ferreira, E.V.D.O., Ferraz, A.V. (2015). Responses of clonal eucalypt plantations to N, P and K fertilizer application in different edaphoclimatic conditions. Forests 7(1):2. Available at http://doi:10.3390/f7010002.

Oda, S., Menck, A.L.M., Vencovsky, R. (1989). Problemas no melhoramento genético clássico do eucalipto em função da alta intensidade de seleção. IPEF, 41:42.

Payn, K.G., Dvorak, W.S., Janse, B.J., Myburg, A.A. (2008). Microsatellite diversity and genetic structure of the commercially important tropical tree species Eucalyptus urophylla, endemic to seven islands in eastern Indonesia. Tree Genetic and Genomic 4:519–530. Available at http://dx.doi.org/101007/s11295-007-0128-7.

Potts, B.M., Dungey, H.S. (2004). Hybridization of Eucalyptus: Key issues for breeders and geneticists. New Forest 27:115–138. Available at http://dx.doi.org/101023/A:1025021324564.

Pupin, S., dos Santos, A.V.D.A., Zaruma, D.U.G., Miranda, A.C., Silva, P.H.M., Marino, C.L., Sebbenn, A.M., Moraes, M.L.T. (2015). Productivity stability and adaptability in open pollination progenies of Eucalyptus urophylla S.T. Blake. Scientia Forestalis 43: 127-134

Pupin, S., Rosse, L.N., Souza, I.C.G., Cambuim, J., Marino, C.L., Moraes, M.L.T., Sebbenn, A.M. (2017). Analysis of Mendelian inheritance and genetic linkage in microsatellite loci of Eucalyptus urophylla S.T. Blake. Genetics and Molecular Research 16(3): https://doi.org/10.4238/gmr16039713

Resende, M.D.V. (2007). Software SELEGEN-REML/BLUP: sistema estatístico e seleção genética computadorizada via modelos lineares mistos. Colombo: Embrapa Florestas.

Resende, M.D.V. (2016). Software Selegen-REML/BLUP: a useful tool for plant breeding. Crop Breeding and Applied Biotechnology 164:330–339. Available at http://dx.doi.org/10.1590/1984-70332016v16n4a49.

Sebbenn, A.M. (2002). Numero de arvores matrizes e conceitos genéticos na coleta de sementes para reflorestamentos com espécies nativas. Revista do Instituto Florestal 14:115–132.

Silva, P.H.M., Lee, D.J., Miranda, A.C., Marino, C.L., Moraes, M.L.T., de Paula, R.C. (2017). Sobrevivência e crescimento inicial de espécies de eucalipto em diferentes condições climáticas. Scientia Forestalis 75: 563-571. Available at http://dx.doi.org/10.18671/scifor.v45n115.13

Silva, P.H.M., Campoe, O.C., de Paula, R.C., Lee, D.J. (2016). Seedling growth and physiological responses of sixteen eucalypt taxa under controlled water regime. Forests 7(6):110. Available at http://doi.org/10.3390/f7060110.

Silva, P.H.M., Shepherd, M., Grattapaglia, D., Sebbenn, A.M. (2015). Use of genetic markers to build a new generation of Eucalyptus pilularis breeding population. Silvae Genetica 64:170–181. Available at https://doi.org/10.1515/sg-2015-0016.

Silva, P.H.M., Miranda, A.C., Moraes, M.L.T., Furtado, E.L., Stape, J.L., Alvares, C.A., Sentelhas, P.S., Mori, E.S., Sebbenn, A.M. (2013). Selecting for rust (Puccinia psidii) resistance in Eucalyptus grandis in São Paulo state Brazil. Forest Ecology and Management 303:91–97. Available at http://dx.doi.org/101016/jforeco201304002.

Silva, P.H.M., Poggiani, F., Libardi, P.L., Gonçalves, A.N. (2013a). Fertilizer management of eucalypt plantations on sandy soil in Brazil: initial growth and nutrient cycling. Forest Ecology and Management 301:67–71. Available at http://dx.doi.org/10.1590/s0100-204x2016000900001.

Steane, D.A., Conod, N., Jones, R.C., Vaillancourt, R.E., Potts, B.M. (2006). A comparative analysis of population structure of a forest tree, Eucalyptus globulus (Myrtaceae), using microsatellite markers and quantitative traits. Tree Genetic and Genomics 2:30–38. Available at http://dx.doi.org/10.1007/s11295-005-0028-7.

Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T.A., Wingfield, B.D., Roux, J. (2008). Eucalypt pests and diseases: growing threats to plantation productivity. South Forest Journal of Forest Science 70:139–144. Available at http://dx.doi.org/10.2989/SOUTH.FOR.2008.70.2.9.537.