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Initiation of breeding programs for three species of Corymbia: Introduction and provenances study

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Abstract: The objective of this study was to establish populations to start the breeding program of three species of Corymbia that are not widely planted in Brazil yet. To this end, basic density, bark content and growth of seedlots/provenances were evaluated three years after planting. The experiments consisted of 14 treatments for Corymbia citriodora subsp. variegata (CCV), 15 for C. maculata (CM), and five for C. henryi (CH). The species exhibited high survival, indicating adaptation to the Cwa climate while average coefficients of determination of seedlots exhibited intermediate values. The CCV from the Richmond Range (28^o 55' S) exhibited the highest productivity. The mean annual increment was low (22 to 26 m³ha⁻¹), but the basic density (573 to 613 kg m⁻³) was high compared to the standard for eucalypts in Brazil. The bark content was close to 15% and varied between and within species.

Keywords: Bark content, basic density, growth, new species, provenance effect

INTRODUCTION

Eucalypt plantations in Brazil account for more than 35% of the commercial reforestation worldwide with a single taxon corresponding to more than 85% of the Brazilian plantation. *Eucalyptus urophylla* and its hybrids with *E. grandis* are widely planted (Assis et al. 2015, Silva et al. 2019).

Species of the genus *Corymbia* have growth potential under several Brazilian climatic conditions. However, their potential has only been explored recently (Silva et al. 2017, Tambarussi et al. 2018, Araujo et al. 2021). Diversification of species and genera for commercial planting is a way to reduce plantation vulnerability to biotic and abiotic stresses. Some species of the genus *Corymbia* have great potential due to tolerance to drought and frost (Assis et al. 2015) and different resistance/tolerances compared to species of the genus *Eucalyptus* (Dianese et al. 1986, Brawner et al. 2011, Silva et al. 2016).

Currently, besides pure species, there is a growing interest in the Corymbia hybrids to establish commercial plantations to minimize the biotic stress effects (Lee 2007). In Brazil, the cross of *C. torelliana* (CT) with *C. citriodora* (CC) resulted in the hybrid known as "torelliodora" deemed promising for tropical and subtropical regions since the growth rate of elite families is higher in the hybrid than in the parental species (Lee et al. 2009). This high growth rate at the beginning of the production cycle remains until the commercial cut age and

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PHM Silva et al.

after (Kumar et al. 2010). Notably, the potential of Corymbia hybrids was observed and reported in Brazil in the 1980s (Assis et al. 2015), but the work stopped due to lack of investments, and the few existing hybrids were obtained from spontaneous hybridization.

The expansion of hybrid combinations can be an option to maintain productivity gains, aiming at heterosis and complementing characteristics/traits between species. Breeding of the Corymbia hybrids in Australia shows a substantial additive genetic variance with each parental species, contributing to the genetic gains across various traits including growth, frost tolerance and pathogen resistance with low genotype-by-environment interactions (Lee et al. 2009). For this to be realized in Brazil, germplasm is needed to supply pollen for hybridization, so the species can advance breeding through recurrent selection. One of the first steps is to establish an appropriate base population to examine the variation within species, that is, the performance of different provenances (Eldridge and Davidson 1988).

The main objective of this study was to establish base populations for the beginning of a breeding program with three species of the genus *Corymbia* (*C. henryi*, *C. maculata*, and *C. citriodora* subsp. *variegata*), which are not widely known in Brazil. The specific objectives were to: i) Evaluate the productivity of different provenances of these three species, three years after planting; ii) Check the effect of seedlot on survival and growth (DBH, height, and volume) under the same environmental conditions; iii) Characterize the species regarding the basic density of wood and bark content, and iv) Establish seed production areas after stratified thinning within each species.

MATERIAL AND METHODS

The experiments were implemented in Itatinga, São Paulo State (lat 23° 03' S, long 48° 38' W, alt 850 m asl) in October 2016. The region has a Cwa climate (Alvares et al. 2013), with average annual temperature and precipitation of 19.4 °C and 1,308 mm, respectively. The randomized complete block experimental design consisted of a linear plot of 10 plants, 14 and 15 treatments (seedlots) in nine blocks for *C. citriodora* subsp. *variegata* (CCV) and *C. maculata* (CM), respectively, and five treatments in ten blocks for *C. henryi* (CH), spaced 3 x 1.5 m in all three experiments. The study used Australian populations (Table 1) obtained by the Cooperative Program for Forestry Improvement (PCMF) of the Forest Research and Studies Institute (Instituto de Pesquisa e Estudos Florestais, IPEF).

Species	Source/Provenance	Seedlot CSIRO (number of mother trees)	Latitude (S)	Longitude (E)	Altitude (m)
CCV	Barakula SF	19664 (11)	26º 16'	150º 32'	300
	Leyburn SF	20420 (8)	28º 02'	151º 37'	500
	Monto SF	19694 (9)	24º 49'	150º 56'	475
	Mount Moffat NP	19666 (10)	24º 53'	147º 59'	1000
	Murphy Range	19691 (10)	25º 17'	149º 11'	420
	Paddys Land	20396 (16)	30º 14'	152º 1'	1100
	Quaid Rd N QLD*	21110 (15)	16º 45'	145º 26'	500
	Richmond Range	20596 (10); 20582 (57); 20597 (57); 20583 (10)	28º 55'	152º 44'	350
	Saddler Springs	19665 (15)	25º 06'	148º 04'	700
	Woondum**	1122	26º 25'	152º 73'	-
	Woondum	19426	26º 25'	152º 73'	-
CM	Barclays Deniliquin***	20541 (41); 20658 (19); 20772 (25); 20884 (13)	35º 29'	145º 14'	100
	Bodalla SF	19422 (10); 20598 (12)	36º 16'	150º 01'	60, 100
	Bunyip VIC	21069 (12)	38º 03'	145º 42'	100
	Holbrook***	20662 (5); 21004 (8)	35º 32'	147º 23'	343
	Mystery Bay	20599 (15)	36º 17'	150º 06'	60
	Southern NSW	20997 (33); 21061 (48); 21062 (22); 21141 (52); 21154 (4)	36º 01'	146º 13'	165
СН	Barclays Deniliquin***	20786 (7); 20664 (5); 20539 (9)	35º 29'	145º 14'	100
Ö	Holbrook***	21040 (9); 20663 (4)	35º 32'	147º 23'	-

Table 1. Description of provenances for Corymbia citriodora subsp. variegata (CCV), C. maculata (CM) and C. henryi (CH)

* C. citriodora subsp. citriodora; ** Seedlot from Dendros Seeds; *** Seed Orchard; SF – State Forest; NP – National Park

Initiation of breeding programs for three species of Corymbia: Introduction and provenances study

The survival rate, height (HT, m), and diameter at breast height (DBH, cm) were evaluated 33 months after planting. The mixed model methodology (REML/BLUP) was used to estimate variance components. The components of variances for the DBH, HT and VOL traits for all three species were estimated by the following model:

$$y = Xb + Zg + Wp + e$$

Where *y* is the vector of individual observations; *b* is the vector of block effects (assumed as fixed); *g* is the vector of the effects of seedlot (random); *p* is the vector of the plot effects (random); *e* is the vector of random errors, and *X*, *Z* and *W* are the incidence matrices of the respective effects. At 36 months, the cubage of 20 trees was determined. The Schumacher and Hall (Logarithmic) (Schumacher and Hall 1933) was the best model for estimating the total volume with bark (VOL, m³), generating equations to estimate volume for CCV (1), CH (2) and CM (3), with adjusted determination coefficients (R^2_{adj}) of 0.993, 0.975 and 0.984, respectively.

$$ln(v) = -9.304162 + 1.62735 \times ln(dbh) + 0.929336 \times ln(h) + e$$
(1)

$$ln(v) = -9.684853 + 1.69836 \times ln(dbh) + 1.020040 \times ln(h) + e$$
(2)

$$ln(v) = -9.423250 + 1.69061 \times ln(dbh) + 0.913325 \times ln(h) + e$$
(3)

The form factor F of an individual stem was computed by dividing the harvested aboveground volume (equations 1, 2 and 3) by the measured cylindrical volume ($\frac{\pi * DBH^2}{4} \times HT$; DBH and HT in m). The coefficient of determination of the seedlot ($\hat{C}^2_{seedlot}$) was calculated as:

$$\hat{C}_{Seedlot}^{2} = \frac{\hat{\sigma}_{Seedlot}^{2}}{\hat{\sigma}_{Seedlot}^{2} + \hat{\sigma}_{plot}^{2} + \hat{\sigma}_{e}^{2}}$$

where $\hat{\sigma}_{seedlot}^2$ is the variance of the seedlot, $\hat{\sigma}_{plot}^2$ is the variance of the plot and $\hat{\sigma}_{e}^2$ is the residual variance.

The average coefficient of determination of the seedlot ($\hat{C}^2_{\text{\tiny Seedlot}})$ was calculated as:

$$\hat{c}_{Seedlot}^{2} = \frac{\hat{\sigma}_{Seedlot}^{2}}{\hat{\sigma}_{Seedlot}^{2} + \frac{\hat{\sigma}_{plot}^{2}}{nBlock} + \frac{\hat{\sigma}_{e}^{2}}{nBlock*nPlant}}$$

where $\hat{C}_{seedlot}^2$ is the variance of the seedlot, $\hat{\sigma}_{plot}^2$ is the variance of the plot and $\hat{\sigma}_e^2$ is the residual variance. *nBlock* and *nPlant* are the numbers of blocks and plants within plots, respectively.

The selection differential (SD) was obtained as:

 $SD = i\sigma_{p}$

where σ_{o} is the phenotypic standard deviation and *i* is the selection intensity (*SI*) calculated as:

$$r = \frac{z}{p}$$

where z is the height of the ordinate at the truncation point of the normal distribution curve and P is the proportion of selected individuals (50%), that is, kept in the test after thinning.

At 36 months, basic density and bark content parameters were determined using the 10 largest trees per species removed by thinning. The restriction criterion was based on tree health, i.e., individuals with any "defect" were discarded. The trees were cut down and discs at heights of 12.5% and 62.5% of the total height were removed to calculate tree basic density with and without bark, bark basic density, and bark thickness. The basic density is the ratio between the mass and the saturated volume of the samples (Equation 4) (Bruder et al. 2016), thus yielding the weighted density (Equation 5).

$$\rho_b = \frac{M_a}{V} \tag{4}$$

Where ρ_b is the wood basic density (kg cm⁻³); M_o is the mass/weight of dried disk (g); and V is the volume of the saturated disk.

$$\rho_{b(weighted)} = \frac{A_1 \rho_1 + A_2 \rho_2 + A_3 \rho_3}{A_1 + A_2 + A_3}$$
(5)

Where $\rho_{b(weighted)}$ is the weighted basic density (kg cm⁻³); A_i is disk area at position *i*; and ρ_i is the density of each disk at position *i*.

Crop Breeding and Applied Biotechnology - 22(1): e40012211, 2022

PHM Silva et al.

In all three populations, transgressive trees (t) performing above the mean (μ) plus twice the standard deviation (σ) of the species DBH seedlots were selected as sources of seeds and pollen.

 $t = \mu + 2\sigma \tag{6}$

The mean annual increment (MAI) was determined from the quotient between the volume of wood accumulated per area (ha), and the planting age.

RESULTS AND DISCUSSION

The three species exhibited high survival, above 95% (Table 2), indicating high adaptation, without significant differences for survival regarding seedlots/provenances. Similar studies have reported comparable survival values for CH (93%) in the region (Silva et al. 2017), as well as CCV (93%) and CT (95%) in the same location (Araujo et al. 2021). However, it is noteworthy that CCV survival rates under different Brazilian climatic conditions vary widely from 50 to 96% three years after planting (Silva et al. 2017). The trial was implemented in a region with Cwa climate and deep latosol suitable for many eucalypt species (Flores et al. 2016). The mild climate, as well as the lack of strong biotic and abiotic stresses, resulted in no selection pressure for the survival traits, thus decreasing the possibility of finding significant differences in survival rates among different seedlots.

Among the studied seedlots, a significant effect (at 1% probability) was observed only for the DBH of the CCV species, whereas no significant effect was observed for the studied traits or in the other species (Table 3). This result indicates that the breeding program may continue without targeting specific lots while making it possible to work with interpopulation genetic diversity. Normally, genetic variation is expected to be lower among than within populations (Clark 2010). However, different eucalypt species such as *E. pilularis* (Carnegie et al. 2004), *E. pellita* (Nieto et al. 2016), *E. grandis* (Marco 1991, Ferreira 2016) and *E. urophylla* (Hodge and Dvorak 2015), which exhibit an effect of provenance resulting from the differences among the regions of origin, despite being the same species, and evolutionary differences resulting

Table 2. Mean and standard deviation of DBH: diameter at breast height (cm); HT: total height (m); VOL: individual volume (m³); MAI: mean annual increment; F: form factor for three *Corymbia* spp., before and after thinning, at 33 months after planting

Creation .		CCV CM		СН			
Species		Before Thinning					
Survival (%)		95	96	96			
DBH (SD)		8.33 (3.56) / 10.84 (1.43)	8.57 (2.08) / 11.03 (1.32)	8.67 (3.35) / 10.90 (1.35)			
HT (SD)		10.95 (3.89) / 12.97 (1.21)	11.00 (2.48) / 12.94 (1.22)	11.15 (3.77) / 12.80 (1.67)			
VOL (SD)		0.028 (0.020) / 0.048 (0.013)	0.029 (0.021) / 0.056 (0.0182)	0.032 (0.024) / 0.055 (0.019			
MAI of population (m ³ ha ⁻¹ y ⁻¹)/ MAI of selected trees		22.74 / 39.30	23.78 / 46.05	26.14 / 44.84			
F		0.49	0.45	0.46			
			After thinning				
	DBH	2.28 (0.80)	2.27 (0.83)	2.43 (0.86)			
Selection differential (Selection Intensity)	HT	2.10 (0.80)	2.15 (0.83)	2.24 (0.86)			
	VOL	0.02 (0.80)	0.02 (0.83)	0.02 (0.86)			

CCV= Corymbia citriodora subsp. variegata; CM = C. maculata; CH= C. henryi.

Table 3. Analysis of deviance and likelihood ratio test (LRT) for the DBH, total height and volume

Trait	Deviance	Species					
Irait		CCV	СН	СМ			
		5.38*	0.38	0.10			
DBH		12.02*	0	4.94*			
НТ	LRT _{seedlot}	3.82	0.69	2.28			
	LRT _{plot}	21.65*	2.85	2.77			
VOL	LRT _{seedlot}	3.81	0.18	0.08			
VOL		23.60*	0.14	15.58*			

* Significant at 1% probability by LRT; LRT_{seedlot}: likelihood test for seedlot; LRT_{plot}: likelihood test for plot. CCV= Corymbia citriodora subsp. variegata, CM = C. maculata, CH = C. henryi.

Initiation of breeding programs for three species of Corymbia: Introduction and provenances study

from different environments generate different performances among provenances and should be considered in breeding programs (Eldridge and Davidson 1988, Williams and Woinarski 1997, Florence 2004, Silva et al. 2019). Nevertheless, it is noteworthy that this study did not test the species fully (all provenances), which could show a difference in the results due to the lack of provenance with different performances in the analysis (Eldridge et al. 1994).

Of the species studied, CCV and CM have the largest natural distributions and, consequently, the highest number of provenances, but only CCV had the provenance effect. Further, the CM is the most southern species occurring in Australia, found on the coastal region in the state of New South Wales, between 31.75° and 37° S latitudes (Boland et al. 2006, Shepherd et al. 2012), theoretically with greater potential for the southern region of Brazil. CCV also occurs naturally in an area on the Australian east coast and southern Queensland, from Carnarvon Gorge to Maryborough, and into New South Wales in Grafton, between 24° and 31.75° S (Shepherd et al. 2012). CH occurs in a more restricted range,

Position	Seedlot	Provenance	g	u + g
		Corymbia citriodora subsp. Variegate		
1	20596	Richmond Range	0.799	9.124
2	20582	Richmond Range	0.375	8.700
3	20597	Richmond Range	0.278	8.603
4	20583	Richmond Range	0.198	8.523
5	19426	Woondum and Wondai	0.136	8.461
6	19665	Saddler Springs	0.038	8.363
7	19691	Murphy Range	0.022	8.347
8	1122	Woondum	-0.016	8.309
9	19666	Mount Moffat NP	-0.028	8.298
10	19694	Monto SF	-0.087	8.239
11	20420	Leyburn S.F.	-0.163	8.162
12	19664	Barakula SF	-0.249	8.076
13	20396	Paddys Land	-0.629	7.697
14	21110	Quaid Rd N QLD	-0.698	7.627
		Corymbia maculate		
1	20658	Barclays Deniliquin	0.111	8.685
2	20599	Mystery Bay	0.066	8.640
3	21141	Southern NSW	0.051	8.624
4	20884	Barclays Deniliquin	0.037	8.611
5	20662	Holbrook	0.029	8.603
6	20541	Barclays Deniliquin	0.017	8.591
7	20772	Barclays Deniliquin	-0.003	8.571
3	21154	Southern NSW	-0.008	8.566
Э	21004	Holbrook	-0.011	8.563
10	21061	Southern NSW	-0.025	8.549
11	20997	Southern NSW	-0.030	8.544
12	21062	Southern NSW	-0.032	8.542
13	21069	Bunyip VIC	-0.043	8.530
14	20598	Bodalla SF	-0.047	8.527
15	19422	Bodalla SF	-0.111	8.463
Corymbia henryi				
1	20664	Barclays Deniliquin	0.189	8.856
2	20786	Barclays Deniliquin	-0.016	8.651
3	20663	Holbrook	-0.027	8.641
4	20539	Barclays Deniliquin	-0.039	8.629
5	21040	Holbrook	-0.051	8.616

Table 4. Ranking of seedlots obtained from the Best Linear Unbiased Prediction (BLUP) for the diameter at breast height (DBH) for Corymbia spp

u = Overall Mean; g = genotypic value (BLUPs).

PHM Silva et al.

between 27.40° and 29.60° S, and is encompassed by the CCV range. At locations where the species are sympatric, the two species are in panmixis (Ochieng et al. 2010).

The CCV of Richmond Range origin (28° 55' S latitude) performed better, ranking in the first four places of the seedlots (Table 4). Self et al. (2002) evaluated the susceptibility of spotted gum trees from eight different origins to Quambalaria shoot blight using an inoculation test and reported that the Richmond Range origin is the most resistant to this disease, while significant differences were observed among provenances. However, Pegg et al. (2011) observed that provenance is an unreliable indicator of Quambalaria shoot blight resistance since results were highly variable within species (provenance and family levels).

The evaluated traits of the three species exhibited low variability according to the coefficient of determination of the seedlot estimates ($\hat{C}^2_{seedlot}$) (Table 5). The growth of the individuals maintained in the test after thinning is less than a standard deviation relative to the original population mean (selection intensity – *i* < 1; Table 2). As a result, the selection differential for growth was not high for the three species. This result is due to the population structure formed by seed bulk generating confusion between a part of the genotypic variance in the residual variance due to the non-structuring of the test in progenies. However, selection within species should be investigated, as high genetic variability was observed within species of the genus (CCC and CCV) in the same study area, in a progeny trial (Araujo et al. 2021).

The average coefficients of determination of seedlots ($\hat{C}^2_{seedlot}$) exhibited low to intermediate values for the studied traits, and the highest was for DBH (0.641) in CCV. Thus, despite the low variability observed among seedlots, provenance selection may be successfully carried out based on average provenance. However, high selection intensities should be avoided because it could restrict the genetic base, generating something similar to the bottleneck or founder effects in the base population, decreasing the effective size of the population, which is essential for conservation and improvement (Vencovsky 1987). It should be noted that gains in the initial stage of improvement are higher than in the more advanced stages (Allard 1999), even with lower selection intensities that maintain a broad genetic base.

The wood volumetric mean annual range from 22 to 26 m³ ha⁻¹ is considered low compared to commercial eucalypt plantations in Brazil that, with adequate management and environment, reach 46 m³ ha⁻¹ yr⁻¹ at the same age, as observed for *E. grandis* and *E. urophylla* (Marco 1991, Silva et al. 2019), with the largest volumetric increments observed in *E. grandis*. Some points should be highlighted: (i) lower improvement level; (ii) the growth curve may be different from those for the species of the genus *Corymbia*; and, (iii) the species of the genus *Corymbia* have lower volumetric productivity, but higher basic density.

The productivity is expected to increase in the next generation since a selection differential of 16.6 46 m³ ha⁻¹ yr⁻¹ was used for CCV, 22.8 46 m³ ha⁻¹ yr⁻¹ for CM, and 18.7 46 m³ ha⁻¹ yr⁻¹ for CH. These values represent 73, 92 and 72% of the species average in the experiments, high values despite the moderate intensity of selection applied (~ 0.8; Table 2). This selection differential reflects the thinning conducted at the age of three, which reached a 50% average thinning of individuals for all three species.

At 36 months, the mean basic density values were 573, 598 and 613 kg m⁻³ for CM, CCV and CH, respectively. These density values are higher than those of the species of the genus *Eucalyptus* used commercially, such as *E. urophylla*

	CCV			СМ			СН		
Parameters	DBH (cm)	HT (m)	VOL (m ³)	DBH (cm)	HT (m)	VOL (m ³)	DBH (cm)	HT (m)	VOL (m³)
$\hat{\sigma}^2_{plot}$	0.546	0.646	6.10E-05	0.312	0.204	4.50E-05	0.018	0.263	5.00E-06
$\hat{\sigma}^2_{Seedlot}$	0.251	0.174	1.70E-05	0.023	0.111	2.00E-06	0.029	0.064	2.00E-06
$\hat{\sigma}_{e}^{2}$	7.241	5.995	5.08E-04	7.065	6.278	5.26E-04	6.346	5.408	5.35E-04
$\hat{\sigma}_{p}^{2}$	8.038	6.815	5.86E-04	7.400	6.594	5.73E-04	6.393	5.735	5.42E-04
$\hat{C}^2_{Seedlot}$	0.031	0.026	0.028	0.003	0.017	3.21E-03	0.005	0.011	3.36E-03
$\hat{C}^2_{Seedlot}$	0.641	0.557	0.573	0.169	0.547	0.145	0.310	0.445	0.236

Table 5. Variances and coefficients of determination of seedlots for Corymbia citriodora subsp. variegata (CCV), C. maculata (CM) and C. henryi (CH) at three years of age for the diameter at breast height (DBH), total height (HT), and volume (VOL) traits

 $\hat{\sigma}^2_{seculat}$: variance due to seedlot effect; $\hat{\sigma}^2_{plot}$: variance due to plot effect; $\hat{\sigma}^2_e$: residual variance; $\hat{\sigma}^2_p$: phenotypic variance; $\hat{c}^2_{seculat}$: coefficient of determination of the seedlot; $\hat{C}^2_{outring}$: average coefficient of determination of the seedlot

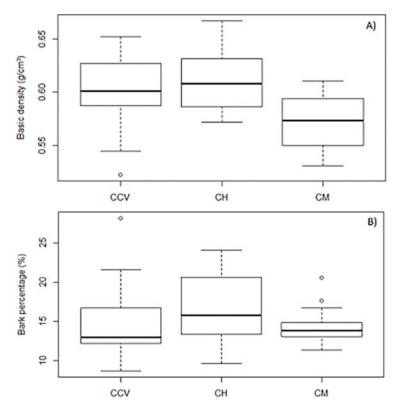


Figure 1. Boxplots for wood basic density without bark (A) and bark content (B) 36 months after planting for three *Corymbia* spp. (CCV= *Corymbia citriodora* subsp. *variegata*, CM = *C. maculata*, CH= *C. henryi*).

(Duc Kien et al. 2008, Trugilho 2009), *E. grandis* (Bhat et al. 1990, Trugilho 2009, Gouvêa et al. 2011), *E. saligna* (Batista et al. 2010) and the hybrid "*E. urophylla* x *E. grandis*" (Bison et al. 2006, Hsing et al. 2016), which vary between 450 kg m⁻³ and 550 kg m⁻³ at the age of six years, with the highest values observed for *E. urophylla*. A higher basic density can compensate for lower volumetric productivity, as both are essential for producing biomass. It is highlighted that the basic density increases until the cutting age, between six and seven years.

The average ratio between the bark and wood volumes varied between 14 and 16.5% for the three species (Figure 1), with CH and CCV showing the greatest variations within the species. In comparison with other species of the genus *Eucalyptus*, the bark percentage in the genus *Corymbia* is higher than those of species used commercially, whose value may be less than 10% of the volume (Ramalho et al. 2019). Because high bark volume is not good for the industrial process, breeding programs should aim at reducing it, which is possible due to existing genetic variability for the trait within the species of the genus *Corymbia* (Paludeto et al. 2020) and verified here for CH and CCV. Nevertheless, it is necessary to verify the relationship of this variable (bark volume) with other important traits.

CONCLUSIONS

The survival rate between species or seedlots did not vary significantly while the mortality rate was low (<5%), demonstrating that the studied species are suitable for the region classified as Cwa. The productivity of different seedlots and provenances was also not significantly different for CM and CH, whereas the CCV from the Richmond Range was the most productive, followed by that from Woondum. The basic density was close to 600 kg m⁻³ and the bark content was close to 15% for the three species at 3 years of age, with variation between trees that could probably be exploited by breeding programs.

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Initiation of breeding programs for three species of Corymbia: Introduction and provenances study

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