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Growth and survival of *Eucalyptus viminalis* in a frost-prone site in southern Brazil, and implications for genetic management

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Abstract

Background: As the climate in southern Brazil is cold with frequent frosts, *Eucalyptus* species that can resist these climatic conditions are needed for commercial plantations. This study aims to evaluate provenances and families of *Eucalyptus viminalis* Labill. and compare them to 11 other *Eucalyptus* spp. to select superior genotypes with high production potential and resistance to frost.

Methods: A total of 58 open-pollinated families from 16 provenances in Australia were planted in a frost-prone site in Irati, Paraná state, Brazil, using a randomised block design, with three replicates, linear plots of three plants, and a 2 × 2 m spacing, for a total experimental area of one hectare. Another eleven *Eucalyptus* species were planted in linear plots ranging from four to 59 plants alongside the experiment.

Results: Survival for *E. viminalis* ranged from 0 to 68%, indicating genetic variability for frost resistance in this species. The variation among provenances was high (56%) for total genetic variation, indicating relatively high additive genetic differentiation among them. The other half of the total additive genetic variation was within (24%) and among families (20%), showing good variability among genotypes of each provenance.

Conclusions: In relation to the other 11 species, *E. viminalis* shows promise for frost-prone sites as the results are comparable to other species used in this region and clones from breeding programmes developed for these conditions. Thus, *E. viminalis* offers the potential for selecting superior genotypes to be cloned for immediate genetic gains, as well as for the next generation of breeding.

Keywords: Bayesian analysis; multi-species conservation; provenance and progeny trial; tree breeding; selection gain; spatial modeling.

Introduction

In the subtropical regions of Brazil, growth in the establishment of *Eucalyptus* plantations has been limited due to the occurrence of severe and frequent frosts and the minimal number of eucalypt species that are adapted to this climatic condition (Müller et al. 2017; Frigotto et al. 2020). Predicting the occurrence and intensity of frosts to address this problem becomes impractical, given the five- to seven-year cycle of *Eucalyptus* cultivation (Diniz et al. 2021), and the fact that several frosts can occur throughout a single production cycle (Alvares et al. 2018). Thus, it is vital to produce genetic materials that are resistant to the adverse conditions found in these regions and can meet economic and productive demands (Wendling et al. 2021).

Some eucalypt species and their genotypes are known to present the ability to limit the damage caused by frost (resistant) or grow despite frequent frosts and withstand the damage they cause (tolerant). The species with good productivity that have stood out for use in the southern states of Brazil are *Eucalyptus benthammi* Maiden et Cambage, *Eucalyptus dunnii* Maiden, and *Eucalyptus saligna* Sm. (Lengowski et al. 2020); in the southeastern US the species used is *E. benthammi* (Hall et al. 2019); and in southern China the species used are *Eucalyptus amplifolia* Naudin, *E. benthamii*, *E. dunnii*, and *Eucalyptus dorrigoensis* (Blakely) L.A.S.Johnson & K.D.Hill (Arnold et al. 2015). In Europe, previous studies recommend *Eucalyptus nitens* (H.Deane & Maiden) Maiden and *Eucalyptus gunni* Hook. f. in countries such as Portugal, Spain, and France (Cerasoli et al. 2016).

Eucalyptus viminalis Labill. is a species that can reach 90 m in height, with smooth, powdery bark that is white to pale brown and sheds in long ribbons. Due to the presence of lignotubers (Ladiges et al. 1974), the species regrows easily. Like most eucalypt species, *E. viminalis* occurs in Australia, where it extends from Tasmania (TAS) to the border between New South Wales (NSW) and Queensland. The best quality stands are found in Victoria (VIC) and NSW, where the terrain is mountainous with rigorous winters and frequent frosts, a fact that favors the planting of the species in southern Brazil (Flores et al. 2016). The wood is light yellow or pink in color, with high basic density (up to 0.748 g.cm^{-3}) compared to other eucalypts, and it is widely used for the production of cellulose, fiber, and particle board, in sawmills, and as poles. *Eucalyptus viminalis* shows several favorable traits, such as stem form, high productivity, and frost tolerance, and has been planted in several countries (Gonzaga et al. 1983; Louppe et al. 2009). Due to its wood quality traits, growth, and resistance to water stress, disease, and frost, in addition to effective regeneration through resprouting (Gonçalves et al. 2013), the species is indicated for interspecific hybridisation based on reciprocal recurrent selection (Santos et al. 2013). The species has also performed well in tests with different *Eucalyptus* species in Argentina, where *E. viminalis* was ranked among the most productive species, with good wood quality, survival, and stem form (Cappa et al. 2010).

The introduction of new provenances and species with an adequate genetic base may help to meet the demand for more productive materials in *Eucalyptus* spp. cultivation in the ecological conditions of subtropical regions (Pinto Junior & Silveira 2021). Therefore, it is important to assess the behavior of these different provenances, as they may present diverse adaptive potential (Kageyama 1983; Cruz et al. 2020; Chaves et al. 2021).

Genetic and phenotypic parameters used to predict expected gains with the selection of superior genotypes and the best provenances in forest species, have been estimated with the restricted maximum likelihood/best linear unbiased predictor (REML/BLUP) methods, and/or Bayesian models via Markov Chain Monte Carlo (MCMC). This is mainly due to the precision that these methods offer in estimates obtained from unbalanced classifications (Resende 2002; Araújo et al. 2014; Konzen et al. 2017; Munhoz et al. 2021; Oliveira et al. 2021; Flôres Júnior et al. 2021). In this context, the comparison between species from different trials may be biased, as the effect of the environment has a significant impact on the manifestation of phenotypic expression. Ideally, the assessment of multiple species should be done in a combined experiment (Santos et al. 2013); however, experiments commonly contain only one species and its progeny. In these cases, models with spatial effects within sites can be adjusted to circumvent the lack of perfect randomisation (Rodriguez-Alvarez et al. 2018), mapping the residual environmental effects of each plot by variograms, and enabling a more accurate comparison between diverse but spatially proximal experiments. This comparison of genotypic performance is valuable, as it enables the validation of a recommended genotype of a new species by comparing it to other species that are known to be suitable, in the present case, for frost resistance in other regions. The Institute for Forestry Research and Studies (IPEF) has developed a robust, ongoing multi-species assessment program in different regions of Brazil, with several species and strategic provenances of *Eucalyptus* and *Corymbia* (Baroni et al. 2020; Araujo et al. 2021). The present research is part of this ongoing programme and aims to evaluate provenances and progenies of *E. viminalis* and compare them to other *Eucalyptus* spp. to inform the selection of superior materials with high productive potential and resistance to typical climatic conditions in the subtropical region of Brazil. In addition, we outline future strategies for the genetic improvement of this potential eucalypt species for such region.

Methods

Study area and experimental design

Study area and trial site

The studied population was established in December 2017 in Irati, Paraná (PR), Brazil, at the State University of the Central-West (coordinates 25º32’25”S and 50°39’51”W; elevation of 812 m; Figures 1 & 2). The soil at the study site is classified as dystrophic Tb Haplic Cambisol (CXbd) (EMBRAPA 2018). The climate in the region is humid subtropical without a dry season (Cfb), with severe and frequent frosts and evenly distributed

FIGURE 1: (**A**) Experimental area of the provenance and progeny test of *Eucalyptus viminalis*; (**B**) *Eucalyptus viminalis* during frost; (**C**) *Eucalyptus benthamii*; and (**D**) *Eucalyptus longirostrata* during frost. All images are from the experimental trial in Irati, PR.

rainfall; the average temperature in the hottest month does not reach 22 °C and in the coldest month it is greater than 10 °C, with precipitation from 1100 to 2000 mm (Köppen & Geiger 1928).

This region of Paraná is the coldest region in Brazil, with frosts occurring frequently throughout the winter. The minimum and maximum temperature data (see Additonal File) were obtained from the National Meteorological Institute (2021) for the period from December 2017 to October 2021. During this period, 75 days (2.68% of days) reached a minimum temperature below 5 °C and plant survival after eight months of age (after three periods of frost – Table 1) was 84.7%. Data for frost occurrence (per day) were taken from INMET (2022) (Table 1).

Genetic material and field layout

A total of 58 open-pollinated families in 16 provenances from Australia were planted (Table 2). The experiment was designed in randomised blocks with three replicates, linear plots of three plants, and a 2 × 2 m spacing, with double border rows of trees surrounding the plots. The total experimental area was 1 ha. Simultaneously, 11 other *Eucalyptus* species (Table 2) were planted adjacent to the *E. viminalis* experiment in linear plots ranging from four to 59 plants (see Supplementary Material S1 for a detailed map of the experiment in the field). These species were planted to act as a control (Table 2). All plants of each of the plots of the different species consisted of a mix of seeds from trees of the same population.

Trait assessment

Diameter at breast height (DBH, cm) was estimated by measuring the circumference at breast height (CBH, cm) collected with a measuring tape and the result divided by π. Total height of the tree (HT, m) was measured with a Vertex IV® hypsometer (Långsele, Sweden). DBH and HT were measured when plants were 46 months old (3.83 years), which is an age close to the length of *Eucalyptus* rotation cycles used in Brazil, and considered a reliable length of time for early selection practices

TABLE 1: Frost occurrence dates, temperature, and intensity in the *Eucalyptus viminalis* experimental area in Irati, Paraná state (PR), Brazil.

Day/Month/Year	Minimum temperature of 1200 UTC (°C)	Frost Intensity
20/05/2018	2.8	Weak
21/05/2018	0.6	Strong
26/08/2018	-0.2	Strong
03/08/2019	0.1	Strong
26/05/2020	1.8	Weak
27/05/2020	1.6	Weak
28/05/2020	3.0	Weak
30/05/2020	3.6	Weak
03/07/2020	0.4	Strong
22/08/2020	-2.3	Strong
23/08/2020	2.2	Moderate
25/05/2021	0.5	Strong
29/06/2021	-1.8	Strong
30/06/2021	-2.0	Strong
01/07/2021	-0.8	Strong
19/07/2021	-3.3	Strong
20/07/2021	-2.1	Strong
21/07/2021	0.0	Strong
22/07/2021	3.1	Weak
28/07/2022	-0.7	Strong
29/07/2022	-3.4	Strong
30/07/2022	-3.9	Strong
31/07/2’022	-1.0	Strong
20/05/2022	0.6	Strong
21/05/2022	1.2	Moderate
22/05/2022	2.0	Moderate
30/06/2022	1.4	Moderate
01/07/2022	2.9	Moderate
30/07/2022	1.2	Moderate

(Lima et al. 2011; Castro et al. 2016). The stem volume (VOL, $m^3\ tree^{-1}$) was estimated as:

$$V = 0.0000018 \times DBH^{1.77298} \times \mathrm{HT}^{1.37336}$$

as used previously for *E. dunnii* (Figueiredo Filho & Amaral 2014).

Statistical model and genetic parameters

Models were fitted in the hierarchical Bayesian framework in the R software environment (R Core Team 2020). To avoid using Monte Carlo Markov Chains, parameters were fitted through Integrated Nested Laplace Approximation (INLA) in the INLA package in R (Rue et al. 2009). The statistical model consisted of:

$$\boldsymbol{y} = \boldsymbol{\mu} + \mathbf{Z}\boldsymbol{p} + \mathbf{W}\boldsymbol{f} + \mathbf{M}\boldsymbol{a} + \mathbf{S}\boldsymbol{u} + \boldsymbol{\varepsilon}$$

where $\boldsymbol{y}$ is a vector containing the response variable, in this case wood volume; $\boldsymbol{\mu}$ is the estimated intercept; $\boldsymbol{p}$ is the vector of estimated genetic effects of provenances; $\boldsymbol{f}$ is the vector of estimated family effects nested within provenances; $\boldsymbol{a}$ is the vector of estimated individual additive genetic effects nested within families and nested within provenances; $\boldsymbol{u}$ is the vector of estimated spatial effects based on the position of each plant; and $\boldsymbol{\epsilon}$ is the vector of residual effects. $\mathbf{Z}$, $\mathbf{W}$, $\mathbf{M}$ and $\mathbf{S}$ are incidence matrices for the respective effects. The genetic effects were parameterised respectively in the linear mixed model described above, with the following probability distributions:

$$p \sim N(0, \boldsymbol{I}\sigma^2_p)$$

$$f \sim N(0, \boldsymbol{I}\sigma^2_f)$$

$$a \sim N(0, \boldsymbol{A}\sigma^2_a)$$

$$\epsilon \sim N(0, \boldsymbol{I}\sigma^2_\epsilon)$$

considering estimated variance among provenances (σ^2_p), variance among families within provenances (σ^2_f), variance among individuals within families within provenances (σ^2_a), environmental variance (σ^2_ϵ) and Identity Matrix ($\boldsymbol{I}$) as fitted in a Bayesian approach with a prior *LogGamma*(1,1).

The relationship among individuals is parameterised based on single-generation pedigree information ($\boldsymbol{A}$) (Wright 1922), thus capturing the relationship at the half-sibling level. Such parametrisation is well known in animal breeding and is computationally efficient since the sparse relationship matrix ($\boldsymbol{A}^{-1}$) can be calculated directly (Henderson 1976).

The spatial term was set using the following parameterisations: Stochastic Partial Differential Equations (SPDE) in which the spatial term is defined as:

$$\mathrm{u} \sim \mathrm{N}(0, \boldsymbol{Q}\,\sigma^2_u),$$

where $\boldsymbol{Q}$ is a spatial covariance structure built via SPDE (Lindgren et al. 2011; Lindgren & Rue 2015) and defined by the function:

$$W(s) = (\kappa^2 - \Delta)^{0.5\alpha} x(s),$$

where $W(s)$ is Gaussian spatial white noise process, κ is scale parameter, Δ is the Laplacian ($\sum_{i=1}^{2} \partial^2/\partial k_i^2$), and α is a smoothing parameter ($\alpha = \nu + 0.5\gamma$) with dimension domain γ.

The heritability was estimated at all levels for genetic effects: narrow-sense heritability as:

$$\hat{h}^2_{(a)} = \sigma^2_a/(\sigma^2_p + \sigma^2_f + \sigma^2_a + \sigma^2_\epsilon)$$

TABLE 2: Details of *Eucalyptus viminalis* and other *Eucalyptus* spp. trials for wood volume (VOL) at 46 months of age, including provenance, location, and descriptive attributes of the populations.

Species	Origin	Source	Latitude	Longitude	Elevation (m)	No. families	No. living plants	Survival* (%)	Average (m^3 $tree^{-1}$) (sd)
E. viminalis	Glenbog SF	CSIRO	36°38’	149°26’	1000	2	6	29	1.41 (1.03)
	Wingello SF	CSIRO	34°44’	150°10’	600	1	1	17	6.24 (-----)
	Mount Sunday VIC	CSIRO	37°20’	146°26’	960	2	5	25	2.88 (2.45)
	Proctors Road TAS	CSIRO	42°57’	147°19’	40	1	2	33	0.29 (0.09)
	Tallaganda SF	CSIRO	35°26’	149°34’	900	2	2	13	3.60 (4.30)
	Tallaganda SF	CSIRO	35°58’	149°35’	900	2	2	13	3.79 (2.37)
	Nimmitabel NSW	Kylisa (bulk)	36°29’	149°16’	1075	1	1	11	0.17 (-----)
	Noojee VIC	Kylisa (bulk)	37°52’	146°00’	275	13	36	27	3.96 (3.09)
	Georgetown TAS	Kylisa (bulk)	41°06’	146°82’	10	1	0	0	-
	Uriarra ACT	Kylisa	35°15’	148°55’	625	3	4	22	1.72 (1.35)
	Warren NSW	Kylisa	31°42’	147°48’	200	1	2	22	2.92 (2.96)
	Martins Creek VIC	Kylisa	-	-	-	12	66	48	3.91 (3.62)
	Timbarra VIC	Kylisa	37°07’	147°59’	198	4	11	28	1.99 (2.12)
	Errinundra VIC	Kylisa	37°17’	148°53’	440	8	40	40	4.31 (4.30)
	Bald Hills VIC	Kylisa	37°26’	143°51’	477	4	10	30	1.12 (1.13)
	Federation’ Range	Kylisa	37°28’	145°52’	1483	6	43	68	3.90 (2.23)
E. benthamii	Sso Kowen-Pure Seed	Company	35°18’	149°18’	650	1	34	58	5.04 (6.02)
E. badjensis	Deua Np	IPEF (bulk)	-	-	-	1	6	17	2.39 (1.59)
E. botryoides	Orbost	IPEF (bulk)	37°43’	148°15’	80	1	3	75	0.72 (0.86)
E. cladocalyx	Sso Hamilton VIC	IPEF (bulk)	37°49’	142°04’	203	1	15	52	1.62 (1.23)
E. deanei	Kedumba Valley	IPEF (bulk)	33°49’	150°23’	140	1	7	78	2.93 (2.89)
E. longirostrata	-	IPEF (bulk)	-	-	-	1	21	100	4.77 (2.31)
E. macarthurii	Paddys River, NSW	IPEF (bulk)	-	-	-	1	23	46	1.82 (2.42)
E. nobilis	Styx River SF	IPEF (bulk)	-	-	-	1	24	50	3.27 (2.93)
E. obliqua	Daylesford	IPEF (bulk)	-	-	-	1	4	15	2.25 (0.95)
E. occidentalis	Old Newgate Road	IPEF (bulk)	-	-	-	1	5	13	1.52 (1.60)
E. smithi	Narooma	IPEF (bulk)	36°10’	150°04’	150	1	7	21	1.85 (1.60)
*E. benthamii*_Clone	-	Company	-	-	-	1	10	83	9.26 (8.51)

SD: standard deviation;* rate of survival for families within the origin site; “-“ data is unavailable.

family level heritability as:

$$\hat{h}^2_{(fa)} = \sigma^2_a + \sigma^2_f / (\sigma^2_p + \sigma^2_f + \sigma^2_a + \sigma^2_\epsilon)$$

and provenance level heritability as:

$$\hat{h}^2_{(pfa)} = \sigma^2_p + \sigma^2_f + \sigma^2_a / (\sigma^2_p + \sigma^2_f + \sigma^2_a + \sigma^2_\epsilon)$$

In addition, a measure of effective population size (N_e) for selected individuals was calculated according to the following equation (Resende & Bertolucci 1995):

$$N_e = 4N_f\bar{K}_f / (\bar{K}_f + 3 + (\sigma^2_{Kf} / \bar{K}_f))$$

Where N_f is the number of families; $\bar{K}_f$ is the average number of selected individuals per progeny; σ^2_{Kf} is the variance of the number of selected individuals per family.

The experiment used in the present study was a preliminary analysis to support two other subsequent aims: (1) to select high-performance families to be used in breeding programmes; and (2) to conserve genetic variability in this *E. viminalis* population pool in Brazil.

Results

Descriptive results

Survival at 46 months varied widely ranging from 0 to 68% for *E. viminalis*, and for the other *Eucalyptus* species from 13% for *E. occidentalis* to 100% for *E. longirostrata* (Table 2). High survival rates were also found for *E. botryoides* (75%), *E. deanei* (78%), and *E. benthamii* (83%), which indicates the presence of genetic variability for cold resistance in these species. Of the survival rates for *E. viminalis* from the 16 tested provenances, Mt Sunday and Georgetown TAS showed 0%, while the other provenances ranged from 11 to 68%. Federation Range showed the best survival among the *E. viminalis* provenances. The locations (Figure 2) of the provenances occur naturally within coordinates that range between latitudes 31°42' to 42°57'; and longitudes 143°51' to 150°10'and with elevation ranging from 10 to 1483 m. This wide environmental variation can explain the diverse results obtained for survival and wood volume. Compared to the other species planted in the same location, *E. viminalis* showed a higher mortality. When we evaluated the average individual volume there was also considerable variation, which was expected since the test includes a diversity of *E. viminalis* provenances. The three provenances that presented the best performance were Federation Range, Uriarra ACT, and Bald Hills VIC, in that order. However, some of the provenances presented individuals with good performance when compared to *E. benthamii*, which is a species with good growth that is adapted to conditions of intense cold. This demonstrates that there is a possibility of obtaining gains with the selection of the best individuals and the establishment of a breeding programme for the species. We found some *E. viminalis* families with individuals that had the same growth in wood volume as other species (Additional File).

Estimated parameters

Most of the observed genetic variance was among provenances ($\sigma^2_p = 0.185$) (Table 3). The variance observed among provenances was high, corresponding to 56% of the total genetic variation (Figure 3). The other half of the total genetic variation was retained within families (24%) and among progenies (20%).

Breeding selection

The results of the two selection strategies (i.e., selection aimed at breeding improvement or conservation) are presented in Table 4. The selection of 10, 20, 30, 40, 50, and 60 individuals were defined and applied to both strategies. These values are based on the operational capacity of deploying new experiments in the next phase of the breeding programme.

To obtain enhanced productivity, selection was made across families, with the only criterion being the highest predicted values. For conservation, selection was within and between families, identifying the best families and then the best individuals within these families. The best individual of the best family was selected, followed by the best individual of the second-best family, and so on. After selecting the best of each family, the second best was selected, and so on.

In selection for production, which was more restrictive, provenances ranging from three to six individuals were included for selection of the 10 to 60 best across all provenances. In these provenances, Errinundra VIC, Martins Creek VIC, and Noojer VIC, appear to offer the best performance with four to five

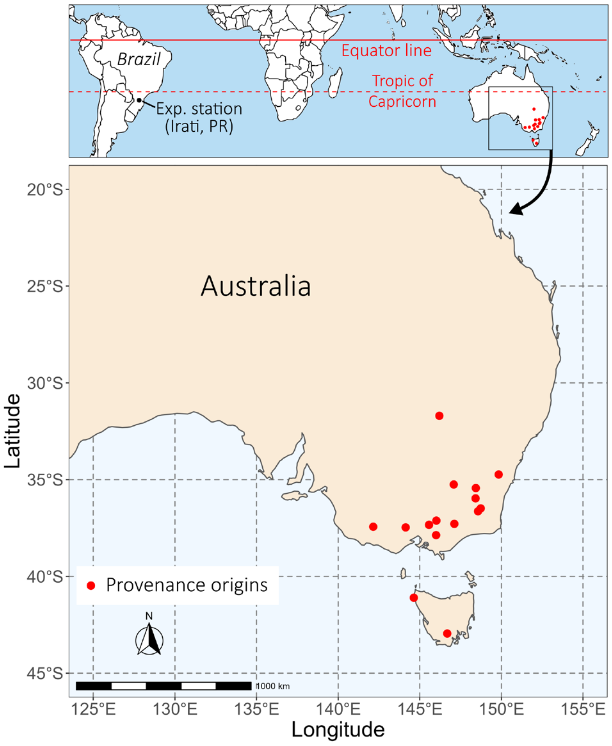

FIGURE 2: Provenance in Australia of *Eucalyptus viminalis* seeds collected for use in this study. The experimental station is located in Irati, Paraná state, Brazil.

TABLE 3: Estimated genetic variance and heritability for wood volume (VOL) in *Eucalyptus viminalis* population at 46 months of age.

‘	**Estimated Parameter**	**Mean**	**HPD (95%)**
Genetic	σ^2_p	0.19	0.08-0.32
	σ^2_f	0.08	0.05-0.11
	σ^2_a	0.07	0.05-0.09
Non-Genetic	σ^2_u	0.05	0.03-0.08
	Spatial range	2.84	1.52-5.61
	σ^2_ϵ	0.04	0.02-0.06
Heritability	$\hat{h}^2_{(pfa)}$	0.89	0.82-0.95
	$\hat{h}^2_{(fa)}$	0.40	0.25-0.55
	$\hat{h}^2_{(a)}$	0.18	0.11-0.27

HPD = highest probability density interval; σ^2_p = variance within provenance; σ^2_f = variance among provenances and families; σ^2_a = variance among provenances and individuals within families; σ^2_u = spatial variance; σ^2_ϵ = residual variance; $h^2_{(pfa)}$ = heritability at *pfa*; $h^2_{(fa)}$ = heritability *fa*; and $h^2_{(a)}$ = heritability at the individual level (*a*).

families showing the highest results. The N_e results for these three provenances were 12.88, 16.64, and 24.25, respectively, with the low values being the result of the selection of only a few provenances and families within the production-focused strategy.

Selection aimed at production obtained predicted gains of 104% in relation to the general average. This very high value is due to the average being relatively low (4.01 m^3 in 1000 plants). When compared to the commercial clone adapted to the study conditions, we found negative gains from -23% to -37% for the selected *E. viminalis* individuals.

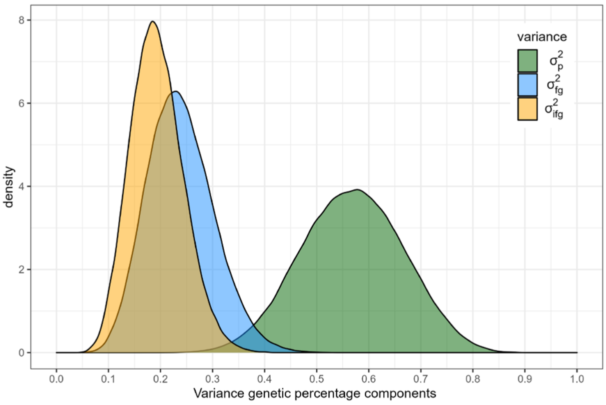

FIGURE 3: Percentage of genetic variance for a *Eucalyptus viminalis* population for wood volume (VOL) at 46 months age: provenance in green; families (half-sibs) in blue; and individuals in yellow.

In selection focused on conservation, the goal was to select the best individuals within the families to help maximise the N_e. Thus, it is possible to obtain gains relative to the average volume of 24% to 50%. Relative to the *E. benthamii* clone, negative gains of -37% to -52% were found, suggesting much lower values in terms of volume yield. However, the N_e was well above the average, and in the range of 20.00 to 59.01 individuals (Table 4). The individuals selected for conservation will be recombined with each other to generate more variability that can be tested in further progeny trials for local environmental conditions.

In the selection-for-production scenario, the behavior of *E. viminalis* is very similar compared to the other studied species (Figure 4), where the selection response for *E. viminalis* (green line) and all other species (blue line) showed a minimal difference for individual

TABLE 4: Two selection strategies, one for genetic conservation and the other for recombination for production, and effective population size (N_e).

Selection Target	N	N_p	N_f	K_f	σ^2_{Kf}	N_e	**BLUP (m^3 tree^{-1} × 1000)**	**Gains (%)**	**Comparative Gains (%)**		
								E. viminalis	***E. longirostrata***[1]	***E. benthamii***[2]	**Clone**[3]
Production*	20	3	9	2.00	2.22	11.78	8.77	82	28	32	-23
	30	4	13	2.14	3.36	16.60	8.17	70	19	23	-29
	40	5	17	2.35	3.24	23.77	7.75	61	13	16	-32
	50	6	20	2.50	3.00	29.85	7.43	54	8	12	-35
	60	6	25	2.73	3.16	39.61	7.17	49	4	8	-37
Conservation**	20	12	20	1.00	0.00	20.00	7.23	50	5	9	-37
	30	14	30	1.00	0.00	30.00	6.46	34	-6	-3	-44
	40	15	40	1.00	0.00	40.00	5.99	24	-13	-10	-48
	50	15	50	1.00	0.00	50.00	5.65	17	-18	-15	-51
	60	16	58	1.03	0.03	59.01	5.56	16	-19	-17	-52

* for production, the best-ranked individuals are selected across families. ** for conservation, the best individual is selected within families. N: number of individuals selected; *Np* = number of provenances in the selection; N_f = number of families; K_f = average number of selected individuals per family; σ^2_{Kf} = variance of the number of selected individuals per family.

[1] Selection compared with total average of *E. longirostrata* (N=21, BLUP =6.87); [2] Selection compared with total average of *E. benthamii* (N=34, BLUP =6.66); [3] Selection compared with total average of *E. benthamii* commercial clone adapted to these conditions (N=1, BLUP =11.46).

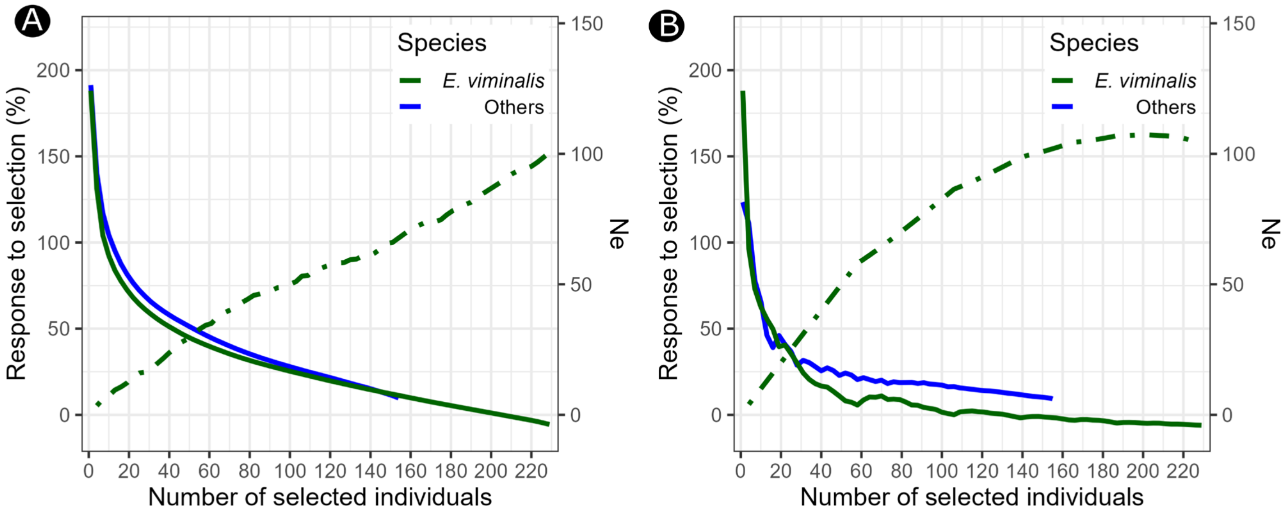

FIGURE 4: Selection trend for wood volume (VOL). (**A**) depicts selection gains considering the best individual, independent of provenance; (**B**) depicts selection for conservation, i.e., selecting the best individuals within families. The blue line is the selection differential for other species and the green line is for *E. viminalis*. The dotted green line is N_e for *E. viminalis* at different selection levels.

selection. These results indicate that the variability present in *E. viminalis* can produce individuals with similar performance to other species currently being used in commercial plantations.

Discussion

Suitability of *E. viminalis* for frost-prone conditions compared to other *Eucalyptus* spp.

Some of the *E. viminalis* provenances showed individuals with high performance compared to other eucalypt species, particularly in comparison to *E. benthamii*, which is a species commonly used in southern Brazil with good growth that is adapted to conditions of severe frost (Graça et al. 1999). As such, the results suggest the possibility of obtaining genetic gains with the selection of the best individuals and the establishment of a breeding programme for these prospective individuals.

In southern Brazil, commercial plantations of *Eucalyptus* spp. have been established using frost-tolerant germplasm since the severe frosts that occurred in the 1970s (Oliveira & Pinto Junior 2021). For all tested species (except *E. longirostrata*) planting in this region has been recommended due to the similar climatic conditions between the regions where the species occurs in Australia and subtropical regions in Brazil. However, *E. viminalis* is currently in high demand, as it can be crossed with other species to achieve high-quality wood for pulp and fiber board (Cappa et al. 2010). In Irati, Paraná, several frosts can occur every year (Table 1 and Figure S1) which cause significant damage to the tropical *Eucalyptus* species that generally have higher levels of productivity. Plantations of *E. viminalis* have been recommended since the 1970s (Sturion et al. 1988; Higa et al. 1997), but to date their implementation has been minimal. Higa et al. (1997) tested 20 subtropical *Eucalyptus* species in Paraná and found that *E. viminalis* from Forest Lands SF, NSW, was among the provenances that produced the most wood at eight years of age. More provenance and progeny tests have been carried out in Paraná for the species (Sturion et al. 1988); however, no large plantation has been established to examine its productivity at an industrial scale.

Beyond its tolerance to frost, there are several other reasons why *E. viminalis* should be planted in southern Brazil, the most important being its high basic wood density (Sturion et al. 1988) and the coppice shoot quality and quantity after thinning (Higa & Sturion 2000). However, as new studies on *E. viminalis* are carried out, important information about their resistance to frost will become available along with other environmental considerations, such as different soil management treatments and/or tree spacing (Resende et al. 2018). It is also important to highlight the possible interactions between wood quality and tree growth in cold conditions, as this will certainly have an impact on wood growth patterns, and consequently stem form (Cao et al., 2020; Legowski et al., 2020).

Is there sufficient genetic variability in the *E. viminalis* population?

The genetic variation identified in this study suggests wide variability among families from each *E. viminalis* provenance. The high levels of variability found between provenances in this trial is expected since its base population has not yet been genetically improved. Further, materials were collected from sites across a wide geographical distribution, which although similar (below the Tropic of Capricorn – see Figure 1) have different climates and conditions to those in which the provenances were exposed (Kageyama & Jacob 1980). Our results corroborate the work by Konzen et al. (2017), who found a marked genetic variance within *Eucalyptus* spp. and *Corymbia* spp. provenances, ranging from 63.6% to 73.1%. The variation observed within *E. viminalis* provenances indicates its potential for improved frost resistance, as well as higher productivity and improved wood quality. Genetic variation between provenances of the same species can be quite useful, since these differences can result in distinct behaviors in a given environment when diverse geographic populations are used as a seed source (Brune 1978).

Furthermore, the values obtained for heritability were considered intermediate $\hat{h}^2_{(a)} = 0.18$ and $\hat{h}^2_{(fa)} = 0.40$ and high $\hat{h}^2_{(pfa)} = 0.89$. Ziegler and Tambarussi (2022) indicate that values ranging from $0.028 < \hat{h}^2(a) \leq 0.36$ are moderate, and $\hat{h}^2(a) > 0.53$ are very high. Individual heritability is consistent with values generally found in the literature for *Eucalyptus* populations (Resende 2002; Henriques et al. 2017; Paludeto et al. 2020) and are classified as intermediate. A high estimate indicates that there are high levels of genetic control at the provenance, family, and individual levels. Significant heritability values will also favor greater genetic selection gains, as discussed further in the following subsection.

The existence and magnitude of genetic diversity is fundamental at the beginning of breeding programmes, and throughout their development, since it is of paramount importance for the definition of strategies for conservation, management, and genetic improvement of a species (Duarte et al. 2015; Brandão et al. 2015). Existing genetic variation indicates that the selection will be effective for conservation when applied between and within families of each provenance, and for the selection of superior genotypes when applied between and within families of the best provenance.

Selection practices for the next *E. viminalis* breeding cycle

Several selection methods can be used in a tree breeding programme, and it is up to the breeder to verify which methods meet their short-, medium-, and long-term demands. However, it is always important to consider not only the expected genetic gains, but also the maintenance of genetic variability that will enable long-term gains (Vencovsky 1987; Costa et al., 2016; Araujo et al. 2021). For example, in the practice of forest tree breeding, the recurrent selection process is highly dependent on the existing genetic variability (as described in the previous subsection). Therefore, the continuation of a new selection cycle must contain individuals selected for the trait of interest, but also those that contain enough genetic variability so that subsequent cycles continue to be successful (Yamashita et al. 2018).

The appropriate size of a selected population should be based on two considerations: (1) what are the best individuals, i.e., those that provide the greatest genetic gains; and (2) its effective population size (N_e). To conduct a more parsimonious selection in relation to these two parameters (selection gain vs. N_e), we can choose to perform such selection at the meeting point between the two. For example, in the individual selection (Figure 4A), this occurred when we selected the best 50 individuals. Furthermore, the individual selection demonstrated that it is more robust since it reached a higher N_e even without including the provenances in the selection criteria. When we conduct selection considering individuals from the best families (Figure 4B), the N_e curve plateaus only when selecting at least 190 individuals, which is impractical for a recurrent forest selection programme.

The gains predicted from selection in relation to the species average varied from 49 to 82% in the strategy aimed at obtaining new, improved cultivars, and 16 to 50% in the strategy aimed at genetic conservation. This was expected as these studied *E. viminalis* populations are considered wild, without any breeding or improvement for Brazilian conditions. In comparison, Costa et al. (2016) obtained gains for volume ranging from 15.5 to 20.5% for *E. benthamii*. It is important to note that *E. benthamii* shows better performance in terms of productivity as it has been subjected to improvement programmes. The site where our trial was implemented is located in a region that typically produces timber from *E. benthamii*; thus, the clone used in this experiment is a traditional regional material with superior performance. Nevertheless, this does not undermine the potential shown here for *E. viminalis.* Without much effort put into genetic improvement, we found that *E. viminalis* can provide materials that are competitive with current *E. benthamii* clones used in the region after only a few cycles of recurrent selection.

In other studies, Silva et al. (2018) obtained gains ranging from 25.7% to 26.6% for an *E. urophylla* progeny, which has been the focus of most breeding programmes in Brazil. Meanwhile, Araújo et al. (2021) designed several improvement and conservation strategies for *E. dunnii*, in which the selection method and number of selected individuals varied, and obtained estimated gains ranging from 8 to 11%. In order to define the best strategy for the species, their study sought a balance between genetic gains and effective size, which is similar to the aims of the present study.

In addition to the improvement and conservation of the species, as proposed herein, another strategy that can be used to accelerate the development of new, more productive cultivars is hybridisation, which can be done with species that show better performance in productivity. Subsequently, the best individuals obtained in these crosses are cloned. As such, complementarity of the species traits used in crosses can be achieved (Assis 2014). Associated with the strategies discussed herein, is the integration of ideal silvicultural practices, such as tree spacing, fertilisation, and weed competition control (Binkley et al. 2017). For *E. viminalis*, this is particularly important, as the species is still poorly studied, and this type of information is lacking. With this, the ideal is to construct a species improvement programme combined with the development of management practices to achieve success with the species or its hybrids in the region.

It is important to highlight that, other studies with larger experimental scopes, including different environment testing, should be conducted for better comparison between species and the examination of species × environments interaction. Furthermore, the number of individuals per family was limited due to the poor availability of seminal propagules. However, the research results presented here can be very helpful for conducting initial *E. viminalis* breeding programmes, both for regions with frequent frosts, and to compare the performance of *E. viminalis* materials with other eucalypts traditionally used in southern Brazil.

Conclusions

Based on the preliminary tests presented in this study, there is potential for the commercial use of *E. viminalis* in cold and frost-prone regions in Brazil. The results for the best individuals are comparable to other species used in this region and to clones from breeding programmes grown in similar conditions.

Competing interests

The authors declare that there are no conflicts of interests.

Author contributions

RMLC - Writing original draft, review & editing; EDBS - Conceptualisation, Formal Analysis, Writing original draft, review & editing; JFFS - Writing original draft, review & editing; VAJ - Data curation, Investigation, RTR Methodology, Supervision, Writing original draft, review & editing; EVT - Conceptualisation, Project administration, Resources, Writing original draft, review & editing.

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Supplemental Information

Data archiving statement phenotype data (https://figshare.com/articles/dataset/Selection_of_Eucalyptus_viminalis_for_frost_compared_to_species_of_Eucalyptus_spp_to_the_subtropical_region_of_Brazil/17284874)

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