

Temperatures and substrates for germination and vigor of *Erythroxylum pauferrense* Plowman seeds

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ABSTRACT. *Erythroxylum pauferrense* is an endemic understory plant species of the Northeast Region of Brazil. The species is of great importance to the region and so ecophysiological studies are needed for its preservation. The objective of the present study was to determine the best substrates and temperatures for testing germination and seed vigor of *E. pauferrense*. An experiment was performed comprising a completely randomized design in a 5 x 4 factorial scheme, with five temperature regimes (20, 25, 30, 35°C constant and 20-30°C alternating) and four types of substrates (paper, vermiculite, sand and commercial substrate). The following characteristics were evaluated: germination percentage, first germination count, germination speed index, mean germination time, seedling length and dry mass (root and shoot). Paper and vermiculite substrates combined with constant temperatures of 20, 25, 30 and alternated between 20-30°C, provide greater seed germination and vigor while 35°C reduces seed physiological quality.

Keywords: *guarda-orvalho*; Erythroxylaceae; forest seeds; ecophysiology; seedlings; atlantic forest.

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Introduction

The family Erythroxylaceae Kunth contains 240 species in four genera, of which only *Erythroxylum* is widely distributed geographically. This genus contains about 230 species, approximately 180 of which are found in the Neotropical Region (Costa-Lima, Loiola, & Jardim, 2014). About 120 species of *Erythroxylum* have been recorded for Brazil, with their greatest diversity in forest environments in the Atlantic Forest domain and, mainly, in the Northeast Region where 40 species have been recorded (Loiola, Agra, Baracho, & Queiroz, 2007).

Erythroxylum pauferrense Plowman is an endemic understory plant species of the Northeast Region of Brazil where it occurs only in the state of Paraíba, with its type locality being in the municipality of Areia. The species is found in forest interiors among environments known as '*Brejos de Altitude*' (Loiola et al., 2007). The species is important for the preservation of endemic genetic resources of the region and as a food source for fauna during various periods of the year, which serve to propagate seeds in disturbed environments like those of '*Brejos de Altitude*'.

Most forest species are propagated via seeds, so knowledge of the ecophysiological requirements for germination is indispensable since adequate development for the establishment of species depends on the tolerance of seedlings to different environmental conditions (Silva, Matos, Farias, Sena, & Silva, 2017). Knowledge of the conditions necessary for normal germination is also important for the artificial germination of seeds because species respond in different ways to numerous factors, including dormancy, environmental conditions, water availability, luminosity, and substrate (Carvalho & Nakagawa, 2012).

Substrate directly influences germination by providing physical support for the seeds and adequate conditions, such as structure, aeration and water availability, not only for germination but also for the subsequent development of seedlings (Oliveira, França, Torres, Nogueira, & Freitas, 2016). Temperature is another important factor for germination because it directly influences the biochemical reactions that regulate the metabolism of the germination process and, consequently, influences germination speed and final germination percentage (Gonçalves et al., 2015). Thus, the behavior of seeds, specifically those of forest species, varies as a function of different temperatures and substrates. Accordingly, there is no optimum and uniform temperature for the germination of seeds of all species, although the range of 20 to

30°C has been recommended as ideal for the germination of seeds of various subtropical and tropical species (Guedes et al., 2011). Specific studies have provided information on the influence of substrates and temperatures on the germination of forest seeds (Oliveira, Ribeiro, Pereira, & Silva, 2013; Bassaco, Nogueira, & Cosmo, 2014; Gonçalves et al., 2015; Shibata, Pavelski, Miranda, & Oliveira, 2016; Silva et al., 2017).

Although Brazil has a great diversity of species of Erythroxylaceae, information on the development, propagation, physiology and ecology of these species is still scarce. Considering the lack of specific information for establishing criteria for testing germination and vigor, the objective of the present study was to determine the best conditions of substrate and temperature for performing these tests with *Erythroxylum pauferrense*.

Material and methods

The research was carried out in the Laboratory of Seed Analysis (LAS) of the Agricultural Science Center of the *Universidade Federal da Paraíba* (CCA/UFPB), in the municipality of Areia, state of Paraíba, Brazil. The seeds of *E. pauferrense* used for the study were harvested from 20 mother plants, located in the *Parque Estadual Mata do Pau-Ferro* (6° 58' 12" S and 35° 42' 15" W) (Areia, Paraíba, Northeast Region of Brazil). The seeds were processed by manual extraction of the adhered pulp, and then dried naturally in the shade for 24 hours.

The experimental design was completely randomized with treatments distributed in a 5 x 4 factorial scheme with four replicates of 25 seeds each. The treatments comprised five temperature regimes (constant 20, 25, 30, 35°C and alternating 20-30°C, with photoperiod of 8 hours) and four substrates [blotting paper, vermiculite, sand and commercial substrate (Bioplant®)].

The water content of the seeds was determined by the oven method at a temperature of $105 \pm 3^\circ\text{C}$ for 24 hours, using four replicates of five grams of seeds each (Brasil, 2009). The seeds used for testing germination and vigor were previously submitted to disinfestation with 2% sodium hypochlorite solution for five minutes.

The germination test was performed in a BOD germination chamber (Biological Oxygen Demand) with the seeds sown in gerbox-type acrylic boxes containing one of the substrate treatments, where the seeds were placed on blotting paper, vermiculite, sand or commercial substrate with seeds sown 0.5 cm deep and covered with a thin layer of said substrates (except paper) — moistened with distilled water up to 60% of the retention capacity or, in the case of the blotting paper, an amount equivalent to 2.5 times its dry mass (Brasil, 2009). The number of germinated seeds was observed daily from the 6th day to the 16th day post-sowing. The germination criterion used was the emergence of cotyledons with the consequent appearance of the hypocotyl. The percentages of normal seedlings (strong and weak), abnormal seedlings, dormant seeds and dead seeds were calculated.

The first germination count (FGC) was performed by counting the number of seeds germinated on the first day of the standard germination test and recording the data on the 6th day post-sowing. The germination speed index (GSI) was calculated according to Maguire (1962) using means of daily counts of the number of seeds germinated until the 16th day post-sowing. Mean germination time (MGT) was calculated according to Labouriau (1983) using daily counts of the number of seeds germinated.

Lengths of the aerial part (LAP) and root (LR) of normal seedlings of each replicate were measured at the end of the germination test using a graduated ruler. The result was expressed in cm seedling⁻¹. Seedling dry mass was obtained by drying them in a forced air circulation oven at 65°C until reaching constant weight. The aerial part and root were separated and packed in kraft paper bags and dried. The aerial parts and roots were later weighed using an analytical balance with a precision of 0.001 g. The result was expressed in g seedling⁻¹.

Data for normal seedlings, abnormal seedlings, dormant seeds and dead seeds were transformed as $\sqrt{(x + 0.5)}$ and submitted to analysis of variance, with the means being grouped by the Scott-Knott test. Means of the original non-transformed data are presented.

Results and discussion

The water content of the seeds was 42.94%, which is considered high due to the seeds exhibiting recalcitrant behavior, since they lose their viability in a short period of time. Species of the family Erythroxylaceae tend to exhibit recalcitrant characteristics, with seeds maintaining a critical level of water content, while lower levels cause viability loss (Silva, Oliveira, Cesarino, & Vieira, 2014).

Table 1 shows the occurrence of significant interactions between temperature and substrate ($p < 0.05$), indicating that there is at least one combination of these two factors that optimizes germination percentage. Bassaco et al. (2014), Gomes, Oliveira, Ferreira, and Batista (2016) and Silva et al. (2017) also reported a significant interaction between temperature and substrate for seed germination of other forest species of the Atlantic Forest.

Mean values for normal seedlings (strong and weak), abnormal seedlings, dormant seeds and dead seeds revealed that the highest germination percentages were obtained on paper at 20, 25, 30 and 20-30°C, vermiculite at 20 and 30°C, sand at 25, 30 and 20-30°C, and commercial substrates at 20-30°C (Table 2). Alternating temperatures produced the highest germination percentages for the substrates used, except for commercial substrate (47.20% total germination) (Table 2). This finding is probably related to the similarity between this treatment and the conditions found during seed formation since the ideal temperature for seed germination is directly associated with the ecological characteristics of the species (Larcher, 2006). No satisfactory germination occurred at 35°C, regardless of the substrate used, while the commercial substrate affected germination regardless of temperature, producing a greater number of abnormal seedlings and dormant and dead seeds than the other substrates. These results show that seeds of *E. pauferrense* are able to germinate over a wide temperature range, which is important for reproduction and perpetuation of the species.

Temperature alternation of 20-30°C favors germination because it facilitates the entry of water into the internal tissues of the seeds from tegument rupture. Silva, Cesarino, Sader, and Lima (2008) and Silva et al. (2014) found the best temperatures for the germination of coca (*Erythroxylum squamatum* Sw.) seeds to be between 25 and 30°C.

The substrates of paper, vermiculite and sand had the highest percentages of germination (% normal seedlings) at temperatures of 20, 25, 30 and temperature alternation of 20-30°C (Table 2). These results may be due to the adequate balance between moisture and aeration of the substrates, allowing the ideal conditions for water to enter the seed and, consequently, promote germination. The germination results revealed that the seeds of *E. pauferrense* are adapted to variation in temperature, as has been found by other studies with forest species including *Amburana cearensis* (Allemão) A. C. Smith (Guedes et al., 2010) and *Piptadenia moniliformis* Benth. (Azerêdo, Paula, Valeri, & Moro, 2010).

The highest mean values for the first germination count were with paper at 25°C and vermiculite at 30° and 20-30°C, while the lowest were for commercial and sand substrates (Table 3). These results demonstrate that paper (at 25°C) and vermiculite (at 30 and 20-30°C) provide better conditions for seeds to absorb water and trigger germination. Water plays an essential role in the cell metabolism of seeds by acting on enzymatic activity, solubilization and transport of reactants, and hydrolytic digestion of stored reserve substances (Marcos Filho, 2015).

The highest values for the germination speed index (GSI) were obtained with paper and vermiculite substrates at all temperatures tested. Sand presented similar results at temperatures of 25 and 30°C (Table 3). This germination behavior may be indicative of specific enzymatic mechanisms of the species that operate at different temperatures as a result of ecological adaptations of the species to the environment (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). Thus, these combinations were found to be the best by promoting the highest percentages of germination in a shorter amount of time, which is in agreement with the findings of Nogueira, Ribeiro, Freitas, Gurgel, and Nascimento (2013) and Barrozo et al. (2014). Paper substrate at a temperature of 25°C produced the highest GSI for seeds of *Diptychandra aurantiaca* (Mart.) (Oliveira et al., 2013) Studying seeds of *Parkia platycephala* Benth., Silva et al. (2017) found that the temperature of 30°C provided the best results for GSI in vermiculite substrate.

Table 1. F-test and mean values for germination (G), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), length of the aerial part (LAP), length of the root (LR), length of the seedlings (LS), aerial part dry mass (APDM), root dry mass (RDM) and total dry mass (TDM) of seedlings of *Erythroxylum pauferrense* as a function of temperatures (T) and substrates (S).

Source of variation	F-test									
	G	FGC	GSI	MGT	LAP	LR	LS	APDM	RDM	TDM
T	107.3**	155.1**	83.7**	5.4**	133.2**	139.1**	162.9**	74.5**	23.8**	62.2**
S	234.0**	222.9**	210.0**	7.1**	154.1**	183.5**	200.3**	34.3**	36.3**	35.1**
TxS	9.3**	31.5**	5.9**	8.4**	7.4**	11.2**	9.01**	6.8**	5.8**	6.40**
Mean	76.1	45	2.72	7.28	3.53	1.52	5.05	0.04	0.01	0.05
CV (%)	7.58	9.82	11.01	11.38	10.23	12.22	11.30	13.71	12.2	12.40

**significant at 1% by the F-test.

Table 2. Percentage of normal seedlings (strong and weak), abnormal seedlings, dormant seeds and dead seeds of *Erythroxylum paufferense* as a function of temperatures and substrates.

Variables (%)	Substrates	Temperatures (°C)					Mean
		20	25	30	35	20 - 30	
Normal seedlings	Paper	92 aA	95 aA	98 aA	76 aB	92 aA	90.60
	Vermiculite	97 aA	87 aB	97 aA	64 bC	92 aB	87.40
	Sand	78 bB	91 aA	87 bA	54 cC	85 aA	79.00
	Commercial	54 cC	60 bB	48 cC	4 dD	70 bA	47.20
	Mean	80.25	83.25	82.50	49.50	84.75	
	CV (%)	7.58					
Strong normal	Paper	77 bB	73 bB	93 aA	76 aB	82 aB	80.20
	Vermiculite	92 aA	87 aA	96 aA	52 bB	90 aA	83.40
	Sand	69 bB	89 aA	84 aA	20 cC	64 bB	65.20
	Commercial	41 cA	37 cA	38 bA	4 dB	41 cA	32.20
	Mean	69.75	71.50	77.75	38.00	69.25	
	CV (%)	14.54					
Weak normal ¹	Paper	15 aA	22 aA	5 bB	0 cB	12 aA	10.80
	Vermiculite	5 aA	0 bB	1 bB	12 bA	2 bB	4.00
	Sand	9 aB	2 bB	3 bB	34 aA	21 aA	13.80
	Commercial	13 aB	23 aA	11 aB	0 cC	29 aA	15.20
	Mean	10.50	11.75	5.00	11.50	16.00	
	CV (%)	30.58					
Abnormal seedlings ¹	Paper	5 bB	3 aB	0 bB	12 bA	3 bB	4.60
	Vermiculite	2 bB	4 aB	0 bB	16 bA	1 bB	4.60
	Sand	5 bB	1 aB	0 bB	11 bA	5 bB	4.40
	Commercial	15 aB	5 aC	26 aA	36 aA	14 aB	22.75
	Mean	6.75	2.66	6.50	18.75	5.75	
	CV (%)	30.63					
Dormant seeds ¹	Paper	2 bA	1 bA	0 cA	2 cA	1 aA	1.20
	Vermiculite	0 bB	2 bB	1 cB	11 bA	3 aB	3.40
	Sand	6 aB	2 bB	4 bB	16 bA	5 aB	6.60
	Commercial	10 aB	12 aB	9 aB	23 aA	6 aB	12.00
	Mean	4.50	4.20	3.50	13.00	15.00	
	CV (%)	22.93					
Dead seeds ¹	Paper	1 cB	1 bB	2 bB	10 cA	2 aB	3.20
	Vermiculite	1 cA	7 bA	2 bA	9 cA	5 aA	4.80
	Sand	11 bB	6 bB	9 aB	19 bA	5 aB	10.00
	Commercial	23 aB	23 aB	16 aC	37 aA	10 aC	21.80
	Mean	9.00	9.25	7.25	18.75	5.50	
	CV (%)	24.98					

Values within columns followed by the same lower case letter and values within rows followed by the same upper case letter do not differ significantly by Scott-Knott test at 5% probability; ¹Data transformed as $\sqrt{(x + 0.5)}$.

Table 3. First germination count (FGC), germination speed index (GSI) and mean germination time (MGT) of seeds of *Erythroxylum paufferense* as a function of temperature and substrate.

Variables	Substrates	Temperatures (°C)					Mean
		20	25	30	35	20 - 30	
FGC (%)	Paper	59 aC	92 aA	69 bB	11 bD	71 bB	52.50
	Vermiculite	44 bB	73 bA	82 aA	43 aB	81 aA	64.60
	Sand	25 cB	81 bA	73 bA	4 bC	11 dC	38.80
	Commercial	7 dB	7 cB	33 cA	3 bB	31 cA	16.20
	Mean	33.75	53.66	64.25	15.25	48.50	
	CV (%)	9.82					
GSI	Paper	3.58 aA	3.94 aA	3.85 aA	2.18 aB	3.60 aA	3.43
	Vermiculite	3.43 aA	3.49 aA	3.89 aA	2.46 aB	3.75 aA	3.40
	Sand	2.41 bB	3.71 aA	3.50 aA	1.51 bC	2.30 bB	2.68
	Commercial	1.08 cC	1.45 bB	1.92 bA	0.15 cD	2.18 bA	1.35
	Mean	2.62	3.14	3.29	1.57	2.96	
	CV (%)	11.01					
MGT (days)	Paper	6.50 bB	6.03 bB	6.42 aB	9.43 aA	6.38 bB	6.95
	Vermiculite	7.32 bA	6.27 bA	6.32 aA	6.61 bA	6.16 bA	6.53
	Sand	8.76 aA	6.15 bB	6.27 aB	9.29 aA	9.81 aA	8.05
	Commercial	9.90 aA	9.43 aA	6.44 aB	4.75 cC	7.38 bB	7.58
	Mean	8.12	6.97	6.36	7.52	7.43	
	CV (%)	11.38					

Values within columns followed by the same lower case letter and values within rows followed by the same upper case letter do not differ significantly by Scott-Knott test at 5% probability.

The tested temperatures and substrates affected the mean germination time of the seeds, with shorter germination times in the commercial substrate at 35°C (Table 3), conditions that also produced a low percentage of normal and high percentage of abnormal seedlings. However, at other temperatures the commercial substrate delayed germination and, consequently, extended the germination time of seeds. The occurrence of this effect shows that temperature and the ideal substrate promote faster germination, indicating conditions close to ideal for the temporal distribution of the germination of the seeds (Oliveira, Souza, Carvalho, & Ojeda, 2017).

The highest mean length of the aerial part (LAP) of seedlings was observed for vermiculite at constant temperatures of 25 and 30°C, and for sand at 25°C (Table 4). Azevedo, Bruno, Gonçalves, and Quirino (2010) also found vermiculite and sand at temperatures of 30 and 20-30°C to produce the greatest seedling length for *Crescentia cujete* L.

Sand at constant temperatures of 30°C produced longer primary roots (LR) and thus provided better conditions for the development of the root system of seedlings of *E. pauferrense*. According to Silva et al. (2017), seedlings with fully developed roots are the result of the vigor of the seeds that produced them. These authors also emphasized that more rapid and uniform emergence will occur, adapting to different field conditions and achieving normal development.

Vermiculite provided the greatest growth of *E. pauferrense* seedlings at all temperatures (Table 4). Thus, it is evident that vermiculite is of fundamental importance to the growth of seedlings and provides ideal conditions for testing vigor and germination (Guedes et al., 2011).

The highest values for aerial part dry mass (APDM) were obtained when seeds were submitted to constant temperatures of 25 and 30°C with vermiculite or sand as a substrate, and with alternating temperatures with vermiculite substrate (Table 5). The highest values for root dry mass (RDM) were obtained with sand at 20, 25 and 30°C, vermiculite at 25 and 20-30°C, and paper at 30 and 20-30°C (Table 5). The highest values for total dry mass (TDM) were recorded at constant temperatures of 25 and 30°C in sand and vermiculite, and with alternating temperatures with vermiculite (Table 5).

Thus, the greater accumulation of dry mass by both the aerial part and the root occurred in conditions of higher temperatures and with the substrates vermiculite and sand, which offered favorable conditions for seedling growth. According to Guedes et al. (2011), adequate development of seedlings, with regard to length and dry mass, can be explained by the favorable characteristics of the substrate used, such as proper aeration and good water retention capacity.

Table 4. Length of the aerial part (LAP), length of the root (LR) and length of the seedlings (LS) of seedlings of *Erythroxylum pauferrense* as a function of seeds submitted to different temperatures and substrates.

Variables	Substrates	Temperatures (°C)					Mean
		20	25	30	35	20 - 30	
LAP (cm seedling ⁻¹)	Paper	3.40 aC	4.23 bB	5.35 bA	0.72 cD	4.24 bB	3.58
	Vermiculite	4.07 aC	6.46 aA	6.94 aA	2.30 aD	5.45 aB	5.04
	Sand	3.26 aC	6.35 aA	5.42 bB	1.30 bD	3.73 bC	4.01
	Commercial	1.32 bB	1.56 cB	2.15 cA	0.10 cC	2.31 cA	1.48
	Mean	3.01	4.65	4.96	1.10	3.93	
	CV (%)	10.23					
LR (cm seedling ⁻¹)	Paper	2.26 aA	1.55 bB	2.46 bA	0.31 bC	2.12 aA	1.74
	Vermiculite	2.38 aA	2.32 aA	2.32 bA	0.64 aB	2.18 aA	1.97
	Sand	1.80 bC	2.42 aA	2.74 aA	0.29 bD	2.16 aB	1.88
	Commercial	0.51 cA	0.58 cA	0.47 cA	0.02 bB	0.80 bA	0.48
	Mean	1.73	1.71	2.00	0.31	1.81	
	CV (%)	12.22					
LS (cm seedling ⁻¹)	Paper	5.66 bB	5.78 bB	7.81 bA	1.02 bC	6.35 bB	5.24
	Vermiculite	6.44 aC	8.78 aA	9.26 aA	2.94 aD	7.62 aB	7.00
	Sand	5.06 bB	8.77 aA	8.16 bA	1.58 bC	5.89 bB	5.89
	Commercial	1.82 cA	2.14 cA	2.61 cA	0.12 cB	3.12 cA	1.96
	Mean	4.44	6.37	6.96	1.41	5.74	
	CV (%)	11.65					

Values within columns followed by the same lower case letter and values within rows followed by the same upper case letter do not differ significantly by Scott-Knott test at 5% probability.

Table 5. Aerial part dry mass (APDM), root dry mass (RDM) and total dry mass (TDM) of seedlings of *Erythroxylum pauferrense* as a function of seeds submitted to different temperatures and substrates.

Variables	Substrates	Temperatures (°C)					Mean
		20	25	30	35	20 - 30	
APDM (g seedling ⁻¹)	Paper	0.0279 aC	0.0386 bB	0.0525 bA	0.0127 bD	0.0470 bA	0.0357
	Vermiculite	0.0286 aB	0.0659 aA	0.0705 aA	0.0327 aB	0.0649 aA	0.0525
	Sand	0.0305 aB	0.0600 aA	0.0664 aA	0.0187 bC	0.0403 bB	0.0431
	Commercial	0.0285 aB	0.0278 cB	0.0439 bA	0.0263 aB	0.0350 bA	0.0323
	Mean	0.0288	0.0480	0.0583	0.0226	0.0468	
	CV (%)	13.71					
RDM (g seedling ⁻¹)	Paper	0.0164 bB	0.0143 bB	0.0197 aA	0.0053 aC	0.0201 aA	0.0152
	Vermiculite	0.0169 bA	0.0187 aA	0.0132 bB	0.0107 aB	0.0184 aA	0.0156
	Sand	0.0219 aA	0.0216 aA	0.0233 aA	0.0069 aB	0.0185 aA	0.0184
	Commercial	0.0066 cA	0.0072 cA	0.0103 bA	0.0088 aA	0.0105 bA	0.0086
	Mean	0.0154	0.0154	0.0166	0.0079	0.0168	
	CV (%)	12.20					
TDM (g seedling ⁻¹)	Paper	0.0442 aB	0.0528 bB	0.0722 bA	0.0180 bC	0.0670 bA	0.0508
	Vermiculite	0.0454 aB	0.0845 aA	0.0836 aA	0.0433 aB	0.0832 aA	0.0680
	Sand	0.0524 aB	0.0816 aA	0.0896 aA	0.0255 bC	0.0587 cB	0.0616
	Commercial	0.0351 aB	0.0350 cB	0.0542 cA	0.0350 aB	0.0454 dA	0.0409
	Mean	0.0443	0.0635	0.0749	0.0305	0.0636	
	CV (%)	12.01					

Values within columns followed by the same lower case letter and values within rows followed by the same capital letter do not differ significantly by Scott-Knott test at 5% probability.

Conclusion

Germination and vigor of *E. pauferrense* seeds are influenced by temperature and substrate;

Paper and vermiculite substrates had higher germination percentages and germination speeds for seeds of *E. pauferrense* at temperatures of 20, 25, 30 and temperature alternation of 20-30 °C;

The temperature of 35°C and commercial substrate are not recommended for testing germination and vigor of seeds of *E. pauferrense*.

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