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ARTICLE

Non-parametric survival analysis in seed germination of forest species

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ABSTRACT: In statistical analyses of seed germination experiments, it is essential to ensure that the assumptions of the chosen methods are not violated to obtain valid and reliable results. When these assumptions are violated, alternative methods should be considered, one of which is survival analysis. This study aimed to evaluate the application of this methodology, using a nonparametric approach, to assess the germination of *Cecropia pachystachya* and *Jacaranda caroba*. In the first experiment, alternating temperatures of 30/20 °C and 25/15 °C were applied under two light conditions (light and darkness). In the second, germination was assessed at constant temperatures ranging from 15 to 35 °C. Germination reached or exceeded 50% in both species under alternating temperatures and light conditions. In continuous darkness, *J. caroba* exhibited a higher germination speed and percentages, whereas *C. pachystachya* was unaffected by light conditions. The recommended alternating temperature for both species was 30/20 °C. The optimal constant temperatures were 25 °C for *J. caroba* and 30 °C for *C. pachystachya*. It is concluded that the nonparametric survival analysis technique effectively discriminates seed germination of the studied species.

Index terms: *Cecropia pachystachya*, censored data, *Jacaranda caroba*, photoperiod, temperature.

RESUMO: Em análises estatísticas de experimentos de germinação de sementes, é essencial garantir que as pressuposições dos métodos escolhidos não sejam violadas para obter resultados válidos e confiáveis. Quando essas pressuposições são violadas, métodos alternativos devem ser considerados, um dos quais é a análise de sobrevivência. Este estudo teve como objetivo avaliar a aplicação desta metodologia, usando uma abordagem não paramétrica, para avaliar a germinação de *Cecropia pachystachya* e *Jacaranda caroba*. No primeiro experimento, temperaturas alternadas de 30/20 °C e 25/15 °C foram utilizadas sob duas condições de luz (claro e escuro). No segundo, a germinação foi avaliada em temperaturas constantes variando de 15 a 35 °C. A germinação atingiu ou ultrapassou 50% em ambas as espécies sob temperaturas e condições de luz alternadas. No escuro contínuo, *J. caroba* exibiu maior velocidade e porcentagem de germinação, enquanto *C. pachystachya* não foi afetada pelas condições de luz. A temperatura alternada recomendada para ambas as espécies foi de 30/20 °C. As melhores temperaturas constantes foram 25 °C para *J. caroba* e 30 °C para *C. pachystachya*. Conclui-se que a técnica de análise de sobrevivência não paramétrica discrimina efetivamente a germinação de sementes das espécies estudadas.

Termos para indexação: *Cecropia pachystachya*, dados censurados, *Jacaranda caroba*, fotoperíodo, temperatura.

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INTRODUCTION

In some studies, the seed germination is statistically evaluated using mean tests to determine the best treatment or regression analysis to examine the response's behavior about explanatory variables. One reason for using these techniques in seed science is the low residual variability of cultivated species, leading to higher germination standards and often results in non-verifying normality assumptions (Carvalho et al., 2018). However, there needs to be more evidence of the appropriateness of these methods, and the assumptions must always be verified.

Under certain circumstances, the limitations of traditional statistical techniques become apparent. Data transformations are often necessary to meet the normality assumptions required by specific tests, standardize variance, or normalize the data for statistical models. However, these transformations can lead to a loss of the original meaning, as the transformed numerical values lose their real significance and can be difficult to interpret, particularly in regression models (Warton and Hui, 2011; Sileshi, 2012; Stroup, 2015). This highlights the need for a more robust and interpretable approach in research.

Another issue is that these techniques fail to assess the behavior of germination over time. Additionally, during germination tests, seeds often rot or fail to germinate. This information, which is frequently discarded, is essential and must be included in the statistical analysis to draw accurate conclusions. One statistical technique that incorporates this information is Survival Analysis.

Survival analysis comprises a set of statistical techniques and models used to analyze experiments where the response variable is the time until an event of interest occurs (Colosimo and Giolo, 2024). This period is also referred to as the failure time, with "failure" denoting the occurrence of the event in question, in this case, germination. An essential characteristic of survival data is the presence of censoring, which refers to the partial observation of the response. Censoring is considered incomplete observation and is characterized by data where the event has yet to be verified, either because the study concluded without the event of interest occurring, as with hard seeds, or due to an unrelated cause. This means the actual time until the event is longer than the observed period.

In the presence of censoring, commonly used statistical techniques become unfeasible as they do not accommodate partial observations (Onofri et al., 2010; McNair et al., 2012; Collett, 2023). All observations in a study, whether complete or partial, must be used in the statistical analysis. Even if incomplete, censoring provides essential information about the event's timing, and omitting it can lead to biased conclusions (Colosimo and Giolo, 2024). Therefore, statistical methods incorporating complete and partial observations into the analysis are essential.

In non-parametric survival analysis, the data does not need to assume a specific probability distribution, such as the normal distribution. This approach is suitable for seed germination data from forest species natives, which have yet to undergo genetic improvement and can exhibit significant variability in their germination.

Some peculiarities of the seed formation process of some native Brazilian species may limit the analysis of the true seed germination potential (Santana et al., 2018). As a result, low germination percentages are often incorrectly attributed to certain species. In this context, survival analysis techniques provide the most appropriate and robust methods for analyzing germination data (McNair et al., 2012).

This study aims to evaluate the use of this methodology, under a non-parametric approach, for the germination of *Cecropia pachystachya* and *Jacaranda caroba* seeds under different light conditions and temperatures.

MATERIAL AND METHODS

The germination data used in this work are from the species *C. pachystachya* (Embaúba) and *J. caroba* (Caroba) and were obtained from a database of functional characteristics of seeds and germination experiments on vegetation from Brazilian rocky outcrops made available free of charge by Ordóñez-Parra et al. (2023).

Database description

The seeds of *C. pachystachya* and *J. caroba* were collected in *Campos Rupestre*, 1150 m above sea level, in the Serra do Cipó (19°17'S, 43°33'W), Minas Gerais, Brazil.

After collection and extraction, the seeds were aseptically cleaned to perform two germination experiments in a controlled environment using BOD (Biochemical Oxygen Demand) chambers. The experimental unit consisted of a Petri dish (9 cm in diameter) with 25 seeds containing two sheets of filter paper moistened with a 2% Nystatin solution, which was used as an antifungal agent.

In the first experiment, germination was assessed for each species. A completely randomized design, with four replications, was used in a factorial scheme, combining two temperatures (30/20 and 25/15 °C) and two light conditions (daily photoperiod of 12/12h and under continuous darkness). The Petri dishes were covered with two sheets of aluminum foil for germination in the darkness, and germination counting was performed under a green safety light.

The second experiment evaluated the effect of constant temperatures on seed germination. Five constant temperatures (15, 20, 25, 30, and 35 °C) were used for each species. The choice of temperatures was based on the daily and seasonal temperature variation in the *Campos Rupestres* (Oliveira et al., 2015; 2020).

All the Petri dishes were checked for seed germination every 24 hours for 30 days. Radicle protrusion was the criterion for germination.

Survival Analysis

In survival analysis, the survival function plays a fundamental role. In the nonparametric approach, the Kaplan-Meier estimator is used to quantify the probability distribution of a failure in a population (Kaplan and Meier, 1958). The survival function is defined as:

$$S(t) = P(T > t) \tag{1}$$

where *S(t)* is the probability of a seed not germinating by a specific time T, i.e., the probability of an observation surviving to time t.

Consequently, the cumulative distribution function is defined as:

$$F(t) = 1 - S(t) \tag{2}$$

where *F(t)* is the probability that germination occurs.

The Kaplan-Meier estimator used to calculate nonparametric estimates of the survival function is defined as:

$$\hat{S}(t) = \prod_{j:t_j<t} \left(1 - \frac{d_j}{n_j}\right) \tag{3}$$

where $t_j < t = t_1 < t_2 \ldots < t_k$ are the k distinct and ordered germination times; d_j is the number of germinations at t_j, j = 1, ..., k; and n_j is the number of individuals at risk at t_j, i.e., the individuals that have not germinated and have not been censored up to the instant immediately prior to t_j.

The cumulative distribution functions *F(t)* were estimated from the $\hat{S}(t)$ and graphs were drawn for the germination curves. The percentiles were also estimated to check the germination percentage over time.

Once the germination curves had been determined, the null hypothesis was tested: H_0: $S_1(t)$=$S_2(t)$, that these curves are the same for two groups of individuals. For this purpose, was used the Log-rank test (Mantel, 1966), given by:

$$T = \frac{\left[\sum_{j=1}^{k} \left(d_{2j} - w_{2j}\right)\right]^2}{\sum_{j=1}^{k} \left(V_j\right)_2} \tag{4}$$

where d_{2j} is the number of germinations that occur in the second group due to cause *j*, which follows a hypergeometric distribution; w_{2j} is the mean of d_{2j}; $(V_j)_2$ is the variance of d_{2j}; T, under the null hypothesis H_0: $S_1(t)$=$S_2(t)$, has a $\chi 2$ distribution with 1 degree of freedom for large samples.

The Wilcoxon test (Gehan, 1965) can be an alternative to the Log-rank test. While the Log-rank test assigns the same weight to the entire time axis, focusing on longer times, the Wilcoxon test emphasizes the information at the beginning of the germination curve where the number at risk is significant, allowing early germinations to be given more weight than later germinations (Colosimo and Giolo, 2024). The Wilcoxon test is given by:

$$S = \frac{\left[\sum_{j=1}^{k} u_j \left(d_{2j} - w_{2j}\right)\right]^2}{\sum_{j=1}^{k} u_j^2 \left(V_j\right)_2} \quad (5)$$

where $u_j = n_j$, d_{2j} is the number of germinations that occur in the second group due to cause *j*, which follows a hypergeometric distribution; w_{2j} is the mean of d_{2j}; (V_j) is the variance of d_{2j}; S, under the null hypothesis H_0: $S_1(t)=S_2(t)$, has a χ2 distribution with 1 degree of freedom for large samples.

All statistical analyses were carried out using the *survival* package version 3.7-0 (Therneau, 2024) of the R software version 4.4.1 (R Core Team, 2024).

RESULTS

Alternating temperatures

The results of seed germination for the studied species exposed to different alternating temperatures and light conditions are presented in Table 1. Under darkness conditions, the germination percentages of *C. pachystachya* seeds increased by 8%, and *J.* caroba exhibited a more pronounced increase of 26% when comparing 25/15 °C with 30/20 °C temperatures. In light conditions, the germination percentages increased by 15% and 11% for *C. pachystachya* and *J. caroba*, respectively, in response to these temperature changes.

The final germination percentages of *C. pachystachya* seeds were very similar in both light conditions within each temperature studied (Figures 1A and 1B). However, the germination curves displayed significant differences ($p < 0.05$), as indicated by the Wilcoxon test. This significant difference can be attributed to a higher germination speed observed in seeds subjected to the presence of light, achieving 45% germination in 13 and 9 days at 25/15 °C and 30/20 °C, respectively (Table 2). In continuous darkness, the same germination percentages were reached only after 18 and 13 days, respectively (Table 2). This underscores the effect of light conditions on germination curves.

Under the same light conditions (Figures 1C and 1D), alternating temperatures had a significant effect ($p < 0.05$) on the germination curves for both tests. Another important information is the median germination time (T_{50}), which represents the time required to achieve 50% germination or the 50th percentile. The alternating temperature of 30/20 °C yielded the fastest germination speed and highest percentages, reaching T_{50} in 10 and 15 days under light and continuous darkness, respectively (Table 2).

Table 1. Percentage of failures (germination) and censoring (non-germination) of *C. pachystachya* and *J. caroba* seeds in response to different alternating temperatures (25/15 °C and 30/20 °C) and light conditions (darkness and light).

Treatment	Darkness		Light	
	Failure (%)	Censoring (%)	Failure (%)	Censoring (%)
C. pachystachya (25/15 °C)	54	46	49	51
C. pachystachya (30/20 °C)	62	38	64	36
J. caroba (25/15 °C)	60	40	66	34
J. caroba (30/20 °C)	86	14	77	23

n = 100 seeds per treatment.

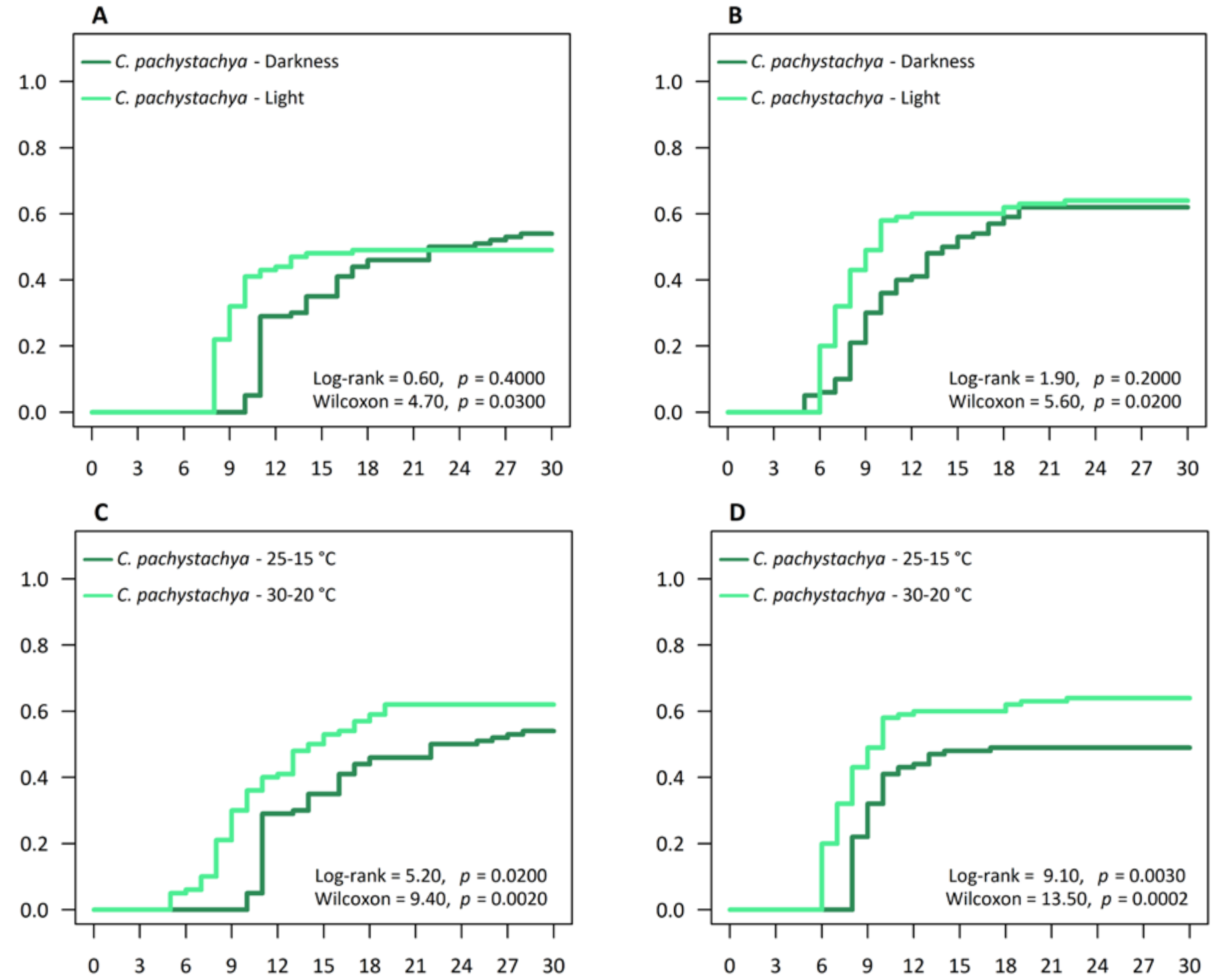

Figure 1. Seed germination probability of *C. pachystachya* at alternating temperatures and light conditions, estimated by the nonparametric Kaplan-Meier estimator. (A) 25/15 °C, (B) 30/20 °C, (C) darkness, and (D) light.

Table 2. Germination time (days) of *C. pachystachya* and *J. caroba* seeds at alternating temperatures and light conditions, considering percentiles from 5 to 90.

Treatment	Percentile																	
	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5
Darkness																		
C. pachystachya (25/15 °C)	–	–	–	–	–	–	–	–	24	18	16	15	14	11	11	11	11	11
C. pachystachya (30/20 °C)	–	–	–	–	–	–	19	17	15	13	12	10	10	9	8	8	8	6
J. caroba (25/15 °C)	–	–	–	–	–	–	30	26	21	19	18	18	17	16	16	14	12	11
J. caroba (30/20 °C)	–	20	12	11	10	10	10	9	8	8	7	7	6	6	6	6	6	6
Light																		
C. pachystachya (25/15 °C)	–	–	–	–	–	–	–	–	–	13	10	10	9	9	8	8	8	8
C. pachystachya (30/20 °C)	–	–	–	–	–	–	15	10	10	9	8	8	7	7	7	6	6	6
J. caroba (25/15 °C)	–	–	–	–	–	27	23	21	19	18	16	15	14	13	12	11	10	9
J. caroba (30/20 °C)	–	–	–	18	14	13	11	11	10	10	10	9	9	8	7	6	6	6

For *J. caroba*, light conditions influenced the germination curves differently at the two alternating temperatures (Figures 2A and 2B). At 25/15 °C, the light favored germination, with a percentage of 65% over 27 days. In contrast, at 30/20 °C, continuous darkness favored germination, achieving 85% in 20 days (Table 2).

Significant differences were observed between temperatures in the different light conditions (Figures 2C and 2D). The highest germination percentages for *J. caroba* seeds occurred at an alternating temperature of 30/20 °C. In this temperature, from days 6 to 12, the germination curve was steeper, with higher germination in this interval, achieving T_{50} in 8 and 10 days under darkness and light, respectively (Table 2). On the other hand, germination was slower at 25/15 °C, with T_{50} achieved in 21 and 19 days under the same conditions.

Table 3 presents an alternative approach to analyzing the data using classical methods (ANOVA and mean testing). For *C. pachystachya*, significant differences between alternating temperatures were observed only in the Germination Speed Index (GSI). However, for *J. caroba*, significant differences were observed in both germination percentages and GSI. The 30/20 °C temperature yielded higher percentages for both species than 25/15 °C, while light conditions were not statistically significant.

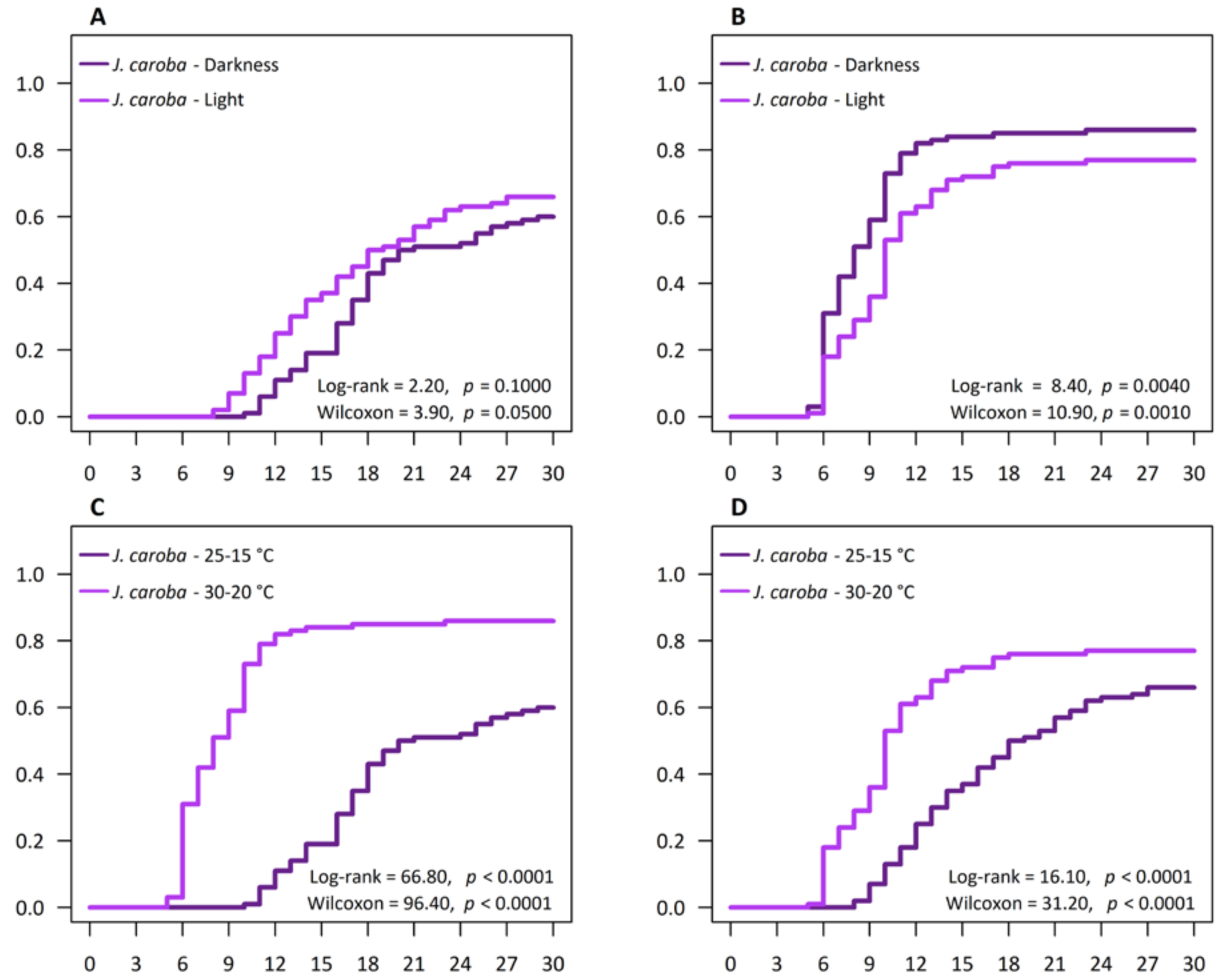

Figure 2. Seed germination probability of *J. caroba* at alternating temperatures and light conditions, estimated by the nonparametric Kaplan-Meier estimator. (A) 25/15 °C, (B) 30/20 °C, (C) darkness, and (D) light.

Table 3. Germination (%) and germination speed index (GSI) of *C. pachystachya* and *J. caroba* seeds in response to different alternating temperatures (25/15 °C and 30/20 °C) and light conditions (darkness and light), analyzed by the traditional technique (analysis of variance and means compared by the Tukey test).

Treatment	*C. pachystachya*		*J. caroba*	
	Germination (%)	GSI	Germination (%)	GSI
darkness	58.00 a[1/]	1.28 a	71.50 a	1.88 a
light	56.50 a	1.72 a	73.00 a	1.70 a
25/15	51.50 a	1.19 b	63.00 b	1.07 b
30/20	63.00 a	1.81 a	81.50 a	2.51 a

[1/] Means followed by the same letters on the column do not differ significantly by the Tukey test ($p > 0.05$).

Table 4. Percentage of seed failures (germination) and censoring (non-germination) of *C. pachystachya* and *J. caroba* in response to constant temperatures.

Temperature (°C)	*C. pachystachya*		*J. caroba*	
	Failure (%)	Censoring (%)	Failure (%)	Censoring (%)
15	52	48	24	76
20	70	30	74	26
25	70	30	79	21
30	77	23	69	31
35	67	33	55	45

n = 100 seeds per treatment

Constant Temperatures

Table 4 presents the seed germination results for the studied species at different constant temperatures. An increase in germination was observed for *C. pachystachya* up to 30 °C, while the optimum germination temperature for *J. caroba* was up to 25 °C. After these values, there is a decrease in germination percentages.

Figure 3A shows that *C. pachystachya* seeds reached 50% or more germination at the constant temperatures tested. Comparisons between these temperatures showed significant differences in at least one test, except for 25 and 35 °C (Table 5). At 15 °C, 30 days were required to obtain 50% germination (Table 6). In the same period, at 20 °C, germination was 70%. This species' highest germination speed and percentages (75%) were observed at 30 °C for 16 days.

For *J. caroba*, germination speed increased with temperature (Figure 3B). This trend was similarly reflected in germination percentage, with temperatures above 15 °C resulting in percentages above 50%. The results in Table 5 indicated significant differences in all temperature comparisons, except between 20 and 35 °C. The highest initial germination speed was observed at 30 °C, while the maximum germination percentage (75%) occurred at 25 °C in 12 days (Table 6).

A regression analysis was performed to compare the methodologies, as shown in Figure 4. The second-degree models, with coefficients of determination above 0.86, were the best fit. In this approach, for the *C. pachystachya*, the germination estimates were 75.2%, achieved at 27.6 °C, and the highest GSI was achieved at 29.7 C. The germination estimates for *J. caroba* were 81.4% at 26.4 °C, and the highest GSI was obtained at 27.6 °C.

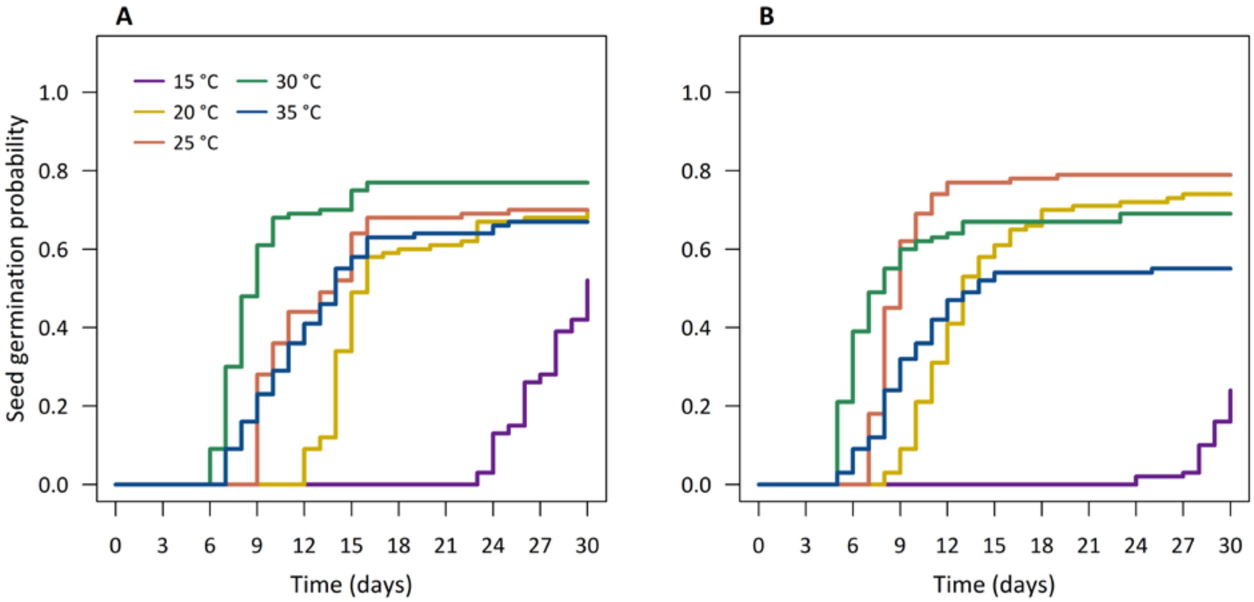

Figure 3. Seed germination probability of *C. pachystachya* (A) and *J. caroba* (B) at constant temperatures estimated by the nonparametric Kaplan-Meier estimator.

Table 5. Log-rank and Wilcoxon tests to evaluate the significance of germination curves at constant temperatures for *C. pachystachya* and *J. caroba*.

Species	Log-rank test		Wilcoxon test	
	χ2	p-value	χ2	p-value
C. pachystachya				
15 °C vs 20 °C	26.10	< 0.0001	46.30	< 0.0001
15 °C vs 25 °C	28.40	< 0.0001	50.80	< 0.0001
15 °C vs 30 °C	48.30	< 0.0001	74.00	< 0.0001
15 °C vs 35 °C	22.90	< 0.0001	41.90	< 0.0001
20 °C vs 25 °C	4.10	0.0400	14.10	0.0002
20 °C vs 30 °C	22.00	< 0.0001	49.20	< 0.0001
20 °C vs 35 °C	2.50	0.1000	10.10	0.0010
25 °C vs 30 °C	13.20	0.0003	29.20	< 0.0001
25 °C vs 35 °C	0.00	0.8000	0.00	0.9000
30 °C vs 35 °C	11.30	0.0008	21.50	< 0.0001
J. caroba				
15 °C vs 20 °C	77.70	< 0.0001	86.60	< 0.0001
15 °C vs 25 °C	94.30	< 0.0001	102.00	< 0.0001
15 °C vs 30 °C	62.00	< 0.0001	72.30	< 0.0001
15 °C vs 35 °C	31.10	< 0.0001	39.20	< 0.0001
20 °C vs 25 °C	17.00	< 0.0001	42.30	< 0.0001
20 °C vs 30 °C	4.70	0.0300	23.00	< 0.0001
20 °C vs 35 °C	1.40	0.2000	0.20	0.7000
25 °C vs 30 °C	0.30	0.6000	6.50	0.0100
25 °C vs 35 °C	16.00	< 0.0001	15.20	< 0.0001
30 °C vs 35 °C	10.90	0.0010	18.80	< 0.0001

Table 6. Germination time (days) of *C. pachystachya* and *J. caroba* seeds at constant temperatures, considering percentiles from 5 to 90.

Treatment	Percentile																	
	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5
C. pachystachya																		
15	–	–	–	–	–	–	–	–	30	30	29	28	28	26	26	25	24	24
20	–	–	–	–	30	23	19	16	16	15	15	15	14	14	14	14	13	12
25	–	–	–	–	28	16	15	15	14	13	11	10	10	9	9	9	9	9
30	–	–	–	16	14	10	9	9	9	8	8	8	8	7	7	7	7	6
35	–	–	–	–	–	24	16	15	14	13	12	11	11	10	9	8	8	7
J. caroba																		
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30	29	28	28
20	–	–	–	–	19	17	15	14	13	13	12	12	11	11	10	10	10	9
25	–	–	–	12	11	10	9	9	9	9	8	8	8	8	8	7	7	7
30	–	–	–	–	–	13	10	9	8	7	7	6	6	6	5	5	5	5
35	–	–	–	–	–	–	–	28	14	12	11	10	9	9	8	8	7	6

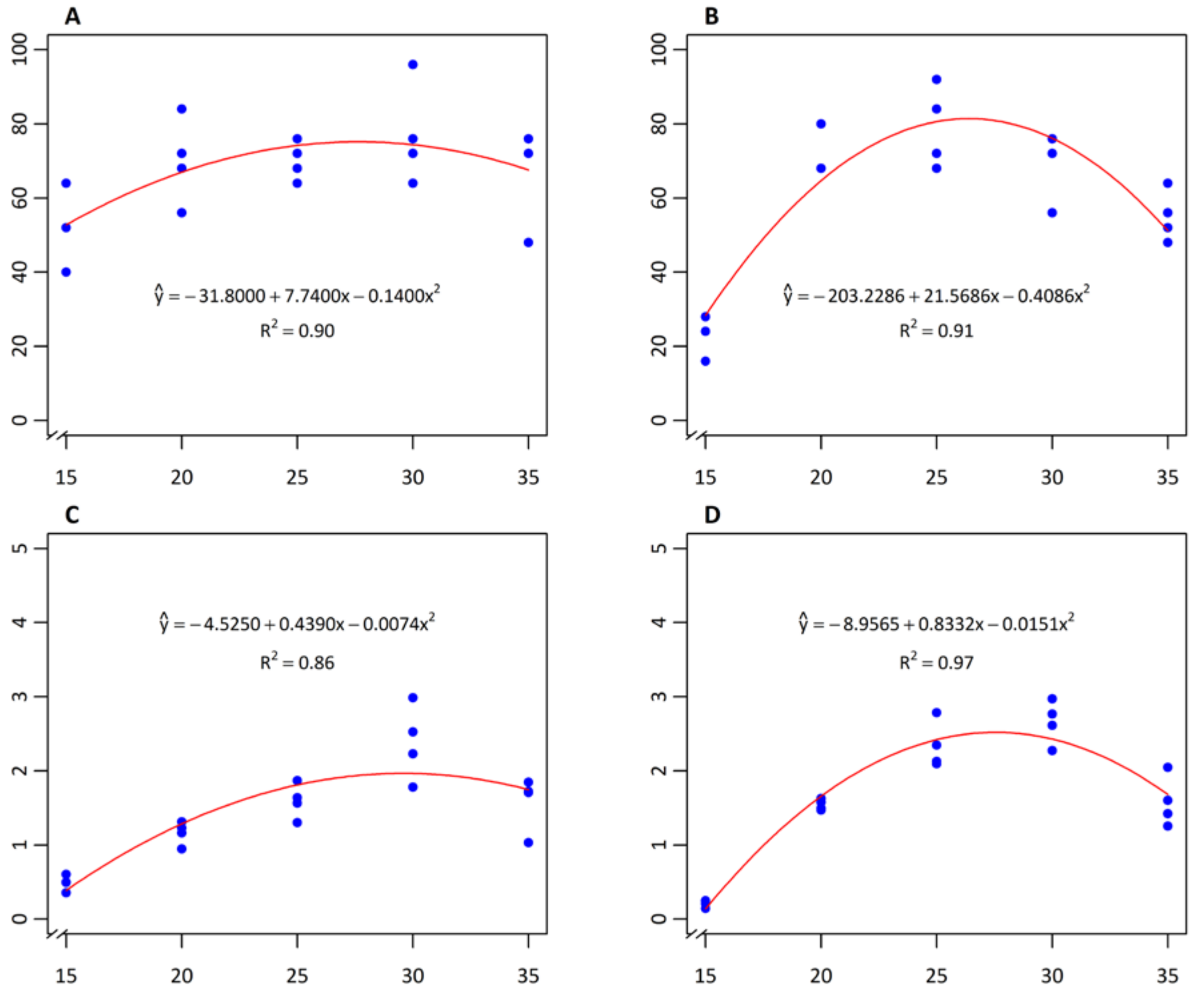

Figure 4. Germination (%) and germination speed index (GSI) of *C. pachystachya* (A, C) and *J. caroba* (B, D) seeds in response to different temperatures, analyzed by the traditional technique (analysis of linear regression).

DISCUSSION

In germination studies, counting the time elapsed until seeds germinate is essential because this information can be used to infer seed vigor. The interpretation of germination curves generated by the Kaplan-Meir estimator is straightforward. It offers an alternative way to interpret the results, facilitating an understanding of the effects of the factors studied in each species. T_{50} and other percentiles can be easily obtained, are conceptually understandable, and show the time required to reach any germination percentage of interest.

This work demonstrated the importance of using the two tests to compare germination curves. Figures 1, 2, and Table 5 show significant differences in one test, while the other did not. According to Colosimo and Giolo (2024), the explanation for these differences is that the Log-rank test assigns the same weight to the entire germination time axis, focusing on longer times. In contrast, the Wilcoxon test emphasizes information at the beginning of the germination curve, where the number of seeds not yet germinated is significant, allowing early germination to receive more weight than late germination.

Compared with traditional techniques (Table 3 and Figure 4), survival analysis provided more information about the results. Classical techniques provide inferences about the final germination percentages. In contrast, survival analysis provides this and other information, allowing inferences about the speed and the germination percentage over the time studied.

The approach presented in this work, being nonparametric, is very flexible and can be used in all types of germination/emergence studies. It eliminates the need to verify the assumptions of the analysis of variance and use of data transformation, which could lead to problems of scale modification, especially in regression models. It can consider censored observations (non-germinated seeds) that contain partial information about the experiment's results. Classical techniques do not consider this partial information, which can bias the conclusions (Onofri et al., 2010; McNair et al., 2012; Collett, 2023; Colosimo and Giolo, 2024).

Traditional analysis methods may still be appropriate for seed germination data but have not shown the same flexibility as survival analysis. In particular, the ANOVA approach, tests of means, and linear regression did not provide a good description of germination trends over time. It did not account for censoring (ungerminated and rotten seeds), which can be problematic in some situations.

In this study, using the classical analysis, it was not possible to verify a significant difference in light conditions during germination (Table 3). However, through survival analysis, it was verified that light conditions combined with alternating temperatures have a significant effect on germination curves (Figures 1 and 2). These results reinforce that more detailed and precise information about the experiment is obtained with this methodology.

When we relate the results obtained with the physiological aspect, *C. pachystachia,* and *J. caroba* seeds demonstrated germination capacity at different temperatures and light conditions. These factors demonstrate that these species can occupy other locations, covering open areas and more closed forest formations, thus explaining their occurrences in different phytophysiognomies of the Cerrado and Atlantic Forest.

Under alternating temperatures of 30/20 °C, the seeds of *C. pachystachia* and *J. caroba* reached 50% germination in a few days (between eight and 15 days), indicating the capacity to produce many seedlings in a short time. (Figure 1 and Table 2). The rapid germination process of these species at alternating higher temperatures may be related to the collection environment (*Campos Rupestres*). Their adaptation to this location since the short average germination time may be related to the rapid colonization of the environment (Bewley et al., 2013).

Alternating temperatures can cause changes in the balance of substances that promote and inhibit germination or alter the physical properties of the seed coat, allowing the seeds to hydrate and favor their germination (Bewley et al., 2013; Marcos-Filho, 2015; Zeballos et al., 2023). Furthermore, smaller seeds, such as those of *C. pachystachia* and *J. caroba*, benefit from temperature variations, germinating more quickly and reducing competition for establishment in clearings (Liu et al., 2013).

In tropical ecosystems, temperature strongly influences germination success and acts directly on the seed's metabolic processes (Gomes et al., 2023). Adapting the species to the environmental conditions determines the optimum temperature, which is the one at which the highest percentage of seed germination is obtained in the shortest time, i.e., the highest germination speed (Marcos-Filho, 2015). Generally, tropical pioneer species have a high optimum germination temperature between 20 and 30 °C (Borges and Rena, 1993). In this study, the temperature of 15 °C caused delayed germination of *C. pachystachia* and *J. caroba* seeds. This fact may be related to slower respiration rates or increased cell membrane rigidity (Bewley et al., 2013)

The instructions for analyzing forest seeds (Brasil, 2013) recommend that batches of *C. pachystachia* seeds be analyzed at a constant temperature of 25 or 30 °C, with germination starting around nine days and finishing in 34 days. These recommendations corroborate the results found in this study, in which the optimum temperature was 30 °C, with 50% of germination occurring in 9 days.

J. caroba is one of the species that requires a germination test protocol, as it is not included in the instructions for analyzing forest seeds (BRASIL, 2013), reinforcing the importance of studying the germination ecology of this species. For the five bignoniaceae of the genus Jacaranda present in this official document from the Ministry of Agriculture, a temperature of 25 °C is indicated in germination tests. This study reinforced this trend for the genus Jacaranda since the optimal temperature for *J. caroba* when analyzing constant temperature was 25 °C (Figure 3 and Table 6), where the species obtained better results in the study with alternating temperatures.

This statistical approach provided a good evaluation of seed germination of *C. pachystachia* and *J. caroba*, showing potential use in evaluating seed germination of forest species. In addition to nonparametric methods, further research using parametric and semiparametric survival models may bring this methodology's real potential to seed science.

CONCLUSIONS

Based on the results obtained, it is concluded that the nonparametric survival analysis technique was effective in discriminating the germination of *C. pachystachya* and *J. caroba* seeds in different light conditions and temperatures. This methodology can be efficiently applied in seed science.

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