

Do the elaiosome and seed coat color affect the seed quality of *Mabea fistulifera* Mart?

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ABSTRACT: *Mabea fistulifera* Mart. (Euphorbiaceae) is a native tree recommended for the restoration of degraded areas. The objective was to evaluate the quality of *M. fistulifera* seed lots through germination and vigor tests. The germination test was conducted in a factorial design (3 x 2 x 2), involving three seed colors (red, orange, and brown), with and without elaiosome, and two substrates (paper roll - PR and top of paper - TP). The conductivity test was arranged in a factorial design (3 x 2), with three seed colors and with and without elaiosome. Survival analysis was performed, along with log-rank and Wilcoxon tests, to compare germination curves. Brown seeds exhibited the highest conductivity and lowest germination, while red seeds showed the lowest conductivity and highest germination. On the PR substrate and without elaiosome, orange seeds had higher initial germination; brown seeds germinated more, and red and orange seeds showed greater deterioration. Red and orange seeds exhibit better physiological quality. Seed color is a reliable indicator of vigor and quality in *M. fistulifera* seeds. Paper is an effective substrate for germination. The absence of elaiosome may influence water absorption, but its effect on germination is not evident.

Index terms: *canudo-de-pito*, germination, seed vigor, survival analysis.

RESUMO: *Mabea fistulifera* Mart. (Euphorbiaceae) é uma arbórea nativa recomendada para recuperação de áreas degradadas. Objetivou-se avaliar a qualidade de lotes de *M. fistulifera* por meio da germinação e testes de vigor. O teste de germinação foi instalado em fatorial (3 x 2 x 2), sendo três cores de sementes (vermelha, alaranjada e marrom), com e sem elaiossomo e dois substratos (Rolo de Papel - RP e Sobre Papel - SP). O teste de condutividade foi montado em fatorial (3 x 2), sendo três cores de sementes e com e sem elaiossomo. Foi realizada análise de sobrevivência e testes de log-rank e wilcoxon para comparação das curvas de germinação. As sementes marrons obtiveram maior condutividade e menor germinação, enquanto as vermelhas apresentaram a menor condutividade e maior germinação. No RP e sem elaiossomo, as alaranjadas apresentaram maior germinação inicial, as marrons germinaram mais e as vermelhas e alaranjadas tiveram maior deterioração. As sementes vermelhas e alaranjadas possuem melhor qualidade fisiológica. A coloração da semente é um bom indicador de vigor e qualidade em *M. fistulifera*. O papel é um substrato eficaz para germinação. A ausência do elaiossomo pode influenciar a absorção de água, porém, não é evidente sua influência na germinação.

Termos para indexação: *canudo-de-pito*, germinação, vigor de sementes, análise de sobrevivência.

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INTRODUCTION

Mabea fistulifera Mart., commonly known as canudo-de-pito, is a deciduous, pioneer tree species native to Brazil, belonging to the Euphorbiaceae family. It is predominantly found in transitional areas between the Semideciduous Seasonal Forest and the Cerrado (Lorenzi, 2020). Due to its high adaptability and efficiency in occupying competitive environments with greater solar incidence, it is more common on forest edges and in areas with significant anthropogenic impact (Carvalho and Ribeiro, 2018; Lorenzi, 2020). The species adapts to acidic and low-fertility soils and has a high regrowth capacity in degraded regions affected by fire in the Atlantic Forest (Souza et al., 2015; Lorenzi, 2020; Christo et al., 2021).

Due to its good adaptation to adverse conditions, *Mabea fistulifera* is recommended for the restoration of degraded areas (Meira-Junior et al., 2015; Arato et al., 2017; Giácomo et al., 2019; Leão et al., 2022; Cortes et al., 2023) and for phytoremediation programs for soils contaminated with hormonal herbicides (Barroso et al., 2022). With the growing encouragement of forest restoration, there is an increasing need to understand the germinative behavior of tree species and the physical and physiological characteristics of their seeds to ensure the use of quality inputs, especially for species adapted to anthropized areas, such as *M. fistulifera* (Gomes-Júnior and Lopes, 2017). Therefore, conducting germination and vigor tests is crucial for characterizing seed lots' quality and physiological potential (Vieira and Krzyzanowski, 2020).

Vigor encompasses a set of characteristics that determine the physiological potential of seeds. It indicates the performance potential of a seed lot, both in the field and under storage conditions, explaining why different lots of the same species may yield different results (Marcos-Filho, 2015). Moreover, vigor elucidates that from the point of maturity, seeds begin the deterioration process (Marcos-Filho, 2015; Brito et al., 2015; Silva et al., 2021; Moulay et al., 2023).

Among vigor tests, the electrical conductivity test is efficient in detecting differences in the physiological potential of seeds (Azeredo et al., 2016). Its advantages include greater efficiency and speed, as it measures the intensity of seed deterioration (Torres et al., 2015) by detecting the increased amount of electrolytes leached in the seed-soaking solution (Azeredo et al., 2016; Vieira and Krzyzanowski, 2020), which is related to the integrity of cell membranes (Silva et al., 2014; Haesbaert et al., 2017; Vieira and Krzyzanowski, 2020).

In certain genera of the Euphorbiaceae family, differences in seed coloration are common (Carvalho and Nakagawa, 2000) and may be associated with their physiological quality (Dalanhol et al., 2014; Brito et al., 2015; Silva et al., 2021; Moulay et al., 2023). This is a morphological characteristic of the seed coat (Silva et al., 2021) and may be related to variations in the maturation stage, which can exist among seeds from the same fruit (Dalanhol et al., 2014; Silva et al., 2021). It can also result from differences in seed size, dry matter, lipid, and moisture content (Brito et al., 2015), genetic and environmental factors, and phenotypic plasticity (Silva et al., 2021).

The seeds of *M. fistulifera* possess a structure known as an elaiosome, a lipid-rich, nutritious appendage common in species of the Euphorbiaceae family (Carvalho and Nakagawa, 2000; Oliveira and Paoli, 2014). The seeds of this family are often oblong to oval, arillate or with an elaiosome (Souza and Lorenzi, 2008), and are, in general, exotegmic and albuminous (Oliveira and Paoli, 2014). This structure attracts ants, which disperse the seeds (Sasidharan and Venkatesan, 2019; Fernandes et al., 2020; Munguía-Rosas and Álvarez-Espino, 2022). During transport, the elaiosome is removed (Fernandes et al., 2020), but the effect of this manipulation on germination and plant establishment remains unclear. Some studies report positive effects from elaiosome removal (Sasidharan and Venkatesan, 2019; Fagundes et al., 2022; Munguía-Rosas and Álvarez-Espino, 2022), while others report negative (Fernandes et al., 2018) and even neutral effects (Fernandes et al., 2020).

The aim of this study was to evaluate the influence of the elaiosome, seed coloration, and different paper substrate methodologies (paper roll and top of paper) on the quality and vigor of *Mabea fistulifera* seeds.

MATERIAL AND METHODS

The work was conducted at the *Universidade Federal de Viçosa*, Viçosa Campus, in the Department of Forest Engineering, within the facilities of the Forest Seed Analysis Laboratory (LASF). The seeds were collected in Viçosa, Minas Gerais (20° 46' 05.84" S 42° 52' 46.11" W) in September 2022. After harvest, the fruits were placed in trays to allow for spontaneous opening through explosive dehiscence and were covered with a light fabric to prevent seed loss while maintaining ventilation. Afterward, the seeds were stored for 90 days in a cold chamber at 10 °C and 60% humidity.

The seeds were manually sorted into three color classes: red, orange, and brown. The classification was based on visual assessment, considering the predominant color of each seed (Figure 1A). The elaiosome was manually removed from a portion of the seeds, as it is easily detachable (Figure 1B).

For the germination test, a paper substrate was used, adopting the paper roll (PR) and top-of-paper (TP) methods. For the PR method, the seeds were placed on two sheets of germitest (germination testing) paper, covered by a third sheet, and arranged in the form of rolls, which were then placed in plastic bags. In the TP method, the seeds were placed on top of two sheets of germitest paper in plastic Petri dishes. The paper was sterilized and moistened with distilled water in the amount of 2.5 times its dry weight, according to the Rules for Seed Testing (Brasil, 2009). The seeds were placed in a BOD (Biochemical Oxygen Demand) germination chamber at 25 °C for 24 days. Daily monitoring was conducted to determine germination through radicle protrusion.

The experiment was set up in a completely randomized design in a 3 x 2 x 2 factorial scheme with four replications. Each treatment was a combination of the three seed color classes (red, orange, and brown), the presence or absence of the elaiosome, and the type of substrate used (PR and TP). The experimental unit consisted of 25 seeds.

For the electrical conductivity test, the seeds were placed in plastic cups containing 75 ml of deionized water and kept in a BOD germination chamber at 25 °C in the absence of light for 24 hours. After the soaking period, the electrical conductivity of the water solution was measured using a conductivity meter, expressed in $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$. The experiment was conducted in a completely randomized design with four replications of 25 seeds in a 3x2 factorial scheme, where each treatment was a combination of the three seed color classes (red, orange, and brown) and the presence or absence of elaiosome.

To provide additional information related to seed coat colors, 50 seeds of each color class were evaluated with the X-ray test to assess the internal morphology. The digital Faxitron equipment, model MX-20 (Faxitron X-ray Corp., Wheeling, IL, U.S.A), was used for this purpose. Each seed was individually placed on an acrylic plate and subjected to a voltage of 29 kV for 10 seconds. Subsequently, the seeds were classified as full, empty, and physically or insect-damaged (Brasil, 2009) and then subjected to the germination test in Petri dishes (TP), maintained in a BOD chamber at 25 °C for 26 days.

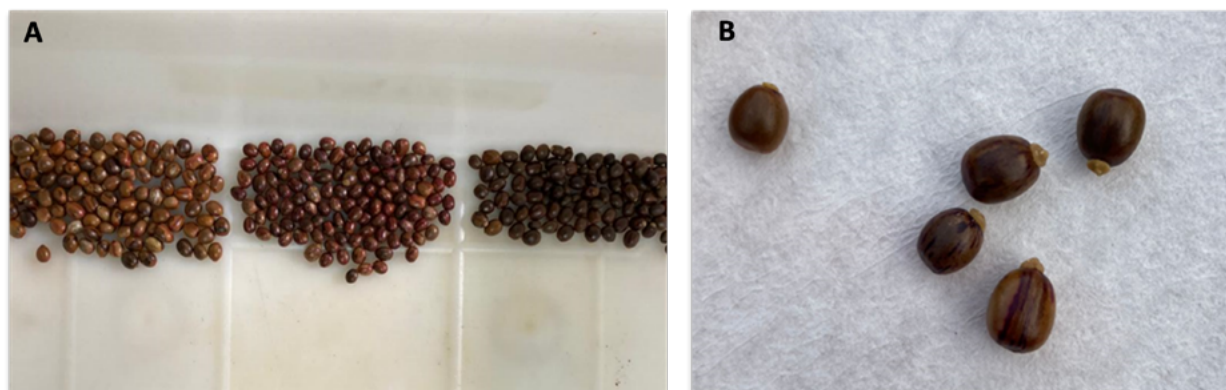


Figure 1. *M. fistulifera* seeds classified into three color classes from left to right: orange, red, and brown (A) and seeds with the elaiosome (B).

Germination assessment was performed using survival analysis, employing the non-parametric Kaplan-Meier estimator to estimate the survival function, aiming to determine the probability of failure (germination) over time (Mamani et al., 2024). The survival function is described by the following equation:

$$S(t) = P(T > t), t \geq 0$$

where $S(t)$ is the probability that the observation survives (does not germinate) up to a time t , meaning it does not fail.

To determine the probability of failure (germination), the function is described as:

$$F(t) = 1 - S(t)$$

where $F(t)$ is the cumulative distribution function that indicates the probability of a failure (germination) occurring by a certain time t .

The most used non-parametric technique in survival analysis is the Kaplan-Meier estimator (Kaplan and Meier, 1958), which is described as:

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

where $t_1 < t_2 < \dots < t_k$, are the distinct failure times, d_j is the number of failures in the interval (t_{j-1}, t_j) , and n_j is the number of individuals at risk at t_j , meaning the observations that have not germinated and have not been censored just before t_j .

Graphs were plotted with the germination curves derived from the cumulative distribution function based on the estimated seed survival functions.

To compare the germination curves, non-parametric Log-rank (Mantel, 1966) and Wilcoxon (Gehan, 1965) tests were used at a 5% significance level. The null hypothesis tested was that there is no significant difference between the two germination curves ($H_0: S_1(t) = S_2(t)$). The Log-rank test is described as:

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

where d_{2j} is the number of failures at time j , w_{2j} is the average number of failures, and $(V_j)_2$ is the variance of d_{2j} . The statistics follow a chi-squared distribution with 1 degree of freedom for large samples.

The Wilcoxon test statistic is described as:

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

where u_j is the weight of the test, with $u_j = n_j$. This weighting allows for greater emphasis on the initial values of the survival curve, as the number of individuals at risk is higher in this interval, thereby assigning greater importance to failures early on (Duarte et al., 2023; Colosimo and Giolo, 2024). In this test, d_{2j} is the number of failures at time j , w_{2j} is the average number of failures, and $(V_j)_2$ is the variance of d_{2j} . The test statistic follows a chi-squared distribution with 1 degree of freedom for large samples.

For the electrical conductivity test, the obtained data were subjected to analysis of variance, and the means were compared using Tukey's test at a 5% significance level.

All statistical analyses were performed using R software version 4.4.1 (R Core Team, 2024), with the survival package version 3.7-0 (Therneau, 2024) for the survival analysis and the ExpDes.pt package version 1.2.2 (Ferreira et al., 2021) for the analysis of variance.

RESULTS AND DISCUSSION

Red seeds achieved the highest germination percentage compared to other colors in the paper roll (PR) substrate, with a rate of 96% (Figures 2A and 2B). In the top of paper (TP) substrate, orange seeds exhibited the highest germination rates (Figures 2C and 2D), with seeds containing elaiosomes reaching 95% germination (Figure 2C) and those without elaiosomes achieving 92.4%, which is very close to the germination rate of red seeds at 92% (Figure 2D). In all treatments, brown seeds had lower germination rates. Overall, there was no significant difference between the germination of red and orange seeds, with a significant difference observed only for seeds with elaiosomes in the TP substrate (Table 1). However, both reached over 90% germination, with only a 3% difference.

There is an indication that red and orange seeds reached physiological maturity, exhibiting higher germination capacity and vigor (Carvalho and Nakagawa, 2000). This hypothesis was supported by the electrical conductivity test, where both colors showed significantly lower average electrical conductivity values compared to brown seeds (Figure 3A), which had the highest value at $70.7 \mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$. This indicates a greater amount of electrolytes leached in the soaking solution, implying a reduced capacity for membrane reorganization (Vieira and Krzyzanowski, 2020), leading to higher nutrient loss and, consequently, lower vigor and germination (Azeredo et al., 2016). Regarding the influence of the elaiosome, there was no significant difference in the average electrical conductivity values (Figure 3B).

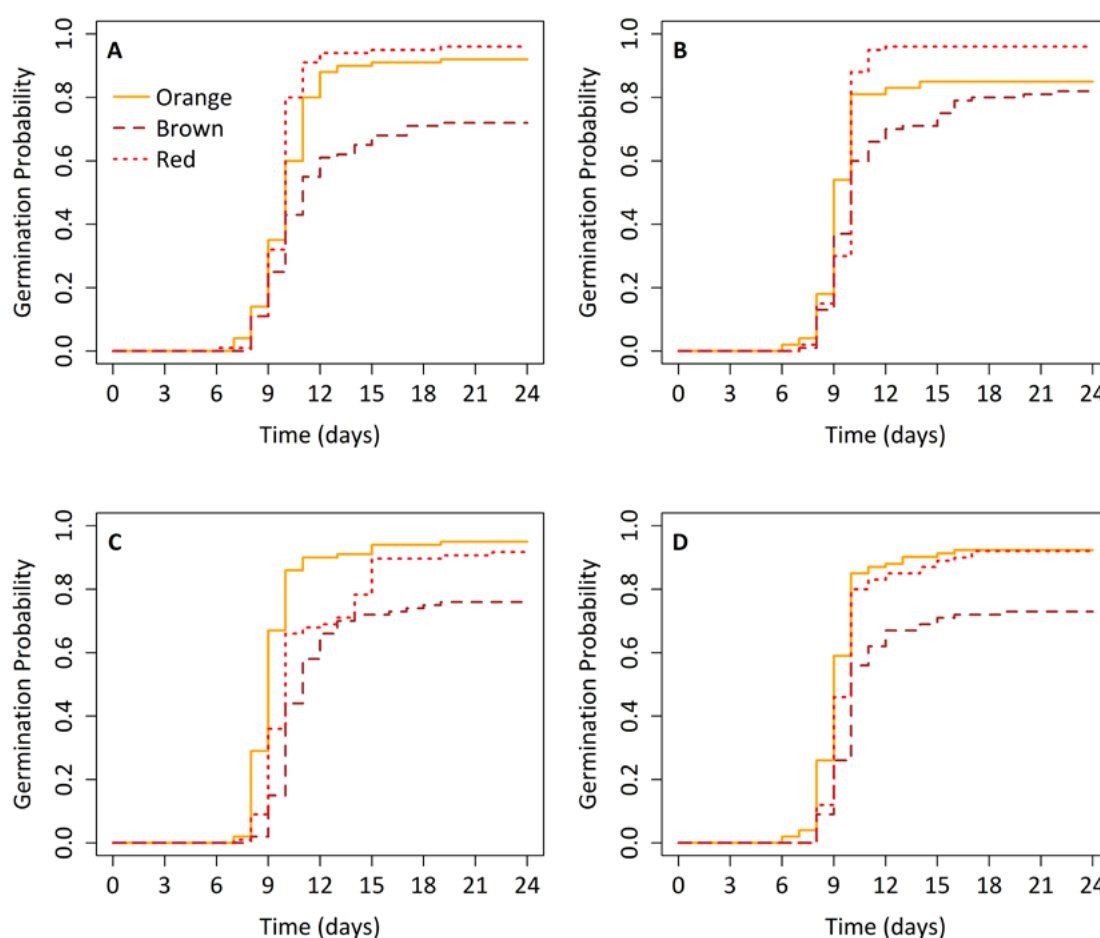


Figure 2. Germination Probability per day estimated by the Kaplan-Meier method for the orange, brown, and red *M. fistulifera* seeds, in two substrates, and with and without elaiosome. A – seeds with elaiosome on PR substrate; B – seeds without elaiosome on PR substrate; C – seeds with elaiosome on TP substrate; D – seeds without elaiosome on TP substrate.

Table 1. Log-rank and Wilcoxon tests for comparing germination curves of orange, red, and brown *M. fistulifera* seeds in two substrates (PR – paper roll and TP – top of paper), and with and without elaiosome.

Log-rank Test			Wilcoxon Test		
Treatments	χ^2	<i>p</i> -value	Treatments	χ^2	<i>p</i> -value
Seeds with elaiosome in PR	31	< 0.0001		20.7	< 0.0001
Orange vs. Red	2.7	0.1	Orange vs. Red	1.8	0.2
Orange vs. Brown	15.5	< 0.0001	Orange vs. Brown	10.8	0.001
Red vs. Brown	27.1	< 0.0001	Red vs. Brown	19.3	< 0.0001
Seeds without elaiosome in PR	7.5	0.02		9.3	0.01
Orange vs. Red	0.1	0.7	Orange vs. Red	2.6	0.1
Orange vs. Brown	3.6	0.06	Orange vs. Brown	7	0.008
Red vs. Brown	9.2	0.002	Red vs. Brown	4.7	0.03
Seeds with elaiosome in TP	51	< 0.0001		67.1	< 0.0001
Orange vs. Red	15.9	< 0.0001	Orange vs. Red	22.7	< 0.0001
Orange vs. Brown	49.3	< 0.0001	Orange vs. Brown	62.4	< 0.0001
Red vs. Brown	9.8	0.002	Red vs. Brown	11.5	0.0007
Seeds without elaiosome in TP	30.3	< 0.0001		31.6	< 0.0001
Orange vs. Red	2.9	0.09	Orange vs. Red	5.4	0.02
Orange vs. Brown	26.4	< 0.0001	Orange vs. Brown	28.5	< 0.0001
Red vs. Brown	15.7	< 0.0001	Red vs. Brown	13.7	0.0002

Bold values indicate significant differences ($p \leq 0.05$).

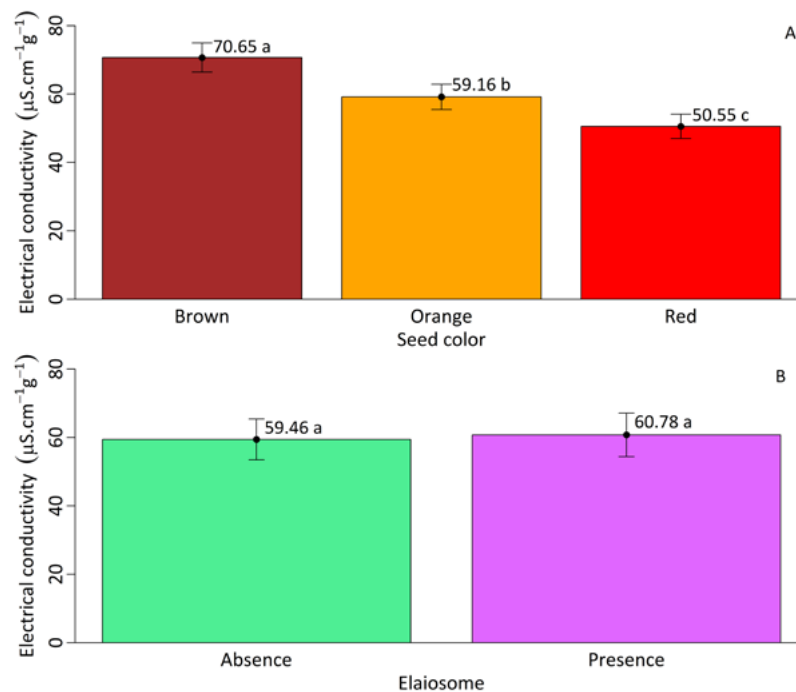


Figure 3. Tukey test and 95% confidence interval for the electrical conductivity ($\mu\text{S.cm}^{-1}.\text{g}^{-1}$) of *M. fistulifera* seeds according to seed colors (orange, brown, and red) (A) and the presence and absence of the elaiosome (B). Means followed by the same letter do not differ significantly by Tukey's test ($p > 0.05$).

Studies conducted with *Jatropha curcas* L. (Brito et al., 2015), *Retama sphaerocarpa* (L.) Boiss (Moulay et al., 2023), and *Bowdichia virgilioides* Kunth (Dalanhol et al., 2014; Silva et al., 2021) indicate that seeds can be classified based on seed coat color, which is related to their physiological quality and maturation stage of the species (Silva et al., 2021). The progression of color change in seeds, where the seed becomes darker with increased deterioration (Silva et al., 2021), suggests a transition from the maturation point to a less physiologically active and viable stage (Moulay et al., 2023). Thus, brown seeds appear to be beyond the optimal maturation point, initiating a deterioration process.

Regarding the influence of elaiosome on germination, a significant difference was observed only in the paper roll (PR) substrate, except for red seeds (Figures 4B and 4C). Orange seeds without elaiosomes exhibited higher initial germination (Figure 4B), as indicated by the Wilcoxon test. Brown seeds were the only ones where survival curves differed significantly in both tests, with seeds lacking elaiosomes showing the highest germination in PR (82%) (Figure 4C). Leal et al. (2017) concluded that the absence of elaiosome may accelerate and improve water imbibition by the seed, thus increasing germination, as it is located near the micropyle (Carvalho and Nakagawa, 2000), one of the most important structures for water penetration into seeds (Marcos-Filho, 2015).

However, Fernandes et al. (2018) suggested that the removal of this structure in *Mabea fistulifera* seeds may lead to greater pathogen entry, reducing germination. Munguía-Rosas and Álvarez-Espino (2022) observed the opposite in *Cnidoscolus aconitifolius* (Euphorbiaceae) seeds, in which elaiosome removal decreased fungal attacks. However, a study by Fernandes et al. (2020) concluded that seed manipulation by ants did not affect the germination, establishment, and survival of *M. fistulifera* seedlings, which highlights the uncertainty regarding the influence of this structure on germination.

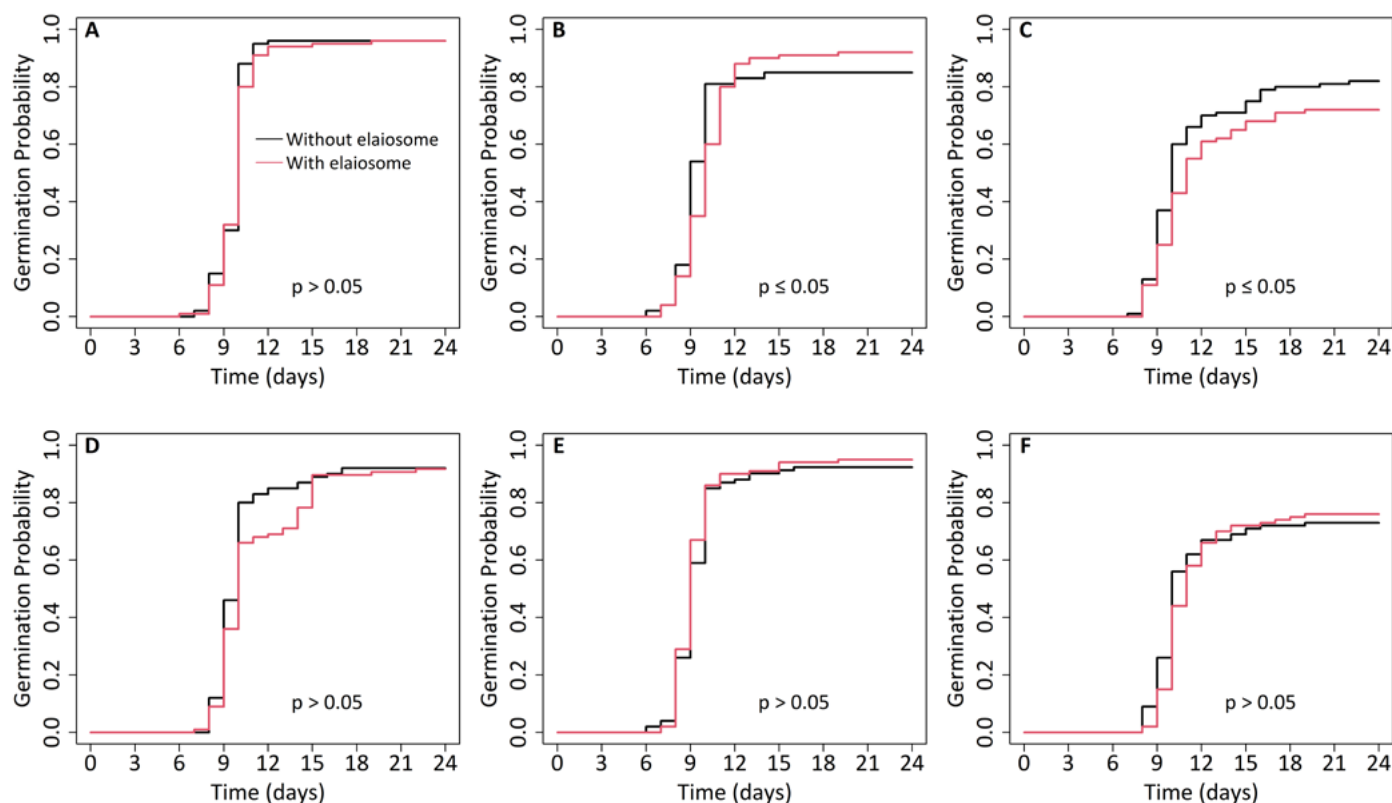


Figure 4. Germination Probability per day estimated by the Kaplan-Meier method for the *M. fistulifera* seeds with and without elaiosome, in three seed colors, and in two substrates. A – red seeds on PR substrate; B – orange seeds on PR substrate; C – brown seeds on PR substrate; D – red seeds on TP substrate; E – orange seeds on TP substrate; F – brown seeds on TP substrate.

Only among brown seeds did the presence of the elaiosome show a significant difference in germination. Due to their lower vigor, these seeds are beyond the physiological maturity point and, consequently, are likely to require more water compared to red and orange seeds. Thus, the absence of elaiosome may facilitate greater water absorption, benefiting germination, particularly in the paper roll (PR) substrate, which provides a larger contact surface (Marcos-Filho, 2015) and maintains higher moisture.

When examining the percentage of seed deterioration, only orange and red seeds without elaiosomes in the paper roll (PR) substrate exhibited deterioration above 20%, with red seeds reaching the highest percentage at 25% (Table 2). All other treatments had deterioration rates below 10%, with most occurring in seeds lacking elaiosomes.

Therefore, it is possible that the absence of this structure made the seeds more susceptible to fungal and pathogen entry, and the excess moisture, also provided by the paper roll (PR) methodology, could have reduced the germination percentage, leading to seed deterioration. Accelerated imbibition and excess water can cause damage to seeds (Marcos-Filho, 2015). Therefore, the elaiosome could provide some protection for the seed, both against excess moisture and infection. Since brown seeds likely have lower water content due to having surpassed the physiological maturity point, this could explain their lower rate of seed deterioration.

After the evaluation using X-ray and the germination test, it was found that red seeds had the highest germination percentage (94%), followed by orange seeds (92%) and brown seeds (84%). A total of 11 empty seeds were identified (Figures 5B and 5C), of which 7 were brown and 4 were orange; 2 seeds were physically damaged (1 brown and 1 red), and 2 red seeds were damaged by insects (Figure 5C). Red seeds showed more insect-damaged seeds, which may be attributed to their higher vigor and greater reserves. This observation aligns with findings by Brito et al. (2015), who concluded that when seeds of *Jatropha curcas* L. (Euphorbiaceae) reached their physiological maturity, they had the highest dry matter accumulation and lipid content, as well as the greatest germination potential and vigor, which makes the seeds more attractive to insects. Similarly, Branco et al. (2002), in their analysis of oak acorn germination, concluded that acorns subjected to extensive insect damage exhibited lower dry weight and reduced seedling vigor due to endosperm loss, which resulted in fewer reserves available for development. These results aligned with findings from the other vigor tests that were performed, where red and orange seeds demonstrated higher vigor and germination.

The paper proved to be an appropriate substrate for germination tests for *Mabea fistulifera* seeds, achieving high germination rates. It is the most recommended type of substrate (Santos et al., 2022) and is also widely used for forest species due to its water absorption capacity and aeration (Figliolia, 2015).

Regarding the substrate methodology, Santos et al. (2022) investigated the relationship between temperature and substrate in *Mabea fistulifera* and recommended the on-paper substrate methodology for lower ranges of alternating temperatures and the between-paper substrate methodology for higher ranges to reduce water loss due to evaporation caused by increased temperatures.

Table 2. Percentiles of deterioration time (days) for red and orange *M. fistulifera* seeds with and without elaiosome in paper roll (PR) substrate.

Percentiles	2	15	20	25
Elaiosome				
Red seed in PR				
With elaiosome	-	-	-	-
Without elaiosome	14	14	14	19
Orange seed in PR				
With elaiosome	-	-	-	-
Without elaiosome	19	22	23	-

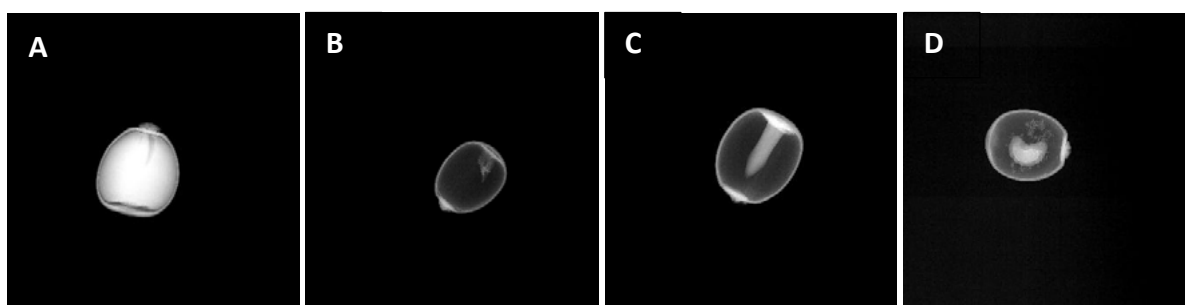


Figure 5. *M. fistulifera* seeds classified by radiographic image analysis: A – full seed (seed containing all essential tissues for germination); B and C – empty seeds (seeds with less than 50% of the tissues); D – seed damaged by insect.

Red and orange seeds are the most suitable for germination and establishment of plantings, although brown seeds also achieved good germination rates, indicating the species' great adaptation potential and reinforcing the importance of its use in the restoration of degraded areas. Brown seeds can also be used for seedling planting or direct sowing; however, calculations should be made to determine the additional number of seeds required, given their lower germination rates.

This study can contribute to the advancement of research on the species *M. fistulifera*, as well as on the Euphorbiaceae family, given that it is not extensively studied, with limited research in the field of plant reproduction within this family (Oliveira and Paoli, 2014). Therefore, this study can support the development of future research on other species from similar forest environments.

CONCLUSIONS

Seed coloration is a good indicator of vigor and quality in *Mabea fistulifera* seeds. Paper is an effective substrate for the germination of *M. fistulifera*. The absence of the elaiosome can influence seed water absorption. It is recommended to conduct germination tests with seeds that contain elaiosome. However, further investigation is needed to better understand the actual influence of elaiosome on germination and seedling establishment.

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AUTHORS' CONTRIBUTION

Vivian Silva Santos contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Visualization, Writing - original draft; Marciel Lelis Duarte contributed with: Conceptualization, Methodology, Formal analysis, Writing—review & editing; Sara Silvério contributed with: Investigation, Data Curation; Lausanne Soraya de Almeida contributed with: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing; Sebastião Martins Filho contributed with: Conceptualization, Methodology, Formal analysis, Supervision, Resources, Writing—review & editing.

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