REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

Morphology and germination of *Aegiphila brachiata* Vell.: potential species for ecological restoration in atlantic forest

Morfologia e germinação de *Aegiphila brachiata* Vell.: espécies potenciais par a restauração ecológica em floresta atlântica

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ABSTRACT
Aegiphila brachiata is a forest native Brazilian species, known as peloteiro, with high capacity for biomass production and resistance to disturbances, having potential use in degraded ecosystems. Seeing the scarcity of studies on the species, we aimed to determine the morphobiometric characteristics of fruits, seeds and seedlings; and evaluate its germination potential. We determined physical characteristics of fruits and seeds, morphological description of seeds, germination stages and seedling development. Germination was in a completely randomized design (factorial scheme), with three temperatures (20, 25, and 30°C) and two methods of overcoming dormancy (partial and total endocarp removal). Fruits are indehiscent, fleshy, with persistent calyx. The pyrene is constituted by endocarp and seeds. Germination is hypogeal, and seedling cryptocotyledonary. Results show that removing the endocarp with the unprecedented methodology used is a suitable method to overcome mechanical dormancy (with 25 and 30°C), showing potential of the method for applicability in several species.

Keywords: lamiaceae, mechanical dormancy, native species, overcome dormancy seed, peloteiro, semilla.

RESUMO
Aegiphila brachiata é uma espécie nativa brasileira, conhecida como peloteiro, com elevada capacidade de produção de biomassa e resistência a perturbações, tendo potencial utilização em ecossistemas degradados. Vendo a escassez de estudos sobre a espécie, procurámos determinar as características morfobiométricas dos frutos, sementes e plântulas; e avaliar o seu potencial germinativo. Determinámos as características físicas dos frutos e sementes, a descrição morfológica das sementes, as fases de germinação e o desenvolvimento das plântulas. A germinação foi realizada de forma completamente aleatória (esquema factorial), com três temperaturas (20, 25, e 30°C) e dois métodos para superar a dormência (remoção parcial e total do endocarpo). Os frutos são indeiscentes, carnudos, com cálice persistente. O pireno é constituído por endocarpo e sementes. A germinação é hipogeal, e as plântulas criptocotídicas são criptocotídicas. Os resultados mostram que a remoção do endocarpo com a metodologia sem precedentes utilizada é um método adequado para superar a dormência mecânica (com 25 e 30°C), mostrando o potencial do método para a aplicabilidade em várias espécies.

Palavras-chave: lamiaceae, dormência mecânica, espécies nativas, superar semente de dormência, peloteiro, semilla.

1 INTRODUCTION

Brazilian forests are a refuge for many species with the potential to use for environmental reforestation; however, to select the species to compose these projects it is necessary to know some characteristics, and among them is their seeds feature. This information may contribute to quality seedlings propagation, which could accelerate the environmental restoration process. Furthermore, knowledge related to native forest species also is of great importance from an ecological and economic point of view (Rocha et al., 2014; Garrett et al., 2020). Despite this, this type of research is rare for most native essences.

An example of this is *Aegiphila brachiata* Vell. (Lamiaceae), popularly known as “peloteiro”, which is a native species occurring in Brazil, mainly in the Mixed Ombrophilous Forest (França, 2003; Harley et al., 2015). The peloteiro tree has great potential for plantations intended for degraded ecosystem recovery (Maggioni et al., 2020; Foladori-Invernizzi et al., 2021); however, there is still a lack of information about the species. Its seeds have a low germination potential because of a physical barrier caused by the endocarp, which prevents water absorption and radicle emission; moreover, there is no germination after 150 days of sowing, and treatments with scarified seeds resulted in 100% of mortality due to the embryo rotting (Maggioni et al., 2020), an issue common to several species. Based on this, organized research is crucial to identify and improve the efficiency of species' seminal propagation.

Research about morphology, reproductive organs, and seed germination contributes to the generation of cultivation technologies and species conservation. The knowledge of morphological structures in native forest species helps in the taxonomic identification and the understanding of species autecology, dispersion mechanisms, biological cycle, regenerative process, growth, and development, in addition to the propagation and expansion of native seeds and seedlings trade (Batista et al., 2011; Alves et al., 2013; Gogosz et al., 2015). Besides, studies related to fruits and seeds biometry may provide important information in determining dispersal characteristics, differentiation of species from the same genus, and genetic variability within populations (Santos et al., 2009; Gonçalves et al., 2013).

Furthermore, it is worth noting the importance of producing knowledge regarding seed germination potential based on laboratory tests under controlled conditions (Duarte et al., 2015). Characteristics such as viability, dormancy, environmental conditions (water, light, temperature), and substrates, in addition to possible barriers to germination, determine different responses for each species, which makes the knowledge of these conditions a priority in forest seed studies (Carvalho & Nakagawa 2012).

Given the above, together with the lack of information regarding peloteiro, the present study aimed to determine seed physical features, fruit, and seed biometry; fruit, seed, and seedling morphology; and evaluate the germination potential of *Aegiphila brachiata* seeds submitted to mechanic dormancy overcoming methods at different temperatures.

2 MATERIAL AND METHODS

2.1 FRUIT HARVEST AND PROCESSING

Peloteiro ripe fruits, yellow in color, were collected from the ground, shortly after their fall, from February to March 2020. Harvest was carried out in fragments of the Mixed Ombrophilous Forest, located in Campo do Tenente and Bocaiúva do Sul, in the State of Paraná, Brazil. After pulping in a sieve and running water, the pyrenes (endocarp + seed) were placed to dry in a natural laboratory environment for loss of water excess (approximately 24 hours).

2.2 PHYSICAL CHARACTERIZATION AND BIOMETRY

For seed physical characterization, we determined: moisture content (%) in four replications of 25 seeds by oven method at 105 ± 3 ºC for 24 hours. The thousand seed weight was obtained using eight repetitions of 100 seeds weighed on an analytical balance, according to the methodology described in Seed Analysis Rules (Brasil, 2009a). From these data, we also determined the number of seeds per kilogram and the 1000 seed dry mass.

For biometric characterization of *A. brachiata* fruit, pyrene, and seed we used 50 units randomly taken from the lot; with a digital caliper and an analytical balance we measured: length, diameter, width, thickness, and mass; in addition, the number of seeds per fruit was count. Pulp percentage (mesocarp + epicarp) was determined based on the difference between the fruit's total mass and pyrene mass, dividing the pulp mass by the fruit mass. Then, we determined minimum and maximum values, means, standard deviation, and coefficient of variation for each variable.

2.3 FRUIT, SEED, AND SEEDLING MORPHOLOGY

For the description and morphological illustration of fruits and seeds, we used 50 units randomly taken from the lot. We described fruits in type, dehiscence, characteristics of epicarp (texture, color), mesocarp (consistency and color), and endocarp (consistency and color). For seed description, seeds were removed from the pyrene, being described externally and internally.

The external aspects observed were color, texture, tegument consistency, and hilum and micropyle position. For internal morphology, seeds were hydrated in distilled water and sectioned with a scalpel blade in transversal and longitudinal sections, identifying and illustrating the presence or absence of endosperm, embryo type, and embryonic axis color and shape.

Ten seeds (without endocarp) were germinated in plastic trays containing vermiculite substrate and kept in a Biochemical Oxygen Demand germination chamber at 25 °C and constant white light to characterize the seedlings and germination stages. Developmental observations were carried out daily after the beginning of germination, and seedlings were observed from the 1st to the 15th day after germination. In this step, the following vegetative elements were described and illustrated: type of germination, hypocotyl, epicotyl, cotyledons, and eophyll. The illustrations were made manually, and the denominations used to describe fruits, seeds, and seedlings were based on Barroso et al. (1999) and the Illustrated Glossary of Morphology (Brasil, 2009b).

2.4 DORMANCY OVERCOMING AND TEMPERATURES FOR SEED GERMINATION

Seeds were sterilized with sodium hypochlorite solution (2.5% active chlorine) for five minutes as a pre-germinative treatment. To overcome mechanical dormancy, we developed an unprecedented methodology, that consists of partial and total removal of endocarp using a type C 2" sergeant clamp (Figure 1). Each pyrene was fixed in the sergeant clamp and subjected to a pressure of 180 to 360° clockwise, counting one click we had the breakage of the endocarp for partial removal and two clicks for endocarp total removal.

Figure 1. Mechanic methods of dormancy overcoming in *Aegiphila brachiata* Vell.: (A) C 2" sergeant clamp; (B) partial endocarp removal; (C) total endocarp removal. Note: pyr: pyrene.

The effects of temperature and dormancy overcoming on seed germination were evaluated under three constant thermal regimes: 20, 25, and 30 °C, associated with two dormancy overcoming methods: partial and total endocarp removal. We carried out the germination test in gerbox boxes filled with vermiculite (approximately 30 g of fine particle size) and moistened with 100 ml of distilled water. The vermiculite was sterilized in a drying oven at 200 °C for two hours. The boxes were placed in the Mangelsdorf germination chamber, under constant white light. We replenished water whenever necessary.

The experiment was carried out in a completely randomized design, in a 3 x 2 factorial arrangement (temperature x dormancy overcoming methods), with eight replications of 25 seeds. We evaluated daily until the absence of germination for three consecutive days. The germination criterion adopted was the protrusion of the primary root, approximately 3 mm in length. After the end of the experiment, the seeds that did not germinate were classified as hard seeds or seeds attacked by fungi.

The germination percentage, germination speed index (GSI) (Maguire, 1962), and mean germination time (MGT) (Laboriau, 1983) were calculated. We tested the treatment variances for homogeneity by the Bartlett test and, later, submitted to Analysis of Variance (ANOVA) ($p < 0.01$ and $p < 0.05$), and means were compared by the Tukey test.

3 RESULTS AND DISCUSSION

3.1 PHYSICAL CHARACTERIZATION, BIOMETRY, AND MORPHOLOGY

The moisture content of *A. brachiata* seeds was 14.65%, with a thousand seed weight equal to 440.82 g (CV% = 2.58%), resulting in 2,269 seeds per kilogram and 376.23 g of thousand seed dry biomass. *A. brachiata* fruits have an average of 27.2 mm in length, 17.86 mm in diameter, and 5.2 g of fresh mass with 90.60% pulp (Table 1). Fruits have fleshy, indehiscent, and oval, with smooth and shiny surfaces, green when unripe (Figure 2A) and yellow when ripe (Figure 2B). The calyx is persistent, and the fruit detaches from the long pedicel when ripe. Also, the pericarp is thin, the mesocarp fleshy, and the endocarp woody (Figure 2B-C). Each fruit has one to three pyrenes (Table 1).

Table 1. Biometric characterization of fruits, pyrenes, and seeds of *Aegiphila brachiata* Vell.
Note. SD = standard deviation; CV = coefficient of variation

Variable	**Minimum**	**Mean**	**Maximum**	**SD**	**CV (%)**
			Fruit		
Length (mm)	22.54	27.23	38.12	2.20	8.07
Diameter (mm)	16.10	17.86	23.03	1.88	10.51
Total mass (g)	3.17	5.24	9.47	1.15	22.06
N° seeds/fruit	1.00	1.14	3.00	0.67	52.44
Mass seed/fruit (g)	0.25	0.49	1.04	0.16	32.69
Pulp (%)	86.37	90.60	94.52	1.82	2.01
-			**Pyrene**		
Length (mm)	12.00	13.46	15.54	0.80	5.94
Diameter (mm)	6.50	8.12	9.30	0.53	6.53
Mass (g)	0.25	0.56	0.66	0.08	14.29
-			**Seed**		
Length (mm)	9.10	9.96	11.60	0.52	5.22
Width (mm)	4.70	5.30	6.00	0.27	5.09
Thickness (mm)	3.10	3.94	4.50	0.32	8.12

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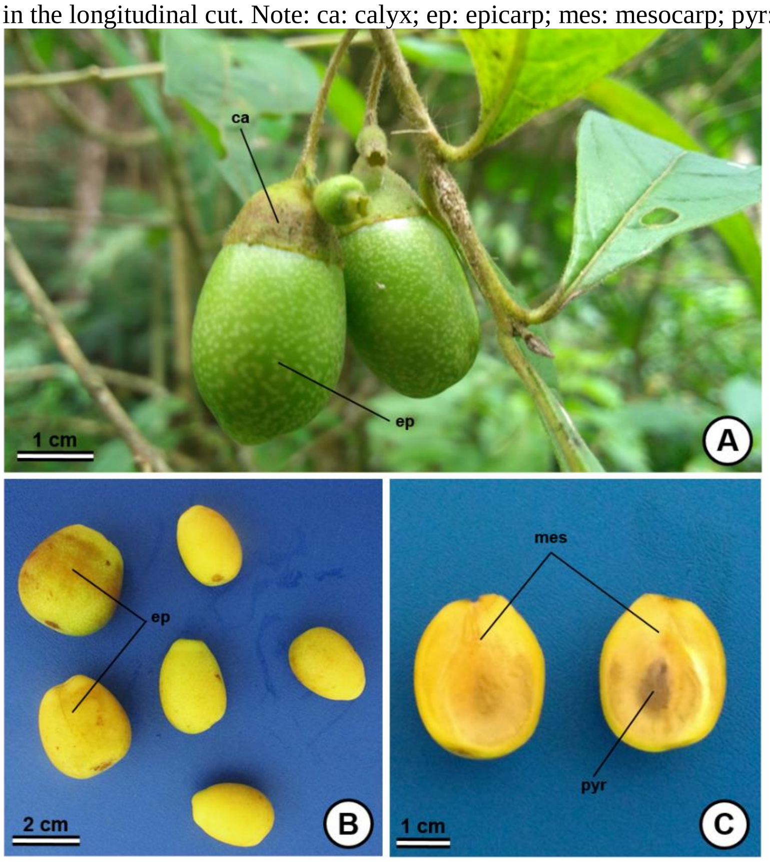
Figure 2. Fruits of *Aegiphila brachiata* Vell.: (a) branch with green fruits attached to the calyx; (b) ripe fruits; (c) ripe fruit in the longitudinal cut. Note: ca: calyx; ep: epicarp; mes: mesocarp; pyr: pyrene.



The pyrene is constituted by the endocarp and the seed; it has an oblong geometric shape, with a rounded base and acuminate apex. The endocarp, which surrounds the seed, is corneal, with light brown color and white streaks in the longitudinal direction and, when hydrated, does not show any apparent modification (Figure 3A). The pyrenes have an average of 13.4 mm in length, 8.1 mm in diameter, and 0.56 g of fresh mass (Table 1). The endocarp is approximately 1.7 mm thick. The thick endocarp imposes a restriction on embryo expansion, forming a rigid barrier that closes the micropyle, preventing the primary root emergence (Figure 3B).

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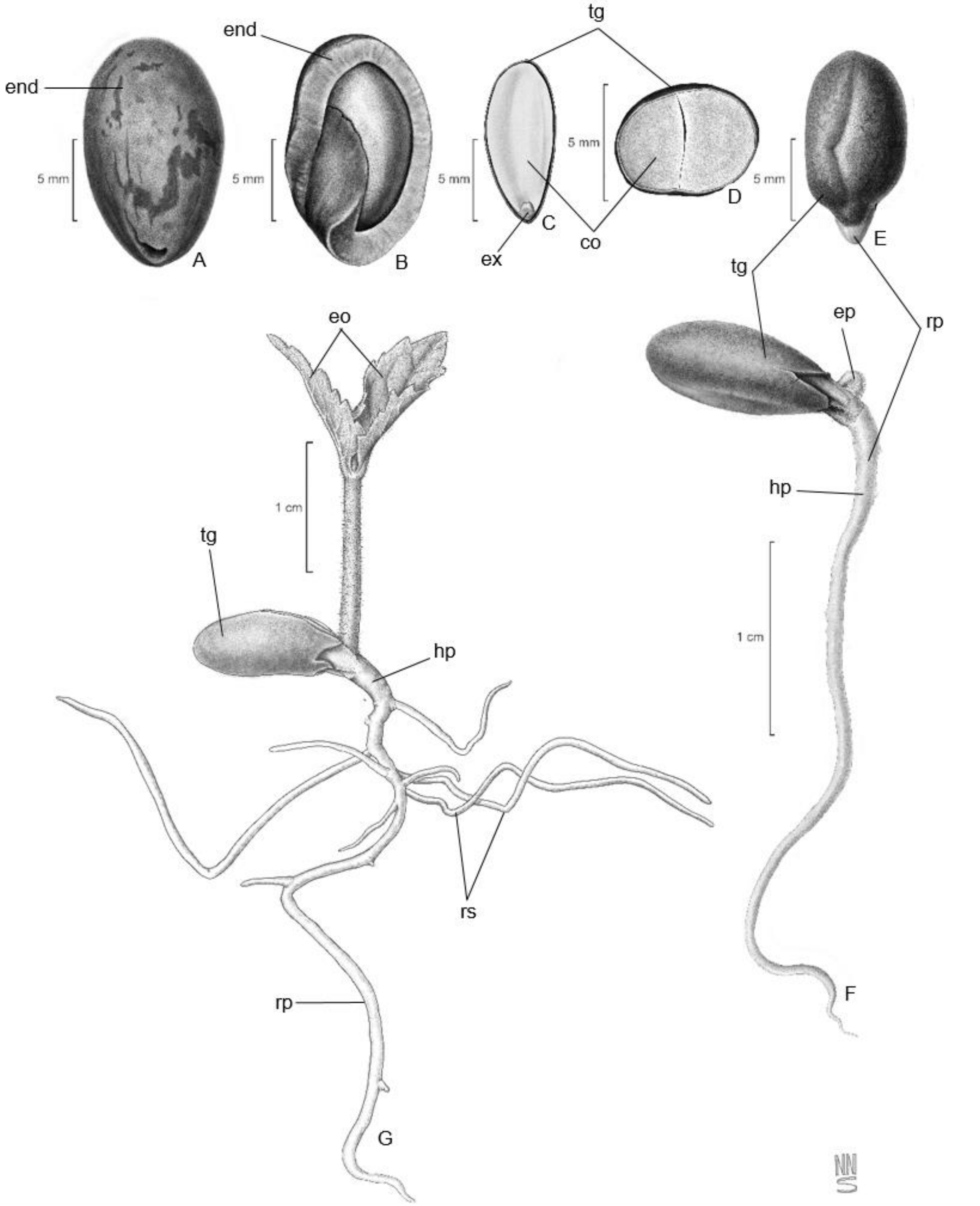
Figure 3. Seed and seedling of *Aegiphila brachiata* Vell.: (a) pyrene; (b) internal appearance of the endocarp; (c) seed in longitudinal section; (d) seed in cross-section; (e) primary root protrusion; (f) start of the areal part growth; (g) seedling. Note: end: endocarp; tg: tegument; ex: embryo axys; co: cotyledon; rp: primary root; ep: epicotyl; hp: hipocotyl; eo: eophyll; rs: secondary root.



Drawn by Natanael Nascimento.

Seeds without the endocarp have an average of 9.96 mm in length, 5.30 mm in width, and 3.94 mm in thickness (Table 1); they have a thin tegument, light brown color, and do not have endosperm – classified as exalbuminous. The hilum region is apical and oblong, and the micropyle is visible in a magnifying glass. The embryo is axial, with a short hypocotyl-radicular axis compared to the cotyledons, which are continuous, spatulate, fleshy, and whitish (Figure 3C-D). Germination is hypogeal, and seedlings are cryptocotyledon type – cotyledons do not emerge from the seed coat and remain inside it until the end of germination process (Figure 3E-G).

At the beginning of germination occurs the primary root emission, which is cylindrical and whitish (Figure 3E). Approximately nine days after beginning the germination, primary roots are about 3 cm long, starting the ascension of the light green epicotyl (Figure 3F). The seedling (15th day after germination) has a very elongated hypocotyl, thicker at the base and thinner at the apex, with long secondary roots and few ramifications (Figure 3G). The first pair of eophylls is simple, dark green, elliptical, with an asymmetrical base, acuminate apex, and serrated margin (Figure 3G). We observed the presence of simple trichomes, visible in a magnifying glass, spread throughout the epicotyl, and dispersed by eophylls (Figure 3F-G).

3.2 DORMANCY OVERCOMING AND TEMPERATURES FOR SEED GERMINATION

There was no interaction between the factors analyzed for germination percentage (G) and germination speed index (GSI) variables; however, there was a significant difference between dormancy breaking methods and temperatures (Supplementary Table S1). Regarding the dormancy overcoming method, total endocarp removal was more efficient than partial removal, with a higher germination percentage (28.5 %) and higher germination speed index (0.87) (Table 2). As for different temperatures, the lowest percentage of germination and GSI occurred at 20 °C (6.75 % and 0.31, respectively), differing statistically from the others (Table 2).

Table 2. Germination percentage (G) and germination speed index (GSI) of *Aegiphila brachiata* Vell. seeds submitted to different dormancy overcoming methods and temperatures.

Variable	Dormancy overcoming		Temperature (°C)		
	Total	Partial	20	25	30
G	28.50 a	13.17 b	6.75 b	26.75 a	29.75 a
GSI	0.87 a	0.50 b	0.31 b	0.84 a	0.89 a

Note: Means followed by the same letter in the line do not differ significantly by the Tukey test (p < 0,05).

The best results for mean germination time (MGT) are related to the lowest germination rates; that is, the lower the MGT value, the more efficient the treatment. Thus, we verified that total removal of the endocarp at 25°C provided the best result (9.63). Contrarily, totally removing endocarp at 20°C proved to be less efficient, with the highest MGT value (21.95) (Table 3).

Table 3. Mean germination time (MGT) of *Aegiphila brachiata* Vell. seeds submitted to different dormancy overcoming methods and temperatures.

Temperature (°C)	Dormancy overcoming methods	
	Total endocarp removal	Partial endocarp removal
20	21.95 aA	13.12 bA
25	9.63 aB	14.84 aA
30	10.72 aB	12.21 aA

Means followed by the same letters, lowercase in the line and uppercase in the column, do not differ significantly by the Tukey test (p < 0,05).

The knowledge of physic, biometrics, and morphologic characteristics of seeds is essential to understand species ecology and being successful in seedling production for economic or conservation purposes since seeds are the most used propagules for seedling production (Rego et al., 2009; Duarte et al., 2019; Maggioni et al., 2020). Our results related to seed physical characterization were close to those reported in the literature (Maggioni et al., 2020). The morphological description of *A. brachiata* is unprecedented in literature, and our study is the first to describe and illustrate seeds and the early development of the species.

The seed's and seedlings' characteristics are closely linked to the autecology of the species. In general, hypogea germination seeds, with reserve cotyledons, and cryptocotyledon seedlings are associated with species that form seedling banks in forests and can survive in understory conditions for long periods (Ressel et al., 2004); this survival is associated with reserve cotyledons, and as observed in our study, in *A. brachiata* they are protected by the endocarp. Also, a seedling morphology study in arboreal species from the Araucaria Forest showed that most pioneer species had trichomes in eophylls, while this characteristic was not present in late secondary species (Gogosz et al., 2015). These results corroborate our study, in which trichomes presence was observed in *A. brachiata* seedling, classified as an initial stage of succession species (Colonetti et al., 2009).

There are no reports in the literature that allow understanding *A. brachiata* dispersion and germination in its natural environment. Because they are relatively large fruits, it is believed that they are not eaten by small birds nor by toucans, as these fruits fall to the ground when they ripen. Thus, it is assumed that species' fruits are consumed by mammals, such as bats and crab-eating foxes, but detailed studies are needed to confirm this hypothesis.

Based on morphology it is clear that *A. brachiata* seeds have mechanical dormancy; this affirmation and the results observed in the germination test are related to the presence of the rigid endocarp (Maggioni et al., 2020). This cause of dormancy is associated with the mechanical action of tissues surrounding the embryo, in this case, the endocarp, which imposes restrictions on embryo expansion (Marcos-Filho, 2015). This type of dormancy is widely distributed in different species and families, as is the case with olive, pecan and walnut (Sotomayor-León & Caballero, 1990; Raoufi et al., 2020; Liu et al., 2022).

Thus, the barrier to embryo growth should be “weakened” or eliminated to allow root protrusion; endocarp removal favors embryo growth and provides advantages in early development and plant growth, and can be a strategy for species seedling production (Silva et al., 2019). Finding an efficient method for this barrier breakage is fundamental when considering plant production, and thus the importance of the results obtained in this study and their applicability to other species that may suffer from mechanical dormancy.

In addition to mechanical dormancy, some authors suggest that there is not only a purely mechanical impediment to embryo growth and that the action of chemical inhibitors may occur together (Marcos-Filho, 2015); some restrictions to the metabolism can inhibit embryonic development, which, in this way, would not exert enough pressure to break the endocarp barrier, although this was not tested in our study. Thus, new studies may be carried out to evaluate other possible impediments to *A. brachiata* seeds germination, given the large number of seeds classified as hard.

Literature shows a great divergence in the best conditions for forest species to express their germination potential; this indicates that each species presents a different germination behavior and is necessary to know the specific demands of each one. In our results, it is clear that

A. brachiata seeds need pre-germination treatments to overcome the mechanical dormancy imposed by the endocarp, furthermore higher temperatures (25 and 30 °C) were more favorable for germination. Also, the temperature of 20 °C is not indicated for presenting a low germination percentage, low GSI, and high value of MGT.

Temperature is a factor of great influence on the percentage, speed, and uniformity of germination since it controls biochemical reactions that regulate the entire metabolic process of germination (Marcos-Filho, 2015). Generally, higher temperatures accelerate metabolic processes, and lower temperatures slow down these processes (Bewley et al., 2013). For Atlantic Forest species, such as *A. brachiata*, studies show that the most frequent optimal temperature for germination is 25 °C (Brancalion et al., 2010).

Regarding overcoming dormancy, in seeds with partial endocarp removal, the radicle needed a greater force for its emission and growth since the endocarp's remaining part still imposed resistance to the primary root protrusion. Also, seeds with partial endocarp removal had a higher incidence of fungi with seed rot. Thus, it becomes clear in our study that the most suitable method to overcome dormancy in *A. brachiata* was total endocarp removal.

Although the germination percentages seem low, our results are promising since the species' seeds do not germinate without endocarp removal; in our study, seeds without endocarp germination started on the fourth day after sowing. In addition, the tool used is low cost and easy to handle, and few seeds were damaged in the process of endocarp removal, showing its potential as a wide-use method not only to overcome dormancy in *A. brachiate,* but also to apply it to other species.

4 CONCLUSIONS

Aegiphila brachiata fruit is fleshy and indehiscent with a persistent calyx; the seed is exalbuminous with axial embryo and spatulate cotyledons; germination is hypogeous, and seedling cryptocotyledonary. Seeds have mechanical dormancy due to the endocarp presence. Total endocarp removal by the proposed unprecedented method using sergeant clamp is the most suitable method to overcome mechanical dormancy, and temperatures of 25 and 30 °C are recommended for species germination in laboratory, showing the potential of the method for applicability in several species.

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