



***Bertholletia excelsa* seeds in the Cerrado-Amazon transition region: morphometry, colorimetry, viability and germination**

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ABSTRACT: The objective of this study was to describe morphometric characteristics of fruits and seeds and evaluate the viability and germination of seeds of *Bertholletia excelsa* stored on litterfall and under similar microenvironmental conditions to those found in their natural habitat, in a Cerrado-Amazon transition area, Brazil. The morphometric characterization consisted of measurements of diameters and latitudinal and longitudinal circumferences of fruits, exocarp and mesocarp thickness, fruit and seed weights per fruit, number of seeds per fruit, and seed thickness, width, and length. Seed moisture content was determined by the ratio between the fresh and dry weights. Colorimetric characterization was performed for mesocarp, outer and inner seed coats, and internal part of the seeds. Seed viability was evaluated using the tetrazolium test and germination was evaluated using intact seeds without seed coat. Morphometric variability was found for fruits and seeds of *B. excelsa*, regardless of the air humidity. Fruit color and seed outer coat color changed as the relative air humidity decreased. Variations in relative air humidity during the storage period resulted in a significant decrease (45.31%) in seed moisture content and compromised of the seed physiological quality, decreasing seed viability and germination capacity. *B. excelsa* seeds are sensitive to variations in air humidity. Storing seeds inside the fruit on litterfall and under similar microenvironmental conditions to their natural habitat for up to 96 days under mean relative air humidity above 65% ensures the maintenance of seed moisture above the critical level (30%). Viable seeds with germination potential present moisture contents above 45%.

Keywords: Amazon Rainforest; forest residues; recalcitrance; Brazil nut conservation; extractivism.

Sementes de *Bertholletia excelsa* na transição Cerrado-Amazônia: morfometria, colorimetria, viabilidade e germinação

RESUMO: Este estudo objetivou descrever as características morfométricas dos frutos e sementes, e, avaliar a viabilidade e germinação das sementes da *B. excelsa* armazenadas sob a serapilheira em condições microambientais do seu habitat natural na transição Cerrado-Amazônia brasileira. A caracterização morfométrica incluiu medidas de diâmetros e circunferências latitudinal e longitudinal dos frutos; espessura do exocarpo e do mesocarpo, massa dos frutos e das sementes por fruto, número de sementes por fruto, espessura, largura e comprimento das sementes. A umidade das sementes foi obtida pela razão entre massas. A caracterização colorimétrica ocorreu no mesocarpo, tegumento externo e interno e na parte interna da semente. A viabilidade das sementes foi avaliada pelo teste de tetrazólio e a germinação com sementes destegumentadas e intactas. Observou-se variabilidade morfométrica dos frutos e sementes da *B. excelsa* independentemente da umidade. A cor do fruto e do tegumento externo da semente mudaram de tonalidade com a redução da umidade relativa do ar. As variações da umidade relativa do ar durante o período de armazenamento influenciaram na redução expressiva de 45,31 % no teor de água das sementes e comprometeu a qualidade fisiológica, diminuindo a viabilidade e capacidade de germinação das sementes. As sementes da *B. excelsa* são sensíveis as variações de umidade. O armazenamento das sementes dentro do fruto sob a serapilheira em condições microambientais do seu habitat natural por até 96 dias com umidade relativa do ar média acima de 65 %, garante a manutenção da umidade das sementes acima da umidade crítica de 30 %. Sementes viáveis e com poder germinativo apresentam teor de água acima de 45 %.

Palavras-chave: Floresta Amazônica; resíduos florestais; recalcitrância; conservação da castanha do Brasil; extrativismo.

1. INTRODUCTION

Bertholletia excelsa Humb. & Bonpl., known as Brazil nut tree, is an ombrophilous forest species of the Amazon region. It has a wide geographical distribution, with the largest tree populations in the Brazilian Amazon region and in border

countries, such as Bolivia, Peru, Venezuela, Colombia, and Guianas (SALOMÃO, 2009). This species has a significant social, economic, and ecological importance in the Amazon Basin, as it is important for native populations and interacts with several living organisms in the forest, mainly agoutis,

which is a rodent animal that secondarily disperses its seeds (ZUIDEMA, 2003; WADT et al., 2018; ANDRADE et al., 2019).

These trees dominate the forest canopy; their lifespan ranges from 361 (SCHONGART et al., 2015) to 1,000 years (VIEIRA et al., 2005). Their cylindrical trunk is composed of wood of moderate specific density (SILVA et al., 2017), with the crown developing at the top, where the fruits are formed. The fruit has a lignified indehiscent capsule (shell) that contains a set of seeds; the seed consists of a triangular-shaped lignified integument, with another integument covering the nut (seed coats) (SANTOS et al., 2006). These seeds are internationally traded as Brazil nuts and are included in the human diet due to their desirable nutritional and energetic characteristics (LIMA et al., 2021).

Brazil is one of the main producers and exporters of fresh or processed Brazil nuts, reaching 33,400 Mg, with a production value of R\$ (BRL) 142.4 million (IBGE, 2021). Native local communities are at the base of this production chain, as they are involved in the extraction and commercialization of nuts from native Brazil nut trees, which are activities that contribute to their income and are part of their culture since pre-colonial times (ANDRADE et al., 2019).

Besides the nuts, residues from seed processing can add income to this extractivist activity. Studies have reported the potential of the fruit and seed coat for producing activated charcoal (SOUZA et al., 2021) and composing materials with high mechanical strength (SONEGO et al., 2019; 2021). The seed coat is nutrient-rich and has the potential to compose agricultural substrates for crops (BOUVIE et al., 2016).

B. excelsa has been subjected to a domestication process due to intense exploitation since the colonial period, which resulted in migratory flows, territorial occupation, and exploitation of resources by intentional and unintentional forest management (ANDRADE et al., 2019). This populational dynamic has caused impacts on the natural regeneration of the species (ZUIDEMA, 2003), leading to genetic erosion (BALDONI et al., 2020) and extinction threats for this species (MMA, 2014).

Supporting and incentivizing commercial plantations of *B. excelsa* is an alternative to maintaining the production chain, generating employment opportunities and economic returns. These initiatives can minimize anthropogenic pressure on native Brazil nut groves and enable the conservation of the genetic variability of the species, management practices for existing trees, and natural forest regeneration (TONINI; BALDONI, 2019).

Natural regeneration of this species is dependent not only on fauna activity and management practices, such as opening of canopy and trails, enrichment of native areas with seedlings, and removal of lianas in trunks and crowns of Brazil nut trees (SCOLES; GRIBEL, 2012), but also on physiological quality of seeds, which is connected with embryo maturity regulated by hormonal balance (CAMARGO et al., 2000) and environmental humidity conditions during dispersal (WADT et al., 2018; BORELLA et al., 2020). Therefore, understanding seed morphophysiological characteristics is important for the propagation and conservation of this species.

The slow and uneven germination process is one of the main challenges for propagating *B. excelsa*; it can vary from 6 to 18 months, as its oily seeds make the embryo hydration slow (MÜLLER et al., 1980). Removing the woody seed coat

without damaging the embryo has been a practice to decrease the germination time. However, other factors partly explain the slow germination process of the species, such as the existence of chemical dormancy (presence of inhibiting compounds) and physiological dormancy (embryo immaturity), i.e., seeds presenting no tissues at the advanced stage of cell differentiation at the time of maturation and dispersal (CAMARGO, 1997; CAMARGO et al., 2000).

The tetrazolium test is applied to evaluate seed physiological quality quickly and reliably; it is the most used test, which classifies the quality of seeds of the same lot through viability and vigor indexes. This test identifies seed tissues that present respiratory activity based on the reduction of the salt 2,3,5-triphenyl-tetrazolium chloride or bromide by the activity of dehydrogenase enzymes that catalyze respiratory reactions in the mitochondria during the glycolyze and citric acid cycle, mainly, malate dehydrogenase (FRANCE NETO; KRZYZANOWSKI, 2019). The tetrazolium solution penetrates the seed, reacting with hydrogen ions released by cell respiration, resulting in a reduction of the salt, shown by a red, stable, non-diffusible substance called triphenyl formazan or formazan (ABBADE; TAKAKI, 2014).

In this context, the objective of this study was to describe morphometric characteristics of fruits and seeds and evaluate the viability and germination of seeds of *B. excelsa* stored on litterfall and under similar microenvironmental conditions to those found in their natural habitat, in a transition region between the Cerrado and Amazon biomes, Brazil.

2. MATERIAL AND METHODS

2.1. Study area

The experiment was conducted in a Legal Reserve (RL) not subjected to recent wood exploration or extractivism in a Cerrado-Amazon transition area, in Claudia, MT, Brazil 11°34'19" S, 55°15'57" W, and 391 m of altitude) (Figure 1). The RL belonged to the Continental Farm, which hosts Module 1 of the long-duration ecological research network of the Biodiversity Research Program (PPBio), affiliated with the Federal University of Mato Grosso (UFMT), Sinop campus.

The climate of the region is Aw, tropical hot and humid, according to the Köppen classification, with a rainy season from October to April and a dry season from May to September (SOUZA et al., 2013). The mean monthly temperature varies from 24.9 to 27.7 °C; the mean monthly relative air humidity varies from 52% to 86%; and the mean annual rainfall depth is 1,945 mm, concentrating more than 1,700 mm in the spring and summer seasons. The soils in northern Mato Grosso state under areas with the presence of *B. excelsa* are deep and well-drained with a flat to undulating topography and are classified as Typic Hapludult and Typic Hapludox (SPERA et al., 2019; ALVES et al., 2022).

Micrometeorological conditions in the evaluation period (rainfall depth and mean and minimum relative air humidity) were obtained based on data from the Environment and Plant Interaction Research Group (www.gpambienteplanta.com). A mean air temperature of 25.66 °C was recorded in the study region during the evaluation period. The mean relative air humidity varied from 84.77% to 61.35% from the rainy to the dry season; the accumulated rainfall depth from January to April 2018 was 1,068.63 mm (Figure 2).

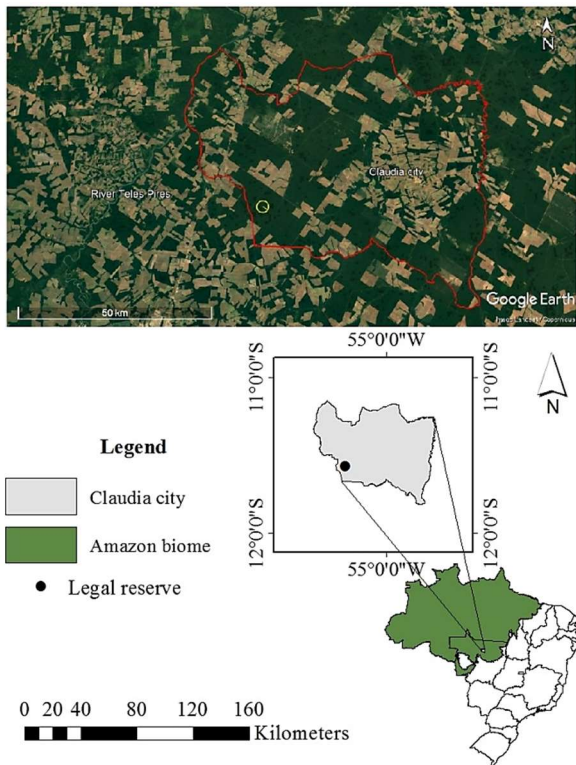


Figure 1. Location of *Bertholletia excelsa* mother trees in the Legal Reserve. Cláudia, MT, Brazil.
 Figura 1. Localização das matrizes da *Bertholletia excelsa* na Reserva Legal, em Cláudia-MT, Brasil.

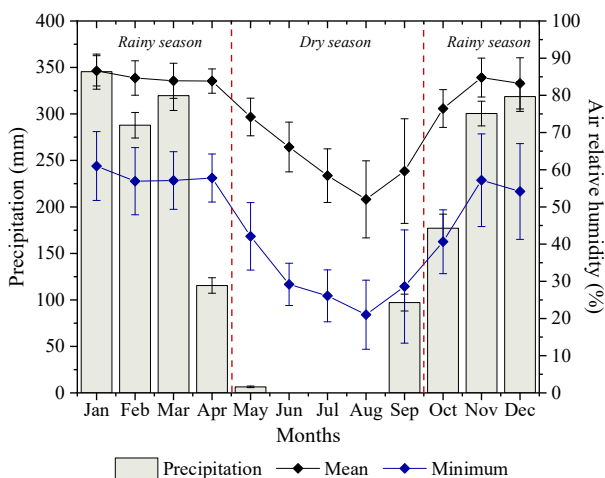


Figure 2. Mean monthly and standard deviation of rainfall depths and mean and minimum relative air humidity between January 01, 2018 and December 31, 2018 in Sinop, MT, Brazil.
 Figura 2. Médias mensais e desvio-padrão da precipitação, umidade relativa do ar média e mínima entre 01/01/2018 e 31/12/2018, em Sinop-MT, Brasil.

2.2. Fruit sampling and harvesting

The dispersal of *B. excelsa* fruits in this region occurs during the rainy season (October to March), enabling the maintenance of seed moisture for some months. Thus, fruits dispersed in January and February 2018, from 100 mother trees in a radius of 1.5 km within the RL (Figure 1), were placed at the base of some mother trees in the center of the collection area and left in direct contact with the litterfall to better maintain moisture under natural conditions (Figure 3).



Figure 3. *Bertholletia excelsa* fruits stored on the litterfall of a Brazil nut grove in Cláudia, MT, Brazil.
 Figura 3. Frutos da *Bertholletia excelsa* armazenados sob a serapilheira no castanhal, em Cláudia-MT, Brasil.

The morphometry and colorimetry of fruits and seeds and moisture content, viability, and germination of seeds were simultaneously analyzed. Ten evaluations (harvests) were carried out for each analysis, with a mean interval of 21 days (0, 15, 44, 63, 96, 127, 142, 159, 178, and 192 days after storage; DAS) from March 02 to September 10, 2018. The fruits were processed with the aid of a hole saw coupled to a drill and the seed coats were removed with a knife.

2.3. Fruit and seed morphometry

The *B. excelsa* fruit and seed morphometric characterization was carried out following the methodology proposed by Borella et al. (2017). The quantitative variables obtained were: fruit diameter and latitudinal and longitudinal circumferences (cm); exocarp and mesocarp thickness (mm); fruit and seed weights per fruit (g); number of seeds per fruit; and seed thickness, width, and length (mm) (Figure 4). Morphometric measurements of fruits and seeds were performed using a tape ruler, digital caliper, and semi-analytical (centesimal) and analytical (millesimal) digital balances. Visual qualitative descriptions of the fruit and seed physical integrity were carried out between harvests.

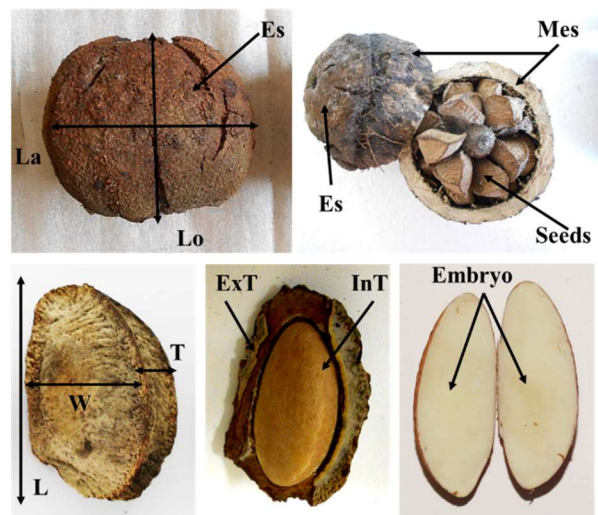


Figure 4. Fruits and seeds of *Bertholletia excelsa*. Cláudia, MT, Brazil. Eq = latitudinal measure; Lo = longitudinal measure; Es = exocarp; Mes = mesocarp; T = thickness; W = width; L = length; ExT = outer seed coat; InT = inner seed coat.
 Figura 4. Frutos e sementes da *Bertholletia excelsa*, Cláudia-MT, Brasil. Em que: Eq: medida latitudinal; Lo: medida longitudinal; Es: exocarp; Mes: mesocarp; T: espessura; W: largura; L: comprimento; ExT: tegumento externo; InT: tegumento interno.

2.4. Seed moisture

The *B. excelsa* seed moisture was determined immediately after harvesting the fruits. The set of seeds was weighed to obtain the fresh weight and, after drying the seeds in a forced air circulation oven at 105 °C for four days or until constant weight, they were weighed again in a digital analytical balance to obtain the dry weight and moisture content (Eq. 1) according to the Rules for Analysis of Seeds (RAS) (BRASIL, 2009).

$$M = \frac{(IW - FW)}{FW} \cdot 100 \quad (01)$$

where: M is the moisture content (%); IW is the initial weight (g); FW is the final weight (g).

2.5. Fruit and seed colorimetry

The colorimetric characterization of fruits and seeds of *B. excelsa* was carried out on the external part of the fruits, outer and inner seed coats, and inner part of the seeds (Figure 4). The colorimetric standards were obtained through the CIELAB system of the International Commission of Illumination (Comission Internationale de l'Eclairage), using the device Chroma Meter CR-400/410, calibrated with the illuminant C ($Y = 87.0$; $x = 0.3163$; $y = 0.3234$), which provides the colorimetric variables: L^* - lightness in a scale from 0 (black) to 100 (white), a^* - hue in the red (+a) to green (-a) axis, and b^* - hue in the yellow (+b) to blue (-b) axis, both scales from +60 to -60; and the variables C - chromaticity or saturation in a scale from 0 to 60 and h - hue angle, both derivatives from values of a^* and b^* (CAMARGOS; GONÇALEZ, 2001).

2.6. Seed viability

The viability of *B. excelsa* seeds was tested using tetrazolium, which is a colorless solution of 2,3,5-triphenyl-tetrazolium chloride or bromide that acts in tissues with respiratory activity. Seeds without seed coats were subjected to asepsis using a 2% sodium hypochlorite solution (NaClO) for three minutes.

A longitudinal cut between the poles of each seed was made for imbibition in 200 mL of distilled water by direct immersion inside Gerbox boxes placed in a BOD chamber at 30 °C without photoperiod for 24 hours. The hydration of seeds was carried out by imbibition in the tetrazolium solution, at the concentration of 0.5% for 24 hours, in a dark environment at 30 °C, as recommended by Borella et al. (2020). After applying the treatments, the seeds were washed in running water and kept on moistened paper towel until the end of the evaluations.

The red carmine color hue of the seeds was evaluated following the methodology of fruit and seed colorimetric characterization described in item 2.5. The colorimetric readings were carried out at positions A and C – apical and basal parts, respectively, and B – middle part (embryo) of the seed (Figure 5). The ΔE index was calculated (Eq. 2); it consists of the initial and final color variation due to the application of tetrazolium solution to the seeds.

$$\Delta E = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*} \quad (02)$$

where: Δ is the variation between the initial and the final or partial readings.

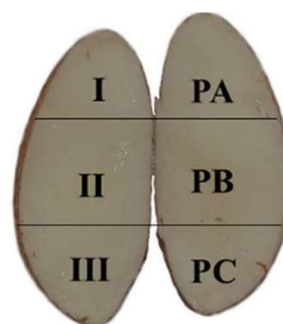


Figure 5. Reading position of colorimetric variables of *Bertholletia excelsa* seeds.

Figura 5. Posição de leitura das variáveis colorimétricas das sementes de *Bertholletia excelsa*.

The percentage of penetration was evaluated by attributing scores of 1 (without penetration, visible only in the cut region – 10% to 20% of the seed); 2 (surface penetration – 20% to 40% of the seed); and 3 (partial penetration – 40% to 60% of the seed) after a new longitudinal cut in the other half of the seeds. Viable and unviable seeds were then classified and counted.

2.7. Seed germination

B. excelsa seeds with apparently good phytosanitary status were selected for sowing and evaluation of the germination process per harvest. Seeds of this species present a lignified tegument, with a high physical-mechanical strength that hinders water absorption and consequently its germination, requiring months to the occurrence of the process. Therefore, intact seeds (without removal of the seed coat) were used, as well as seeds subjected to a mechanical process to overcome dormancy, with total removal of the seed coat with the aid of pliers and knives.

Seeds without mechanical damage were sown to a depth of 2.0 cm in washed sand on an above-ground bed made of corrugated fiber-cement roofing tiles, with dimensions of 10.0 × 0.6 × 0.03 m (length, width, and depth), placed at 1.0 m above ground, and lined in the East-West direction. The bed had upper, front, and side covers made of black polyolefin screen with 80% shading. Irrigation was carried out manually as needed to keep the seeds hydrated.

2.8. Statistical analysis

The results were subjected to descriptive statistics and analysis of variance at 5% significance level. The number of replications per analysis was as follows: i) morphometry = 20 fruits and 20 seeds (replications) per harvest; ii) seed moisture = 5 fruits or 5 sets of seeds (replications) per harvest; iii) colorimetry = 5 fruits and 5 seeds per fruit (replications) per harvest; iv) viability = 22 seeds per replication and three replications per harvest; and v) germination = 20 seeds (replications) with and without seed coat per harvest. The graphical representations were developed in the Origin® 6.0 program.

3. RESULTS

3.1. Fruit and seed morphometry

The *B. excelsa* fruits presented high variability in morphometric characteristics, with latitudinal and longitudinal diameters varying from 0.0749 to 0.1229 m and from 0.0659 to 0.1285 m, and latitudinal and longitudinal circumferences varying from 0.2370 to 0.4250 m and from

0.2670 to 0.4130, respectively (Figure 6A-D). Mesocarp thickness varied from 0.0039 to 0.0129 m, reaching a mean of 0.0043 ± 0.0011 m between 0 and 44 days after storage, the period in which they were in the fruits (Figure 6E).

Fruit fresh weight varied from 0.2114 to 0.7301 kg, with decreases from the beginning to the end of storage; the number of seeds per fruit varied from 9 to 24; the fresh weight of the set of seeds presented high variation during storage: 0.0509 to 0.2430 kg (Fig 6F-H).

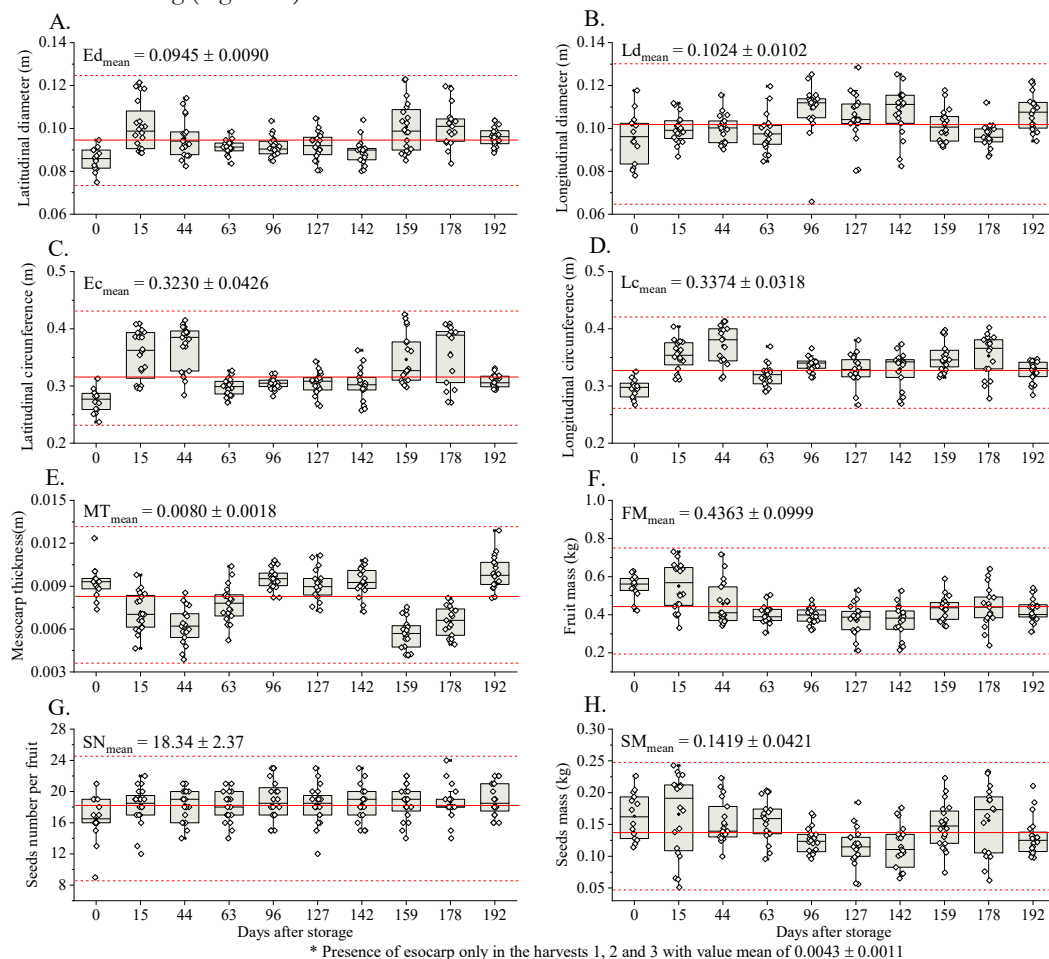


Figure 6. Boxplot of linear measurements and weights of fruits and a number of seeds of *Bertholletia excelsa* harvested in a native forest after 0 to 192 days of storage under natural conditions. Cláudia, MT, Brazil. I interquartile interval (amplitude between upper and lower quartile); ■ mean; – median.

Figura 6. Boxplot das medidas lineares e massas dos frutos e número de sementes da espécie *Bertholletia excelsa* colhidas em floresta nativa entre 0 e 192 dias após armazenamento nas condições naturais, em Cláudia-MT, Brasil. Em que: I intervalo interquartil (amplitude entre o quartil superior e inferior); ■ média; – mediana.

3.2. Seed moisture variation

The *B. excelsa* seed moisture at 0 days of storage on the forest litterfall was 61.65%, with exponential decreases over time. A significant decrease in seed moisture (25.01%) was found after 127 days of storage, reaching 16.34% after 192 days, at the end of storage (Figure 8).

3.3. Fruit and seed colorimetry

The external part of fruits and outer and inner seed coats of *B. excelsa* presented low lightness (L^*), with values close to 0 (black). The parameters a^* and b^* showed the predominance of red and yellow colors for fruit and outer and inner seed coats over the storage period (Table 1).

The inner part of the seeds exhibited pigmentations close to green and yellow and high lightness and hue angle, denoting a lighter hue than the other seed parts; the color

The *B. excelsa* seeds presented larger dimensions in length, varying from 0.02 to 0.06 m, followed by width varying from 0.02 to 0.04 m and thickness varying from 0.01 to 0.03 m (Figure 7A-C). In general, the ratios between the dry weights of the set of seeds and the fruit were between 0.06 and 0.33 and this ratio decreased from the beginning to the end of storage (Figure 7D).

saturation (C) was lower in the fruit and inner part of the seed (Table 1).

3.4. Seed viability and germination

The seed positions A, B, and C (apical, middle, and basal seed parts, respectively) presented no significant differences ($p > 0.05$) for colorimetric variables before or after applying the tetrazolium test. Thus, colorimetric readings were grouped by mean values and standard deviations in each harvest to confirm the viability and vigor of seeds through colorimetry and penetration of the tetrazolium solution.

Seed lightness (L^*) after the tetrazolium test varied from 48.72 to 24.45, denoting low lightness, mainly after 96 DAS. The seed pigmentation a^* and b^* denoted red and yellow colors varying from 42.37 to 34.46 and from 20.05 to 10.90, respectively.

Seeds with light red hues were found between 0 and 63 DAS, and seeds with darker red hues were found after 96 DAS, which affected the seed color variation (ΔE) (Table 2).

Color saturation (C) varied from 36.16 to 46.60; the hue angles (h^*) were low (17.28° to 26.32°), representing a more intense and bright red color (Table 2). The penetration of the tetrazolium solution into the *B. excelsa* seeds was not uniform. Most seeds presented no penetration between 0 and 96 DAS, i.e., showed visible color only in the region of disruption of

tissues; a higher percentage of seeds with surface penetration was found at 159 and 178 DAS; and partial penetration in most seeds were found at 142 and 192 DAS (Figure 9).

Viable seeds were those that presented light red carmine color and vigorous tissues, which were characteristics found in more than 50% of the seeds between 0 and 96 DAS; unviable seeds were those that presented white milky and soft tissues, which were characteristics found in most seeds after 127 DAS (Figure 9).

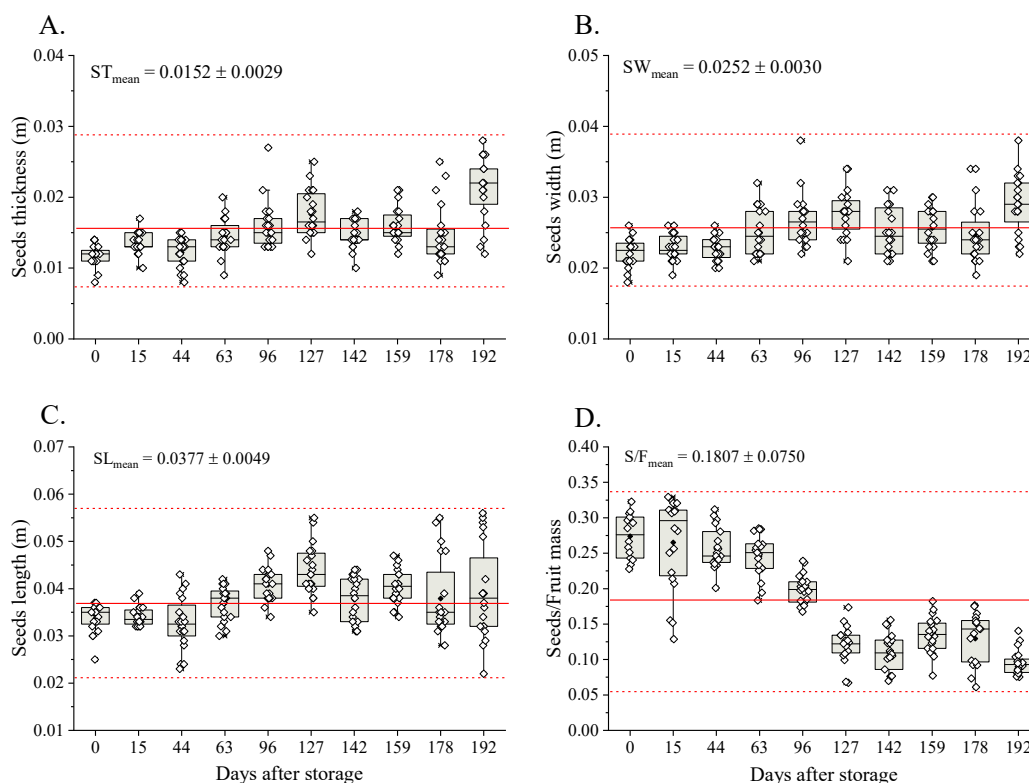


Figure 7. Boxplot of linear measures of seeds and seed dry weight ratio of *Bertholletia excelsa* fruits harvested in a native forest between 0 and 192 days after storage under natural conditions. Cláudia, MT, Brazil. I interquartile interval (amplitude between the upper and lower quartiles); ■ mean; – median.

Figura 7. Boxplot das medidas lineares das sementes e razão das massas secas de sementes por fruto da espécie *Bertholletia excelsa* colhidas em floresta nativa entre 0 e 192 dias após armazenamento sob condições naturais, em Cláudia-MT, Brasil. Em que: I intervalo interquartil (amplitude entre o quartil superior e inferior); ■ média; – mediana.

Table 1. Colorimetric characterization and color representation of *Bertholletia excelsa* fruits and seeds after different periods of storage under natural conditions. Cláudia, MT, Brazil.

Tabela 1. Caracterização e representação colorimétrica dos frutos e sementes da *B. excelsa* em diferentes períodos de armazenamento em condições naturais, Cláudia-MT, Brasil.

Fruit (Mesocarp)							Outer seed coat						
Harvest/DAS	L*	a*	b*	C	h	Color representation	Harvest/DAS	L*	a*	b*	C	h	Color representation
1/0	20.94	4.62	7.94	16.27	71.45		1/0	28.89	5.31	11.59	18.19	68.93	
2/15	18.11	2.89	4.20	9.19	59.42		2/15	24.53	3.66	6.68	12.80	62.97	
3/44	30.43	3.69	10.77	10.97	70.39		3/44	36.19	6.17	15.02	16.25	67.66	
4/63	34.51	4.76	12.87	13.73	69.03		4/63	40.07	7.04	17.85	19.97	70.57	
5/96	37.21	5.24	14.24	15.19	69.62		5/96	40.58	6.56	16.90	18.13	68.74	
6/127	33.29	4.75	12.46	13.34	69.13		6/127	39.80	7.54	18.62	20.10	68.13	
7/142	37.37	4.82	13.72	14.55	70.70		7/142	41.13	10.37	17.59	20.45	60.29	
8/159	39.32	5.97	14.44	16.25	67.97		8/159	46.88	13.01	21.63	25.24	67.04	
9/178	33.14	6.00	23.09	12.14	57.02		9/178	48.87	13.19	22.21	25.84	59.36	
10/192	37.48	7.23	16.34	17.92	66.47		10/192	46.66	12.32	21.08	24.43	59.58	
Mean	32.18	5.00	13.01	13.96	67.12		Mean	39.36	8.52	16.92	20.14	65.33	
SD	8.24	1.88	8.41	4.06	9.38		SD	9.27	3.85	5.88	5.57	7.02	

Inner seed coat							Seed						
Harvest/DAS	L*	a*	b*	C	h	Color representation	Harvest/DAS	L*	a*	b*	C	h	Color representation
1/0	47.96	13.62	26.05	29.76	60.13		1/0	87.24	-1.18	14.34	14.07	94.59	
2/15	41.01	11.14	15.91	29.43	62.11		2/15	85.60	-0.93	15.85	15.87	93.59	
3/44	49.99	13.69	26.28	29.73	61.66		3/44	85.71	-0.90	13.48	13.52	94.31	
4/63	47.40	13.21	23.70	27.24	61.42		4/63	87.59	-1.19	13.64	13.70	95.08	
5/96	46.81	14.55	26.00	29.81	60.61		5/96	87.83	-0.96	12.60	12.64	94.31	
6/127	47.22	14.34	23.47	27.56	58.26		6/127	87.79	-0.56	13.58	13.62	94.51	
7/142	49.15	15.21	25.47	29.72	59.15		7/142	88.56	-1.00	12.56	12.60	94.52	
8/159	48.99	15.08	25.98	30.05	59.79		8/159	87.92	-1.26	13.56	13.62	95.33	
9/178	46.65	14.37	23.29	27.41	57.94		9/178	87.02	-0.87	14.37	14.41	93.33	
10/192	47.49	14.60	24.14	28.26	58.54		10/192	87.67	-0.91	12.58	12.61	94.08	
Mean	47.27	13.98	24.03	28.90	59.96		Mean	87.25	-0.97	13.65	13.66	94.36	
SD	4.40	1.93	4.59	3.52	3.63		SD	2.63	0.54	2.19	2.20	1.52	

DAS = days after storage; SD = standard deviation. Source: <https://www.nixsensor.com/free-color-converter/>
 DAS é dias após armazenamento; SD é desvio padrão. Fonte: <https://www.nixsensor.com/free-color-converter/>

Table 2. Colorimetric characterization and representation of *Bertholletia excelsa* seeds subjected to the tetrazolium test after different periods of storage under natural conditions. Cláudia, MT, Brazil.

Tabela 2. Caracterização e representação colorimétrica de sementes de *Bertholletia excelsa* submetidas ao teste de tetrazólio em diferentes períodos de armazenamento em condições naturais, Cláudia-MT, Brasil.

Harvest/DAS	L*	a*	b*	ΔE	C	h	Color representation
1/0	44.92	40.38	20.05	2.96	45.03	26.32	
2/15	47.11	38.13	18.74	2.69	42.52	26.30	
3/44	48.72	38.54	17.93	2.72	42.52	24.71	
4/63	48.68	37.61	18.58	2.47	41.92	26.28	
5/96	25.23	38.55	13.94	4.24	41.04	19.54	
6/127	24.64	35.43	11.79	5.74	37.41	17.88	
7/142	24.45	34.46	10.90	5.49	36.16	17.28	
8/159	30.73	42.37	19.10	2.80	46.60	23.95	
9/178	28.93	40.45	17.39	3.03	44.05	23.14	
10/192	26.93	38.43	15.07	4.00	41.31	21.14	
Mean	34.68	38.42	16.27	3.61	41.81	22.56	
SD	11.05	4.79	4.43	1.49	5.94	4.18	

Source: <https://www.nixsensor.com/free-color-converter/>
 Fonte: <https://www.nixsensor.com/free-color-converter/>

B. excelsa seed germination was monitored for 266 days after sowing seeds from the first harvest, at 0 DAS. The mean time for germination was 4 months (116.62 days), varying from 3 to 6 months, showing high germination percentages

at 44, 63, and 96 DAS for seeds without seed coats. There was a low germination percentage of seeds with seed coat, which totaled 3 seeds (Table 3).

Table 3. Germination percentage of *Bertholletia excelsa* seeds subjected to mechanical scarification after different periods of storage under natural conditions. Cláudia, MT, Brazil.

Tabela 3. Porcentagem de germinação das sementes de *Bertholletia excelsa* submetidas a escarificação mecânica e em diferentes períodos de armazenamento em condições naturais, Cláudia-MT, Brasil.

Harvest/DAS	Without seed coat (%)	With seed coat (%)	Total germination (%)	Mean time for germination (day)
1/0	5	0	2	103.0
2/15	10	0	3	112.5
3/44	45	0	15	117.1
4/63	25	5	10	80.0
5/96	45	5	17	172.0
6/127	20	5	8	118.7
7/142	5	0	2	113.0
8/159	0	0	0	0
9/178	0	0	0	0
10/192	0	0	0	0
Total of seeds	31*	3*	34*	116.62 ± 16.56

* number of germinated seeds.

* número de sementes germinadas.

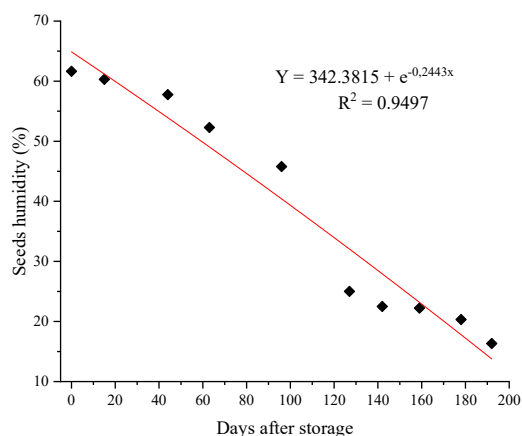


Figure 8. Exponential regression between seed moisture and days after storage in a native forest under natural conditions. Cláudia, MT, Brazil.

Figura 8. Regressão exponencial entre umidade das sementes e dias após armazenamento em floresta nativa sob condições naturais, em Cláudia-MT, Brasil.

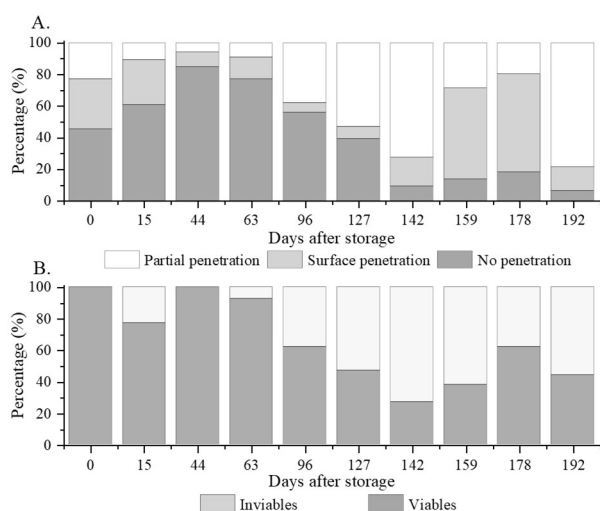


Figure 9. Percentage of penetration of the tetrazolium solution into the seeds (A); and percentage of viable and unviable seeds of *Bertholletia excelsa* harvested in a native forest between 0 and 192 days after storage under natural conditions. Cláudia, MT, Brazil.

Figura 9. Percentual de penetração da solução de tetrazólio nas sementes (A); e percentual de sementes viáveis e inviáveis de *Bertholletia excelsa* colhidas em floresta nativa entre 0 e 192 dias após armazenamento nas condições naturais, em Cláudia-MT, Brasil.

4. DISCUSSION

Despite the Brazil nut tree is represented by a single species (*Bertholletia excelsa*), studies have reported genetic diversity between populations (BALDONI et al., 2020) and between individuals of the same population (CAMARGO et al., 2010; COELHO et al., 2017), and consequently a high phenotypic variability, shown by yield indexes (KAINER et al., 2007; TONINI; PEDROZO, 2014; ROCKWELL et al., 2015), dispersal rates (WADT et al., 2018), morphology (size and shape), fruit weight, and number of seeds (CAMARGO, 2010; BORGES et al., 2016; BORELLA et al., 2017).

In general, most fruits exhibit an elongated shape (60.53%), meaning that they have a larger diameter and longitudinal circumference than the latitudinal circumference. However, Borella et al. (2017) found different

results: more flattened (47.75%) or rounded (40.30%) fruits. Contrastingly, the mean exocarp and mesocarp thickness, as well as the size and number of seeds were similar to those found in the present study.

The absence of exocarp found after 63 days after storage was due to seed deterioration by action of microorganisms (Figure 10). Intensification of attack of microorganisms is associated with high relative air humidity and direct contact of fruits with the soil and litterfall microbiota, which make the decomposition faster, as observed by Borella et al. (2017).

Borges et al. (2016) found fruit weights varying from 0.2288 to 0.9257 kg, mean seed weight per fruit of 0.1054 kg, and a mean number of 17 seeds per fruit. Borella et al. (2017) found lower fruit weights (from 0.0490 to 0.2770 kg), mean seed weight per fruit of 0.1160 kg, and 17.5 seeds per fruit, whereas 18.34 seeds per fruit were found in the present study. Both studies were carried out in the same region of the present study.

Variations in fresh fruit weight and seed weight were due to relative air humidity conditions during the harvest period, as well as due to exocarp deterioration. In addition, the presence of fungi in fruits and seeds may have affected seed weight loss and quality from the beginning to the end of storage.

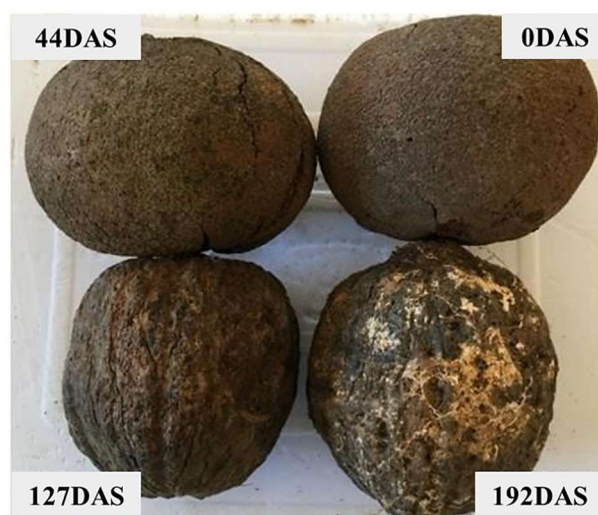


Figure 10. Exocarp deterioration in *Bertholletia excelsa* fruits under natural conditions of relative air humidity. Cláudia, MT, Brazil.

Figura 10. Deterioração do exocarpo nos frutos de *Bertholletia excelsa* em condições ambientais de umidade relativa do ar, Cláudia-MT, Brasil.

B. excelsa seeds exhibit a triserial triangular-angular shape, and an angular base, margin, and apex (SANTOS et al., 2006) requiring measurements of length, width, and thickness. The seed dimensions presented variability, as also found by Muller et al. (1995), who reported seed lengths of approximately 0.04 to 0.07 m. Peres; Baider (1997) found a mean seed length of and width of 0.047 and 0.021 m, respectively.

These morphological variations in fruits and seeds (size, weight, and number of seeds) in native species of the region are common, as they are strongly affected by species genetic diversity, geographical distribution, and edaphoclimatic conditions (CAMARGO et al., 2010; BALDONI et al., 2020).

The dry weight ratios between the set of seeds and fruit decreased from the beginning to the end of storage, mainly after 96 DAS, denoting a direct relation between fruit weight loss and seed weight loss. Decreases in fruit and seed dry weights are connected to the action of microorganisms, mainly on seeds, as their nutrient, protein, oil, and enzyme composition are attractive to fungi and bacteria, resulting in weight loss.

The seeds consisted, on average, of 18% dry fruit and approximately 32% moisture, but, different results were reported by Muller et al. (1995), who found approximately 25%. Zuidema (2003) found approximately 35% and Borella et al. (2017) found approximately 32% on wet basis. The remaining percentage is forest residues that can be used as substrate for crops, such as the case of seed exocarp and tegument, which have important macronutrients (N, K, Ca, S, Mg, and P) and micronutrients (Mn, B, Zn, and Cu), as well as adequate physical attributes, such as density, pH, and C to N ratio for manufacturing agricultural substrates (BOUVIE et al., 2016).

The mesocarp is composed of two types of lignified thick-wall cells (~56%), forming fibers that create a tangled structure, which provides it with high mechanical strength and potential for composing resistant materials (SONEGO et al., 2019;2021). The seed mesocarp and tegument have high C to N ratios (BOUVIE et al., 2016) and can be used for producing activated charcoal for energy production (SOUZA; SILVA, 2021), whereas the exocarp has nutrients and adequate physical attributes (density, pH, and C to N ratio) for manufacturing agricultural substrates (BOUVIE et al., 2016).

The *B. excelsa* fruits and seeds exhibit a color, hue, and lightness pattern that is probably connected to its chemical composition. The hue of the fruit and outer seed coat varied as the relative air humidity decreased. Burtin et al. (1998) explained that wood color varies due to air humidity and temperature, as well as to degradation of the material by photochemical reactions of chemical components in its structure. These variations in brown hues in fruits are also connected to the deterioration of the exocarp and the presence of fungi.

Color variation is used to identify the different fruit maturation stages and evaluate fruit quality without removing fruit samples or using other materials. This information, combined with morphometric characteristics, contributes to the development of classification and selection protocols for fruits and seeds during harvesting and processing. However, seed physiological evaluations through viability and/or germination tests are needed for understanding the propagation of species.

Considering that *B. excelsa* seeds are recalcitrant, i.e., sensitive to long storage periods and decrease in humidity (MÜLLER et al., 1980; BARBEDO, 2018), the seed viability and vigor were affected by the gradual decreases in relative air humidity, which decreased seed water content to critical levels (<30%) after 127 DAS (Figures 8 and 9). This was evidenced in the tetrazolium test after 96 DAS when the seeds started to present tissues with darker carmine red hues, as well as after 142 DAS when seeds presented flaccid tissues with a milky appearance (Figure 11) and significant decreases in germination percentage (Table 3).

A water content below 30% in recalcitrant seeds induces the deterioration processes, such as denaturation of proteins,

changes in peroxidase activities, and damage to the membrane system, which may result in viability losses and low germination rates. Recalcitrant seeds should be stored under high relative humidity to maintain seed moisture above 30% and between 50% and 70% moisture to reach physiological maturity; however, seed contamination by microorganisms should be considered under storage conditions (PAMMENTER; BERJAK, 2014).

Seed recalcitrance is associated with the immature stage; some species present strategies to elongate and anticipate their maturation cycle and, depending on the environmental conditions, the seed dispersal can occur before maturation, producing seeds with levels of different recalcitrance (BARBEDO, 2018). This may be one of the possible reasons for the high levels of recalcitrance of *B. excelsa* seeds, besides genetics and edaphoclimatic conditions.



Figure 11. Red carmine color pattern of *Bertholletia excelsa* seeds subjected to the tetrazolium test. Cláudia, MT, Brazil.

Figura 11. Padrão de coloração vermelho carmim nas sementes de *Bertholletia excelsa* submetidas ao teste de tetrazólio, Cláudia, MT.

The color and penetration of tetrazolium solution into seed tissues indicate the presence of respiratory activity. The red carmine color was exhibited in all seeds evaluated from 0 to 192 DAS, however, with differences in hue (Table 2), penetration (Figure 9), and coverage region (Figure 11) of the solution. Thus, the tetrazolium test used was adequate to identify viable seeds.

Vigorous seeds with a light red carmine color were found in the first harvest; however, there was the penetration of tetrazolium solution only into the tissue cut region and a low germination (Tables 2 and 3). Thus, the recently dispersed seeds were probably immature, with low respiratory activity, and reached maturity over time, as indicated by the increases in germination percentage from 44 to 96 DAS and partial penetration of the solution.

Mature seeds started to present dark carmine hues, a distinction of flaccid tissues, and no respiratory activity (dead tissues) after 127 DAS (Figure 11), as confirmed by the high percentage of unviable seeds and low germination (Table 3). The decrease in air humidity was a determining factor for the loss of physiological quality and degrees of salt penetration in these seeds.

Despite the germination peak from 44 and 96 DAS (45%), the percentage was still low. The *B. excelsa* seeds present minerals, proteins, and high lipid contents in their composition (LIMA et al., 2021), which makes water absorption slow, thus resulting in dehydration. A large

amount of embryo reserves and/or the internal hormonal balance can slow the imbibition process, enzymatic activation, and differentiation of the seed's meristematic tissues of seeds, consequently affecting germination (CAMARGO et al., 2000).

Müller et al. (1980) explain that the dispersal of premature fruits of *B. excelsa* may occur, which may be related to the hormonal balance. In addition, the seeds present no tissues at advanced stage of differentiation at the time of seed maturation and dispersal, such as other common seeds that form plumule, radicles, and cotyledons. These may be some of the possible reasons for the challenging assessment of the physiological quality and germination of these seeds (CAMARGO et al., 2000).

B. excelsa seeds are sensitive to variations in air humidity and there is no efficient protocol to keep them stored for a long time. These seeds can be stored for short periods inside the fruit on litterfall and under similar microenvironmental conditions to their natural habitat for up to 96 days under mean relative air humidity above 65%; these conditions may ensure the maintenance of seed moisture above the critical level (30%), as viable seeds with germination potential present water contents above 45%.

5. CONCLUSIONS

The viability loss of *Bertholletia excelsa* seeds is connected to decreases in air humidity levels during the storage period. The germination does not depend only on the seed moisture, but also on the seed maturity.

Under adequate conditions of seed physiological maturity and moisture, the propagation of *B. excelsa* through seeds should be carried out using seeds without seed coat to decrease the time for germination.

Morphometric characteristics of *B. excelsa* fruits and seeds present high phenotypic variability, denoting that these variations may originate from genetic and microclimate diversities.

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The authors declare no conflict of interest.