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Original Article

Unonopsis guatterioides: morphological and chemical characteristics of its fruits and seeds, and germination processes under experimental conditions

Unonopsis guatterioides: características morfológicas e químicas de frutos e sementes e processos de germinação em condições experimentais

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Abstract

The *Unonopsis guatterioides* (Annonaceae) species provides wood which is used in rural construction projects, has leaves which are used in traditional medicine and its fruit is important in bird diets. This study aimed to evaluate the morphometry of seeds and fruits, their chemical composition, and the germination at different temperatures using seeds subjected to tegumentary dormancy break from fruits collected in a semi-deciduous seasonal forests in Mato Grosso do Sul, Brazil. The fruits under ripening showed a higher number of secondary metabolites and higher phenolic compounds. Morphometry results indicated that most seeds have length between 3.5 and 5.6 (81.3%), width between 8.1 and 9.0 mm (85.0%) and average weight of 0.28 g. The results indicate that there is a greater number of fruits with length ranging between 9.0 and 16.5 mm (74.8%), width between 9.0 and 12.0 mm (60.0%) and average weight of 1.33 g. Germination was strongly affected by temperature, reaching 58.0% and 62.0% of germinability at 30 °C and 25-35 °C, respectively, with seeds showing integumentary dormancy, requiring scarification.

Keywords: Annonaceae, flavonoids, germination, chemical scarification, seed morphometry.

Resumo

A espécie *Unonopsis guatterioides* (Annonaceae) possui madeira utilizada em construções rurais, folhas com uso na medicina tradicional e os frutos são importantes itens na dieta de aves. O objetivo deste trabalho foi avaliar a morfometria das sementes e frutos, sua composição química e germinação em diferentes temperaturas, com sementes submetidas a quebra de dormência tegumentar, com frutos coletados em matas estacionais semideciduais, Mato Grosso do Sul, Brasil. Os resultados obtidos indicaram que os frutos em maturação apresentaram maior número de metabólitos secundários e teor de compostos fenólicos. Em relação as sementes, os resultados de morfometria indicaram que a maior parte das sementes possui entre 3,5 e 5,6 mm de comprimento (81,3%), 8,1 e 9,0 mm de largura (85,0%) e 0,28 g de peso médio. Em relação aos frutos, os resultados indicaram maior número entre 9,0 e 16,5 mm de comprimento (74,8%), 9,0 e 12,0 mm de largura (60,0%) e 1,33 g de peso médio. A germinação foi fortemente afetada pelas temperaturas, atingindo 58,0% e 62,0% de germinação na temperatura de 30°C e 25-35 °C, respectivamente, com sementes apresentando dormência tegumentar, necessitando de escarificação.

Palavras-chave: Annonaceae, flavonoides, germinação, escarificação química, morfometria de sementes.

1. Introduction

The *Unonopsis guatterioides* (A.DC.) R.E.Fr. (Annonaceae) species is found as a tree or shrub, with a length between 1 and 10 meters, found in forest areas of the Amazon, Cerrado and Atlantic Forest biomes (Lopes and Mello-Silva, 2023). It is a secondary, evergreen and heliophile species with flowering between September and October, fruiting between April and May (Lorenzi, 2021), and its seeds are normally dispersed by birds (Oliveira and Paula, 2001), such as curassow (*Crax* sp.) and guan (*Aburria* sp.) (Lorenzi, 2021).

The species has several metabolites of medicinal interest (Silva et al., 2012), in addition to being used in folk medicine, with the leaves used to treat stomach ulcers (for example) (Lorenzi, 2021), which explains the importance of studying its phytochemical and germinative characteristics. However, landscape fragmentation processes have led to a rapid reduction of forest cover in the Brazilian biomes, especially the Cerrado, thereby increasing the risk of different species extinction (Zorzetto, 2021).

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Knowledge about germination processes is essential for the conservation and recovery of degraded areas (Salomão et al., 2003). Thus, evaluating seed behaviour in different substrates and temperatures becomes a tool which enables seedling production (Brancalion et al., 2010). Annonaceae seeds have dormancy morphological (Salomão et al., 2003) and studies have been conducted to determine the factors involved with seed dormancy (Battilani et al., 2007; Lorenzi, 2021). Thus, it is necessary studies on dormancy break in Annonaceae (Salomão et al., 2003).

According to Brancalion et al. (2010), a temperature of 25 °C is the most appropriate for the majority of Brazilian tree species. However, each species has specific requirements and information on the germination processes of a large part of Brazilian trees are still unknown (Brasil, 2009). There is also great diversity regarding the substrate, and the best substrates can be correlated with the specific characteristics of each species, such as the region of origin and the size and moisture content of the seed (Brasil, 2009). The correct choice of temperature and substrate enable accelerating and homogenizing the germination, providing a greater number of seedlings in the shortest space of time (Brasil, 2009; Brancalion et al., 2010).

In addition to factors related to germination, information on the seed is useful in species identification because this structure is slightly modified by the environment (Oliveira et al., 2011). Therefore, the morphometry is fundamental to assist in seedling production and area recovery studies (Andrade et al., 2010). Despite its importance, this information is still scarce, taking into consideration the great biodiversity in the Brazilian biomes (Fonseca et al., 2013), and Pagliarini et al. (2014) described the results of this kind of research as being infrequent. In addition, knowledge about the fruit and seed chemical characteristics also enable indicating their potential use and being able to correlate these characteristics with the seed dispersal processes (Fernandes et al., 2021).

Taking into consideration the fact that relatively little is known about the germination processes of *Unonopsis guatterioides*, this study aimed to evaluate its seed and fruit morphometry, their chemical composition, seed scarification methods and germination in different temperatures.

2. Methods

2.1. Collection area

Ripening and ripe *Unonopsis guatterioides* fruits were collected in semi-deciduous seasonal forests (directly from 12 mother trees) from the Cerrado biome, Corguinho (latitude 19°49'54" South and longitude 54°49'46" West), in Mato Grosso do Sul, Brazil. The average altitude in the region is 320 m and the region's climate is tropical (Aw) with dry winters and rainy summers according to the Köppen and Geiger climate classification. The region has an average temperature of 24.8 °C and average annual rainfall of 1444 mm (SEMAC, 2011). The material was stored in paper bags and transported in thermal boxes to the Interdisciplinary Laboratory for Research in Environmental and Biodiversity Systems (LabPSAB), Universidade Anhanguera-Uniderp, Campo Grande, Mato Grosso do Sul. An exsiccate was mounted and incorporated into the herbarium collection (Herbarium of Laboratory of Plant Morphology) under number 8560.

2.2. Morphometry of seeds and fruits

The intact fruits (n = 1237) and seeds (removed from fruits) (n = 2846) were evaluated for their length (measured between the base and the apex) and width (measurement performed in a median position) using a digital pachymeter (precision 0.01 mm, model Digimatic Caliper 700-113). The Sturges rule (Sturges, 1926) was used to build the frequency histograms, indicating 11 classes for fruits and 12 classes for seeds.

The seeds were subsequently extracted and weighed individually (analytical scale - precision 0.001 g, model AG 200, Gehaka), obtaining the average weight of 2,846 fresh seeds. The pulp of each fruit was also weighed individually, obtaining the average weight of 1,237 fresh fruits. The average weight of pulp and seeds was used to calculate which structure (pulp or seed) accumulates the highest amount of fresh mass, using the simple rule of three.

2.3. Obtaining the ethanol extracts - pulp and seeds

The fruits were visually selected in the laboratory regarding the absence of spots or injuries, then separated into two groups: (1) completely ripe (bark 100% black-violet colour and soft texture); and (2) partially ripe (red-orange colour). After washing in running water to eliminate surface dirt, the pulp and seeds were separately subjected to extraction processes with ethyl alcohol in ultrasound bath (ultrasonic cleaner®) for 60 minutes, followed by static maceration and subsequently kept at rest for 24 h in a refrigerator (Fontoura et al., 2015).

2.4. Phytochemical prospection

Phytochemical tests were performed using the procedures described by Matos (2009), with the extracts obtained. The analyses were performed in triplicate, and the results were compared with the control sample by observing changes in colour and precipitation based on Fontoura et al. (2015). Changes in colour were classified as partial (10%), low (25%), moderate (50%), medium (75%), high intensity (100%), or negative (0%) (Fontoura et al., 2015). The tests with precipitate formation (phenolic compounds and tannins) were carried out in graduated tubes, and the results were classified as partial intensity (<0.2 cm), low (0.21-0.50 cm), moderate (0.51-0.70 cm), and high intensity (0.71-1.0 cm) (Fontoura et al., 2015).

2.5. Determination of the phenolic compounds, flavonoids, carotenoids, pH and soluble solids

Total phenols were measured by the Folin-Ciocalteu method with gallic acid (McDonald et al., 2001). Flavonoids were evaluated by the aluminium chloride method (Chang et al., 2002). Carotenoids were evaluated according to the methodology of Rodriguez-Amaya (1993). The pH reading was done in a potentiometer (pH DM-20, Digimed) and the soluble solids concentration was determined using a refractometer (Model 45 RTD), with results expressed in degrees Brix, and corrected to 20 °C. The experimental design was based on three replications for each evaluated compound, with the mean being calculated followed by the standard deviation.

2.6. Germination

The seeds obtained from ripe fruits were initially submitted to a moisture degree test using the gravimetric method with seeds dried in an oven at 105 ± 3 °C for 24 hours to measure the water mass removed from the seeds by drying, using the arithmetic mean of four samples, with four replicates of 25 seeds (Brasil, 2009).

A sample of seeds obtained from ripe fruit were then submitted to four treatments: mechanical scarification passing the seeds seven times on sandpaper no. 80 (80 grit 3M sandpaper) and then superficially disinfected by immersion in sodium hypochlorite (2%) for three minutes (treatment 1); chemically scarified seeds by immersion for one minute in sulphuric acid 98% (H_2SO_4) and washed in running water (treatment 2); two minutes in sulphuric acid 98% (H_2SO_4) and washed in running water (treatment 3); and we included a control treatment in which intact seeds were used (treatment 4).

Using transparent boxes (11 x 11 x 3.5 cm), the seeds were placed on vermiculite (moistened with distillate water 1.5 times vermiculite mass) and kept in germination chambers Eletrolab EL 202 (temperatures of 20, 25, 30, 35, and 25-35 °C). A photoperiod of 12:12 hours of white light and dark was adopted in all cases, with four replicates of 25 seeds each. The alternating temperature (25-35 °C) was obtained every 12 hours, according to the change of photoperiod.

We measured the germination percentage (germinability) (Labouriau, 1983) and germination was monitored every day for a period of 100 days, with seeds that presented the primary root (approximately 2 mm) being considered germinated. The non-germinated seeds were submitted to the viability of tetrazolium chloride test (salt-triphenyl tetrazolium chloride to 1% in aqueous solution); first, they were placed in a plastic box between two sheets of filter paper soaked in tetrazolium reagent box and kept in the dark at a temperature of 28 ºC. The readings of the results were performed after 24 h, longitudinally sectioning the seeds with a scalpel. Seeds that presented 76 to 100% of their vital areas stained in light carmine red were considered viable, in addition to turgor of the tissues and developed and intact embryo structures (Brasil, 2009).

2.7. Germination - experimental design and statistical analysis

A completely randomized experimental design with four replications of 25 seeds per treatment in 4x5 factorial scheme (4 scarification treatment x 5 temperature) was implemented. The data were subjected to analysis of variance, and when significant, the averages were compared by the Tukey's test (p<0.05). The results obtained from germination did not meet the assumptions of the ANOVA, as the average was zero in some treatments. Thus, the variance is zero and the variances are heterogeneous between treatments (transformation of the results into arcsine did not improve the homogeneity of the variance). Therefore, ANOVA and regression analysis were performed on the untransformed germination percentage (Dong et al., 2020), with significant differences between treatment means identified by the Tukey's test.

3. Results

3.1. Phytochemical prospection

The *Unonopsis guatterioides* fruit showed red-orange (ripening fruits) and black-violet (ripe fruits) peel colour, depending on the ripening stage, contrasting with the green of the leaves, and the fruit is distributed in bunches (Figure 1).

Figure 1. *Unonopsis guatterioides* fruit (A) in the ripening process; and (B) ripe, collected in semi-deciduous seasonal areas forest, Corguinho, Mato Grosso do Sul, Brazil.

The fruit in the red-orange stages (Figure 1) showed lower soluble solid levels (4.9% ± 0.3) in relation to the black-violet colour stage (5.9% ± 0.5), with the values of both stages being higher than those found in the seeds (2.3% ± 0.4). The results also indicated a high intensity of reducing sugars in ripe fruit in relation to ripening fruit (Figure 2).

The flavonoids, a structurally diverse group, and their derivatives, were among the bioactive compounds found with greater intensity in seeds (183.5 ± 12.1 mg g^{-1}), followed by ripe fruit (179.2 ± 17.6 mg g^{-1}) and ripening fruit (158.6 ± 11.5 mg g^{-1}). The phenolic compounds followed a different profile of flavonoids, with higher levels in the ripening fruit (323.3 ± 21.4 mg g^{-1}), followed by ripe fruit (204.9 ± 15.7 mg g^{-1}) and seeds (191.5 ± 19.3 mg g^{-1}). The pH of the ripening fruits (6.3 ± 0.3) was more acidic than those found in the seeds (6.6 ± 0.3) and ripe fruits (6.9 ± 0.5). The results regarding carotenoids indicate the highest levels in ripe fruit (7.9 ± 0.3 mg g^{-1}), ripening fruit (6.3 ± 0.5 mg g^{-1}) and seeds (4.6 ± 0.5 mg g^{-1}).

The ripening fruit showed a greater number of secondary metabolite classes (n = 10), followed by the ripe fruit and seeds (n = 9). Seven classes were common in the ripe and ripening fruits and seeds. Flavonols were only found in ripe fruit, and xanthones and flavones in ripening fruit and seeds. The anthraquinones were only found in the fruits (ripening and ripe), with high intensity (Figure 2). Xanthones, flavones and flavonols found confirm the strong presence of phenolic compounds and flavonoids in the fruits.

3.2. Morphometry

The results indicate that there are a greater number of fruits with length ranging between 9.0 and 16.5 mm (74.8%) and total variation in size from 6.0 to 22.5 mm. In terms of width, most of the fruits have between 9.0 and 12.0 mm (60.0%) and total variation between 1.5 and 18 mm (Figure 3). The average fruit weight was 1.33 g and the pulp represented 51.9% of the total mass, indicating the accumulation of resources in this part of the fruit.

There was greater standardization regarding the seeds, with the majority having length between 3.5 and 5.6 mm (81.3%) and ranging between 1.4 and 9.8 mm. Most had between 8.1 and 9.0 mm (85.0%) width, ranging between 3.6 and 14.4 mm (Figure 4). The average seed weight was 0.28 g.

3.3. Germination

The seed moisture content found (15.0%) indicates seeds in the group between orthodox and recalcitrant seeds. The result of the germination process indicated that there is a need for scarification for primary root protrusion, as the control treatment did not show germination (Table 1).

The results also showed that low temperatures, such as 20 and 25 °C, are not suitable for seed germination, as only a temperature of 20 °C and 25 °C (acid treatment for 2 minutes) resulted in a small number of germinated seeds when compared to the other treatments. Although there was germination, the immersion in sulphuric acid for one minute, and scarification with sandpaper (mechanical scarification) were less suitable. On the other hand, two minutes of acid resulted in the best results, with temperatures of 30 °C and 25-35 °C (Table 1). The tetrazolium chloride viability test performed on the embryos after the end of the experiment indicated that they were no longer viable because the embryos of all the seeds were dead.

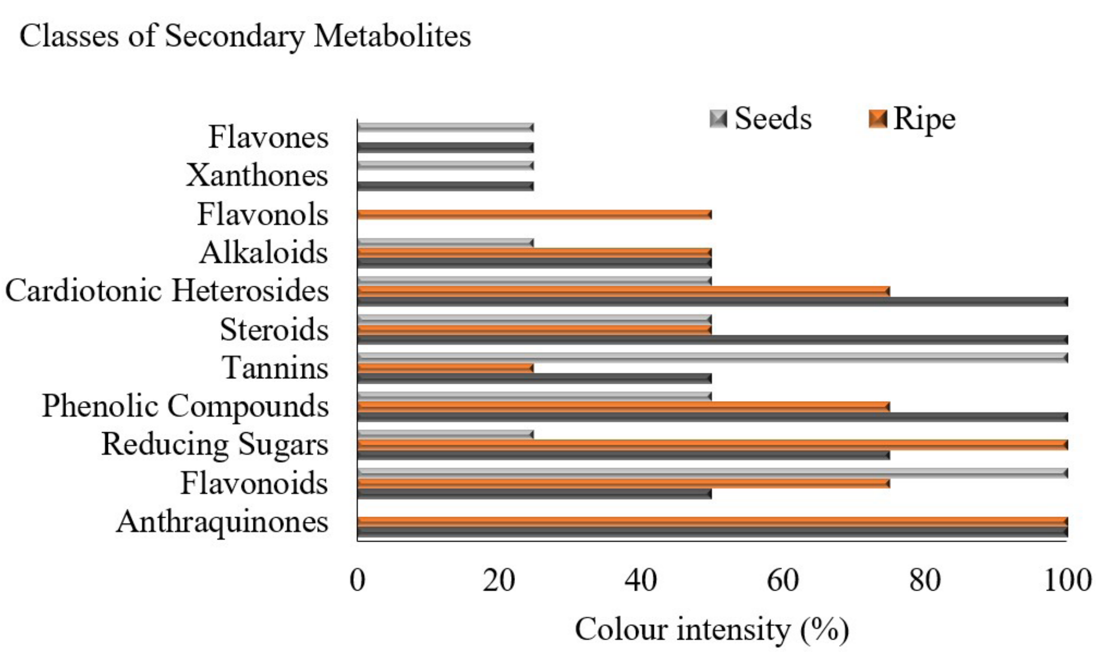

Figure 2. Classes of secondary metabolites (colour intensity) found in the extract of *Unonopsis guatterioides* fruits (ripe and ripening fruit pulp) and seeds, Corguinho, Mato Grosso do Sul, Brazil.

Table 1. Germination, *Unonopsis guatterioides* seeds subjected to chemical and mechanical scarification and maintained at different temperatures in vermiculite substrate. Campo Grande, MS, Brazil, 2021.

Source of variation	***d. f.***	**Sum of squares**	**Mean square**	***F* (*p*)**	
Scarification (F1)	3	15276.80	3819.20	2604.00 (<0.0001)	
Temperature (F2)	4	12271.20	4090.40	2788.91 (<0.0001)	
Interaction F1xF2	12	6700.80	558.40	380.73 (<0.0001)	
Treatments	19	34248.80	0.00000001	-	
Residuals	60	88.00	1.47	-	
Total	79	34336.80	0.00000001	-	
Germination (%)*	20 °C	25 °C	30 °C	35 °C	25-35 °C
Control	0.00 bA	0.00 bA	0.00 dA	0.00 cA	0.00 dA
Acid 1 minutes	0.00 bD	0.00 bD	24.00 cC	32.00 bB	48.00 bA
Acid 2 minutes	4.00 aE	16.00 aD	56.00 aB	36.00 aC	62.00 aA
Sandpaper	0.00 bD	0.00 bD	28.00 bC	32.00 bB	36.00 cA

*Means followed by the same lowercase letter in the column, and upper letter in the line do not differ statistically among themselves by the Tukey's test (*p*>0.05).

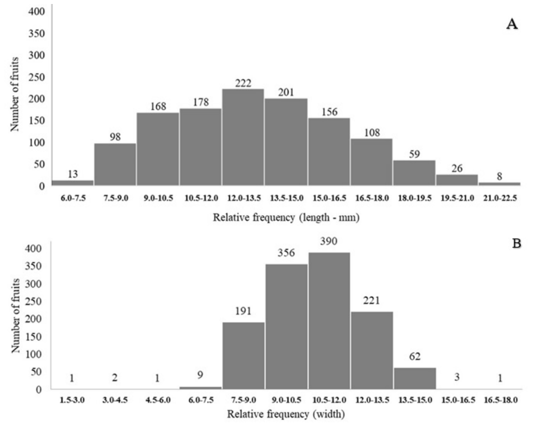

Figure 3. Relative frequencies of fruit morphometric characteristics (A - length and B - width) of *Unonopsis guatterioides* collected in semi-deciduous seasonal forest areas, Corguinho, Mato Grosso do Sul, Brazil.

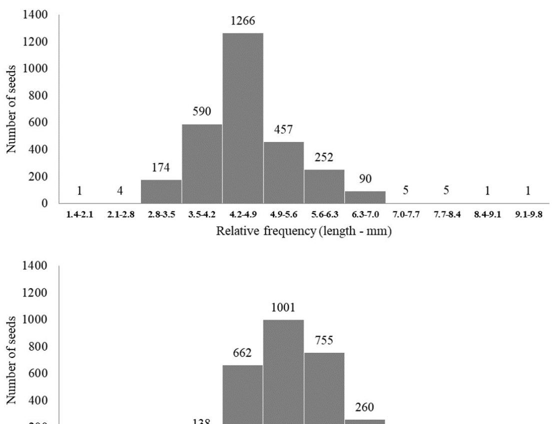

Figure 4. Relative frequencies of seeds morphometric characteristics (length and width) of *Unonopsis guatterioides* collected in semi-deciduous seasonal forest areas, Corguinho, Mato Grosso do Sul, Brazil.

4. Discussion

4.1. Chemical characterization of fruits

Unonopsis guatterioides fruit has important nutritional characteristics, such as the presence of reducing sugars in medium intensity, in addition to phenolic compounds in high intensity. However, they also have high intensity compounds that can select their consumption by certain groups of herbivores, such as anthraquinones and cardiotonic heterosides. Its chemical composition can explain consumption by birds, without mention of consumption by mammals, with Lorenzi (2021) writing that *U. guatterioides* fruit is one of the most attractive to avifauna, as their seeds are dispersed by birds such as Curassow and Guan. Melo et al. (2013) confirm that the dispersion syndrome of the species is ornithochoric.

The observations in the collection area confirm the information by Melo et al. (2013) and Lorenzi (2021), with the consumption of fruits by terrestrial mammals such as *Pecari tajacu* Linnaeus, 1758 (Musk hog) or *Tapirus terrestris* Linnaeus, 1758 (Tapir) found in the region not being observed. A camera trap installed under the canopy of the trees by the researchers during the fruiting period did not record the consumption of fruits by any species of terrestrial mammals. The presence of cardiotonic heterosides observed in medium intensity in ripe fruits collected in the area could hamper their ingestion by mammals, because this metabolite can cause damage to the organism of these animals (Rates et al., 2017).

The preference for the consumption of *Unonopsis guatterioides* fruits by birds, as reported by Melo et al. (2013) and Lorenzi (2021), may be related to their morphologic and chemical characteristics, such as colouring, nutrients and secondary metabolites and the degree of colour saturation can indicate the nutritional quality of the fruits. According to Maruyama et al. (2019), the relationship between plants and frugivorous birds is related by nutritional and morphological characteristics of fruits, with the species preferring fruits with a higher concentration of sugars, which is important for the reproductive season of birds.

The ripe pulp becomes attractive to dispersal agents like birds, and the fruits spread by birds in general are colourful, in a way that there is a contrast with the green background (leaves) when they are ripe and ready for the dispersion (Barnea et al., 1993). For the Annonaceae species, the fruit colouring pattern for ripening and ripe fruit is very variable. *Onychopetalum periquino* (Rusby) D. M. Johnson & N. A. Murray have similar aspects with the species under study. Its fruit is a berry, and it goes from dark green to yellow over the course of its development, until it reaches a red wine colour when ripe (Maas et al., 2007).

Another important feature for foraging refers to the variation of the nutritive elements (Jordano, 2000), which include lipids, amino acids, proteins, carbohydrates and mineral salts. Jordano (2000) also reports that primary carbohydrates, such as glucose and fructose, are the most attractive for frugivorous birds, but in different proportions depending on the ripening stage, and resulting in the choice of ripe fruits for greater efficiency in their use. Birds are the main seed dispersers of the species, and are probably attracted to the fruits due to the carbohydrates, specifically the reducing sugars (monosaccharides) such as glucose and fructose. According to Chitarra and Chitarra (2005), ripening promotes biochemical transformations until reaching the edible stage, with suitable texture and colour for consumption. According to Levey (1987), the sugar concentration in the fruit is common and probably the birds choose the sweeter ones due to easy assimilation of this group. The presence of carbohydrates is confirmed by the concentration of soluble solids, also found in *Onychopetalum periquino* fruits, with values of 12.6% for ripening fruit and 11.4% for ripe fruit (Farias et al., 2011), which differs from the results found in this study.

Fruits of the Annonaceae species are generally investigated for commercial purposes, with emphasis on the *Annona* genus (in Brazil, *A. muricata* L. and *A. squamosa* L.) (Orsi et al., 2012). The fresh pulp of the two species showed a pH of 3.4 and 3.3 (Orsi et al., 2012), indicating higher acidity in relation to the *Unonopsis guatterioides* species. However, the high acidity in *Annona* is compensated by its high value of soluble solids, between 63.0 and 68.6% for the mentioned species. In a study of temperate regions, the high acidity of *Viburnum opulus* var. *Edule* Michx. fruit may be related to the presence of chlorogenic acid, a phenolic compound, as a plant defence against pathogens (Jones and Wheelwright, 1987). Regarding the species under study, *U. guatterioides*, it is possible to suggest that the greater acidity of ripening fruits, along with the lowest value of soluble solids, do not favour its consumption by birds.

Another nutritional characteristic of *Unonopsis guatterioides* fruit important for frugivory is the presence of carotenoids, an important source of provitamin A, such as the α and β carotene pigments that determine the yellow, orange and/or red colours (Rodriguez-Amaya, 1993) found in greater concentration in ripe fruit. The black-violet colour of ripe fruits is probably associated to carotenoids and anthocyanins. Other species of the same family, *Annona crassiflora* Mart., has values of 5.0 mg g^{-1} of carotenoids, which is similar to those found in this study, perhaps indicating a pattern for the family (Cardoso et al., 2013).

In addition to the presence and quantification of nutrients, other important information about the characteristics of the fruits used as food for birds is the existence of toxins, such as some secondary metabolites. Natural toxins of ecological interest are known to have allelopathic and agonists effects against herbivores and pathogenic micro-organisms and are likely involved as mediators of interactions among seed dispersers (Cipollini and Levey, 1997). According to Tsahar et al. (2002), there are two approaches to explain the presence of secondary metabolites in the fruits. The first hypothesis is sustained on the basis of the species evolution, aiming to improve the success of the seed dispersal (adaptive approach). The second one, an adaptive approach, in which the metabolites primarily evolved to prevent herbivores from eating fruits and seeds, with their presence in ripe fruits being a by-product of this function.

Thus, it is necessary to determine the classes of secondary metabolites in these structures to better understand the relationship between the birds' ability to establish and maintain preferences for the ripening fruit and ripe fruit, as well as seed dispersal. In this sense, it is important to highlight the presence of cardiotonic heterosides in high intensity in the ripening fruit. This group of metabolites can cause poisoning and death in mammals when consumed in greater quantity (Rates et al., 2017). Their consumption can be minimized through the effects of anthraquinones, a group recognized as a facilitator of the evacuation process. Therefore, ingesting fruit with the presence of anthraquinones will lead to a faster residue excretion process, avoiding their permanence in the birds digestive system and their fermentation, and can eliminate possible toxic substances, such as the cardiotonic heterosides and alkaloids found in fruits.

The laxative/constipating effects of secondary constituents on the animals which consume the fruits are still discussed. Cipollini and Levey (1997), precursors of these studies, suggest the hypothesis that specific metabolites change the passage rates, facilitating seed elimination through a laxative effect, considering that such action may be associated with a higher seed germination rate. On the other hand, the anthraquinones and other secondary compounds present in green fruits may discourage their consumption by frugivores, before the seeds are ready to be dispersed. It is still possible to suggest the action of anthraquinones in defence of ripe fruits from fungal attack, because according to Harborne (1994), anthraquinones generally contain antimicrobial agents.

On the other hand, flavonoids, a structurally diverse group, and their derivatives, are among the bioactive compounds found with greater intensity and are among the most ubiquitous components of polyphenols which are distributed in various fruits, being known for their antioxidant activities and health benefits for different organisms (Harborne, 1994); this suggests protection of the birds' intestinal tract through a detoxifying system. Barnea et al. (1993) indicate that the presence of flavonoids are common in fruits, such as rutin and quercetin, and are attractive to birds. For Harborne (1994), the flavonoids in plants have the ability to act as a significant barrier against the herbivores' supply. Therefore, it is possible to infer that the seeds store these constituents as a form of protection against predation, justifying its higher concentration in seeds.

According to Barnea et al. (1993), it is possible to point out that non-palatable chemical groups are allocated in unripe fruit, but these groups are neutralized in ripe fruit. The same authors explain that it is possible to find non-palatable groups and often toxic ones in the seed as a form of protection against predation.

The group of phenolic compounds and flavonoids is formed by anthocyanins, anthocyanidins, flavones, flavonoids, xanthones, chalcones, aurones and flavonols (Zuanazzi et al., 2017). Anthocyanins are natural glycosylated pigments, water soluble and are related to most of the shades of the colours blue, purple and red found in flowers and fruits, for example, which is a way to attract birds (Lev-Yadun and Gould, 2008; Zuanazzi et al., 2017). In addition to these colours, black-violet is also regarded as a characteristic colour of fruits consumed by avifauna (Schaefer et al., 2006), and although anthocyanins are not identified, the black-violet coloration indicates the presence of this group. These natural dyes have an important role in several mechanisms for breeding plants, such as seed dispersal, in addition to performing other functions such as defence mechanism (Lev-Yadun and Gould, 2008).

This chemical group (phenolic compounds) is the first to be formed in the synthetic route and is probably involved as a mediator of interactions between seed and fruits dispersers (Lev-Yadun and Gould, 2008; Zuanazzi et al., 2017). The same authors also claim that they act in ripening fruit to repel birds at first because the seeds are still not viable for dispersion; then, their concentration decreases when the fruits are ripe, allowing consumption by frugivores. Cipollini and Levey (1997) support the hypothesis that certain secondary metabolites are retained in fruit pulp to inhibit the seeds germination, and when frugivores remove the pulp from the fruits during consumption, they enable/initiate the seed germination process.

Tannins showed a different profile from other polyphenols, with greater intensity in the ripening fruit compared to ripe ones, in addition to 100% intensity in the seeds. Their astringent characteristics are known and it is possible to suggest that the tannins are free in ripening fruit and the pulps become non-attractive to spreaders; then when the fruits ripen, they bind to proteins and/or carbohydrates, which leads to a decrease in their activity, making them more palatable (Lambers and Oliveira, 2019). The same authors write that the tannins in the seeds are in free form, meaning without forming complexes with proteins and carbohydrates, making them less palatable and protecting them against herbivory. This hypothesis is based on Cipollini and Levey (1997), in which the authors suggest that the tannin-protein complexes and tannin-carbohydrates are formed in ripening fruit and reduce the astringency of tannins. Therefore, the fruit becomes palatable for consumption, facilitating seed dispersal. In addition, tannins and other compounds can interact with each other when consumed together in a way that their negative effects (independent) are minimized or increased (Lambers and Oliveira, 2019).

Another group identified in high intensity (100%) in ripening fruit was steroids, which are considered phyto-hormones (Taiz and Zeiger, 2013). In normal conditions they could induce greater seedling growth after seed germination because the bird droppings would possess scarified seeds rich in phyto-hormones, and thus facilitate seedling establishment. This group was also found in fruits of other Annonaceae species such as *Annona squamosa* Linn. (Sharma et al., 2013). However, the lower intensities were found in ripe fruits and seeds (50% of intensity), and perhaps a greater intensity could be expected in seeds, which did not occur. Perhaps this type of metabolite has other functions when in high intensities in ripening fruit, but have not been described yet.

4.2. Morphometry

The data obtained are partially similar to those mentioned by Battilani et al. (2007) for *Unonopsis lindmanii* Fries, which is currently considered a synonym for *Unonopsis guatterioides*, in which the authors describe fruits from 0.9 to 1.8 cm in length, 0.8 to 1.3 cm in width and mass, more often between 0.5 and 1.0 g. In addition, the seeds were found to be between 0.7 and 1.1 cm in length, 0.6 to 1.1 cm in width and mass, between 0.1 and 0.4 g. Our results indicate that there is greater variability concerning the size and mass for the species. The differences found in relation to the study by Battilani et al. (2007) are probably related to the region of the seeds origin (Serra da Bodoquena, region of limestone rocks), the number of matrices where the collections were carried out (not mentioned), and probably the number of fruits and seeds assessed, which in the case of the authors above was only 100 fruits and seeds (a small number).

4.3. Germination

The seed moisture content is similar to other Annonaceae species, such as *Annona tomentosa* R.E.Fries (12%) and *Xylopia aromatica* (Lam.) Mart. (14%). However, other species of the same family have higher values, such as *Xylopia sericea* A.St.-Hil. (28%) and *Annona crassiflora* (33%) (Salomão et al., 2003).

Regarding the germination process, non-scarified seeds did not germinate in the implementation period of the experiment, while scarified seeds using acid or sandpaper presented primary root protrusion. Although germination occurred in all scarification treatments, the one-minute sulphuric acid immersion and sandpaper scarification were less adequate, while the two-minute acid immersion produced the best results. The suitability of the substrate used for tests with species of the same family had already been mentioned by Ferreira et al. (2010) in evaluating the emergence of *Rollinia mucosa* (Jacq.) Baill (Annonaceae), indicating that this is one of the most suitable substrates, a factor related to its high water retention capacity.

In evaluating the species germination, Battilani et al. (2007) mentioned 3% at a temperature of 25 °C (continuous light, germination chamber) and 70% when in the greenhouse, with the beginning of the process after 45 days and 60 days for the hypocotyl emission. However, the authors do not mention the existing environmental temperatures in a greenhouse covered with a screen (low density polyethylene) or the luminous intensity reached. Battilani et al. (2007) also mention that there is dependence of the photoperiod for germination, since there was a good germination percentage in the greenhouse (but they do not mention how the seeds were sown in the substrate when in the greenhouse). In addition, they indicate the presence of morphological dormancy for the species due to the fact that the germination occurs slowly and irregularly (two to five months), but without mentioning the emergence speed indexes.

According to Bewley et al. (2013), this type of germination occurs in seeds with rudimentary embryos. Van Setten and Koek-Noorman (1992) also describe that Annonaceae seeds have a poorly developed or unripe embryo when dispersed, requiring time for its growth after dispersion. The evaluation of the presence of morphological dormancy was not performed for the studied species. However, the results obtained in this study also indicated that tegumentary dormancy occurs for the *Unonopsis guatterioides* species, with dormancy breaking being necessary and the use of acid is more efficient for the process.

The best condition regarding germination would be scarification, acid for 2 minutes, and alternating temperature. Battilani et al. (2007) and Lorenzi (2021) mentioned a long emergence time for the species, which was confirmed by this study and the presence of tegumentary and physiological dormancy would be responsible for this situation. According to Salomão et al. (2003), species such as *A. crassiflora*, *A. tomentosa*, *X. aromatica* and *X. sericea* have different degrees of dormancy. This process would be related to having a tegument which is impermeable to water and gases, as observed in this study. Salomão et al. (2003) also mention several days for the germination process for some species of this family, such as *A. crassiflora* with 41-75 to 392 days, *X. aromatica* needing 30 days, and *X. emarginata* 30 to 60 days. The same authors mention that the mechanical scarification process is required for breaking the dormancy of species such as *D. lanceolata*, *X. aromatica* and *X. emarginata*, demonstrating the hard tegument effect. Brancalion et al. (2010) consider the range of 25 °C as the most appropriate germination temperature for most species of the Cerrado biome, whereas Zucareli et al. (2007) concluded that 30 °C is the most suitable germination temperature for species of the *Annona* genus.

The results obtained in this experiment confirm the temperature of 30 °C as appropriate and also the alternate ones of 25-35 °C. In accordance with Brancalion and Marcos Filho (2008), alternate temperature regimes may be related to overcoming the tegument impermeability to water, allowing the seeds hydration and their germination. The alternate temperatures are suitable for obtaining good germination rates for certain species (Borges and Rena, 1993), and according to De Vitis et al. (2014), may indicate that the species has adaptation to successional environments (gaps), where pioneer species occur and seeds may require these temperatures to optimize the germination. However, other factors, such as seed scarification, may be necessary to induce germination.

On the other hand, lower temperatures of 20 and 25 °C, and higher of 35 °C were not the most appropriate. Brancalion et al. (2010) mention that many tropical species are sensitive to low temperatures, reducing their germination rates. The obtained results showed that the seeds kept at lower temperatures did not germinate or germinated in small rates. The longer time of seeds in germination chambers can also generate other problems. The embryo examination at the end of the experiment indicated that they were no longer viable, constituting a factor related to the degradation caused by the attack of different fungi, despite the use of fungicides.

According to Bewley et al. (2013), low temperatures slow the metabolic activities, providing a delay in germination process and allowing greater attacks by pathogens. On the other hand, according to the same authors, high temperatures can modify the chemical reactions speed and increase the consumption of reserves (carbohydrates, for example) and the water absorption rate, degrading the seeds and leading to their death. This situation was observed after visualizing non-germinated seeds embryos at 35 °C, which were no longer viable.

One of the objectives of germination tests is to generally evaluate the seeds' physiological potential. Thus, the tested temperatures significantly influenced the germination process of *Unonopsis guatterioides* seeds, and indicates that the species has requirements which enable good germination rates. In addition to these factors, the existence of tegumentary dormancy resulted in a long germination process, demonstrating that the species exhibits characteristics which need to be better known.

5. Final Considerations

The ripening fruit showed a greater number of metabolite classes (n = 10), followed by the ripe fruit and seeds (n = 9). Seven metabolite classes are common to the three samples (ripe and ripening fruit pulp, and seeds), with flavonols only evidenced in ripe fruit, xanthones and flavones in ripening fruit and seeds, and anthraquinones found with high intensity in ripe and ripening fruits. The temperature choice affected the germination rates for the species, which presents a germination process that extends for weeks, reaching 62% in the best conditions. Its seeds have tegumentary dormancy, requiring scarification and temperature of 30 °C to obtain appropriate germination.

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