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Morpho-anatomical study of *Protium altsonii* Sandwith plants

Estudo morfo-anatômico de plantas *Protium altsonii* Sandwith

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Abstract
This study aimed to describe the morphoanatomical characters of fruits, seeds and seedlings, in addition to characterizing the anatomy of leaves and trunk of the species *Protium altsonii* Sandwith. The fruits, leaves and wood were collected in the Sustainable Development Reserve in the Iratapuru River (SDR), Amapá. Cross and longitudinal sections were cut with a rotary microtome. As for the characteristics, it was observed that the fruits are globose to ovoid, with a slightly sharp apex and short stipitate, and they also present one and, more rarely, two pyrenes per fruit. The mature seed of *P. altsonii*, in cross section in the abaxial region, is formed by the seed-coat with collapsed cells and fleshy cotyledonary parenchyma, containing secretory cavities and oil. In understory, the leaves of young plants with two years and six months are compound, imparipinnate and can present 6 to 11 pairs of leaflets. In their turn, the adult leaves are compound, imparipinnate and may present six or more pairs of leaflets per branch. In a cross-section of the wood, there are numerous solitary and multiple vessels; small to medium; vascular elements with a simple perforation plate. The morphological characteristics of the fruit, seed and leaves, in addition to the anatomy of the species under

study allow us to assure that *Protium altsonii* presents phenotypic plasticity as an ecological strategy. The germinative efficiency in field of these species may have been caused by the release of the coumarin present in the cotyledons.
Keywords: *Protium*; Anatomy; RDS Iratapuru; Burseraceae.

Resumo
Este trabalho teve como objetivo descrever os caracteres morfológicos de frutos, sementes e plântulas, além de caracterizar a anatomia de folhas e tronco da espécie *Protium altsonii* Sandwith. Os frutos, folhas e madeira foram coletados na Reserva de Desenvolvimento Sustentável no Rio Iratapuru (SDR), Amapá. Cortes transversais foram feitos com um micrótomo rotativo. Quanto às características, observou-se que os frutos são globosos a ovóides, com ápice levemente pontiagudo e estipitado curto, além de apresentarem um e, mais raramente, dois pirênios por fruto. A semente madura de P. altsonii, em corte transversal na região abaxial, é formada pelo tegumento com células colapsadas e parênquima cotiledonar carnoso, contendo cavidades secretoras e óleo. No sub-bosque, as folhas das plantas jovens com dois anos e seis meses são compostas, imparipinadas e podem apresentar de 6 a 11 pares de folíolos. Já as folhas adultas são compostas, imparipinadas e podem apresentar seis ou mais pares de folíolos por ramo. Em uma seção transversal da madeira, existem numerosos vasos solitários e múltiplos; pequeno a médio; elementos vasculares com uma placa de perfuração simples. As características morfológicas do fruto, semente e folhas, além da anatomia da espécie em estudo, permitem afirmar que *Protium altsonii* apresenta plasticidade fenotípica como estratégia ecológica. A eficiência germinativa em campo dessas espécies pode ter sido causada pela liberação da cumarina presente nos cotilédones.
Palavras-chave: *Protium*; Anatomia; RDS Iratapuru; Burseraceae.

Resumen
Este estudio tuvo como objetivo describir los caracteres morfológicos de frutos, semillas y plántulas, además de caracterizar la anatomía de hojas y tronco de la especie *P. altsonii* Sandwith. Los frutos, hojas y madera fueron recolectados en la Reserva de Desarrollo Sostenible en el Río Iratapuru (SDR), Amapá. Las secciones transversales y longitudinales se cortaron con un micrótomo rotatorio. En cuanto a las características, se observó que los frutos son globosos a ovoides, con el ápice ligeramente afilado y estipitado corto, además de presentar uno y más raramente dos pirenos por fruto. La semilla madura de *P. altsonii*, en corte transversal en la región abaxial, está formada por la cubierta de la semilla con células colapsadas y parénquima cotiledóneo carnoso, que contiene cavidades secretoras y aceite. En el sotobosque, las hojas de las plantas jóvenes con dos años y seis meses son compuestas, imparipinnadas y pueden presentar de 6 a 11 pares de folíolos. Por su parte, las hojas adultas son compuestas, imparipinnadas y pueden presentar seis o más pares de folíolos por rama. En una sección transversal de la madera, hay numerosas vasijas solitarias y múltiples; pequeño a mediano; elementos vasculares con una simple placa de perforación. Las características morfológicas del fruto, semilla y hojas, además de la anatomía de la especie en estudio, permiten asegurar que *Protium altsonii* presenta plasticidad fenotípica como estrategia ecológica. La eficiencia germinativa en campo de estas especies puede haber sido causada por la liberación de la cumarina presente en los cotiledones.
Palabras clave: *Protium*; Anatomía; RDS Iratapuru; Burseraceae.

1. Introduction

The knowledge on the morphology of native species is of great importance for understanding their autoecology, as well as their behavior and distribution in different ecosystems (Cosmo et al., 2010). The morphology of fruits and seeds usually shows little phenotypic plasticity and is, therefore, of great taxonomic importance (Heneidak & Khalik, 2015). Morphological and anatomical studies of seeds and seedlings are important to facilitate research on the soil seed bank, as well as to contribute to the identification of species in studies of natural regeneration of degraded areas and forest communities in general (Araújo Neto et al., 2002; Alves et al., 2014).

The genus *Protium* Burm. f. stands out for the diversity of its species, many of which are adapted to the most varied terrains and soils and are, consequently, very important, especially in heterogeneous environments. Generally, it becomes a group of difficult botanical identification at the species level, which is why several of them are frequently reclassified due to previous descriptions based on unreliable taxonomic characters (Daly and Fine, 2011). In addition, species belonging to this genus have a wide distribution and a long history of use, present in almost the entire state of Amapá (HAMAB), with the Amazon as the primary center of dispersion (Daly, 2015).

The species *P. altsonii* Sandwith has representatives of economic importance in the state of Amapá, known as “breu branco”, which exude a white and volatile resin of musky odor. They are used by the São Francisco Community (SDR), through the Mixed Cooperative of Producers and Extractivists of the Iratapuru River - COMARU, for boat-caulking and insect reppeling; in addition to supplying the company Natura Technological Innovation with 80 tons of "breu" stones per year, which represents an important input for the perfumery industry and a source of income for the community (Tostes et al., 2018). *P. altsonii* is often confused with other species of its own genus and with other species of other genera of the Burseraceae family, such as *Tetragastris Gaertn*. and *Trattinnickia Willd*., which all are called Amazon "breus" because of their similar vegetative characteristics. These species are marked by several synonyms and the use of taxonomic characters that are not very relevant, as they all exude resins from their trunks (Daly, 2015; Tostes, 2015).

Studies on the morphology of the vegetative and reproductive system of *Protium* species are still scarce and incipient (Melo et al., 2007). Chiefly, the combination of seed and young plant characteristics can provide numerous clues for identifying species in the field or in seed samples (Silva et al., 2009; Cosmo et al., 2009).

Information on the characteristics of germination of forest species represent the basis of forestry and sustainable management, since morphological studies of seeds enables the determination of the species existing in soil seed banks and the identification of forest species in the young phase, which contributes to understanding natural regeneration and succession in forest ecosystems (Silva and Costa, 2014).

Thus, the present work aimed to describe the morphological characters of *P. altsonii* Sandwith fruits, seeds and seedlings, as well as to provide information about the germination of this species until the initial growth of its plants.

2. Methodology

2.1 Study area

The fruits were collected from January to April, in the Sustainable Development Reserve of the Iratapuru River - SDR, in Laranjal do Jari, Amapá (from 00° 20' 00" to 00° 30' 00" S and 52° 30' 00" to 52° 34' 00" W). The studied area of the SDR was the Manoel Preto/Anani Hill complex, 9km from the São Francisco village in river and land routes, with altitudes between 200 and 300 meters. The relief is dissected, often in the form of hills. There is a dense mainland forest, with a predominance of sandy to clayey latosols, which are quite weathered (Rabelo, 2008). The climate is tropical, with an average temperature of 27.5 °C and an average rainfall index of 2,158.8 mm (Sobrinho et al., 2012).

2.2 Material origin

There were used five seed-bearing matrices, with the collection of 200 fruits from each one of them (1000 fruits). Once harvested, the fruits were taken to the Seed Laboratory (IEPA). In addition, 5x5m plots were installed in the five matrices to monitor and record the germination and development of seeds that fell and germinated until the seedling stage.

During the collection period, the fruiting and flowering of *P. altsonii* were monitored. All fertile individuals collected were identified by the specialist in Burseraceae, Dr. Douglas Daly from the New York Botanical Garden - USA and incorporated into the HAMAB Herbarium of the Institute of Scientific and Technological Research of the State of Amapá- IEPA, with the numbers 510, 511, 512 and 513.

2.3 Morphological studies

For the morphological description and illustration, 1000 fruits and seeds were used. The morphometric observations were carried out at the Laboratory of Physiology and Plant Biochemistry of the National Institute of Amazonian Research-INPA, with a stereoscopic magnifying glass and the unarmed view, recording the measurements of length, width and thickness, expressed in millimeters, with the use of a digital caliper. To describe the fruits, there were observed external and internal details of the pericarp regarding texture, consistency, color, hairiness, shine, shape, number of seeds per fruit and dehiscence. For the seeds, cross and longitudinal cuts were made with scalpel blades in 100 units, in order to analyze the following variables: external - dimensions, color, texture, consistency, shape and position of the hilum and micropyle; internal - presence or absence of endosperm, type, shape, color, position of the cotyledons, - hypocotyl-root axis and plumule arrangements in relation to the seed. The color to any description was attributed according to the the Amazonian Propagules and Seedlings Guide (2004). After the morphometry of the fruits and seeds, both were manually processed by maceration or threshing until the complete removal of the mesocarp.

2.4 Germination

In order to monitor the germination of *P. altsonii*, 500 seeds were used. These structures were sterilized in sodium hypochlorite at 20% of the commercial solution for 15 minutes and then distributed in humid plastic trays filled with vermiculite and covered with filter paper, which were kept in an Eletrolab germination chamber, with fluorescent lamps of cold white light; 70 PAR light intensity; 12:12 h photoperiod and temperature of 25 °C. Germination was separated by representative lots of each seed-bearing matrix.

For this study, germination was considered as the period from the emission of the radicle to the emergence of the epicotyl. Afterwards, the seedlings were transferred to polyethylene bags containing soil, which were kept in a shaded greenhouse and watered daily. Therefore, daily observations were performed, with the record of all seedling development.

The most vigorous seedlings in the greenhouse were used for the morphological description. Seedling was considered to be the phase in which eophylls (the first laminar structures) were fully formed (Melo et al., 2007). The vegetative elements described and illustrated were: shape, color and surface of the root (main and secondary), of the stem and of the eophyll, adding to the latter the phyllotaxis, the petiole and the buds. After 12 months of observation and description of the seedlings in the field, 500 seedlings (100 per seed-bearing matrix) were collected at the SDR Iratapuru-AP and taken to the Laboratory of Plant Physiology and Biochemistry at INPA to conduct the ecophysiological studies.

2.5 Morpho-anatomical studies

There were used ten mature seeds of *P. altsonii*, which were fixed in F.A.A (70%) for 48 hours, stored in 70% alcohol and dehydrated in a growing ethanol series until their infiltration in historresin (Johansen 1940). The cross and longitudinal sections were cut in a rotary microtome, stained in toluidine blue and safranin and mounted in glycerin for observation and photodocumentation. During germination tests, 10 seeds that did not reach this process were removed for anatomical records.

To verify the chemical nature of the walls and other cell components, sections of fresh material were cut freehand and treated with the following dyes or reagents: Sudan IV for cutinized and/or suberized walls and lipids in general (Jensen, 1962); 0.02% aqueous solution of ruthenium red for pectic substances and mucilage (Jensen, 1962); lugol reagent (iodized potassium iodide) for starch (Johansen, 1940); Dragendorff's reagent for alkaloids (Svendsen and Verpoorte, 1993); saturated picric acid solution for proteins (Johansen, 1940); ferric chloride solution for phenolic compounds and coumarin (Jensen, 1962) and Nadi's reagent for terpenoids (David and Carde, 1964). Observations and photographs were made with a digital camera and a stereomicroscope, in order to obtain a better view of the structures and indument. The terminology used is in conformity with the works of Beltrati (1992), Oliveira (1993), Barroso et al. (1999) and Vidal and Vidal (2000). All illustrations were realized through observations in a stereoscopic magnifying glass and, later, placed in ink.

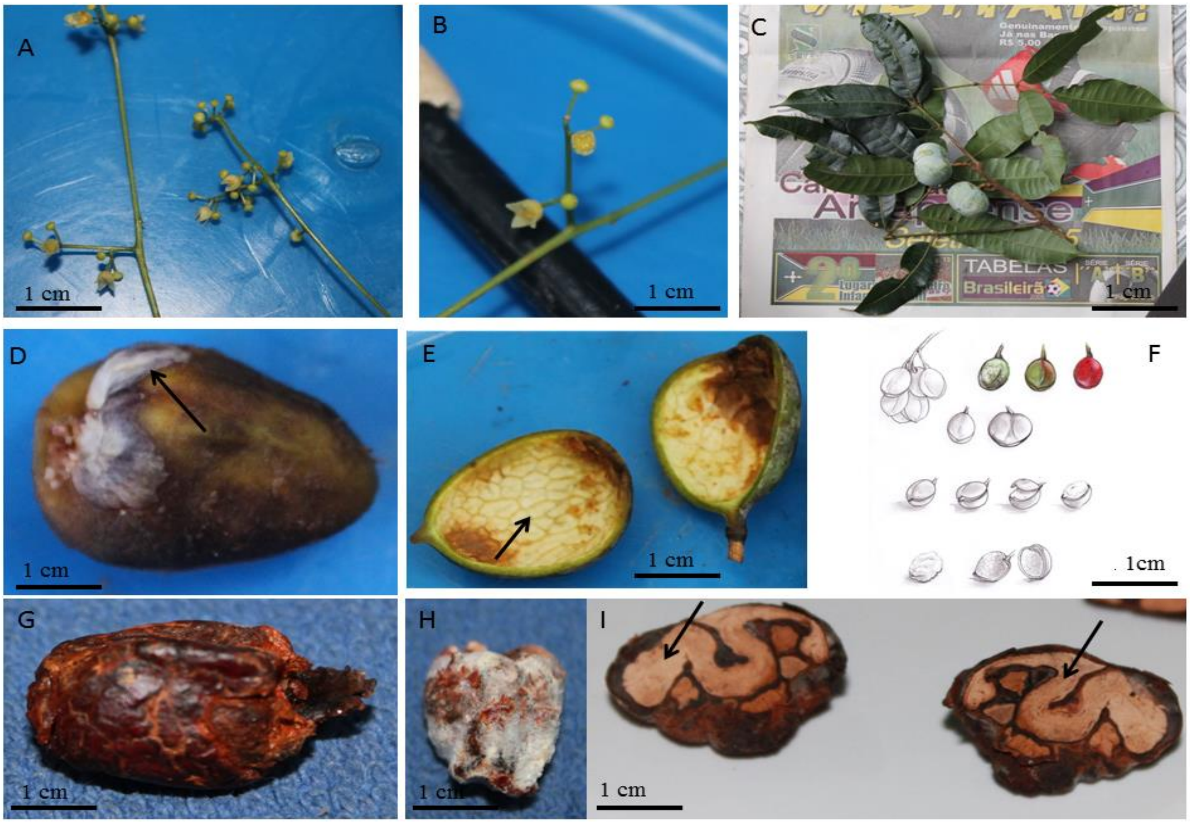
3. Results

P. altsonii presents supra-annual flowering and fruiting in periods of lesser and greater rainfall, respectively. Its flowers are tiny, delicate and yellow, forming a panicle inflorescence (Figure 1 A-B). Its fruits are succulent, fragrant and shaped as phylotrimids, with an epicarp that opens into valves (1, 2 or more) (Figure 1C).

As for the morphology, it is verified that the fruits are globose to ovoid, of average size, with a length of 2.3 ± 2.9 cm, width 2.09 ± 2.9 cm, thickness of 1.6 ± 2.7 cm, a slightly sharp apex, a short stipitate and a weight of 2.1 ± 8.2 g, with one, or more rarely, two pyrenes per fruit (Figure 1C).

Fruits are dehiscent, with initial dark green color - turning red when ripe -, and develop as an axillary panicle inflorescence (Figure 1A-F). They have a slightly coriaceous and smooth epicarp, usually with 2-4 valves, which are internally velvety, white and reticulated, with an average weight of 0.6 ± 3.8 g (Figure 1 E-F). The endocarp is brown, woody, resistant and indehiscent (Figure 1G). The mesocarp with white pseudo aryl is fleshy, soft and sweet, with an average weight of 0.3 ± 2.5 g, which completely envelops the surface of the pyrene (Figure 1H). The pyrene is yellowish-brown, with a firm consistency and ovoid shape, narrowing at one of the poles; its dimensions are 1.9 ± 2.4 cm in length, 1.7 ± 2.1 cm in width, 1.3 ± 1.7 cm in thickness and an average weight of 0.7 ± 2.3 g. The region of funicular connection is characterized by a white spot, which consistency and texture are different from the rest of the pyrene (Figure 1D).

Figure 1. Morphological studies of *Protium altsonii* Sandwith Inflorescence branch (**A**), Detail of small yellow flowers (**B**), Infructescence branch (**C**), and Seed with funicular scar (arrow) (**D**), Reticulated epicarp (arrow) (**E**), Ovoid and immature fruits (**F**). Detail of the endocarp (**G**). Detail of mesocarp remnants in pyrene (**H**). Detail of contorted cotyledons (arrows) (**I**)



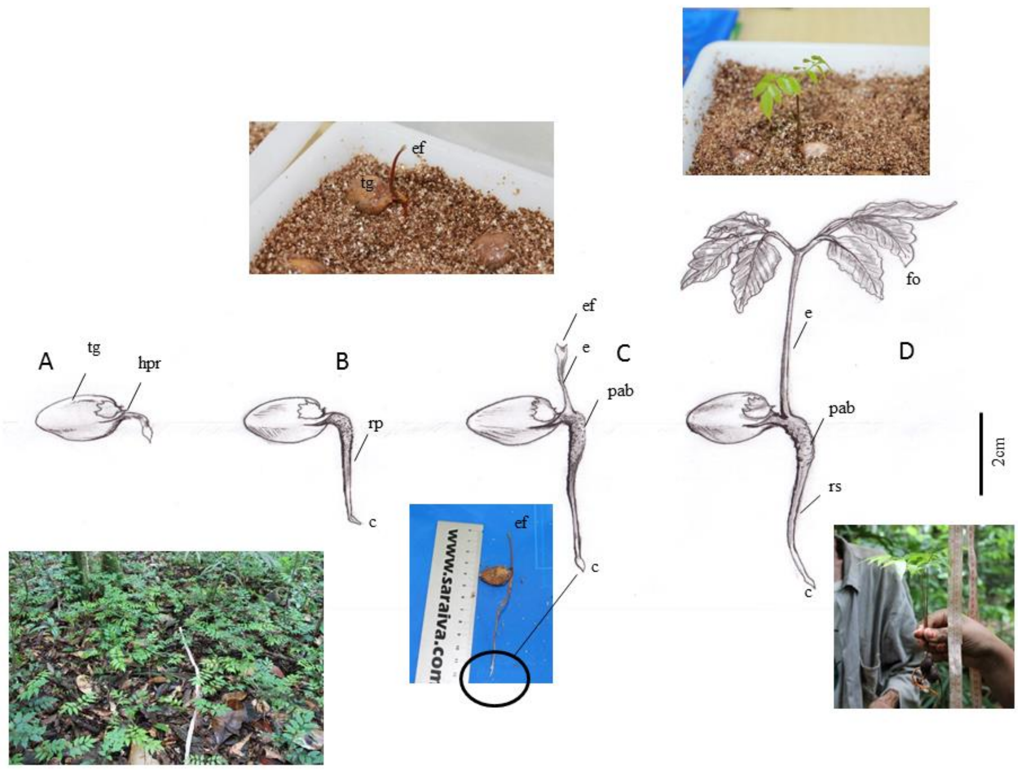
Source: Authors.

The seeds of *P. altsonii* are exalbuminous, dormant (probably due to the resistance of the endocarp), with embryonic immaturity, presence of phenolic substances, and a very low germination rate (2.4%). Additionally, of the seeds that did not germinate (97.6%), 20% were found only with the tegument and 77.6% only with the cotyledons. Twelve germinated and reached the seedling stage. It is important to emphasize that the seeds observed in the field presented a similar development to those studied in the laboratory. However, the germination rate is more efficient in the field, since, of the 5000 seeds there observed, 81.62% germinated, while others suffered herbivory attacks (7.26%) or simply did not bud (11.12%).

P. altsonii presents ecological strategies in the field, where its fruits ripen and its seeds germinate in the period of greatest rainfall. Its pseudo aryl is responsible for keeping the seed hydrated until its removal by birds that feed on this succulent and sweet mass. Although its seeds are recalcitrant, these structures manage to establish this conservative strategy in the environment. Once ruptured, shoot emergence occurs within three weeks, while seedling formation occurs after four weeks. The epicotyl elongates after the radicle protrusion and the expansion of the first two leaves. The leaflets and the stem, when injured, exhale a pleasant balm odor due to resin exudation, a remarkable characteristic of the family.

The morphology of seed germination is hypogean and cryptocotyledonary, with emission of the hypocotyl-root axis after the rupture of the pyrene apex, after 12 days of sowing. The hypocotyl-root axis, in its turn, is hypogeal, cylindrical, short, evident, dark green and approximately 3 cm long (Figure 2A).

Figure 2. Stages from germination to seedling development of *Protium altsonii* Sandwith. Seed with tegument rupture (tg) (**A**) and protrusion of the hypocotyl-root axis (hpr) (**B**). Primary root development (rp) (**C**)**.** Epicotyl formation (e), first eophylls (ef) and absorptive hairs (pab) (**D**). Elongation of the epicotyl (e), formation of leaflets (fo), growth of secondary roots (rs); 12-month-old cryptocotyledonary seedling.



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The primary root protrusion occurs around 14 days after sowing and it is characterized by being short, cylindrical, with tiny absorbent hairs, fast growing, cream-colored and glabrous, becoming thick and brown towards the apex as it develops

(Figure 2B). The white-colored calyptra is located at the apex (Figure 2C). The roots are short, sparse, slender and branched (Figure 2B-C). They present persistent cotyledons, with a light brown color (Figure 2).

Three days after the emission of the hypocotyl-root axis, the projection of the epicotyl occurred. This structure is green and approximately 0.3 cm long, becoming thick and dark brown during its development. The collection is inconspicuous and the first eophylls were emitted within about three days (Figure 2C).

Regarding the seedling morphology, it was observed that *P. altsonii* reaches this stage about 30 days after sowing. The seedling has an axial root system with a cylindrical primary root, yellowish to brown and cylindrical secondary roots, which are yellowish, slender and irregularly distributed along the primary root. The hypocotyl is reduced and dilated, yellowish, glabrous and approximately 0.2 cm long. The axis of the epicotyl is cylindrical, erect, sub-woody, light green to yellow, pubescent and long in relation to the hypocotyl, about 6.5 cm long. In addition, it has two simple eophylls, described as unifoliate, opposite, membranous, oval-elliptic, green, with opaque leaflets, acuminate apex, obtuse to rounded base, entire margin, slightly serrated at the apex, glabrous on the adaxial face and puberulent on the abaxial face. Thin leaf petiole, ca. 0.1cm long, pubescent, short petiolule of ca. 0.1 cm (Figure 2 C-D). At this stage of development, 12-month-old young plants are already considered.

After 12 months of sowing, many young plants still retain their cotyledons, both in the observations of the tests in the greenhouse and in the field (Figure 2D). Furthermore, *P. altsonii* forms a bank of young plants in shaded environments with a high survival rate, with a height of around 5 to 8.5 cm in length and 2 to 2.8 cm in diameter, with the presence of 3 to 4 leaflets completely expanded (Figure 2).

In young plants, the first leaves (3 ± 6 cm) are compound, initially trifoliate (Figure 2D), imparipinnated, opposite and they exude a balsamic odor. These plants present a short, green petiole (0.3 to 0.6cm), pulvinus (ca. 0.1cm), petiolule (ca.0.3 cm), pulvinulus (ca. 0.1cm). The leaflets (ca. 3 cm) are bright green, elliptical to slightly ovate. The leaf blade is green and smooth in both phases, with a rounded base and an acute to acuminate apex; entire margin, but crenated at the apex; pinned venation; secondary brochidodromal venation (Figure 2D). The next leaves have some morphological characteristics similar to those described for the first leaves, except for the petiole (3 ± 5 cm) and leaflets, which appear in number from 5 to 7 (3 ± 10 cm) (Figure 3). The stem is woody, brown and, when wounded, exhales a balmy odor; its roots are quite branched (Figure 3D).

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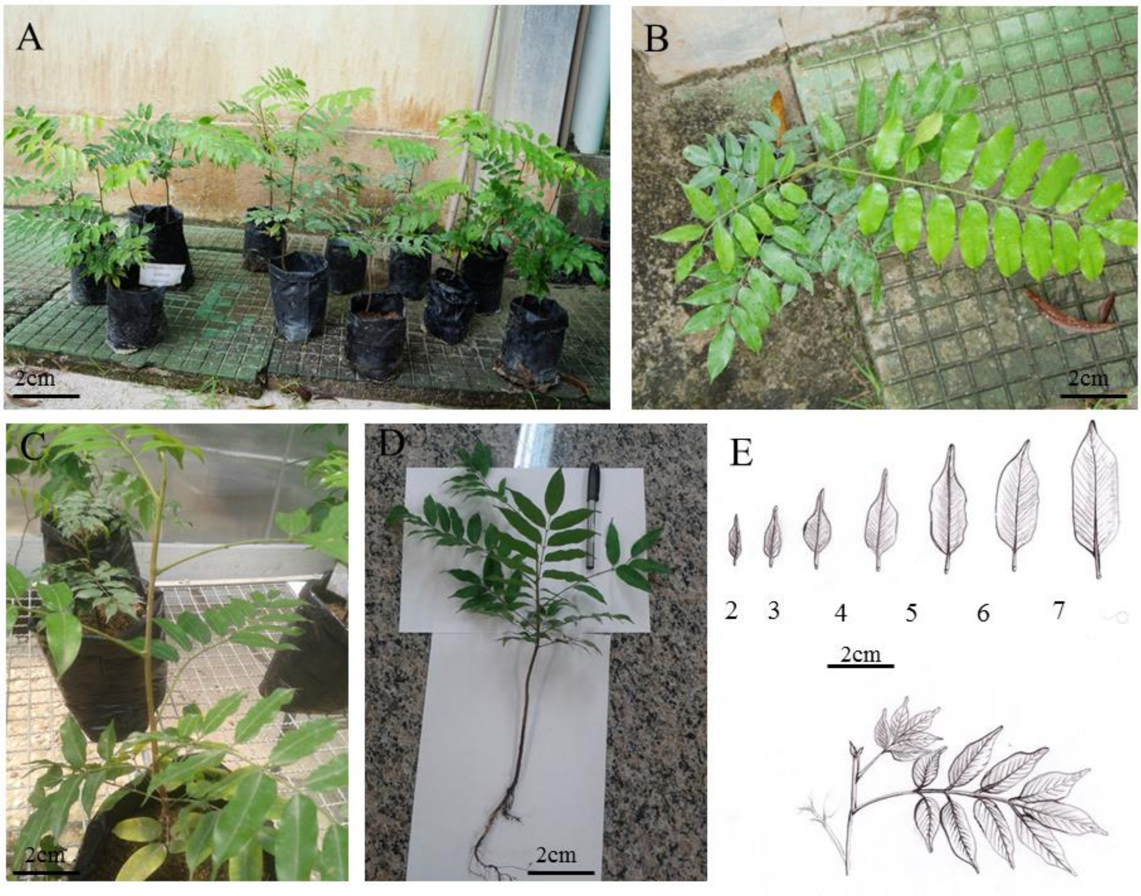
Figure 3. Successive stages of the development of young plants of *Protium altsonii* Sandwith (**A**). Plants aged 24 months in greenhouse (**B**). Compound leaves, imparipinnated (**C**)**.** Leaf architecture (**D**)**.** Root growth (**E**)**.** Leaves at different stages of development.



Source: Authors.

The seed of *P. altsonii*, in a cross-section in the abaxial region, is formed by the seed-coat with collapsed cells, fleshy cotyledonary parenchyma with secretory cavities and other substances. In the adaxial region of the epidermis, in cross section, the cells are contiguous and flattened between the two cotyledons (Figure 4D).

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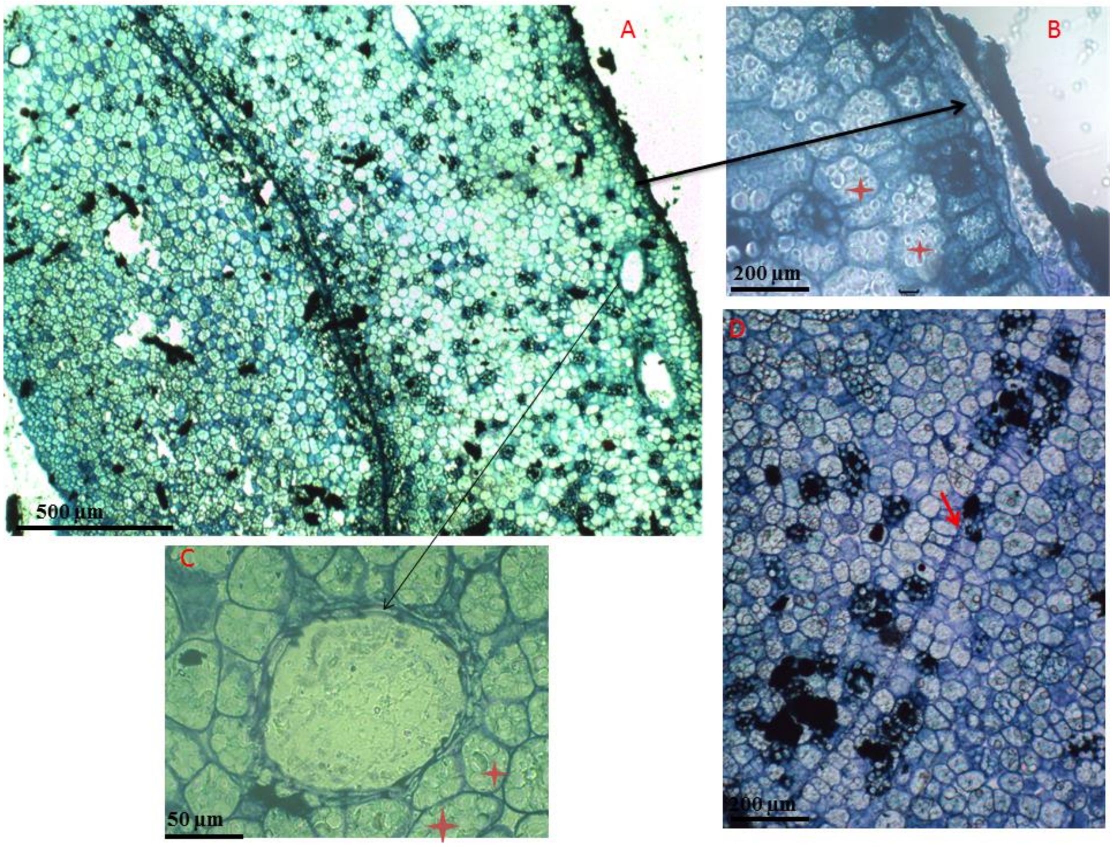
Figure 4. Anatomical studies of the seed of *Protium altsonii* Sandwith. (**A**) Cross section highlighting the seminal envelope, the parenchymal cells of the cotyledons filled with mucilage content and the phenolic compounds. **B**) Detail on the arrow of the reduced endosperm layer and cells with oil (star). (**C**) Cross section of the secretory cavity with epithelial cells and oil droplets (star). (**D**) Cross section of the adaxial region separating the two cotyledons (arrow); presence of coumarin.



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The cotyledons in longitudinal section are contortuplicate (Figure 4) and contain cell reserves with mucilage, oil, lipids, proteins and phenolic substances, such as coumarin and tannins, found in the histochemical tests of the studied species (Figure 4 A-D). Seeds have a reduced endosperm layer, with thin-walled cells (Figure 4A). Additionally, the presence of oil occurs in specialized cavities distributed throughout the cotyledonary parenchyma, formed by epithelial cells (Figure 4 A-C), in addition to individual secretory cells containing oil, mucilage, terpene and phenolic substances (Figure 4 B-D).

During the anatomical cuts, there were found large cotyledons that occupy almost the entire seed. Of the 488 seeds that did not germinate in the laboratory, 10 were evaluated in cross sections and, in only 20%, the presence of an embryonic axis was recorded (Figure 4A). Of the field records, most had embryonic axis (81.62%) and of those that did not germinate (11.12%) the presence of coumarin in the cotyledonary parenchyma was evident (Figure 4D). However, as the cuts were made in the tissue, it was observed that the coumarin disappeared over time.

4. Discussion

It was observed that there is no synchrony between the fruit and the embryo, that is: when the fruits are ripe, the embryos are still in the rudimentary phase at the time of dispersion. In this sense, we suggest that one of the factors that influenced the germination process is embryonic immaturity, as well as the presence of phenolic substances (Daly, 1989). As for *P. altsonii*, the species *P. spruceanum* (Benth.) Engl., *P. aracouchini* (Aubl.) and *P. pilosissimum* Engl. also have a low germination rate, around 10% (Melo et al. 2007). After fertilization, the fruit and the pyrene develop faster than the seed, reaching their maximum size way before the embryo expands and occupies the internal cavity of the pyrene (Daly, 1989).

In a study involving the species *P. widgrenii* Engler, there were observed around 70% of malformed seeds, with typical recalcitrant behavior, since their germination percentage decreased with desiccation (Seiffert et al., 2006). Additionally, recalcitrant seeds are highly susceptible to water loss, which requires storage with high humidity levels (Vidal and Vidal 2000). Seeds of *P. altsonii* also show recalcitrant behavior, with low germination rate.

Different morphological interpretations are attributed to the fruits of this genus, such as drupes, drupaceans, phylotrimid and nuculans (Beltrati, 1992; Barroso et al., 1999; Melo et al., 2007). The most relevant morphological characters for the delimitation of some *Protium* species were found, mainly, in the color, shape and surface of the fruit (Melo et al., 2007). Likewise, the size, shape and type of dehiscence of the fruits are essential characteristics for their classification (Barroso et al., 1999).

Fruit development results in a variety of physiological changes such as water content, color, consistency, odor, flavor and physical dimensions (Dias et al., 2013). In an ecological context, the description of fruits is relevant to understanding the reproductive biology of species (Paoli and Bianconi, 2008). Furthermore, the biometrics of fruits and seeds, as well as the knowledge on the morphology and development of seedlings, are essential to support studies on germination and seedling production for vegetal recomposition (Silva et al., 2014).

There are also different opinions about the definition of a suitable term for the sweetened pulp involving the pyrene, such as aryloid or pseudaryl (Barroso et al., 1999; Beltrati, 1992). The pyrene is a structure that can help in the identification of fruits and seedlings, since, in general, it is persistent in this phase of the life cycle. Among the characters used for the taxonomic description, the following stand out: shape, color, presence or absence of trichomes, texture, consistency and thickness. The pyrenes in Burseraceae are generally ovoid, with an acute apex, cartilaginous (*P. paniculatum* variety *riedelianum*) to bony (*P. subserratum*) consistency, glabrous or pubescent , with a different surface around the funicular scar, of different shapes and sizes (Melo et al., 2007).

The genus *Protium* has a wide morphological diversity of seeds and germination processes, as well as the Burseraceae family as a whole, in which the cotyledons can be whole or lobed and straight, contorduplicated or folded in a vertical and/or horizontal direction; germination can be hypogeal or epigeal and crypto or phanerocotyledonary; and both eophylls and metaphylls can be opposite or alternate and simple, unifoliolate, trifoliolate or pinnate, with full or serrated margins (Melo et al., 2007; Daly, 2011).

The morphological behavior and the different types of germination were observed in nine species of *Protium*, in most of which germination dormancy was registered (Melo et al., 2007). Fruit maturity is not always a necessary condition to indicate that the embryos are able to germinate, but the integrity and full development of the embryo are fundamental for germination (Daly, 2011).

Species with fleshy cotyledons are adapted to shaded environments and form a seedling bank with a higher survival rate (Garwood, 1996). The presence of storage and persistent cotyledons (which in some cases remain in the seedlings for almost a year) should largely meet the resource requirements during the seedling stage (Garwood, 1996). The investment in reserve cotyledons can ensure the survival of shade-tolerant seedlings until a clearing appears, which reduces the possibility of desiccation of the young seedling (Dias et al., 2013; Alves et al., 2013).

It is possible to recognize the peculiarities of each taxon through the seedling morphology. The set of characters based on the composition, margin and apex of the eophylls of *Protium* seedlings, in addition to being very important, are useful for the recognition of species in the field (Melo et al., 2007). These transitory structures contribute to expanding the knowledge of the essential structures of the seedling throughout its development, as well as enabling the characterization of the species, especially those of forest ecosystems (Gurgel et al., 2012).

Secondary metabolites are organic compounds that are not directly associated with the growth, development and reproduction of these organisms, often being synthesized as by-products of the metabolism of primary compounds. These secondary metabolites play a fundamental role in defenses against herbivores, in addition to acting as germination inhibitors (Castro et al., 2004).

Coumarin is a phenolic compound that acts as a natural germination inhibitor, which is widely known and has a wide spectrum of occurrence, besides being responsible for affecting other physiological processes (Perez and Moraes, 1991). Phenolic compounds in vegetables, mainly tannins, are known for inhibiting herbivores in fruits, leaves, seeds or other young plant tissues (Evert, 2006).

Concerning the morphology of the seeds of the genus *Protium*, in *P. unifoliolatum* the seeds are bitegmented and there are several integumentary layers, as well as the endosperm is reduced to a single layer of cells – characteristics that corroborate what was observed in *P. altsonii* (Corner, 1976).

The embryo usually consists of a hypocotyl-root axis, one or more cotyledons and the stem primordium. The root and stem primordia can simply be apical meristems and, sometimes, there is an embryonic root (radicle) at the lower end of the hypocotyl and an embryonic stem above the insertion of the cotyledons, which consists of an axis with non-extended internodes and one or more leaf primordia. This first apical bud is commonly called plumule and its stem part is the epicotyl (Carvalho and Nakagawa, 2000).

Cotyledons are seminal structures of variable shape, which are connected to the embryonic axis and have the functions of absorbing and reserving food from the endosperm and/or perisperm that will be used during germination. The loss of vigor and viability of seeds chiefly involves alterations in the embryonic axis (Carvalho and Nakagawa, 2000). The embryo of these seeds normally forms fleshy cotyledons that store nutritive substances and occupy the largest volume of the seed (Corner, 1976; Appezzato-da-Glória and Carmello-Guerreiro, 2006).

Secretory cavities in fruits and seeds form secretions in cells that eventually break down and release substances within them (Metcalfe and Chalk, 1950). The inner secretory cells have a wide variety of content. They often appear as specialized cells dispersed among others less specialized (Appezzato-da-Glória and Carmello-Guerreiro, 2006). In *P. altsonii*, there is a large presence of substances in the parenchyma tissue, with many secretory cells.

5. Conclusion

Therefore, the morphology of *P. altsonii* is essential to ensure the survival of seedlings in the field. The morphological characteristics of the fruit, the seed, in addition to its anatomy, allow us to assure that this species presents phenotypic plasticity as an ecological strategy. The field germination efficiency of these species may have been caused by the release of the coumarin present in the cotyledons, as the soil is washed by the rain during the period of greatest rainfall. Under controlled conditions, the presence of coumarin inhibited germination.

Furthermore, the tannin present throughout the cotyledonary parenchyma can serve as an anti-herbivory compound, due to its astringent action. The anatomical structure of the *P. altsonii* seed was not found in literature, so the present study represents a contribution to the genus, in addition to indicating the presence of secondary metabolites, such as oil and mucilage.

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