

REPRODUCTIVE PHENOLOGY AND POLLINATION OF THE BRAZIL NUT TREE (*Bertholletia excelsa* HUMB. & BONPL. LECYTHIDACEAE) IN EASTERN AMAZONIA

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ABSTRACT

The brazil nut tree (*Bertholletia excelsa* Humb. & Bonpl. Lecythidaceae) is an economically important fruit tree, endemic to the Amazon Region. Investigations of the reproductive phenology and insect pollinators of *B. excelsa* were carried out in the orchards of Embrapa Amazônia Oriental, in Belém (1°27'S 48°29'W) and in the experimental Field Station of Capitão-Poço (1°27'S 48°29'W), in the eastern region of Pará State, Brazil. The main flowering period occurred from August to November, during the driest months of the year and the main fruiting period lasted from October to December. The maturation period extended throughout the following year with the ripening and dissemination occurring in the rainy season, from February to April. The pollen:ovule ratio was 26,755.29. Indicating obligate xenogamy. The main pollinators are medium- to large-sized bees, belonging to the following species: *Xylocopa frontalis*, *X. aurulenta*, *Epicharis rustica*, *E. affinis*, *Centris similis*, *Eulaema nigrita*, *E. cingulata*, *Bombus brevivillus*, *B. transversalis*. From this study it was concluded that *B. excelsa* is a mellitophilous species dependent on the pollinators activity to ensure its fruit production.

INTRODUCTION

The brazil nut (*Bertholletia excelsa* Humb. & Bonpl., Lecythidaceae) is a large tree, up to 50 m high, native to the Amazon rainforest. It is considered one of the noblest and most valuable plants of the Amazon region, due to the economic value of its nuts, one of the main products in the exportation list of the state of Pará (Almeida, 1963; Müller et al., 1980), as well as timber production from opened plantations (Kanashiro 1992).

The Lecythidaceae family is predominantly Neotropical (Mori 1987; Endress 1994). The majority of the species of this family tend to flower in the dry season. In places without a regular dry season, the phenological events may be influenced by other factors, such as longer duration of the photoperiod within the day (Mori & Prance 1987).

The main flowering period of *B. excelsa* occurs from October to December, showing erect and short-ramified terminal panicles, with zygomorphic flowers with pale-green calyx and yellowish fleshy petals, offering nectar and pollen as rewards for visitors (Moritz 1984). According to (Pinheiro & Albuquerque 1968), the flower/fruit ratio is low, approximately 0.4%. The fruits are large and indehiscent, with seeds dispersed by agoutis (*Dasyprocta* sp.) (Huber in Kanashiro 1997). Many authors emphasize the importance of the pollination agents in the brazil nut fruit set, stating that this low ratio may change in agreement with the activity and efficiency of natural pollinators.

The brazil nut is an allogamous plant with mellitophilous pollination syndrome. The flower structure bears a chamber composed of congruent staminodes, creating a robust structure (ligule) that conceals the stamens and the stigma. This chamber restricts the insects visitors' entrance and selects a specific guild of pollinators with physical vigor and compatible size, required to lift the ligule and to assess the reproductive organs. The main visitors and pollinators of the of brazil nut are the bees, belonging to the following genus: *Bombus*, *Centris*, *Xylocopa*, *Epicharis* and *Eulaema* (Müller et al. 1980; Moritz 1984; Maués & Oliveira 1996; Maués et al. 1996).

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From this study, the phenological patterns and the legitimate pollinators of *B. excelsa* in the northern Brazilian state of Pará were identified in order to obtain subsidies for further studies on management of native pollinators in orchards.

MATERIALS AND METHODS

The field studies were carried out between 1994 and 1997, in experimental orchards in the municipalities of Belém, (1°28'S;48°29'W) and Capitao-Poço (1°46'S 47°28'W), in the eastern Amazon region, north of Brazil. Grafted plants with ages varying between 15 and 20 years old were utilized.

From March 1994 to March 1996, phenological observations were realized every two weeks, taking into account the occurrence, duration and frequency of the following events: flowering (e.g., floral buds and flowers); fruit set (e.g., green fruit, mature fruit and seed drop) and leaf changes (e.g., new and mature leaf; partial and total defoliation), in agreement with the methodology of Fournier and Charpantier (1975). The phenologic records were correlated with meteorological data (e.g., precipitation, temperature, relative humidity and photoperiod) obtained in the Meteorological Station of Embrapa Amazônia Oriental. For these studies, five trees of precocious brazil nut were selected in a 15-years-old orchard, in the experimental area of Embrapa Amazônia Oriental.

During the flowering period, direct observations about the behavior of the insect visitors were accompanied by specimen collection and photographic documentation with a Nikon F90 camera and 35-135mm lens, aiming to support the identification of the main pollinators. The insects were identified in the Entomological Museum of Embrapa Amazônia Oriental, and/or sent to specialists from the Faculdade de Filosofia Ciências e Letras de Ribeirão Preto (FFCLRP-USP). Voucher specimens were incorporated into the Entomological Museum of Embrapa Amazônia Oriental.

The investigations on the floral biology concentrated on the determination of the pollen:ovule ratio, osmophores location, determination of the main receptive stigma area, pollen viability, number of stamens and number of flowers opened daily by inflorescence. According to Cruden (1977), the pollen:ovule ratio is an indicator of the mating system. 20 flowers were used to obtain the ovule number per flower, after dissection of the ovary under stereomicroscope. To estimate the number of pollen grains per flower, three anthers of five flowers were separately picked and macerated with a solution of distilled water and detergent. A sample of this solution was spread in a Neubauer chamber (hemacytometer) and the number of pollen grains was properly scored under microscope. The results were divided by three, multiplied by the dilution factor and by the number of anthers. The osmophores were determined by submerging the flowers for two hours in a solution of neutral red 0,1% and then washing in running water, according to Vogel mentioned by Faria (1989). Afterwards, the areas colored in dark red were verified, which revealed the presence of the scent glands. The stigma receptivity was tested in flowers bagged one day before the anthesis. The stigmas were cut off and submerged in four reagent types: solution of Peroxtesmo KO (Dafni & Maués 1998), Perex Test Merck, Hydrogen Peroxide solution (H₂O₂) 6% and Baker's solution. The pollen viability was also tested in bagged flowers, using Peroxtesmo KO and Baker's solution.

Peroxtesmo KO indicates the presence of an enzyme, peroxidase. The viable pollen or the receptive stigma turns into a blue or purple color, thereby confirming the enzymatic activity. The Perex Test shows the presence and intensity of hydrogen peroxide (H₂O₂), measured through a colored scale varying from pale-yellow to red, with scores for each nuance. Hydrogen peroxide itself indicates the receptivity through the appearance of air bubbles, being a simple and inexpensive method, but if there is any damage on the stigma surface or even pollen germination, it may give a false positive result. Baker's procedure also indicates

the presence of enzymatic activity, through the alcohol-dehydrogenase enzyme. It is a reliable method for demonstrating pollen viability and stigma receptivity. All of these procedures were done in accordance with Dafni (1997), using a Olympus BH-2 microscope and Olympus SZH stereomicroscope.

Photomicrographies of the surface of visited and not visited stigmas (on flowers protected before anthesis with pollen-proof bags) were done with JEOL JSM 5.400 LV Scanning Electric Microscope (SEM), in order to verify the importance of the pollinator's activity on the pollen deposition on the stigma surface.

Some of these procedures were carried out with the aid of a disassembled metallic tower, of 12m height, in order to reach the tree canopy.

RESULTS AND DISCUSSION

In the first year of study, 40% of the studied plants exhibited the flowering phase from March to June, coinciding with the rainy period. However, the most expressive flowering period occurred in the dry season, extending from September to December, when up to 100% of the individuals were flowering (Fig. 1). Precipitation and sun light data are presented in Fig. 2. The same pattern was found in the second year, with two peaks in September and November (80% of the total plants). There is a narrow relationship between the precipitation and the phenological events of tropical species. Mori & Prance (1987) in studying the reproductive phenology of 14 species of Lecythidaceae in French Guyana, verified that the flowering period usually happens in the driest months of the year or in the transition among the dry and rainy months. The flowering type can be classified as "cornucopia" according to Gentry (1974), characterized by a large amount of one-day-long flowers opening each day within a period of three to eight weeks, in the majority of the trees in the population.

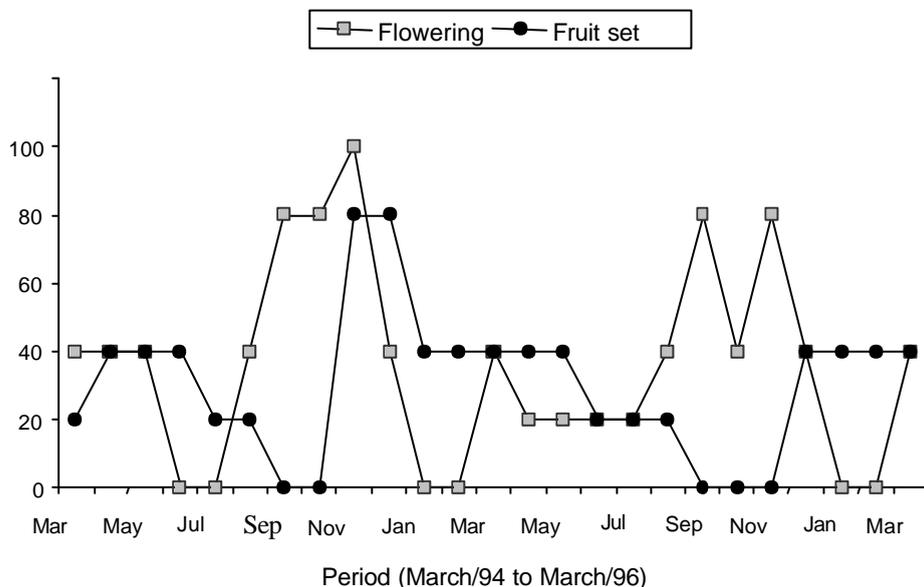


Fig. 1 – Phenological events (flowering and fruit set) of *Bertholletia excelsa* in Belém, from March 1994 to March 1996, expressed by the percentage of trees with flowers and fruits among the studied plants.

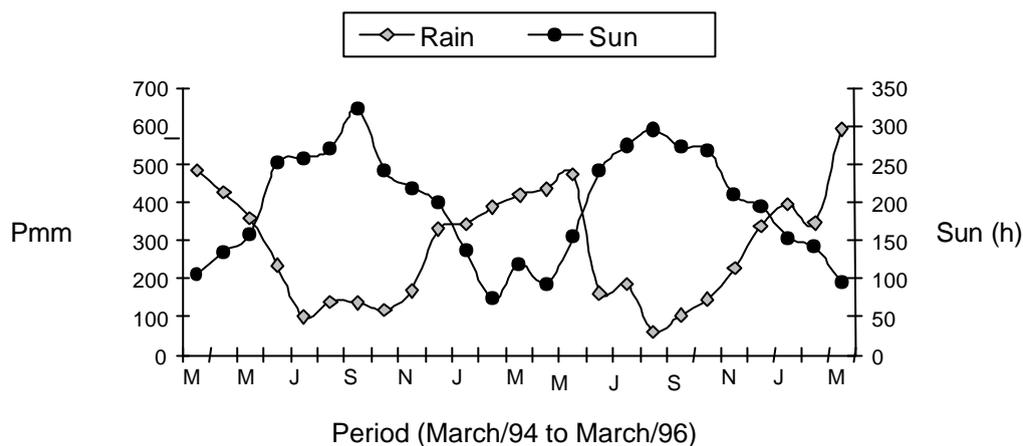


Fig. 2 - Meteorological data (precipitation=mm and photoperiod=hours) from Belém, Para State, Brazil, from March 1994 to March 1996.

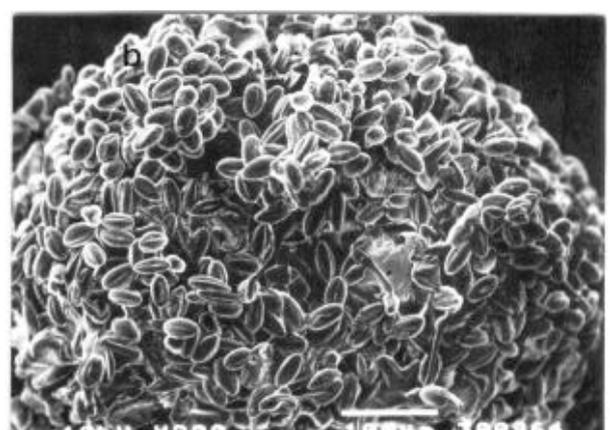
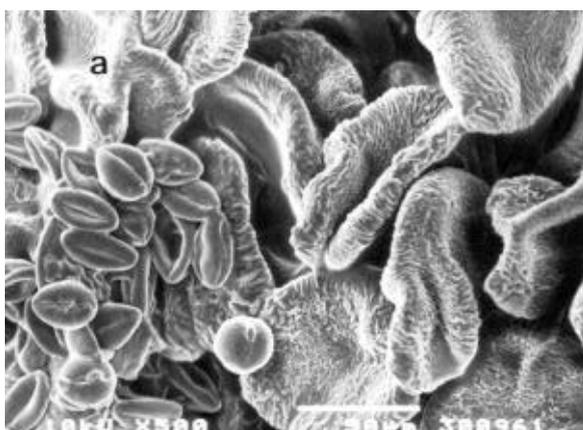
One fruit set cycle extended from November 1994 to May 1996. The fruits of *B. excelsa* present a long development period, with an average of 14 months, therefore it is common to find fruits in different development stages in one plant during the whole year. Seed drop occurred mainly in the beginning of the wet season (January to March). Green fruits drop was particularly verified in the dry season (September to November). The appearance of new leaves preceded the floral buds emergence. Some individuals presented total defoliation in the dry season, especially from October to December. Figure 2 shows the variation on the meteorological data from 1994 to 1996.

B. excelsa presents terminal inflorescences with 15 to 45 cm long and an average of 0.76 (n=182) flowers opened per day (Fig. 3). The anthesis happens slightly before dawn, about 5:30 h, confirming the observations of Müller et al. (1980). Its special morphology permits only the entrance of robust and vigorous insects into the staminode chamber, to collect pollen or nectar. Mori et al. (1978), Müller et al. (1980), Moritz (1984) and Maués & Oliveira (1996), already mentioned that the morphological structure of brazil nut flowers select the pollinator's entry.



Figure 3 –*Bertholletia excelsa* inflorescence.

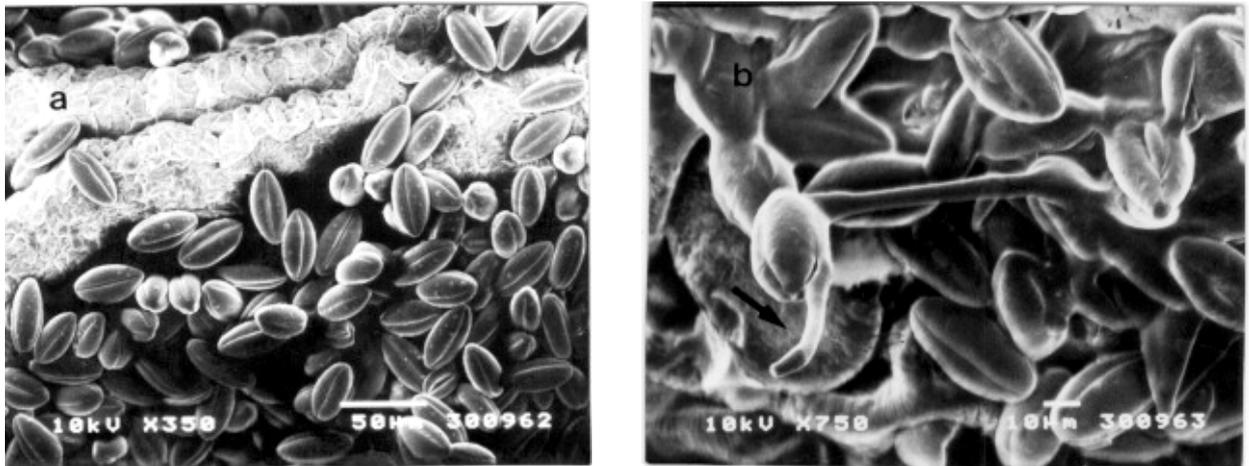
The stigmatic surface of *B. excelsa* carries innumerable papillae. These structures facilitate the adherence of the pollen grains (Fig. 4a). In newly opened flowers, the papillae are turgid and white, changing to pale-brown in the senescence period, and losing the turgidity. The analysis of the photomicrographies demonstrated that the stigmatic surface of the non-visited flowers presented small amounts of pollen, compared to the visited flowers, where the pollen grains almost totally covered the stigma (Fig. 4b). Considering the strong auto-incompatibility system allied this observation, this evidence emphasizes the importance of pollinators' activity for the



reproductive success. Photomicrographies of the pollen morphology and anther dehiscence were also made (Fig. 5a and 5b).

Figure 4. Stigmatic surface of *B. excelsa* – Scanning electron Microscope: a) evidencing the papillae (500x); b) pollen deposition (200x).

The test with neutral red indicated a concentration of osmophores in the internal portion of the petals. The main receptive area of the stigma is placed in the basal portion of the central papillae, confirmed by all the tests performed. There was no response in the tests of pollen viability. However, using Baker's procedure to analyze stigma receptivity, any pollen grain attached to the stigmatic surface turned into a deep blue color, evidencing the viability. For some



reason, the pollen collected directly from the anthers behaved differently from the pollen attached to the stigma, indicating that a reaction (or hydration) may occur, when the contact between the pollen and the stigma is made. Richards (1997) states that in wet stigmas the turgidity of the stigmatic papillae marks the period of the stigma receptivity, helping in the fertilization process by the secretion of a sugary solution that hydrates the pollen. The ovule counting revealed flowers with four and five locules, always presenting five ovules on each locule. The average number of stamens was of 90.3 ± 6.7 ; the mean number of pollen grains was 601,993.98 and the pollen:ovule ratio was of 26,755.29, indicating obligate xenogamy, according to Crudden (1977).

Figure 5. Scanning Electron Microscope photomicrographies: a) Anther dehiscence of *B. excelsa*, exposing the pollen grains (350x); b) pollen germination in the stigma (750x).

The main visitors were bees of the following families: Apidae (*Bombus brevivillus*, *Bombus transversalis*, *Eulaema cingulata*, *Eulaema nigrita*, *Euglossa* sp., *Eufiessa* sp., *Apis mellifera*, *Trigona hialinata*, *Trigona pallens*, *Trigona fuscipennis*, *Tetragona clavipes*) and Anthophoridae (*Xylocopa frontalis*, *Centris similis*, *Epicharis rustica*, *Epicharis (Hoplepicharis) affinis*, *Epicharis* sp.). Other insects such as Chrysomelidae beetles, Sphecidae and Vespidae wasps were also recorded as flowers visitors. The most frequent bees were *Epicharis*, *Xylocopa*, *Eulaema* and *Bombus*. Prance (1976) mentioned that the main pollinators of *B. excelsa* are euglossine bees and Mori & Boeke (1987) agreed with his statement. The legitimate pollinators penetrated in the flowers, forcing the ligule upward, and stayed approximately 10 to 30 seconds collecting nectar. When they left the flower, the thoracic surface was covered with pollen. In visiting other flowers, they promoted the pollination, transferring the pollen to the receptive stigma. These bees are vigorous and robust, some of them being able to fly long distances (up to 20 km) (Janzen 1971), which is extremely important for maintaining the gene flow among allogamous plants in tropical forests.

The insect visitors' activity begins immediately after the anthesis, about 6:00h, and the visiting period can extend to 11:00h. (Table1).

TABLE 1 – Insect visitors and pollinators of brazil nut (*B. excelsa*) in the eastern Amazon region, Pará State, Brazil.

Family	Species	Importance*	Category**	Activity period
Anthophoridae	<i>Xylocopa frontalis</i>	+++	P	6:00 - 11:00
Anthophoridae	<i>Xylocopa aurulenta</i>	+	P	6:00 - 9:00
Anthophoridae	<i>Epicharis rustica</i>	+++	P	6:00 - 9:00
Anthophoridae	<i>Epicharis (Hoplepicharis) affinis</i>	+++	P	6:00 - 10:00
Anthophoridae	<i>Epicharis sp.</i>	+++	P	6:00 - 10:00
Anthophoridae	<i>Centris similis</i>	++	P	7:00 - 8:30
Apidae	<i>Bombus brevivillus</i>	++	P	6:30 - 9:30
Apidae	<i>Bombus transversalis</i>	++	P	6:30 - 9:30
Apidae	<i>Eulaema meriana</i>	+	P	7:00 - 9:30
Apidae	<i>Eulaema nigrita</i>	+++	P	7:00 - 9:30
Apidae	<i>Euglossa sp.</i>	+	P	6:30 - 9:30
Apidae	<i>Eufriesea sp.</i>	+	P	7:00 - 9:00
Apidae	<i>Trigona pallens</i>	-	V	8:00 - 10:00
Apidae	<i>Trigona hyalinata</i>	-	V	8:00 - 10:00
Apidae	<i>Trigona fuscipennis</i>	-	V	7:00 - 10:30
Apidae	<i>Tetragona clavipes</i>	-	V	8:00 - 10:00
Vespidae	<i>Synoeca surinama</i>	-	V	9:00 - 11:00
Vespidae	<i>Polistes infuscatus</i>	-	V	9:00 - 11:00
Vespidae	<i>Polybia sp.</i>	-	V	9:00 - 11:00

* +++: strong; ++: moderate; +: weak ** P: pollinators; V: visitors

The *Trigona* and *Tetragona* bees were classified as pollen thieves and opportunists, according to the classification of Wille (1963). Its presence was frequently observed, and in many times *T. fuscipennis* drove away the natural pollinators (*Bombus* and *Xylocopa*, mainly) when they tried to penetrate in the flower. Similar incident was observed and tested in passion fruit flowers (*Passiflora edulis*) in the state of São Paulo (Sazima & Sazima 1989). These authors verified that *Trigona* bees impeded the carpenter bees' visits, through direct attacks by biting the base of the antennas, legs and wings of the carpenter bees, driving them away. The presence of dead *Trigona* specimens on the flowers was just enough to move away the carpenter bees of the plantation. These bees perforate the flower to collect nectar and pollen without contributing to the pollination process, in a classic robbery behavior. *B. excelsa* flowers are typically mellitophilous, "sensu" Faegri & Pjil (1979). The main pollinators are medium to large-sized bees.

It is important to emphasize that brazil nut yields are conditioned by the pollinator's activity, therefore any imbalance that affects the pollinator's population within plantations or natural areas will reflect directly in the fruit production. The low yields in sapucaia (*Lecythis pisonis*) was attributed to the lack of native pollinators by Mori et al. (1980). Prance & Mori (1987) also highlighted the need for studies on the Lecythidaceae's pollinators, attributing the failure of brazil nut plantations to the pollinators decrease. As an alternative, to avoid the decline of the natural population of pollinators in extensive plantation fields, inter-cropping systems with plants that attract the same pollinators' guild, e.g. passion fruit (*Passiflora edulis*) and the anatto (*Bixa orellana*), would help to supply food during the interval in the flowering periods. It is also recommended that natural vegetation be permitted to grow in strips among the plantation lines, in order to provide nest sites for the bees.

CONCLUSIONS

The main flowering period of the brazil nut occurred during the months of weak precipitation (August to November).

The pollen:ovule ratio corroborates the auto-incompatibility stated for this species in previous studies.

The main pollinators were carpenter bees and bumble bees, families Apidae and Anthophoridae, e.g. *Xylocopa frontalis*, *Epicharis rustica*, *E. affinis*, *Epicharis* sp., *Eulaema nigrita*, *Bombus transversalis* and *B. brevivillus*.

The pollination syndrome was characterized as melittophily.

In order to avoid the decrease of the pollinator's natural population in commercial plantations, and therefore a low fruit production, the development of management programs for the main pollinators will be of major importance for wide-scale cultivation of the brazil nut.

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