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SUMMARY

Bees visiting flowers of *Bertholletia excelsa* (Brazil Nut tree) and *Couratari tenuicarpa* were collected, their behavior described and the pollen found clinging to their dorsal thorax and stored on their legs was identified. Female bees of *Xylocopa frontalis* (Olivier) and males of *Eulaema mocsaryi* (Friese) are apparently effective pollinators of *Couratari* in igapó near Manaus. Female bees of *Euplusia seabrai* Moure *in litt.*, *Epicharis umbraculata* (Fabricius), *Epicharis rustica* (Olivier) and *Eulaema nigríta* (Lepeletier), as well as male bees of *Eulaema cingulata* (Fabricius) and *Eulaema nigríta* are apparently effective pollinators of adult Brazil Nut trees in the Aleixo plantation near Manaus. Only large bees capable of uncurling the floral androecium can effectively pollinate *Couratari* or *Bertholletia*.

Pollen analysis indicated that all bees captured carried pollen of the host tree in question and had been foraging on flowers of plant species common in secondary growth. Secondary growth near the Aleixo plantation supports a bee guild which appeared to effectively pollinate almost every flower on the Brazil Nut tree studied. Proximity to primary forest (and to those Euglossine bee species which occur only in primary forest) therefore does not appear to be necessary for pollination of Brazil Nut trees.

In the Aleixo plantation chronic low fruit set is probably due to some factor other than pollination. Since natural occurrences of Brazil Nut trees to the north and south of Manaus are associated with a higher soil fertility, low production at the Aleixo plantation may be due to deficiencies of the soil.

INTRODUCTION

The androecium of neotropical Lecythidaceae has evolved from an open, radially symmetrical structure as in *Gustavia* and *Grias*, to an increasingly zygomorphic shape with

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a curled hood enclosing the anthers and nectaries, as in **Eschweilera**, **Bertholletia** and **Couratari**. Pollination studies of the family (Mori & Kallunki, 1976; Mori et al., 1978; Prance, 1976; Prance, 1983; Mori & Boeke, in prep.) indicate that this evolutionary trend is a specialization for attracting large Euglossine bees whose long tongues and heavy bodies provide access to nectar and pollen and the ability to uncurl the protective hood.

Bee pollination can provide the outcrossing shown to be required for seed set in all Lecythidaceae species so far studied for this trait: **Gustavia superba** (Kunth) Berg, **Couropita subsessilis** Pilg. and **Lecythis elliptica** H.B.K. (Mori et al., 1978). Müller et al. (1980) and Moritz (1984) have reported **Bertholletia excelsa** H.B.K. (Brazil Nut tree) to be allogamous since self fertilization attains less than the 85% ovule fertilization necessary for fruit set.

Clearly, pollinators' habitat requirements merit consideration for conservation of the economically important Brazil Nut tree. Ferraz (1982) and Kitamura & Müller (1984) reported an alarming reduction in Brazil Nut production in southeastern Pará state associated with large scale deforestation and burning. Prance (1983) and Mori & Prance (in prep.) have shown that the staggered flowering of different species of Lecythidaceae in French Guiana provides a food source for Euglossine bees for at least four months and have suggested that intact forest, with its variety of trees, as well as orchids, is necessary for Euglossines to survive throughout the year. Censuses of Euglossines coming to scent baits in pasture and neighboring forest 80 km north of Manaus (Powell, 1983) have shown that some Euglossine species are restricted to forest and will not cross even narrow strips of pasture, while other species of Euglossines can be captured in open pasture near forest.

In **Couratari**, the most elaborately zygomorphic genus of Lecythidaceae, nectar is produced at the center of the coiled androecium and access from the sides is prevented by the position of the petals, so that only large bees with long tongues can reach the nectar (Fig. 1-A,B,C). **Bertholletia excelsa** is somewhat less zygomorphic. Bees capable of extracting nectar from **Couratari** should therefore also be capable of extracting nectar from **Bertholletia**. Prance (1976) has observed the Euglossine bee **Eulaema meriana** Oliver and **Bombus** sp. obtaining nectar from **Couratari atrovinosa** Prance. Müller et al. (1980) and Moritz (1984) have shown that large **Bombus**, **Centris** and **Xylocopa** (all common in disturbed vegetation far from mature forests) are apparently effective pollinators of short, grafted Brazil Nut trees in a small experimental plantation in Belém.

Here we identify flower visitors to **Bertholletia excelsa** and **Couratari tenuicarpa**, report their behavior and identify the pollen clinging to their dorsal thorax (area of anther and stigma contact) and stored on their legs. The **B. excelsa** tree studied is part of a tall, mature, non-grafted plantation near Manaus resembling a mature forest in gross structure and density but with less undergrowth and no intermediate strata. The **C. tenuicarpa** grows in a natural black water igapó forest at the edge of open water, 15 km northwest of Manaus. By studying the pollen clinging to the dorsal thorax and stored on the legs of captured bees it was possible to determine whether each bee was capable

of transporting the pollen of the host tree, as well as to determine the bee's habitat preference as indicated by pollen remaining from visits to other plants prior to capture.

Voucher material of both the plants and the bees is deposited in the herbarium and entomological collections of INPA, respectively.

MATERIALS AND METHODS

Couratari tenuicarpa grows scattered in very low densities in black water flooded forest near Manaus. Each tree produces a flush of showy purple flowers over a period of four to six weeks from May to July. At this time most of the crown is leafless. The tree has many open flowers at all times of day, and flowers must be open at night since spent blossoms drop into the water at a high rate between 0900 and 1000 hrs.

At 1600 hrs on 10 June 1984 four large bees were observed visiting a specimen of *C. tenuicarpa* near Vivenda do Pontal, about 15 km NW of Manaus. We returned to make collections and closer observations on 16 June during the afternoon and 17 June in the morning. The tree was standing in two meters of water at this time of year, which is the high water period in Manaus. Observations and collections were made by climbing the tree with a simple climbing belt ('peconha') to the top of the bole.

In the INPA palynology lab pollen was scraped from the dorsal portion of the head and thorax and from the corbiculae of bees bearing pollen, then treated by acetolysis. Permanent slides were made of pollen from the flower visitors for comparison with a similar slide made from anthers of the tree studied. SEM photos were also made of pollen from the dorsal area between the head and thorax of one bee from *C. tenuicarpa* and from pollen taken from anthers of the host tree.

Observations of *Bertholletia excelsa* were made in the large plantation on Estrada do Aleixo about 12 km ENE of Manaus, on 10 and 11 December 1984 from 0530 to 1000 hrs. No observations were made later than 1000 hours as most of the flowers are unreceptive to fertilization after that time (Müller et al., 1980). The Aleixo trees were grown from seed and are now 20-25 m tall and 50-80 cm DBH. The species does not occur naturally near Manaus, but natural stands are found 100 km north and 35 km south, across the Solimões. Planted trees near Manaus flower once a year, mostly in the months of November and December. The Aleixo plantation resembles a mature natural forest with a nearly closed canopy in some parts. The tree studied was 18 m tall with many open flowers in the upper crown and a few flowers in the lower branches accessible to an eight meter pole with collecting net.

Pollen analyses by the acetolysis method were carried out the same as for *Couratari tenuicarpa*'s flower visitors. The Brazil Nut tree studied was checked for fruit-set six months after pollinator observations.

RESULTS

Couratari tenuicarpa A.C. Sm. (BN 1337)

Two species of bees were collected: six female *Xylocopa frontalis* (Olivier, 1789) (BN 1338) and two male *Eulaema mocsaryi* (Friese, 1889) (BN 1339). One small black *Trigona* (BN 1340) was captured at 1645 hrs on 16 June attempting to enter between the hood and staminal ring of the androecium, but it was unable to penetrate because it could not lift the hood.

From 1400 to 1700 hrs on 16 June three specimens of *Xylocopa frontalis* and three specimens of *Eulaema mocsaryi* were observed foraging for nectar in the crown of *C. tenuicarpa*. No flower visitors arrived between 0600 and 0630 hrs of the next day during a slight rain with overcast sky. *Eulaema* began working the upper, driest flowers at 0630 and this bee visited about six flowers per minute for twenty minutes before being caught. One *Xylocopa* arrived at 0630 but did not alight until 0700 when the flowers were dry. This species also visited about six flowers per minute, spending about five seconds on each flower. Length of time on the tree for *Xylocopa* varied from a few seconds to ten minutes. Most bees were either caught or disturbed by the collector before ten minutes on the tree had passed, so the bees might have stayed longer. About 15 *Xylocopa* visited the tree between 0630 and 0900 hrs on 17 June, but only two *Eulaema* were seen over this time.

The method of obtaining nectar is similar for both species. These large powerful bees land on the tightly coiled androecium hood and by their weight and strength push the hood down and away from the pistil and staminal ring. They then insert their head and tongue into the androecium, contacting the pistil and staminal ring with the dorsal portions of the head and thorax (Fig. 1-B,C). The tongue of *Xylocopa frontalis* is 9 mm long, that of *Eulaema mocsaryi* is 20 mm long.

Of the two specimens of *Eulaema* captured one was covered with sticky white pollen in the vicinity of the ocelli and area of antenna insertion, on the smooth exoskeleton of the nearly hairless head. The second specimen was very clean with no pollen visible under the stereoscope. Of the six specimens of *Xylocopa* captured, only two were obviously contaminated with pollen in the hairy portion of the dorsal thorax, and on the dorsal posterior portion of the head (Fig. 1-D).

Results of pollen study after acetolysis are presented in Table 1. *Couratari tenuicarpa* dominated the pollen on the dorsal head and thorax of both *Xylocopa* and *Eulaema*. Genera common in secondary vegetation (*Bellucia*, *Alternanthera* and an indet palm) were also encountered. SEM analysis of the pollen on the dorsal thorax of one *Xylocopa* specimen revealed 99% *Couratari tenuicarpa* and about 1% *Cassia* sp. Several *Cassia* species occur commonly in secondary vegetation on terra firme near Manaus.

Bertholletia excelsa H.B.K.

Flowers fall in the late afternoon, but are replaced by a new crop each night. Anthesis is attained at 0430-0500 hrs so that at sunrise many flowers are already open



Fig. 1. Flower and flower visitors of *Couratari tenuicarpa* A.C. Smith: A. Single flower with tightly curled spring-like androecium; B. Androecial hood pried open with thumb to expose staminal ring; C. Male *Eulaema mocsaryi* posed in flower with half of longitudinally sliced androecial hood pried open. Note long tongue of this Euglossina; bee capable of reaching nectaries at end of androecial curl; D. *Couratari tenuicarpa* pollen clinging to head and dorsal thorax of *Xylocopa frontalis*. This portion of the bee contacts staminal ring when head and tongue are forced into androecial curl. Scale bars: A, B & C = 01 cm; D = 02 mm.

and bee activity can be intense.

The following bees were captured in the Brazil Nut tree:

1. *Euplusia*, probably *seabrai* Moure in litt. ♀ (BN 1378): two bees, captured at 0555 and 0630, had heavy pollen dusting on the dorsal thorax and heavy corbiculae loads.
2. *Eulaema nigrita* (Lepelletier, 1841) ♂ (BN 1373): one bee captured at 0645 with an accumulation of pollen in the dorsal area between head and thorax. Apparently very common, these male Euglossines do not collect pollen, so were presumably after nectar.
3. *Eulaema nigrita* ♀ (BN 1377): one bee captured at 0740 with pollen between head and dorsal thorax and pollen loads on the corbiculae.
4. *Eulaema cingulata* (Fabricius, 1804) ♂ (BN 1374): two bees captured in the period 0700-0845, with pollen dusting on the dorsal area between head and thorax. These male Euglossines had no evident pollen on the legs and were presumably collecting nectar.
5. *Epicharis umbraculata* (Fabricius, 1789) ♀ (BN 1375): one bee captured in the period 0700-0845. Members of this genus are commonly observed collecting oil from *Byrsonima* in disturbed secondary vegetation near Manaus. The oil is mixed with pollen to form "bee bread" for larvae. Slight pollen dusting observed on dorsal head and thorax.
6. *Epicharis rustica* (Olivier, 1789) ♀ (BN 1375): two bees collected in period 0700-0900 on the second day of observation when many of this species were seen on the tree. This is also an oil collecting bee. Even from a distance of ten meters the dense yellow pollen dusting on the dorsal thorax was visible on this species.

Three Euglossine species and two Anthophorid species — all large bees — were collected from flowers of the Brazil Nut tree with accessible branches. Euglossines were the dominant flower visitors from 0600 to 0800 while Anthophorids were common from 0800 to 0930.

Flower visits were made in the same manner as observed for *Couratari tenuicarpa*, each visit lasting 5-15 seconds, with 2-5 flowers visited per minute. Bee activity was intense and virtually all flowers on the tree were visited. Many were visited repeatedly, by different bees, during the period of observation. One large Brazil Nut tree with a full crown of flowers was host to approximately 50 large black bees at one time when observed between 0600 and 0630 on 10 December. These were probably the common Euglossine *Eulaema nigrita*.

Eight of the nine individual bees had a predominance of *Bertholletia* pollen on the dorsal area between the head and thorax (Table 1 and Fig. 2), precisely where stigma and anther contact is made. The single female *Eulaema nigrita* captured had a liberal dusting of pollen on the same part of its body, though from a Fabaceae. All bees captured are potentially effective pollinators. All female bees with pollen loads on the corbiculae were carrying a preponderance of *Bertholletia* pollen (again with exception of the female *E. nigrita*). This indicates that *Bertholletia* is providing rewards of both pollen and nectar, the latter exploited by male bees. The two male bees (*Eulaema cingulata* and *Eulaema nigrita*) had a slight pollen dusting on their legs which proved to be mostly *Bertholletia*.

After *Bertholletia* the most frequent pollen types from the dorsal region, and from

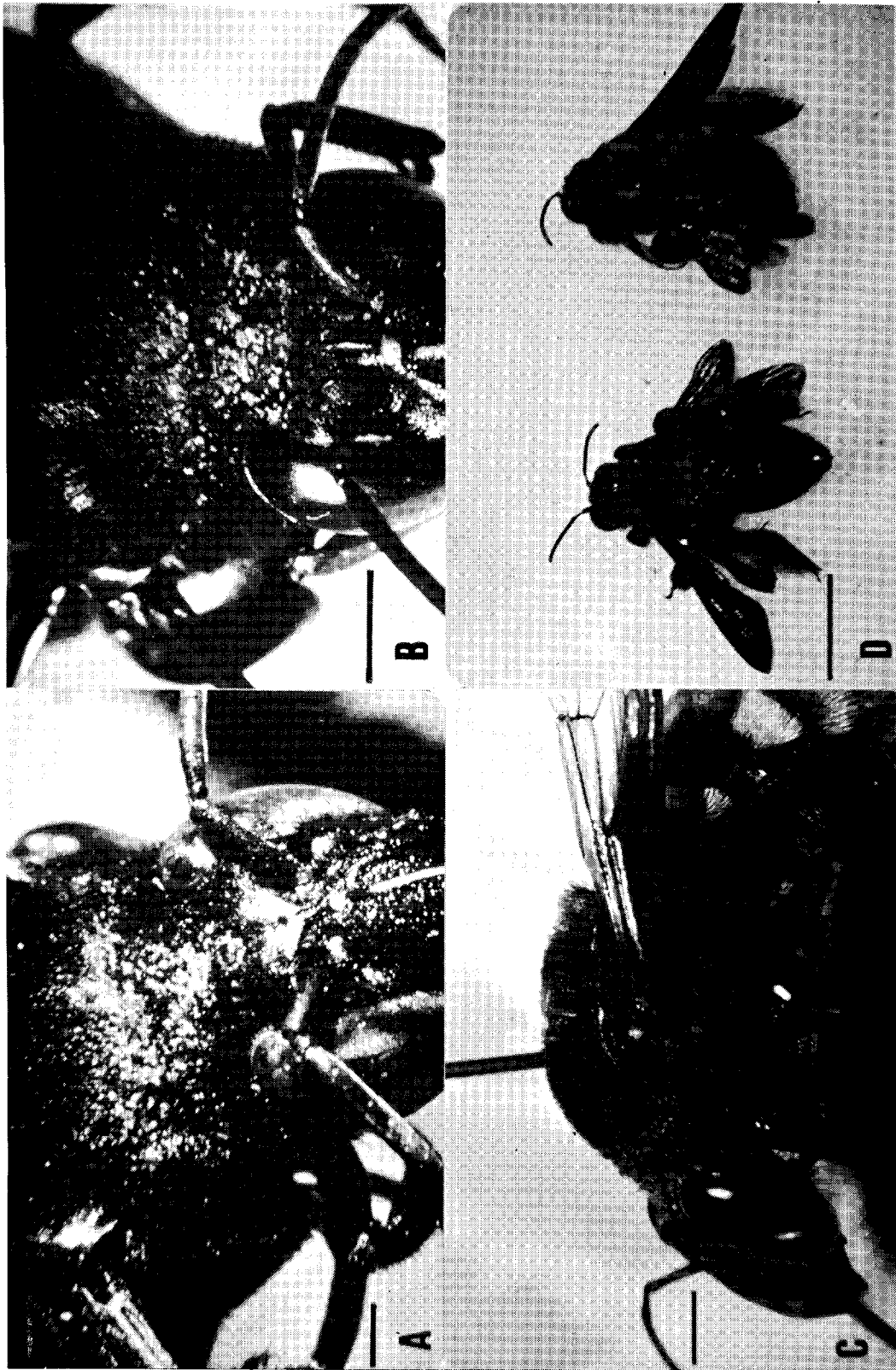


Fig. 2. Bees captured in flowers of *Bertholletia excelsa* H.B.K., all with pollen on dorsal thorax and head: A. *Eulaema nigrita*, male (BN 1373); B & C. *Eulaema cingulata*, male (BN 1374); D. *Epicharis rustica*, females (BN 1376) with oil stored in leg brushes, probably from *Byrsonima* flowers. Scale bars: A. 01 mm; B & C. 02 mm; D. 10 mm.

Table 1. Pollen suites found on bees visiting flowers of *Couratari tenuicarpa* and *Bertholletia excelsa*. Relative abundance of pollen types on prepared slides after acetolysis: (*) frequent; (+) common; (x) occasional.

No of bees checked	POLLEN SOURCE		FLOWER VISITORS	
	Dorsal thorax/head pollen:		Dorsal thorax/head pollen:	
3	<i>Xylocopa frontalis</i> (Olivier) ♀		<i>Xylocopa frontalis</i> (Olivier) ♀	
2	<i>Eulaema mocsaryi</i> (Friese) ♂		<i>Eulaema mocsaryi</i> (Friese) ♂	
4	Pollen from legs:		Pollen from legs:	
2				
	Dorsal thorax/head pollen:		Dorsal thorax/head pollen:	
2	<i>Eulaema cingulata</i> (Fabricius) ♂		<i>Eulaema cingulata</i> (Fabricius) ♂	
2	<i>Euplusia seabrai</i> Moure in litt. ♀		<i>Euplusia seabrai</i> Moure in litt. ♀	
1	<i>Eulaema nigrita</i> (Lepelletier) ♂		<i>Eulaema nigrita</i> (Lepelletier) ♂	
1	<i>Eulaema nigrita</i> (Lepelletier) ♀		<i>Eulaema nigrita</i> (Lepelletier) ♀	
2	<i>Epicharis rustica</i> (Olivier) ♀		<i>Epicharis rustica</i> (Olivier) ♀	
1	<i>Epicharis umbraculata</i> (Fabricius) ♀		<i>Epicharis umbraculata</i> (Fabricius) ♀	
	Pollen from legs:		Pollen from legs:	
1	<i>Eulaema cingulata</i> (Fabricius) ♂		<i>Eulaema cingulata</i> (Fabricius) ♂	
2	<i>Euplusia seabrai</i> Moure in litt. ♀		<i>Euplusia seabrai</i> Moure in litt. ♀	
1	<i>Eulaema nigrita</i> (Lepelletier) ♂		<i>Eulaema nigrita</i> (Lepelletier) ♂	
1	<i>Eulaema nigrita</i> (Lepelletier) ♀		<i>Eulaema nigrita</i> (Lepelletier) ♀	
2	<i>Epicharis rustica</i> (Olivier) ♀		<i>Epicharis rustica</i> (Olivier) ♀	
1	<i>Epicharis umbraculata</i> (Fabricius) ♀		<i>Epicharis umbraculata</i> (Fabricius) ♀	
	Sapotaceae indet			
	Simaba			
	Euphorbiaceae indet			
	Solanum			
	Fabaceae indet			
	Bixa			
	Melastomataceae indet			
	Lecythidaceae indet			
	Proteaceae indet			
	Didymopanax			
	Polygonum ?			
	Croton			
	Miconia			
	Cassia			
	Byrsonima			
	Passiflora coccinea			
	<i>Bertholletia excelsa</i>			
	Phaseolus			
	Cissus			
	Arecaceae			
	Alternanthera			
	Bellucia			
	Couratari			

the corbiculae of females, were *Passiflora coccinea*, *Byrsonima* sp., *Miconia* sp., and *Bertholletia* sp. which suggests that all bees had been foraging in secondary growth on terra firme prior to capture on the *Bertholletia*.

Fruit set in the Aleixo Brazil Nut tree plantation is consistently lower than the 0.4% fruit-set to flower ratio reported by Lima (cited in Müller et al., 1980). Despite apparently effective flower visits to virtually all flowers on the tree, no fruits were set on the study tree in the Aleixo plantation, as indicated by observations six months after the flowering period. This is a common occurrence for trees in the Aleixo plantation.

CONCLUSIONS

Bertholletia excelsa can be effectively pollinated by large Euglossine and Anthophorid bee species found in secondary vegetation far from primary forest (though not necessarily in pastures). Additional collecting should reveal other large bees common to secondary vegetation capable of pollinating the Brazil Nut tree (e.g. *Bombus*, *Centris*, *Xylocopa*). Since *Xylocopa frontalis* and *Eulaema mocsaryi* are capable of pollinating *Couratari tenuicarpa* they should be able to exploit the flowers of the Brazil Nut tree.

Low fruit set on the Brazil Nut trees in the Aleixo plantation is probably not due to a lack of effective pollinators, but rather to some other factor, such as soil. Brazil Nut trees do not occur naturally on Tertiary soils near Manaus and no self-sustaining stands were left by the indigenous peoples who once inhabited the banks of the lower Rio Negro. Natural stands of Brazil Nut trees are encountered on the richer soils some distance from Manaus, for example: south of the Rio Solimões on Pleistocene terrace (relict várzea) soils, 100 km north of Manaus on soils weathered from Paleozoic beds, and further north, on soils weathered from the Brazilian shield.

RESUMO

Foram estudadas as abelhas que visitavam flores de *Bertholletia excelsa* H. B. K. (castanheira) e *Couratari tenuicarpa* A.C. Smith. Foi descrito o comportamento dessas abelhas e identificados os grãos de pólen aderido à parte dorsal do tórax e nas patas. As abelhas femininas de *Xylocopa frontalis* (Olivier) e masculinas de *Eulaema mocsaryi* (Friese) observadas em igapô próximo de Manaus, são aparentemente polinizadores de *Couratari*. As abelhas femininas de *Euplusia seabrai* Moure in litt., *Epicharis umbraculata* (Fabricius), *Epicharis rustica* (Olivier) and *Eulaema nigrita* (Lepeletier), bem como as abelhas masculinas de *Eulaema cingulata* (Fabricius) e *Eulaema nigrita* são aparentemente polinizadores da castanheira estudada em uma plantação na Estrada do Aleixo, próximo a Manaus. Apenas as abelhas maiores, capazes de desenrolar o androceu floral, podem polinizar *Couratari* e *Bertholletia*.

A análise polínica indicou que todas as abelhas capturadas transportavam pólen de

Bertholletia ou *Couratari*. Os outros grãos de pólen encontrados nas abelhas provêm de plantas de capoeira. Observamos que estas abelhas comuns em capoeira visitaram praticamente todas as flores da castanheira permitindo assim, constatar que a presença de mata primária (e aquelas espécies de abelhas Euglossine restritas a mata primária) não é necessária para a polinização da castanheira.

A baixa produção de frutos na plantação do Aleixo deve ser atribuída a outros fatores, e não a polinização. Uma vez que as ocorrências naturais de castanheiras na região estão associadas com melhores condições edáficas, essa baixa produção de frutos na plantação do Aleixo pode ser devido as deficiências do solo.

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