Bees, plants and pollen in Central Amazonia - how surrounding areas contribute to pollination of guarana (*Paullinia cupana* var. *sorbilis* (Mart.) Ducke)

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Project Presentation

This study investigated visiting/pollinating bees in guarana (*Paulinia cupana* var. *sorbilis* [Mart.] Ducke) plantations and surrounding plants, and was developed as a part of the project "Plant-bees interaction networks with North and Northeast fruit trees (12.16 .04.024.00.00)" financed and developed by Embrapa in four Brazilian states in partnership with RCPol (Online Pollen Catalogs Network), various universities, and National Institute for Amazonian Research (INPA), with the general objective of "characterize plant-pollinator interactions of fruit species, with an emphasis on bees, aiming to support crop systems that co-share the most efficient pollinators and increase pollination and sustainability of agroecosystems".

Study Region

This study was carried out in a central Amazonian guarana cultivation area in the Embrapa Western Amazon experimental site (2°53'22.24" S, 59°58'47.34" W), located at Km 29 on the AM 010 highway, near Manaus, Amazonas State, Brazil (Figure 1). The cultivation area covers 7.63 ha.

Vegetation and Climate

The natural vegetation of the study area is upland (terra firme) Amazonian Rainforest (Hopkins 2005) and the climate is humid tropical, AM, with a mean annual temperature of 26.5° C (Köppen 1936). The rainy season generally occurs between January and June, with a noticeable reduction in rainfall between July and September (Antonio 2017).

Material and Methods

Data were collected monthly for 1 year, by two collectors on two consecutive days. These were carried out on the first day from 11:00 to 17:00 and on the second day, from 5:00 to 11:00, between May 2016 and June 2017. The sampled area corresponds to edges of a conventionally managed guarana planta-

tion (Pereira et al. 2005). The edges of the plantation and access roads are bordered by upland Amazon forest with the presence of native and exotic ruderal species used by bees (Figure 1A).

Bee surveys

A transect of approximately 3.5 kilometers in length with 5 meters in width buffer was set between the plantation edge and the adjacent forest, and the roads that connect the guarana crop sites (Figure 1, line in yellow). Along this transect, all flowering plants, including guarana, which had at least three branches with flowers or inflorescences (used operationally as this is the minimum requirement for exsiccates), were evaluated/observed for five minutes, and all floral visitors present on the flowers over this time were collected with entomological nets. Following capture, insects were prepared, stored and labelled properly with information from the insect(s) and the plant visited. Pollen, when present on the body, corbiculae or scopa of each bee, was removed and duly labelled, for later acetolysis. Bees were identified by the authors and are deposited in the Invertebrate collection of INPA and classified following Michener (2007). In this study, we present only the data related to bees collected on guarana and plants visited by them in the surroundings, the target species for this chapter.

Plant and pollen collections

All species of plants sampled during the project had parts (fertile parts) collected for exsiccate and later identification (done in triplicate). In addition, fertile anthers of flowers and flower buds were collected from the respective plants visited to identify pollen

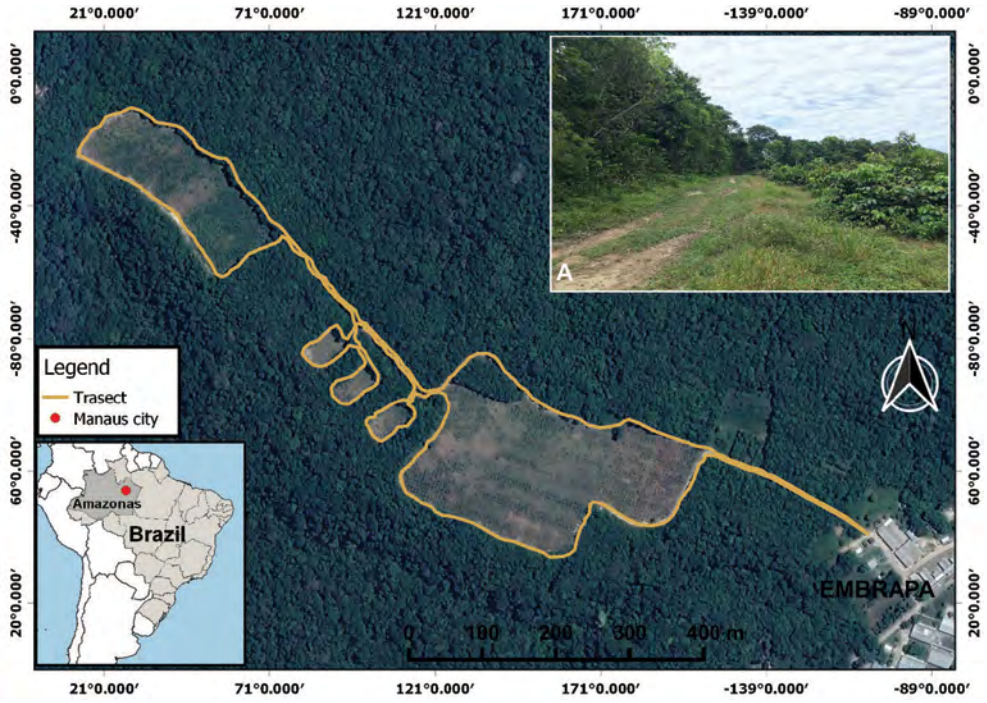

Figure 1. Map of the study area, showing the trasect in guarana plantation at the Embrapa Western Amazon experimental site (Manaus, AM). A: Image of a portion of the study site. Source: GoogleEarth, modified via Qgis version 3.6.3.

types and compose a reference pollen collection to assist in identification of the pollen collected and transported by bees. Collected plants were identified by specialists into the appropriate botanical groups and/or by comparison with material deposited in the herbarium of INPA, where the exsiccates were also deposited. Botanical families were classified following APG IV (2016).

Samples of floral pollen and pollen removed from collected bees were prepared with the acetolysis method of Erdtman (1960) and Silva et al. (2014), which consists of the chemical treatment of the pollen grain, eliminating the cytoplasmic content and substances adhering to the grains, to expose the morphological characteristics useful for identification. Samples of floral pollen, after acetolysis, were mounted on permanent slides with gelatin (Kisser 1935) and sealed with paraffin. Pollen grains were described with the aid of an optical microscope with a camera attached and linked to the reference network of RCPol. Slide preparation methods followed Silva et al. (2014). Samples of acetolyzed pollen from bees were mounted on semi-permanent slides, in triplicate, and pollen present was identified with the aid of an optical microscope and the RCPol reference pollen library.

Results and Discussion

In total, 27 species of flower-visiting bees belonging to 4 families (Apidae, Colletidae, Halictidae and Megachilidae) were sampled (Table 1) visiting flowers of guarana and other plants surrounding the crop during the course of the 1-year study. In addition to guarana, the 20 native plants most used by these bees are listed in Table 2.

Eight bee species were the most abundant (i.e. had the largest number of individuals sampled), showing more than 50 visits to plants, and were responsible for 87% observed interactions. These were: *Apis mellifera* (46%), *Trigona guianae* (12.5%), *Aparatrigona impunctata* (7.6%), *Nannotrigona melanocera* (6%), *Trigona cilipes* (4.8%) and *Melipona (Michmelia) fulva, Frieseomelitta trichocerata, Tetragonisca angustula* (all with 3.5%). The remaining 19 bee species, in sum, were collectively responsible for 12.5% abundance.

Pollen grains were obtained from 21 species of flower-visiting bees. After evaluation on pollen data presented on bee species and their interactions with the plant species, we found that *Apis mellifera* (Figure 2A) had the greatest diversity of pollen types in pollen samples (N = 12), followed by *Melipona (Michmelia) fulva* (N = 9), *Trigona cilipes* (N = 7), *Paratetrapedia basilares* (N = 6) and *Trigona guianae* (N = 5).

Although *Apis mellifera* was the most abundant bee and had the highest diversity of pollen types, it was not the species that exploited the largest number of flowering plant species, visiting ten plant species. Of the native bee species, *Trigona guianae* was the one that interacted most with different species of flowering plants (N = 13). However, pollen samples from this species revealed that these bees exploit only a few plant species, in terms of pollen collected (N=5).

Of the plants most visited by bees, *Hyptis atrorubens* received 39% of all visits (Figure 2B), followed by *Borreria alata* (33%: Figure 2C) and *Paullinia cupana* var. *sorbilis* (8.5%). Together, these three species were responsible for about 80% of all visits. *Hyptis atrorubens* and *Borreria alata* remained on flowers for extended periods across the year, while *Paullinia cupana* var. *sorbilis* flowering was restricted to the July – September period. Such timing was an important factor for the dominance of these species in guarana plants, which, despite having a short flowering period, offers a large amount of pollen and nectar for bees visiting the plantation. The other 18 plant spe-

Table 1. Bees visiting flowers of guarana (*Paullinia cupana* var. *sorbilis*) and other plant species in adjacent habitats near Manaus, Amazonas State, Brazil.

Family	Species
Apidae	*Aparatrigona impunctata* (Ducke, 1916)
	Apis mellifera Linnaeus, 1758
	Cephalotrigona femorata (Smith, 1854)
	Frieseomelitta trichocerata Moure, 1990
	Melipona (Michmelia) fulva Lepeletier, 1836
	Melipona (Michmelia) seminigra merrillae Cockerell, 1919
	Melipona (Michmelia) seminigra seminigra Friese, 1903
	Nannotrigona melanocera (Schwarz, 1938)
	Paratetrapedia basilaris Aguiar & Melo, 2011
	Paratrigona euxanthospila Camargo & Moure, 1994
	Paratrigona melanaspis Camargo & Moure, 1994
	Paratrigona pannosa Moure, 1989
	Paratrigona sp. 1
	Partamona auripennis Pedro & Camargo, 2003
	Partamona vicina Camargo, 1980
	Tetragona kaieteurensis (Schwarz, 1938)
	Tetragonisca angustula (Latreille, 1811)
	Trigona cilipes (Fabricius, 1804)
	Trigona guianae Cockerell, 1910
	Trigona williana Friese, 1900
Colletidae	*Ptiloglossa* sp. 1
Halictidae	*Augochloropsis hebescens* (Smith, 1879)
	Megalopta amoena (Spinola 1853)
	Megalopta sodalis (Vachal 1904)
Megachilidae	*Coelioxys* sp. 1
	Hoplostelis (Rhynostelis) multiplicata (Smith, 1879)
	Megachile sp. 3

Table 2. Main flowering plants found in areas close to guarana (*Paullinia cupana* var. *sorbilis*) cultivation, which were visited by bees and/or had bees carrying their pollen.

Family	Species	Visit	Pollen
Asteraceae	*Pseudelephantopus spiralis* Cronquist	X	X
	Unxia camphorata L.f.	X	
Hypericaceae	*Vismia cayennensis* (jacq.) Pers	X	X
	Vismia japurensis Reichardt	X	
Cyperaceae	*Rhynchospora pubera* (Vahl) Boeck.	X	X
Lamiaceae	*Hyptis atrorubens* Poit.	X	X
Lauraceae	*Nectandra cuspidata* Nees	X	
Fabaceae	*Mimosa pudica* L.	X	X
	Mimosa sensitiva L.	X	
	Stryphnodendron pulcherrimum (Willd.) Hochr.	X	X
	Senna quinquangulata (Rich.) H.S.Irwin & Barneby	X	
	Zornia latifolia DC.	X	
Malpighiaceae	*Byrsonima chrysophylla* Kunth	X	X
Melastomataceae	*Bellucia dichotoma* Cogn.	X	X
	Clidemia hirta (L.) D.Don	X	X
Myrtaceae	*Eugenia stipitata* McVaugh		X
Rubiaceae	*Borreria alata* Aubl.	X	X
	Borreria verticillata (L.) G.Mey.	X	X
Sapindaceae	*Paullinia cupana* var. *sorbilis* (Mart.) Ducke	X	X
Solanaceae	*Solanum paniculatum* L.	X	X
Verbenaceae	*Stachytarpheta cayennensis* (Rich.) Vahl	X	X

cies received the remainder of the visits in a more equitable manner, with no species with more than 3% of the visits. Using only pollen data, we found the plant species with pollen most frequently found on bees were: *Byrsonima chrysophylla* (17%) (Figure 2D), *Borreria alata* (10.5%), *Rhynchospora pubera* (10.5%), *Solanum paniculatum* (9.2%) and *Bellucia dichotoma* (9.2%).

In this study, it became evident that pollen analysis provides complementary information for the study of interactions between bees and plants. It also showed that there were plants, such as *Eugenia stipitata*, not directly sampled, but whose pollen was used as a resource by some bee species.

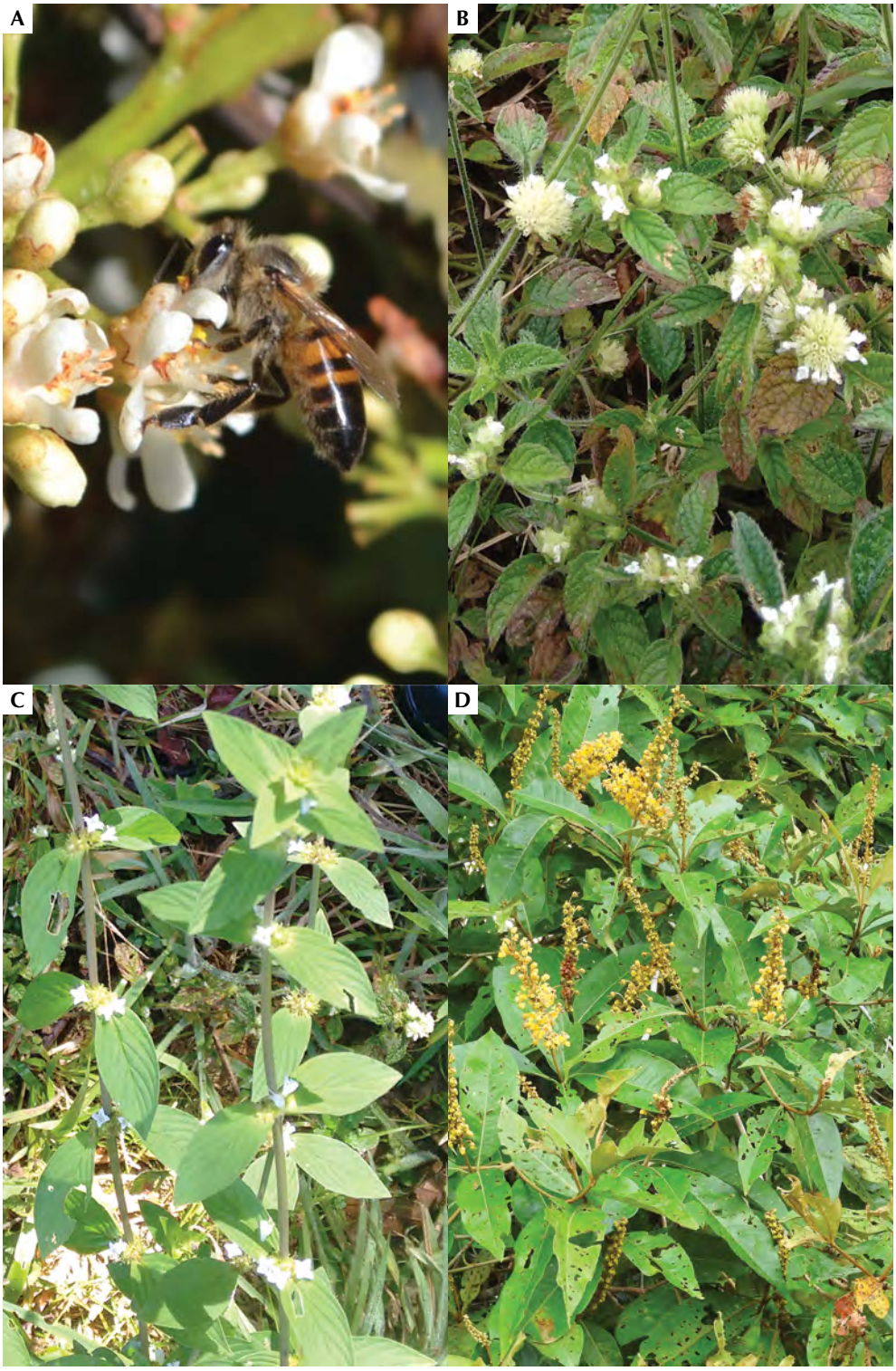

Figure 2. *Apis mellifera* visiting guarana flowers (A), and individuals of *Hyptis atrorubens* (B), *Borreria alata* (C), and *Byrsonima chrysophylla* (D).

It is widely recognized that native bees are essential for pollination and for increasing the productivity in a variety of agricultural crops (Garibaldi et al. 2013). But it is important to note that, in addition to agricultural crops, these bees/pollinators depend on the floral resources offered by other plants present in the vicinity of the crops in question, for food and nutritional diversification. This pollen atlas aims to provide scientific and technical support for the maintenance of these pollinators associated with guarana crops, through the maintenance and/or inclusion of these pollinator-friendly plants in agricultural areas. The adoption of this practice will favor agricultural cultivation and the maintenance of the natural environmentally-based pollination service in the region.

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Figure 3. Staminate (A), pistilate (B) inflorescences and ripe guaraná fruits (C).

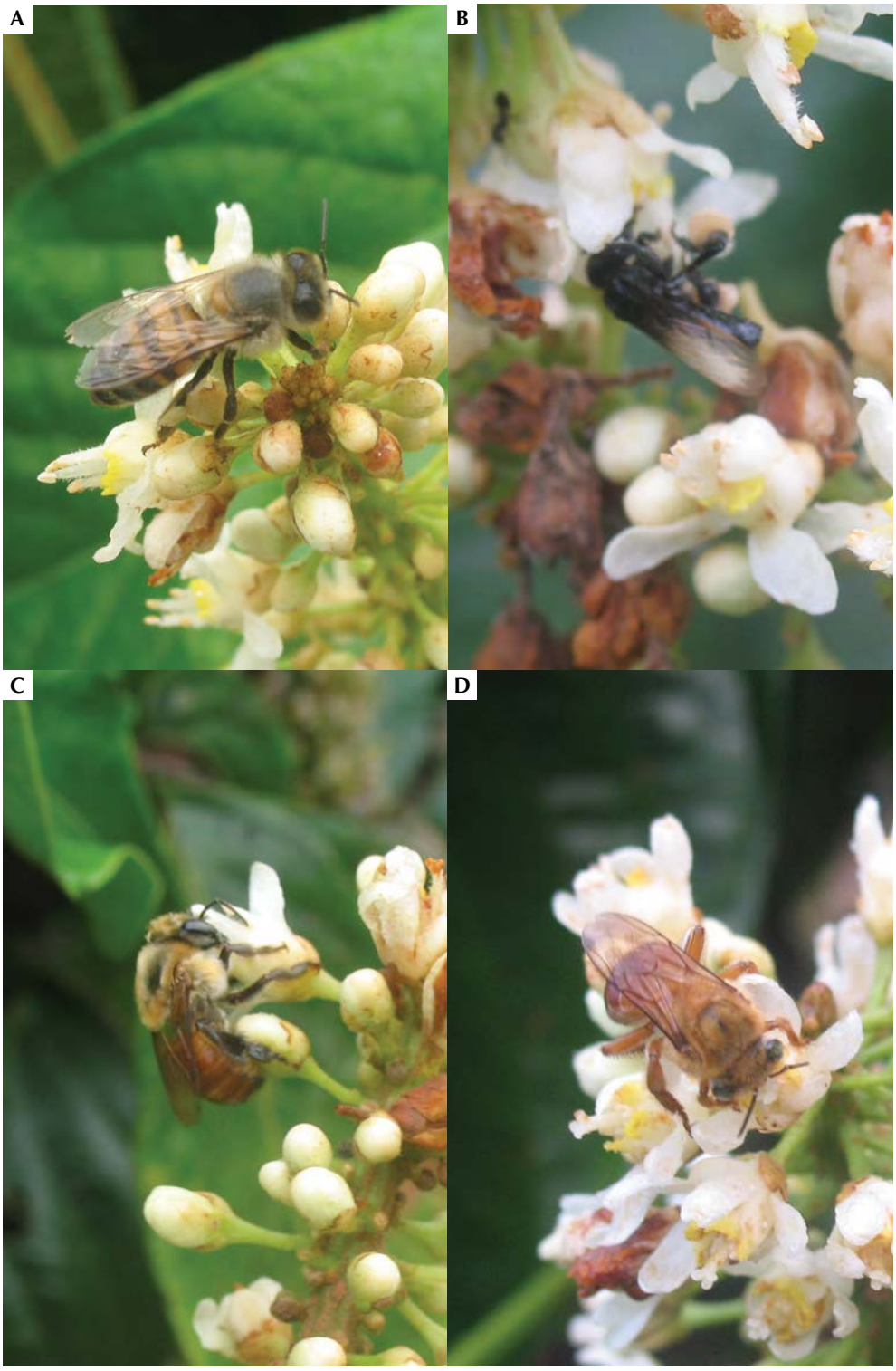

Figure 4. Bees visiting guaraná flowers, the exotic bee Apis mellifera (A) and native stingless bees (B,C,D).

Nest of *Euglossa cordata*