Project Update: August 2023
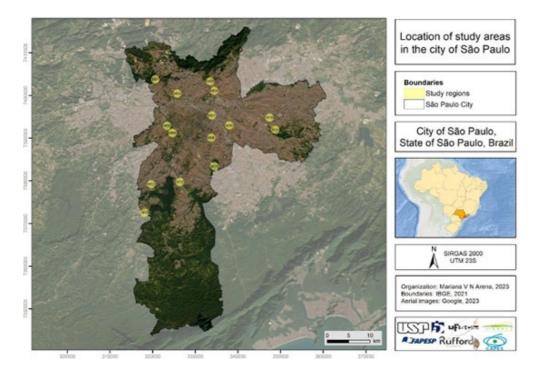
1.   Summary of activities carried out in the first year

1.1 Study areas selection
During the first stage of the project, the selection of possible study areas was carried out based on the search for stingless beekeepers in the city of São Paulo, by establishing contact, establishing partnerships, visiting meliponary farms and mapping the locations. The 15 locations (Figure 1) that represented the best spatial representation of the city were selected, that is, the points were well distributed in neighbourhoods across all zones of the municipality - North (ZN), South (ZS), East (ZE), West (ZW) and Center. To facilitate the layout, the areas were abbreviated as M (meliponary) and ordered from 1 to 15.

1.2 Pilot experiments
1.2 Three study areas were selected to carry out the pilot experiments (M03, M04 and M05). There was variation between the study areas, showing that the release and monitoring methodology can be used in urban areas. To be able to infer whether the variations between the coverage regions occurred due to the landscape around the nests and not to other factors, it is necessary to repeat the experiment with a larger number of study areas, and a greater distribution of release points and distances, to cover a better representation of the landscape around the meliponary. Thus, the pilot study provided the necessary subsidies for the development of the present project proposal, and based on these observations, the experimental design was redesigned. The results of the pilot experiments were consistent and enabled the publication of a scientific article in Frontiers in Sustainable Cities magazine, special edition World Bee Day 2022: Pollinators in Urban Environments. This article was presented in the Qualifying Exam on 03/30/2023, with Approved result.
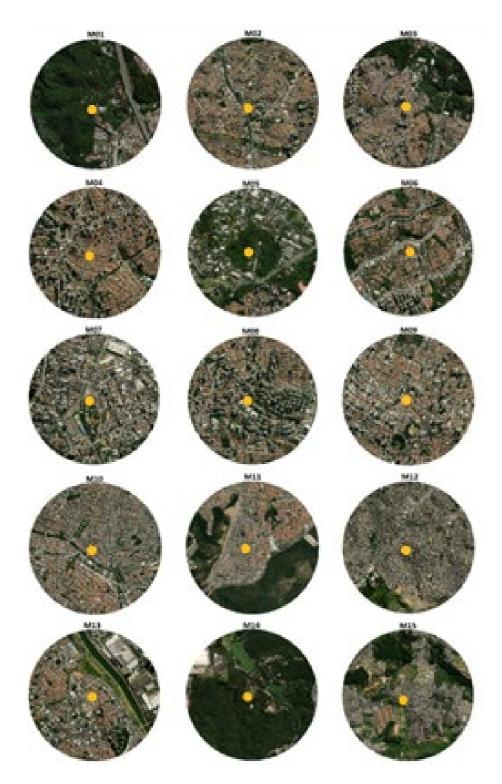
Figure 1: Location of the study areas in the city of São Paulo, with details of the areas. Yellow dots indicate the location of the meliponary. M: Meliponary; Numbers: Identification of the area. M01 - Vila Jaraguá (ZN); M02 - Freguesia do Ó (ZW); M03 - Parque Mandaqui (ZN); M04 - Jardim São Paulo (ZN); M05 – Butantã (ZW); M06 – Caxingui (ZW); M07 – Brás (Center); M08 - Vila Mariana (ZS); M09 – Mooca (ZE); M10 - Vila Nhocuné (ZE); M11 - Jardim Nossa Senhora do Carmo (ZE); M12 – Jardim Ângela (ZE); M13 - Santo Amaro (ZS); M14 - Água Funda (ZS); M15 - Jardim Horizonte Azul (ZS).

1.3 Field experiments

After the confirmations obtained through the pilot experiment, the sample design of the bee release points was organised and the experimental stage of the project began, including the marking, release and monitoring of the bees and the honey and pollen collection.

1.3.1 Experimental design

The experimental design was improved according to the considerations of partner researchers and considering the results obtained in the pilot experiment. Data collection in the field was carried out in 15 meliponaries located in the city of São Paulo, and in each one a nest of *Melipona quadrifasciata* was selected for carrying out the experiments, prioritizing hives classified as healthy by their respective stingless beekeepers.

The surroundings of each meliponary were divided into three coverage regions from generated buffers, with the colony as the central point: 300 m, 600 m and 900 m. The coverage regions were organised into subdivisions of 20 m, which served as a guide for the release of the bees. Additionally, for each meliponary, the 900 m coverage region was divided into nine 40° sections (Figure 2).
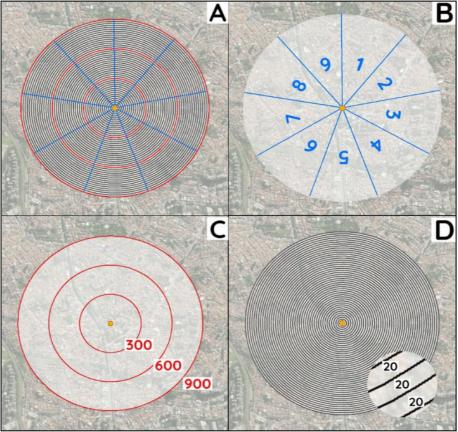

Figure 2: Example of a study area with its divisions and classifications. Orange dot indicates meliponary. Red lines indicate the coverage regions of 300, 600 and 900 m away from the meliponary. Black lines indicate subdivisions every 20 m. Blue lines indicate sections from 40°, 1 to 9. A: Study area indicating coverage regions, subdivisions and sections. B: Study area with details of the sections. C: Study area with details of coverage regions. D: Study area with details of the subdivisions.

During the period of 6 months (spring and summer), for each meliponary, three

collections and releases of 45 bees (n=135) were carried out, in non-simultaneous experiments (only one place per day). Considering that the peak of *M. quadrifasciata* foraging activities occurs until 10:00 am, the release of bees from each nest was carried out on the same day of collection, on sunny days or with low cloudiness, between 08:00 and 09:00. The first release stage (November and December) was carried out in sections 1, 2 and 3, the second release (January and February) in sections 4, 5 and 6 and the third (March and April) were carried out in sections 7, 8 and 9 (blue lines, Figure 2).

For each meliponary, the bees were randomly divided into nine tubes, each with five individuals. A coloured marking was assigned to each tube, from which the bees were marked with a non-toxic water-based pen of different colors (Figures 3 and 4). These tubes were grouped into three groups, each with three tubes, so that bees from three groups (15 individuals) were released within the 300 m coverage region (red lines, Figure 2) - Points (P) 1, 2 and 3 -, others of three groups in the region of 300 to 600 m - P4, P5 and P6 - and finally, others of three groups between 600 and 900 m - P7, P8 and P9.
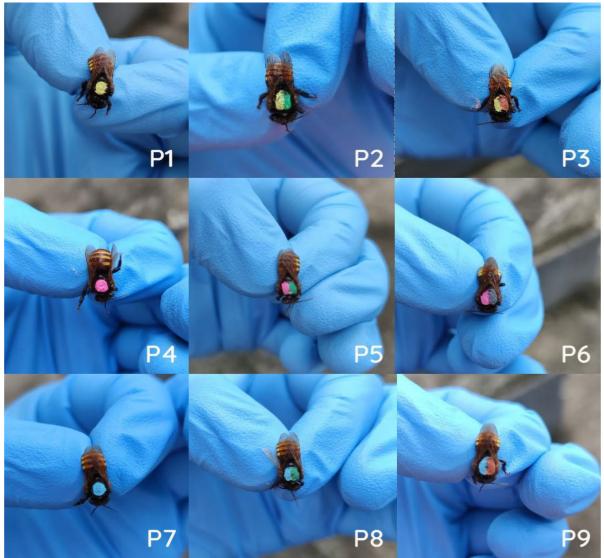

Figure 3: Bees marked in colors representing release points (P) from 1 to 9.

Figure 4: Tube containing five bees marked in yellow, representing the release point P1.

The release section for each group was randomized. For the release of bees within the groups, first a distance was drawn considering the 20 m subdivisions (black lines, Figure 2) as a reference, and the other two releases were made considering 100 m of distance in relation to the first draw. For example, in Figure 5, for the first group (0 to 300 m – points 1, 2 and 3 in pink) the third section (rays in blue) and the distance of 60 m were drawn; thus releases were performed at 60 (point 1, pink), 160 (point 2, pink) and 260 m (point 3, pink). For the fourth group (0 to 300 m, points 1, 2 and 3 in blue) the sixth section and the distance of 20 m were drawn; thus releases were performed at 20 (point 1, blue), 120 (point 2, blue) and 220 m (point 3, blue).
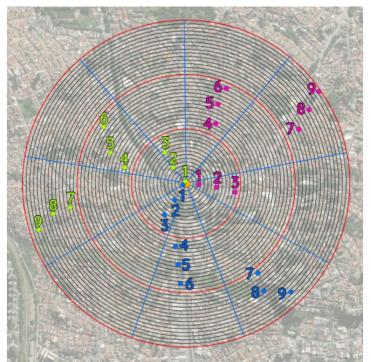

Figure 5: Example of a release scheme for a meliponary (central orange dot) with its

divisions (see fig. 2). Red lines indicate the coverage regions of 300, 600 and 900 m away from the meliponary. Black lines indicate subdivisions every 20 m. Blue lines indicate sections 1 to 9. Pink dots represent the different points of the first release stage (November and December), blue those of the second (January and February) and green those of the third (March and April).

1.3.2 Data collection
Video monitoring was carried out to obtain the returning times of bees to the hive (Figure 6A and B). After the release of the last group of bees, the recording was performed for 3 hours, a period that was established from the pilot experiment, as the interval that the bees need to return to the meliponary. Currently, we have finished the field experiments, together with honey and pollen collection (Figure 6C).
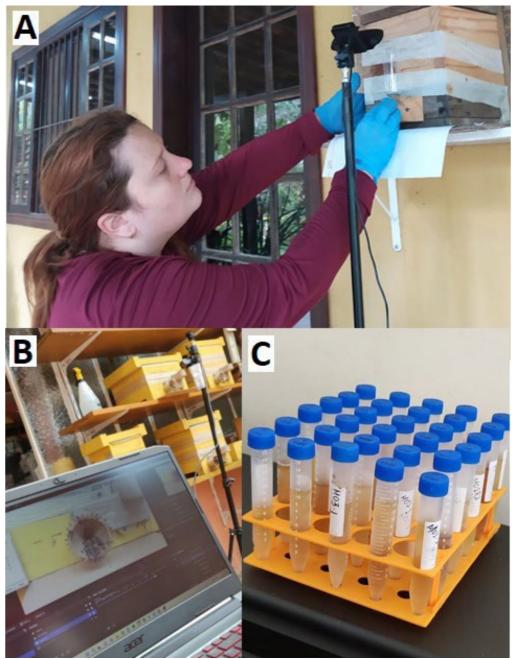

Figure 6: Data collection. A: Assembly of video monitoring equipment. B: Video monitoring in progress. C: Mandaçaia honey samples.

2. Pollen Analysis

Pollen collection was carried out during the data collection period, in spring and summer, directly from pollen combs in bee nests. The samples were stored in Falcon tubes (10 ml) with 70% alcohol. It was not possible to carry out the collection in all colonies and in all periods, as not all of them offered a pollen stock that we could access.

The analysis and identifications were carried out with the help of Dra Claudia Ines da Silva. Acetolysis of the material was performed and slides were mounted for each sample. The identification of the pollen types was carried out with the aid of an optical microscope and using the database of the Online Pollen Catalog Network (RCPol) as a reference. 400 pollen grains were examined per slide and their percentages will be calculated to calculate the frequency of each pollen type in each sample. The results were organised in a table and figures were organised with images of the pollen types identified for each sample (Figure 7).
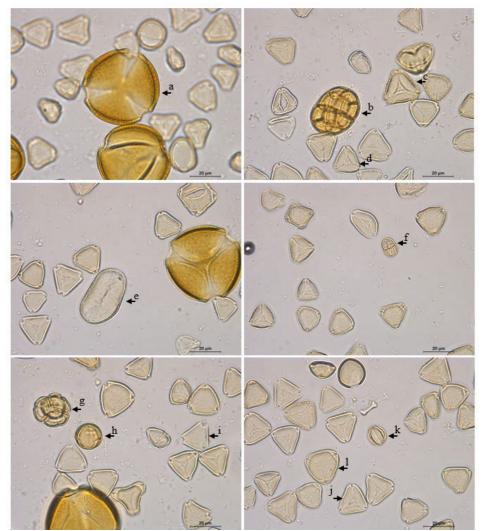

Figure 7: Pollen types identified in the M02 (spring) sample – a: *Leucaena leucocephala*; b: *Anadenanthera* sp.; c: *Eucalyptus moluccana*, d: *Eugenia uniflora*; e: *Arecaceae* sp1; f: *Mimosa caesalpiniifolia*; g: *Ericaceae*; h: *Solanum* sp.; i: *Eugenia pyriformis*; j: *Eugenia brasiliensis*; k: *Tibouchina mutabilis*; l: *Eugenia involucrata*.

3. Honey Analysis

Honey analysis is going to be taken in the Center for Nuclear Energy in Agriculture (CENA) from University of São Paulo. Sample treatment will be performed by microwave assisted sample decomposition using (Microwave ETHOS 1600). Elementary chemical determinations will be performed by Inductively Coupled Plasma Optical Emission Spectrometer (ICP OES). The amounts of B, Al, Na, Mg, P, S, K, Ca, Mn, Fe, Cu, Zn, Mo, As, Cd and Pb will be detected.

4. First Rufford Foundation Learning Event

From April 13th to 17th, 2023 I had the pleasure to take part in the First Rufford Learning Event, which took place in Olho d'Água do Casado, AL (Figures 8 and 9). These were incredible days of immersion, learning and exchanging knowledge. We learned about project finance, theory of change and science communication. We had the opportunity to learn a little about local stakeholders and the work they do. We interacted with many inspiring researchers and their projects. The main message I take away from this experience is about the indisputable importance that different sectors of society need to be united for conservation projects to be effective.

Figure 8: First Rufford Learning Event participants.

Figure 9: Taking part in the First Rufford Learning Event workshop.

5. Other activities

in addition to the activities directly related to the project, I also took part in other assignments favorable to my academic development. I assisted Professor Dr. Isabel Alves dos Santos in a practical class for undergraduate students (BIE213 – Ecology; basic discipline of the Biological Sciences course at USP) and in other activities involving the Bee Laboratory (IB-USP), such as, for example, receiving researchers' visitors. I participated in the evaluation board of six final projects of students at the Federal University of São Carlos. I participated as a debater at the 13th Congress of Initiation in Technological Development and Innovation, 28th Congress of Scientific Initiation at the Federal University of São Carlos and 1st Congress of Scientific Initiation in High School at the Federal University of São Carlos.

Aiming at carrying out the data management plan and making information about the project's development available to the public, in addition to providing a project database, the link https: The site will be updated as data and results are generated. Additionally, to make project news in real time more accessible, more audiovisual content can be accessed via Instagram @mari.ecologia.

6. Plan of activities for the next periods

In the current stage, we began the analysis of the data obtained during the field data, including the analysis of the videos to obtain the displacement times of the bees and the analysis of the collected honey and pollen. We have been working on a manuscript about the results of the pollen analysis. I am also going to present some of the results of the first year of the project on two scientific congresses in October: XIII Encontro sobre Abelhas (FMRP - USP) and the IUFRO 2023 Latin American Conference. Additionally, aiming to improve my technical and academic knowledge, I have been exchanging emails with Dr Lars Chittka from the Research Centre for Psychology at Queen Mary, University of London, to take an internship period with his research group next year.

Year	2023												2024												2025											
Semester	1°						2°						1°						2°						1°						2°					
Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Bibliographic survey	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Field data collection	x	x	x	x	x	x																														
Honey and pollen analysis							x	x	x	x	x	x																								
Mapping and spatial analysis																									x	x	x	x	x	x						
Data analysis							x	x	x	x	x	x													x	x	x	x	x	x	x	x	x	x	x	x
* Internship abroad													x	x	x	x	x	x	x	x	x	x	x	x												
Presentation at congresses		x							x																											
Organization and divulgation of promotional material																															x	x	x	x	x	x
Submission of annual reports								x												x												x				
Submission of the final report																																				x