Project Update: March 2024

1. Summary of activities carried out in the 2nd year

1.1 Field experiments

Data collection in the field was completed based on the new organisation of the sampling design of bee release points described in the previous report. The experimental stage of the project included the marking, release and monitoring of bees and the collection of honey provided for in the original project, in addition to the inclusion of the collection of pollen material.

1.2 Collection and analysis of pollen material

Pollen collection was carried out according to the availability of material inside the bee nest boxes, together with the collection of honey, at the end of spring and summer. The material was collected with the help of straws submerged in the nests' storage pots, in order to sample the resource distributed in all collections during the period and stored in Falcon tubes (15 ml) containing 70% alcohol.

The analysis of pollen material was carried out in collaboration with professor Dr. Cláudia Inês da Silva (founder and coordinator of the Online Pollen Catalog Network – RCPol). To enable better identification of pollen grains, acetolysis of the material was performed (Erdtman 1960; Silva et al. 2014). The material was organised into slides, and for each slide the pollen types present were identified with the aid of an optical microscope and using the Online Pollen Catalog Network (RCPol) database as a reference. 400 pollen grains were examined per slide and their percentages were calculated to obtain the frequency of each pollen type in each sample (Silva et al. 2014).

The composition, diversity, evenness and dominance of plant species identified in the pollens that made up the bees' diet in each month for each meliponary will be analysed. To evaluate the composition, species richness (S) will be analysed, calculating the number of types found in the samples. To evaluate the diversity of plants that made up the diet, the Shannon- Weaver diversity index (H') will be used (Shannon-Weaver 1949). To evaluate the distribution and contribution of each plant species in the diet, Pielou's evenness index (J') will be calculated (Pielou (1966). To verify the dominance of a species in the diet, Berger's dominance index will be used. Parker (D) (Magurran 2004).

1.2 Preliminary results

1.2.1 Video monitoring

At the end of the monitoring stage, a total of 45 releases were obtained in 45 videos lasting 3 to 4 hours each, containing records of the marked bees (according to the release distance) entering the nests and their respective return times. These videos are now being analysed manually, to obtain data on the number of marked bees that returned, and the time taken for each bee to return to the nest (given by the subtraction between the time of release and the time of arrival at the nest). To date, data from four releases have been compiled. In total, 50% (91 of 180) of the bees that were released returned to the nest, with 34 (37% of 91) returning from distances of up to 300 m, 29 (32%) from 300 to 600 m and 28 (31%) from 600 to 900m. The shortest recorded return times were less than a minute, for two bees released at distances of 80 and 180 m, and the longest were 180 minutes, for two bees released

at 740 and 840 m. A positive linear correlation was found between the bees' return time to the nest and the release distance (Figure 1).

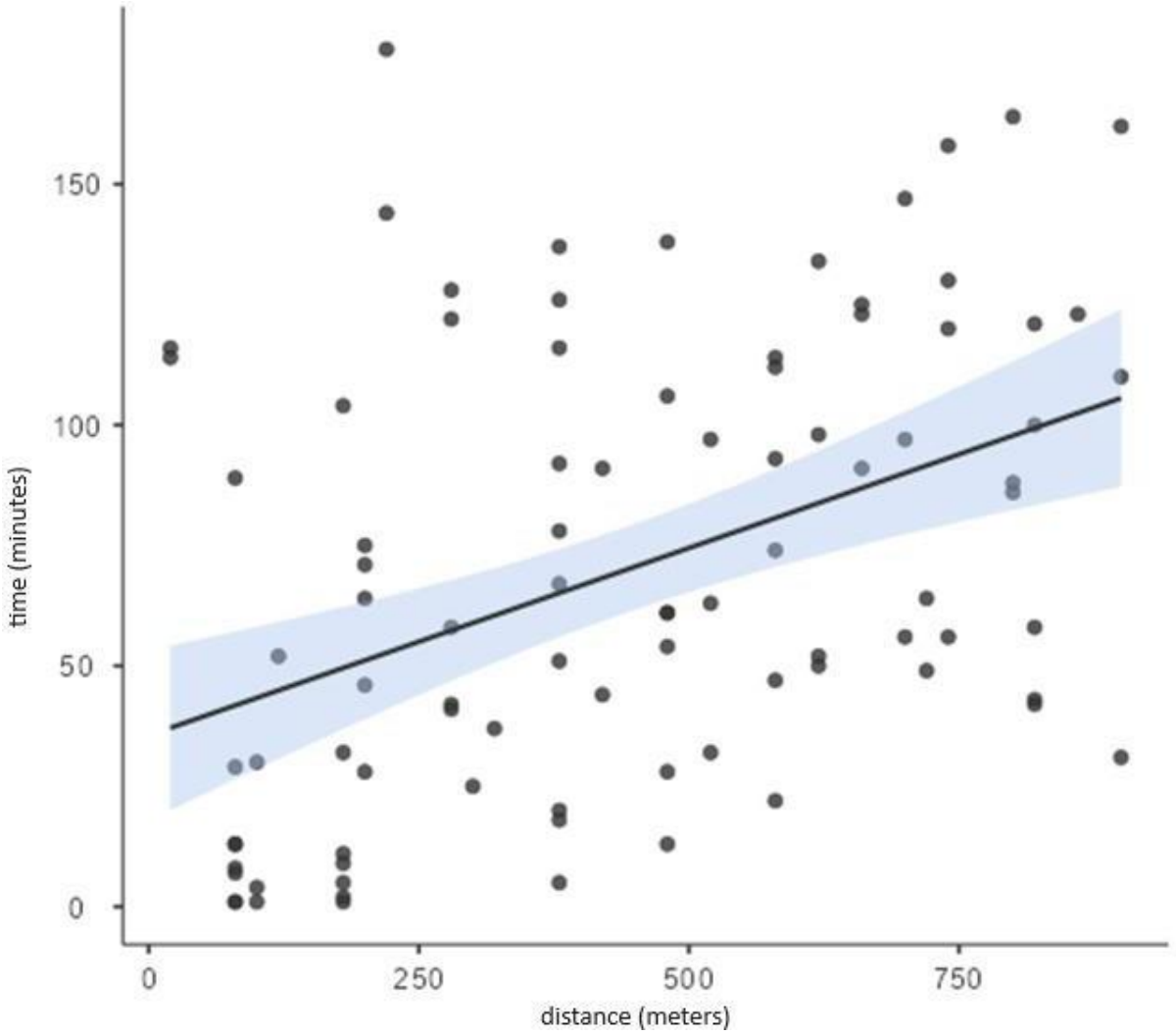

Figure 1: Scatter plot indicating the linear correlation between the bees' return time to the nest and the release distance.

1.2.2 Honey analysis

The analyses of the honey samples were carried out at the Henrique Bergamin Filho Analytical Chemistry Laboratory (CENA-ISP) by Dr Liz Mary Bueno de Moraes. The opening (decomposition) of samples was carried out with the aid of microwave radiation in a closed system using an ETHOS 1600 MICROWAVE and the trace elements of the samples were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP OES). In total, 45 honey samples were collected and analyzed (3 for each bee nest). The concentrations in mg/kg of the elements B, Na, Mg, Al, P, S, K, Ca, Mn, Fe, Cu, Zn, As, Mo, Cd and Pb were obtained (Table 1).

Table 1: Concentrations (mg/kg) and standard deviation of the elements for each honey sample. <LDO: Values below detection limits.

AMOSTRAS	B	Na	Mg	Al	P	S	K	Ca	Mn	Fe	Cu	Zn	As	Mo	Cd	Pb	
M01-1A	0,96 ± 0,01	5,9 ± 0,2	10,8 ± 0,1	2,83 ± 0,03	6,4 ± 0,2	6,0 ± 1,8	187 ± 1	31,3 ± 0,5	1,224 ± 0,006	0,739 ± 0,014	0,059 ± 0,005	< LDO	< LDO	< LDO	< LDO	<	
M01-R2	2,25 ± 0,02	7,2 ± 0,4	8,9 ± 0,1	0,50 ± 0,01	8,2 ± 0,4	7,1 ± 1,0	127 ± 1	16,9 ± 0,3	0,276 ± 0,003	0,468 ± 0,003	0,145 ± 0,007	0,156 ± 0,002	< LDO	< LDO	< LDO		
M01-R3	1,558 ± 0,003	18,1 ± 0,2	20,96 ± 0,02	1,72 ± 0,02	8,8 ± 0,2	9,6 ± 1,2	209 ± 1	38,9 ± 0,3	0,604 ± 0,004	1,270 ± 0,011	0,067 ± 0,009	0,162 ± 0,002	< LDO	< LDO	< LDO		
M02-1	1,32 ± 0,02	7,4 ± 0,1	14,59 ± 0,03	0,53 ± 0,01	6,9 ± 0,9	22,7 ± 0,3	293 ± 2	48,1 ± 0,6	0,166 ± 0,001	0,509 ± 0,006	0,075 ± 0,002	< LDO	< LDO	< LDO			
M02-R2	2,04 ± 0,02	17,9 ± 0,4	15,50 ± 0,03	0,328 ± 0,004	7,9 ± 0,6	15,5 ± 1,9	256 ± 1	42,4 ± 0,4	0,275 ± 0,003	0,407 ± 0,006	0,061 ± 0,005	0,158 ± 0,001	< LDO	< LDO			
M02-R3	1,36 ± 0,01	22,7 ± 0,4	23,5 ± 0,1	0,46 ± 0,01	11,1 ± 0,4	26,9 ± 1,6	360 ± 2	71,5 ± 0,5	0,388 ± 0,001	0,555 ± 0,002	0,065 ± 0,005	0,205 ± 0,003	< LDO	< LDO			
M03-1	0,48 ± 0,01	21,7 ± 0,4	25,9 ± 0,3	1,83 ± 0,01	6,9 ± 0,6	33,1 ± 1,7	390 ± 3	76,1 ± 0,7	0,954 ± 0,000	1,081 ± 0,002	0,081 ± 0,003	0,287 ± 0,001	< LDO	0			
M03-R2	0,31 ± 0,01	10,38 ± 0,02	29,2 ± 0,2	1,18 ± 0,02	10,9 ± 0,5	38,8 ± 1,0	434 ± 1	115,2 ± 1,3	0,535 ± 0,006	1,250 ± 0,011	0,102 ± 0,002	0,217 ± 0,000	< LDO				
M03-R3	0,79 ± 0,01	39,8 ± 0,4	39,5 ± 0,4	0,84 ± 0,02	9,3 ± 0,2	28,6 ± 0,5	437 ± 4	104,2 ± 0,2	1,035 ± 0,004	0,885 ± 0,004	0,102 ± 0,004	0,274 ± 0,002	< L				
M04-1	1,56 ± 0,02	19,6 ± 0,1	23,97 ± 0,04	0,93 ± 0,01	21,7 ± 0,8	26,1 ± 1,3	454 ± 3	59,7 ± 0,9	0,308 ± 0,003	0,868 ± 0,012	0,160 ± 0,004	0,462 ± 0,000					
M04-R2	1,58 ± 0,01	6,0 ± 0,4	17,11 ± 0,01	0,72 ± 0,01	9,2 ± 0,2	24,7 ± 1,0	337 ± 1	56,5 ± 0,4	0,412 ± 0,002	0,575 ± 0,005	0,078 ± 0,003	0,163 ± 0,00					
M04-R3	1,17 ± 0,01	9,4 ± 0,3	25,9 ± 0,1	2,11 ± 0,02	11,0 ± 0,5	40,3 ± 0,1	412 ± 2	90,8 ± 0,8	0,373 ± 0,002	0,872 ± 0,008	0,082 ± 0,003	0,219					
M05-1	1,62 ± 0,01	22,1 ± 0,1	17,9 ± 0,2	0,58 ± 0,01	7,0 ± 0,2	10,4 ± 0,4	240 ± 3	35,5 ± 0,5	0,461 ± 0,001	0,452 ± 0,006	0,127 ± 0,008						

M05-R2	3,03 ± 0,02	19,4 ± 0,2	19,7 ± 0,2	0,60 ± 0,03	10,3 ± 1,2	76,9 ± 2,7	556 ± 3	51,7 ± 0,7	0,422 ± 0,002	0,457 ± 0,006	0,141 ± 0,						
M05-R3	2,08 ± 0,02	50,6 ± 0,2	72,2 ± 0,4	0,63 ± 0,04	10,2 ± 1,2	44,2 ± 1,1	722 ± 2	99,1 ± 0,2	1,405 ± 0,012	0,472 ± 0,006	0,1						
M06-1	0,22 ± 0,01	13,0 ± 0,4	29,4 ± 0,2	2,16 ± 0,04	15,5 ± 0,8	49,0 ± 1,1	452 ± 3	104,4 ± 0,7	0,463 ± 0,005	1,731 ± 0,014							
M06-R2	3,02 ± 0,02	21,8 ± 0,1	36,63 ± 0,04	0,72 ± 0,02	29,2 ± 0,3	42,1 ± 2,6	1885,2 ± 0,2	81,6 ± 0,7	0,263 ± 0,002	0,531 ± 0							
M06-R3	2,57 ± 0,01	53,6 ± 0,5	62,7 ± 0,2	0,53 ± 0,02	13,5 ± 0,5	28,8 ± 0,8	1054 ± 4	87,1 ± 0,6	1,189 ± 0,008	1							
M07-1	0,227 ± 0,005	9,1 ± 0,2	11,2 ± 0,1	0,80 ± 0,03	7,2 ± 1,1	10,5 ± 0,4	130 ± 2	40,9 ± 0,8	0,235 ± 0,001								
M07-R2	0,28 ± 0,01	7,3 ± 0,2	12,9 ± 0,1	4,36 ± 0,03	10,0 ± 0,4	11,0 ± 1,7	136 ± 2	42,3 ± 0,2	0,580 ±								
M07-R3	1,56 ± 0,01	149 1	229,2 ± 0,7	1,46 ± 0,02	12,2 ± 0,3	15,0 ± 2,6	1533 ± 10	270,9 ± 1,6									
M08-1	2,16 ± 0,01	14,8 ± 0,1	13,34 ± 0,02	0,31 ± 0,01	4,2 ± 0,5	11,8 ± 1,3	221 ± 1	33,5 ± 0,3									
M08-R2	1,02 ± 0,01	14,2 ± 0,2	30,6 ± 0,1	0,85 ± 0,01	9,4 ± 0,6	47,9 ± 0,5	773 ± 6	104									
M08-R3	0,98 ± 0,01	19,5 ± 0,1	36,5 ± 0,3	0,70 ± 0,01	6,1 ± 0,9	35,1 ± 0,4	777 ± 5										
M09-1	1,19 ± 0,01	11,9 ± 0,1	34,2 ± 0,1	1,06 ± 0,02	12,3 ± 0,7	37,8 ± 0,3	408 ± 1										
M09-R2	2,43 ± 0,01	13,6 ± 0,3	32,0 ± 0,2	0,67 ± 0,02	12,8 ± 0,5	40,8 ± 1,8											
M09-R3	1,05 ± 0,01	11,9 ± 0,2	20,0 ± 0,1	0,76 ± 0,02	8,7 ± 0,6	21,8 ± 1,5											
M10-1	0,20 ± 0,01	9,5 ± 0,1	31,1 ± 0,2	1,14 ± 0,02	13,8 ± 0,3	39,2 ±											
M10-R2	0,263 ±	10,7 ±	32,6 ±	1,69 ±	20,2												

	0,003	0,4	0,1	0,01	± 0,3			
M10-R3	0,27 ± 0,01	10,9 ± 0,5	34,8 ± 0,1	2,71 ± 0,01	20,7 ± 0,5			
M11-1	2,24 ± 0,01	67,2 ± 0,4	54,4 ± 0,1	1,08 ± 0,04	6,6			
M11-R2	2,61 ± 0,02	53,3 ± 0,4	51,9 ± 0,4	1,022 ± 0,005				
M11-R3	3,08 ± 0,01	62,0 ± 0,1	74,4 ± 0,7	1,09 ± 0,02				
M12-1	0,79 ± 0,01	7,2 ± 0,1	14,4 ± 0,1	0,6				
M12-R2	0,27 ± 0,01	6,2 ± 0,3	20,3 ± 0,1					
M12-R3	0,219 ± 0,005	30,7 ± 0,8	18,3 ± 0					
M13-1	1,63 ± 0,01	13,1 ± 0,4	2					
M13-R2	0,71 ± 0,01	24,6 ± 0,6						
M13-R3	0,354 ± 0,004	27,7 ±						
M14-1	1,50 ± 0,02							
M14-R2	0,95 ± 0,01							
M14-R3	1,150							
M15-1								
M15-R2								

1.2.3 Pollen analysis

From the 15 meliponaries sampled, it was possible to collect 12, with a total of 20 samples. 56 pollen types were identified, of which only 20 represent almost 90% of the pollen grains recorded in the samples, with 75% belonging to the Myrtaceae family (Table 2). The most frequent pollen types were *Eucalyptus citriodora* (29.4%), *Eugenia brasiliensis* Lam. (12.4%), *Eugenia pyriformis* Cambess. (8.6%), *Eugenia uniflora* L. (7.3%), *Psidium guineense* Sw. (7.6%), *Tibouchina mutabilis* (Vell.) Cogn. (7.5%) (Figure 2).

Table 2: Percentages of pollen types identified in the samples collected.

Family	Pollen type	Springs		Summer		Total	
Myrtaceae	*Callistemon* sp.	0.9	86.7	0.0	63.5	0.5	75.1
	Eucalyptus citriodora	17.5		41.3		29.4	
	Eucalyptus moluccana Wall. ex Roxb.	8.7		1.3		5.0	
	Eugenia brasiliensis Lam.	18.8		6.0		12.4	
	Eugenia involucrata DC.	5.6		1.2		3.4	
	Eugenia pyriformis Cambess.	8.1		9.2		8.6	
	Eugenia uniflora L.	13.1		1.5		7.3	
	Myrtaceae sp1	0.0		0.3		0.1	
	Myrtaceae sp2	0.0		0.0		0.0	
	Psiduium guajava	1.5		0.0		0.8	
	Psidium guineense Sw.	12.4		2.7		7.6	
	Syzygium cumini (L.) Skeels	0.0		0.0		0.0	
Fabaceae	*Acacia* sp.	0.0	4.1	0.0	22.0	0.0	13.1
	Anadenanthera sp.	0.6		4.8		2.7	
	Crotalaria sp1	0.0		0.0		0.0	
	Cajanus cajan	0.0		0.0		0.0	
	Leucaena leucocephala (Lam.) de Wit	3.2		6.1		4.6	
	Mimosa caesalpiniifolia Benth.	0.2		11.1		5.6	
	Senna sp1	0.1		0.0		0.1	
	Senna sp2	0.1		0.0		0.0	
Melastomataceae	*Tibouchina granulosa* (Desr:) Cogn.	0.0	5.8	0.1	9.2	0.1	7.5
	Tibouchina mutabilis (Vell.) Cogn.	5.8		9.1		7.5	
Outros		3.4	3.4	5.3	5.3	4.3	4.3
Total		100	100	100	100	100	100

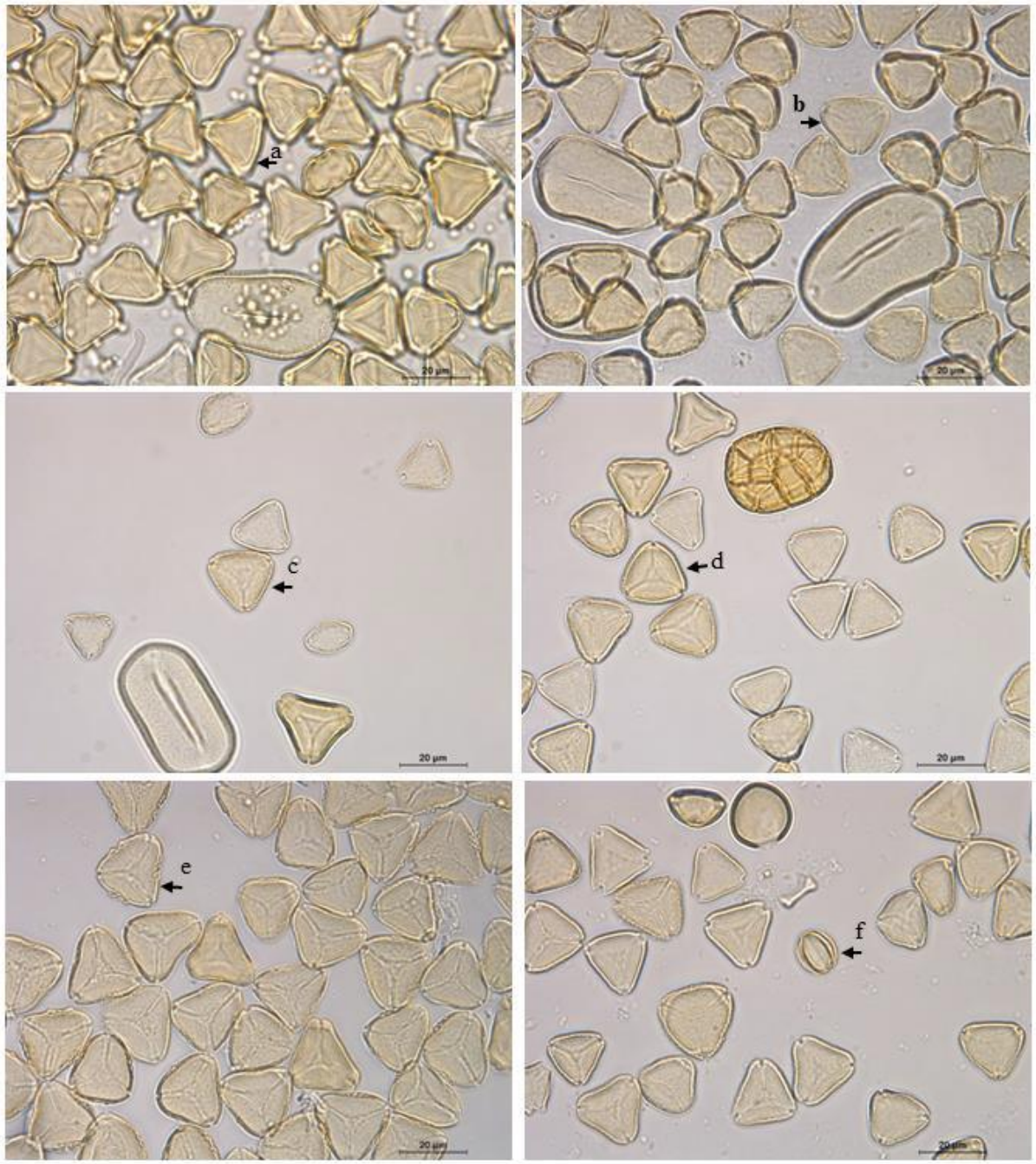

Figure 2: Most frequent pollen types found in samples: a. *Eucalyptus citriodora*; b. *Eugenia brasiliensis*; c. *Eugenia pyriformis*; d. *Eugenia uniflora*; e. *Psidium guineense*; f. *Tibouchina mutabilis*. Images: Cláudia Inês da Silva, 2023.

2. Schedule for the next period

In the next semester (Table 3), from March to September 2024, the research internship period abroad will be carried out. In 2025, mapping of land uses and coverage will be carried out and data analyses will be completed. The thesis will be written and presented, and the final report will be delivered. Participation in conferences and the publication of at least two articles are also planned.

Table 3: Schedule of activities planned for the next months of the project.

Year	2024												2025												2026			
Semester	1°						2°						1°						2°						1°			
Month	J	F	M	A	J	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
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
Field data collection	concluded																											
Qualification exam	concluded																											
Mapping landscape elements										x	x	x	x	x	x	x	x	x										
Honey analysis	concluded																											
Pollen analysis	concluded																											
Data analysis	x	x								x	x	x	x	x	x	x	x	x										
* Internship abroad (project paused)			x	x	x	x	x	x	x																			
Presentation at conferences		x																				x						
Writing and submitting articles														x	x	x												
Delivery of annual reports			x													x												
Thesis Writing													x	x	x	x	x	x	x	x	x	x						
Thesis defense																							x					
Delivery of the final report																												x

3. Participation in events

I presented the preliminary results of the project at three national scientific events. I participated in the XIII Encontro Sobre Abelhas, from October 12th to 14th, 2023 in Ribeirão Preto, SP, with the poster "Pollen preference of mandaçaia bees in the city of São Paulo" (Figure 3a). From October 17th to 19th, 2023, I participated in the IUFRO 2023 Conference, in Curitiba, PR, presenting the poster "Proportion of exotic and native plants in the composition of floristic species in the mandaçaia diet in an urban environment" (Figure 3b). From February 26th to 29th, 2024, I participated in the XXXV Congresso Brasileiro de Zoologia, in Porto de Galinhas, PE, presenting the poster "Preliminary analysis of the capacity and return time of *Melipona quadrifasciata* (mandaçaia) in an urban matrix" (Figure 3c).

Figure 3: Poster presentations at conferences. a) XIII Encontro Sobre Abelhas; b) IUFRO 2023 Conference; c) XXXV Congresso Brasileiro de Zoologia.

4. Internship abroad

From April to September 2024, the abroad internship will be carried out at the Bee Sensory and Behavioral Ecology Laboratory at Queen Mary University of London, under the supervision of Prof. Dr. Lars Chittka. The objective of the internship is to learn more about aspects of bee navigation, to better understand how they forage in a given landscape. The objective is to understand the methodologies used in sensory and behavioral ecology studies in European social bees to be applied in the future to Brazilian species. Semifield research will be carried out with bee colonies (*Bombus terrestris*). Experimental setups will be developed using feeders, artificial flowers and reference points. Experiments with harmonic radar will be carried out in open

experimental fields. Prof. Chittka (host supervisor) has extensive experience in the behaviour, cognition and ecology of bees and bumblebees. His research group has several projects being developed on this subject and has a laboratory with facilities that allow such studies. This exchange represents an incredible opportunity for me to work with a renowned research group and learn in practice the methods that could be applied in the future with native Brazilian bees.

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