Workshop Exposure Assessment Paradigm for non-*Apis* Bees

Stingless Bees

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Brazil

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US EPA Office of Pesticide Programs' building - Arlington, VA (USA)

Meliponines:
Potencial surrogates species

...uncertainty regarding the extent to which honey bees can serve as surrogates for non-*Apis* bee species in the risk assessment for pesticides…

Which bees besides *Apis mellifera* would be directly at exposure?

The first step trying to answer this question was to search in the literature, in order to find what non-*Apis* bees would be associated with the agricultural environment and therefore, possibly at direct exposure

Results of the literature survey

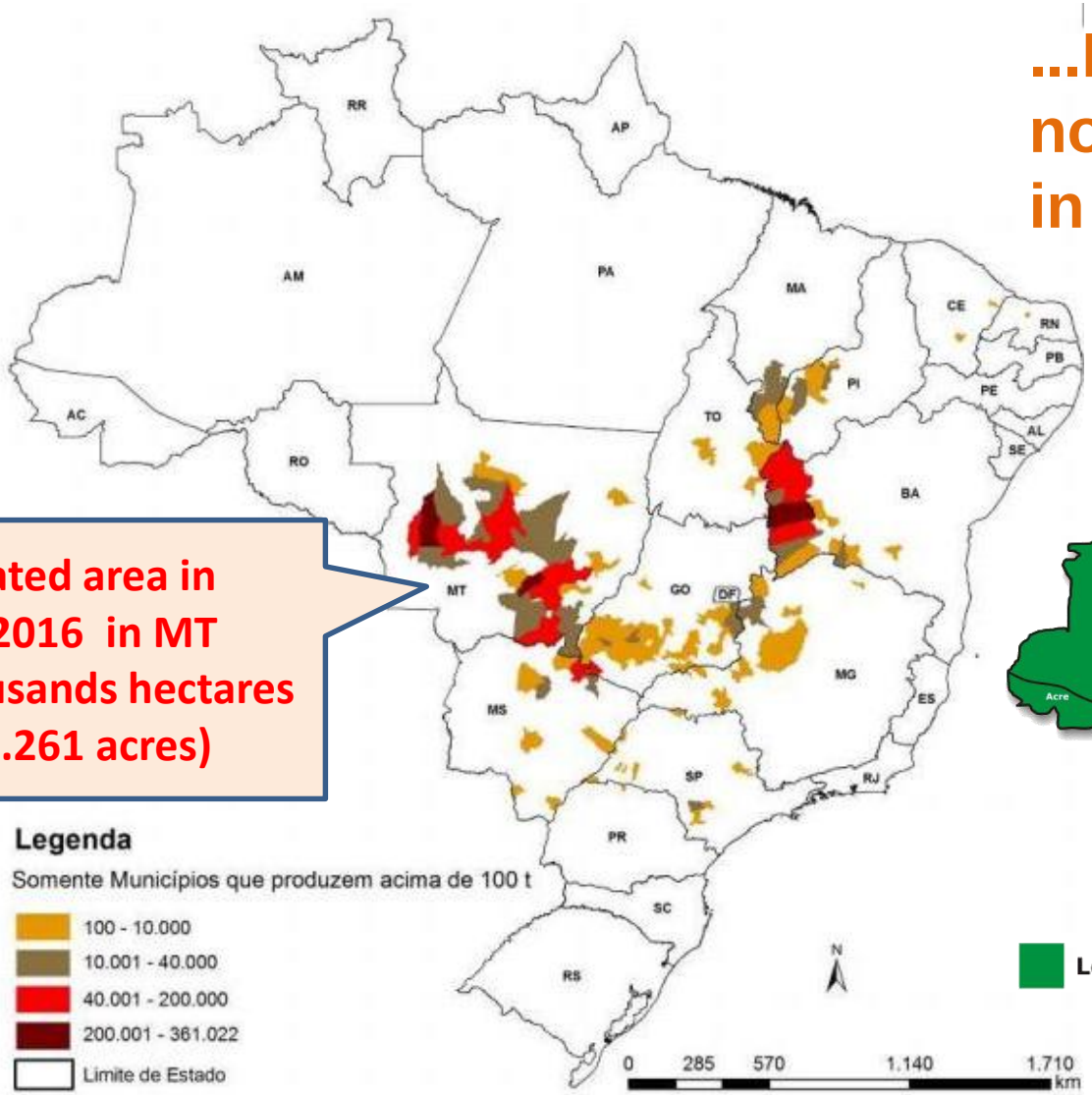
Data found for 40 crops

Top Crops (ranked by economic importance)	Number of species found (total)	Number of stingless bees found	No data for
Soya	11	3	Maize, rice, wheat
Sugar Cane	5	-	
Coffee	7	6	**No bees or lack of research ?**
Cassava	4	3	
Cotton	**127**	22	
Tomato	64	8	
Beans	37	6	
Citrus	18	5	
Apple	65	2	

- **Map of cotton production - 2015**

...Honey bees do not collect pollen in cotton...

Cultivated area in 2015/2016 in MT
586,9 thousands hectares (1.450.261 acres)



Fonte: Conab/IBGE.

Species selected according the matrix criteria:

Social:

- *Trigona spinipes* = 28 points
- *Tetragonisca angustula* = 24 points
- *Nannotrigona testaceicornis* = 22 points
- *Bombus morio* = 19 points
- *Melipona quadrifasciata* = 19 points
- *Melipona scutellaris* = 19 points

Solitary:

- *Xylocopa frontalis* = 20 points
- *Xylocopa grisescens* = 19 points
- *Eulaema nigrita* = 18
- *Centris aenea* = 17
- *Epicharis flava* = 16

Main criteria:

- 1. Geografic distribution
- 2. Association with agricultural environments
- 3. Abundance in crops

Trigona spinipes (Arapuá, Dog Bee)

Trigona spinipes

Pros	Cons
• Wide geographic distribution  • Representative (found in 32 of 40 crops, *Apis mellifera* found in 36 of 40 crops) • Large number of bees	• Not available comercially, very agressive • No methods to manage colonies in laboratory conditions • Protocols for acute toxicity tests available but not standardized • No protocols for semi-field or field tests

Tetragonisca angustula (Jataí)

Tetragonisca angustula

Pros	Cons
• Wide geographical distribution  • Relatively representative (found in 19 of 40 crops) • Very small bee (more sensitive???) • Commercially available and easy to manage	• No protocols for toxicity or semi-field and field tests

Nannotrigona testaceicornis (Iraí)

Nannotrigona testaceicornis

Pros	Cons
• small bee (more sensitive???)	• Geographical distribution in northeast, southeast and south, but not in legal Amazon  • Hives not available commercially • No methods to manage colonies in laboratory conditions • No protocols for toxicity, semi-field or field tests

Melipona quadrifasciata (Mandaçaia)


Melipona quadrifasciata.
Foto: Int. Rã-Bugio para Conservação da Biodiversidade


Melipona quadrifasciata em flor de tomate
Foto: Patrícia Nunes Silva

Melipona quadrifasciata
Foto: Khalil Pereira e Joaquim Pífano

Melipona quadrifasciata

Pros	Cons
• Toxicity can be tested using standardized protocols • Hives available comercially	• Geographical distribution in northeast, southeast and south, but not in legal Amazon  • Appears to be less sensitive than *Apis mellifera* (?)

Melipona scutellaris (Uruçu)

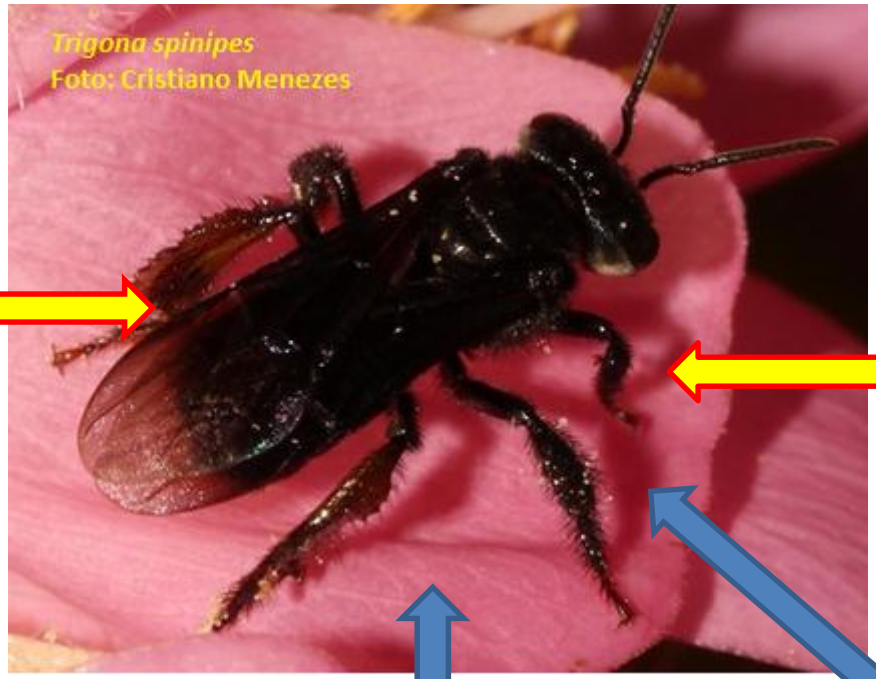
Melipona scutellaris

Pros	Cons
• Biology well known	• Geographical distribution restricted to Northeast
• Toxicity can be tested using standardized protocols (lab/field)	
• Hives commercially available	
• Easy to manage	• Included in the national list of threatened species
• Appears to be more sensitive than *Apis mellifera* (?)	• Method for larvae available but not standardized

Meliponines: Routes of exposure

Routes of exposure: workers

Contact exposure
Direct exposure during spraying application **(size?)**



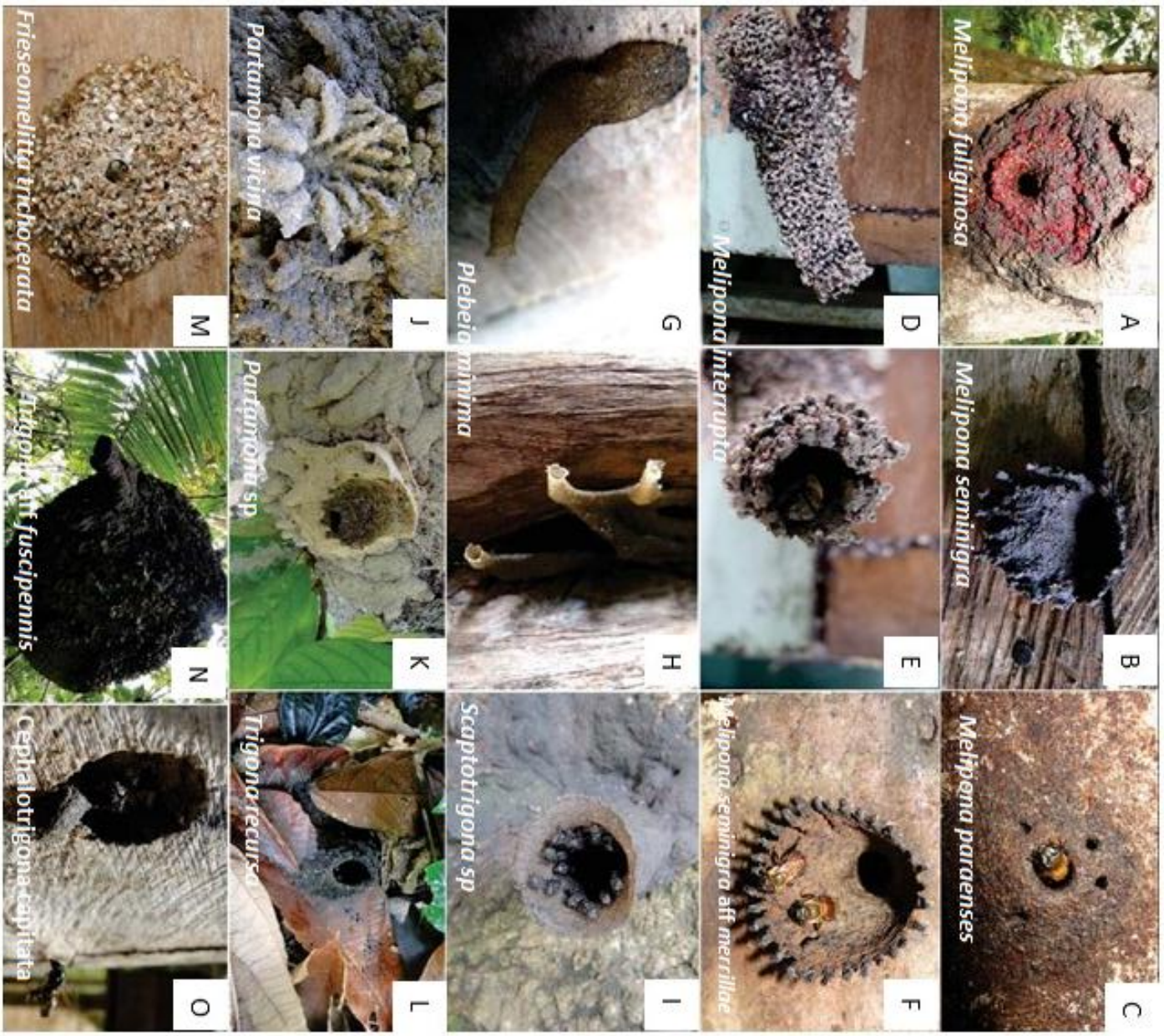
Oral exposure
Consumption of contamined nectar, pollen and/or water

Inhalation
of droplets or gaseous phase of pesticides (TOTALLY GAP...)

Contact exposure
Petals, leaves, soil, **mud, resins, sticks, seeds**, wax, pollen, water

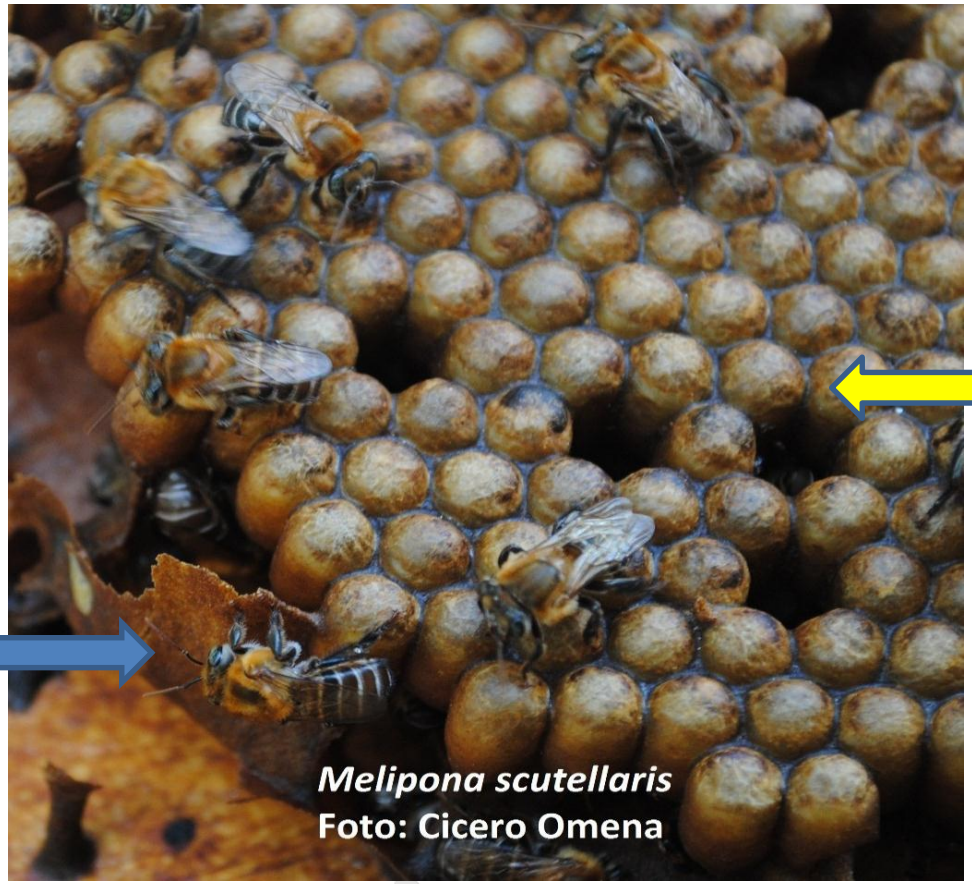
Nest materials

OLIVEIRA. et al. (2013) Guia Ilustrado das Abelhas "Sem-Ferrão"das Reservas Amanã e Mamirauá, Amazonas, Brasil (Hymenoptera, Apidae, Meliponini). Instituto de Desenvolvimento Sustentável Mamirauá



Routes of exposure: in-hive bees

Contact exposure
Contaminated nectar, pollen, mud, resins



Oral exposure:
Consumption of nectar, pollen, water

Larvae are feed with **pollen not processed** (mass provisioning system)

Possible sources of contact exposure inside de nest

Fig. 11. Depósito de resina em ninho de *Melipona* sp.

Life traits

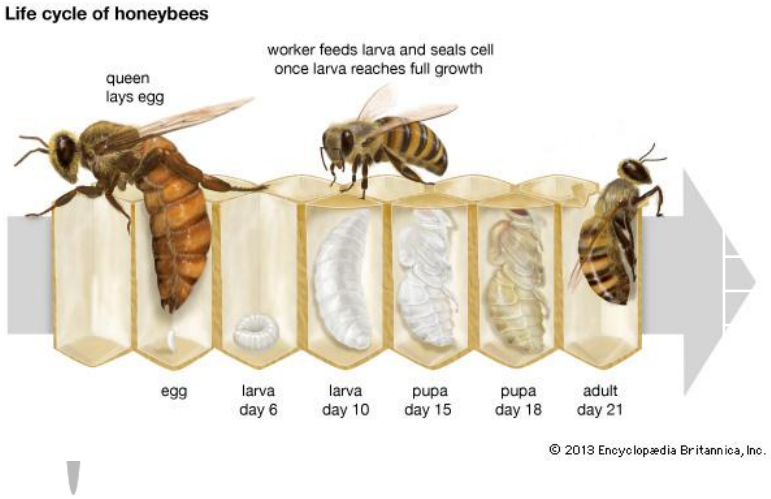
Traits	Apis mellifera (africanized)	Trigona spinipes	Tetragonisca angustula	Nannotrigona testaceicornis	Melipona quadrifasciata	Melipona scutellaris
Foraging range (maximum)	10 km	950 m	600 m		2.7 km (*M. mandacaia*) 4.0 km (*M. subnitida*)	
Nesting period	permanent	permanent	permanent	permanent	permanent	permanent
Body size (mg)	90.0		3.96	13.99	70.55	82.27
Body measur.						
- Total lenght	11.52	5.92	3.68	4.20	9.92	10.72
- Head width	3.68	2.56	1.60	1.92	3.84	4.16
- Torax width	4.16	2.4	1.44	1.92	4.48	4.64

Tetragonisca angustula (Jataí):
Found in 19 of 40 crops...22 times smaller than *Apis mellifera*...(more sensitive???)

Life cycles

Honey Bee	Meliponines
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10
11	11
12	12
13	13
14	14
15	15
16	16
17	17
18	18
19	19
20	20
21	21
22	22
23	23
24	24
25	25
26	26
27	27
28	28
29	29
30	30
31	31
32	32
33	33
34	34
35	35
36	36
37	37
38	38
39	39
40	40

egg | **larvae** | **pupe** | **adult**



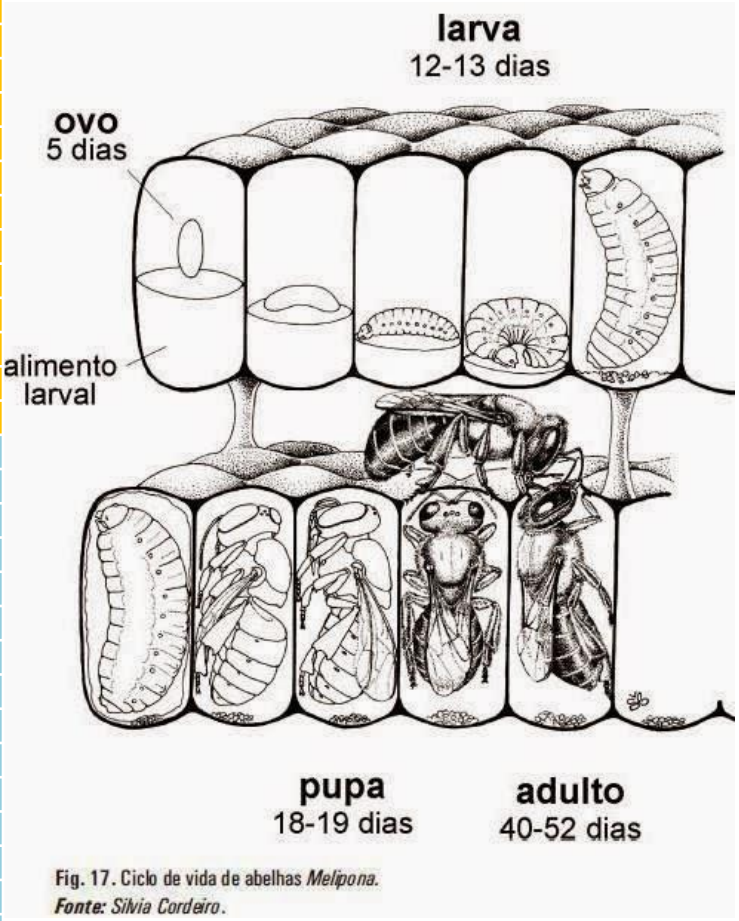


Fig. 17. Ciclo de vida de abelhas *Melipona*.
Fonte: *Silvia Cordeiro*.

Meliponines: Estimates of exposure

Resins and Mud collecting

Tabela 1. Cargas coletadas por operárias de duas colônias de *Melipona seminigra* em Sistema Agroflorestal (BR-174 Km 23, Manaus – AM) no período de junho a Agosto de 2005.

Coleta	Colônia 1		Colônia 2	
	6-7h	13-14h	6-7h	13-14h
Pólen	97,42%	2,58%	97,95%	2,05%
Néctar/Água	68,98%	31,02%	69,29%	30,71%
Resina	54,15%	45,85%	55,22%	44,78%
Barro	22,66%	77,34%	23,19%	76,81%

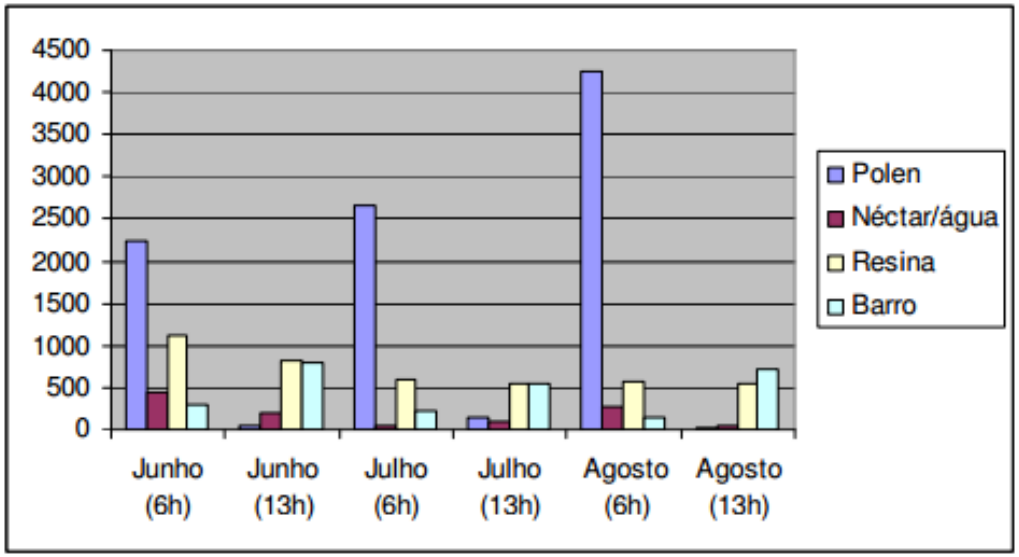
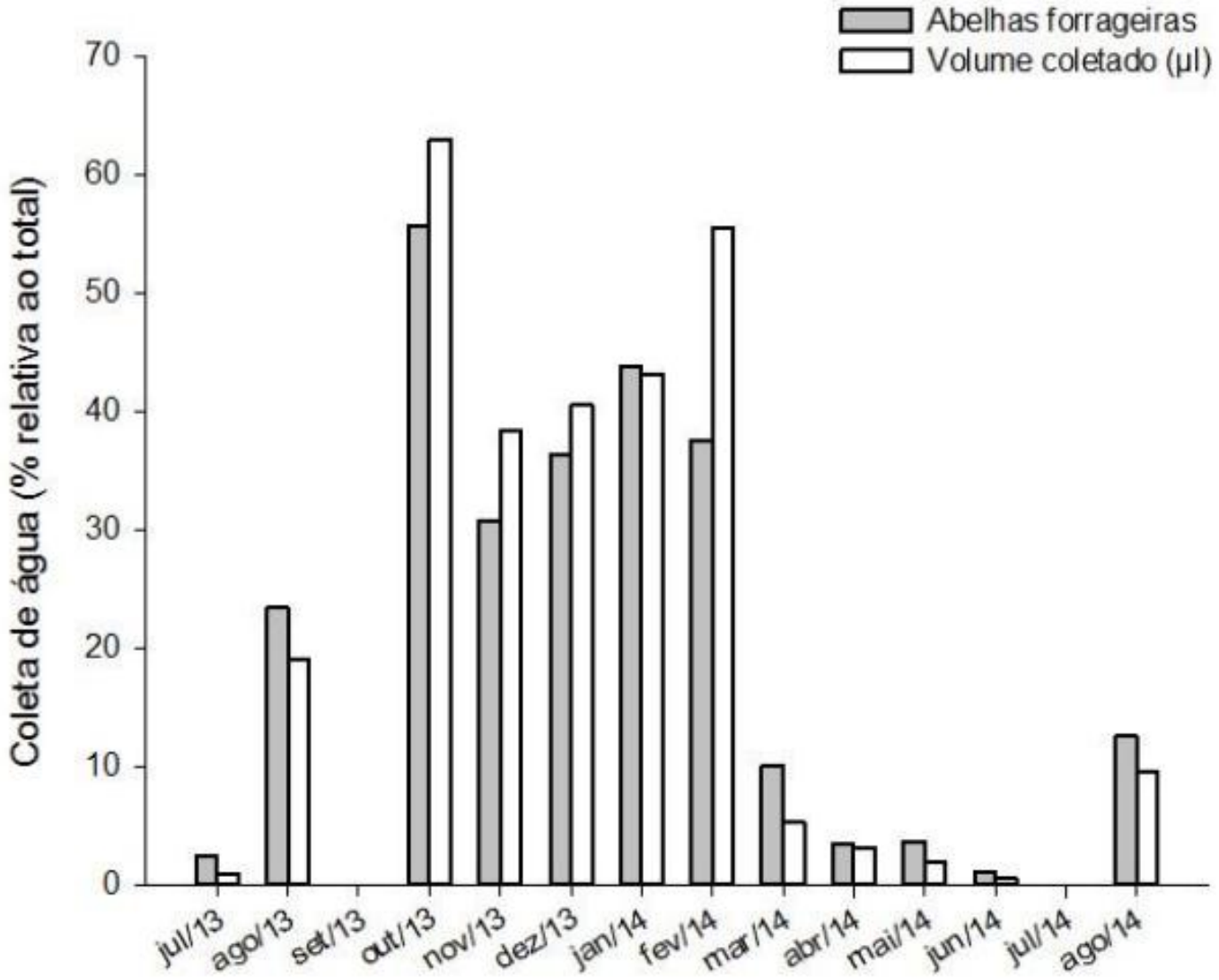


Figura 1. Cargas coletadas por operárias de duas colônias de *Melipona seminigra* e temperaturas médias (período do dia/mês) em Sistema Agroflorestal (BR-174 Km 23, Manaus – AM) no período de junho a agosto de 2005.

Water collecting



Source: Limão, A. A. D. C. (2015). A influência dos fatores bióticos e abióticos no néctar coletado por *Melipona subnitida* (APIDAE, MELIPONINI) na caatinga.

Food consumption

Adults	Nectar consumption bee/day	Pollen consumption bee/day
Apis mellifera	52.5 – 75 mg	6.5 – 12 mg
Scaptotrigona depilis		8 mg
Melipona flavolineata	2.32 mg	6.13 mg

Larvae	Nectar consumption bee/day (?)	Pollen consumption bee/day (?)
Apis mellifera	Day 4: 56 mg Day 5: 117 mg	2,7 mg
Scaptotrigona bipunctata	food consumed: 17,72 mg/bee/day 12% sugar: 2.12 mg 30% polen: 5.13 mg 56% polen: 9.92 mg	

http://dx.doi.org/10.1590/1519-6984.22314

Quantification of larval food and its pollen content in the diet of stingless bees – subsidies for toxicity bioassays studies

Rosa, AS.[a], Fernandes, MZ.[b], Ferreira, DL.[b], Blochtein, B.[b], Pires, CSS.[c] and Imperatriz-Fonseca, VL.[a]*

Table 1. Amount of larval food per cell and its pollen content, in different species of stingless bees.

Bees species	Amount of larval food per cell (mg) (Mean ± SE)	Weight of pollen per cell (mg)(Mean ± SE)
Plebeia droryana	9.4 ± 0.0001	1.3 ± 0.0000
Melipona obscurior	49.8 ± 0.0010	6.0 ± 0.0003
Scaptotrigona bipunctata	37.3 ± 0.0006	1.9 ± 0.0001
Tetragonisca fiebrigi	10.1 ± 0.0002	0.4 ± 0.0000

Route of exposure	Material	Gaps
Contact	Resins Mud Seeds Sticks	Which plants offer? All species collect? How much they collect? No residue data on this materials
Contact	Water Collecting of nectar Collecting of pollen	Quantification not available
Oral - adults	Consumption of nectar	
	Consumption of pollen	Quantification not available
	Consumption of water	
Oral - larvae	Consumption of nectar	Quantification not available
	Consumption of pollen	Quantification available for very few species (no data for the species selected)
	Consumption of water	Quantification not available

Meliponines:
Gaps on effects…

Current Biology

A Brazilian Social Bee Must Cultivate Fungus to Survive

Highlights

- A social bee from Brazil must eat a fungus to survive
- The fungus grows inside brood cells over the larval food
- Fungus is transmitted to other generations via contaminated building materials
- This bee-fungus symbiosis shows parallels to fungus-farming insects

Authors

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In Brief

Menezes et al. report an obligatory relationship between a fungus and a social bee, in which the larvae eat the fungal hyphae that grows inside brood cells. The fungus occurs in the building material of the nest and uses larval food as growth medium. It is transmitted via swarming, suggesting this is the first case of a fungus-growing bee.

http://www.cell.com/current-biology/pdf/S0960-9822(15)01108-2.pdf

SCIENTIFIC REPORTS

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Queens become workers: pesticides alter caste differentiation in bees

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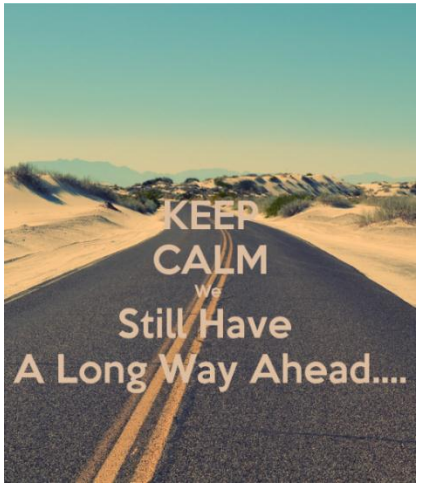
Bees are important for the world biodiversity and economy because they provide key pollination services in forests and crops. However, pesticide use in crops has adversely affected (decreased) queen production because of increased mortality among larvae. Here, we demonstrated that *in vitro*-reared queens of a neotropical social bee species (*Plebeia droryana*) also showed high larval mortality after exposure to an organophosphate pesticide (chlorpyrifos) via larval food. Moreover, most of the surviving larvae that were destined to develop into queens became workers more likely because they ate less food than expected without pesticide skewing thus caste differentiation in this bee species. This adverse effect has not been previously reported for any other social insects, such as honeybees or bumblebees. Queens are essential for breeding and colony growth. Therefore, if our data are applicable to other pantropical social bee species across the globe, it is likely that these bees are at a serious risk of failure to form new colonies.

Next steps

- To publish the bibliographical survey about bees associated with agricultural environments
- To publish the list of gaps and send it to Research Support Foundations
- Propose a interlaboratory program in order to compare the sensitivity of non-*Apis* x *Apis mellifera*
- Assess the need of changes in the risk assessment procedure for bees



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THANK YOU!!!

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