Workshop on Pesticide Exposure Assessment Paradigm for non-*Apis* Bees

January, 10th – 12th, 2017
Arligton, VA, USA

Stingless Bees

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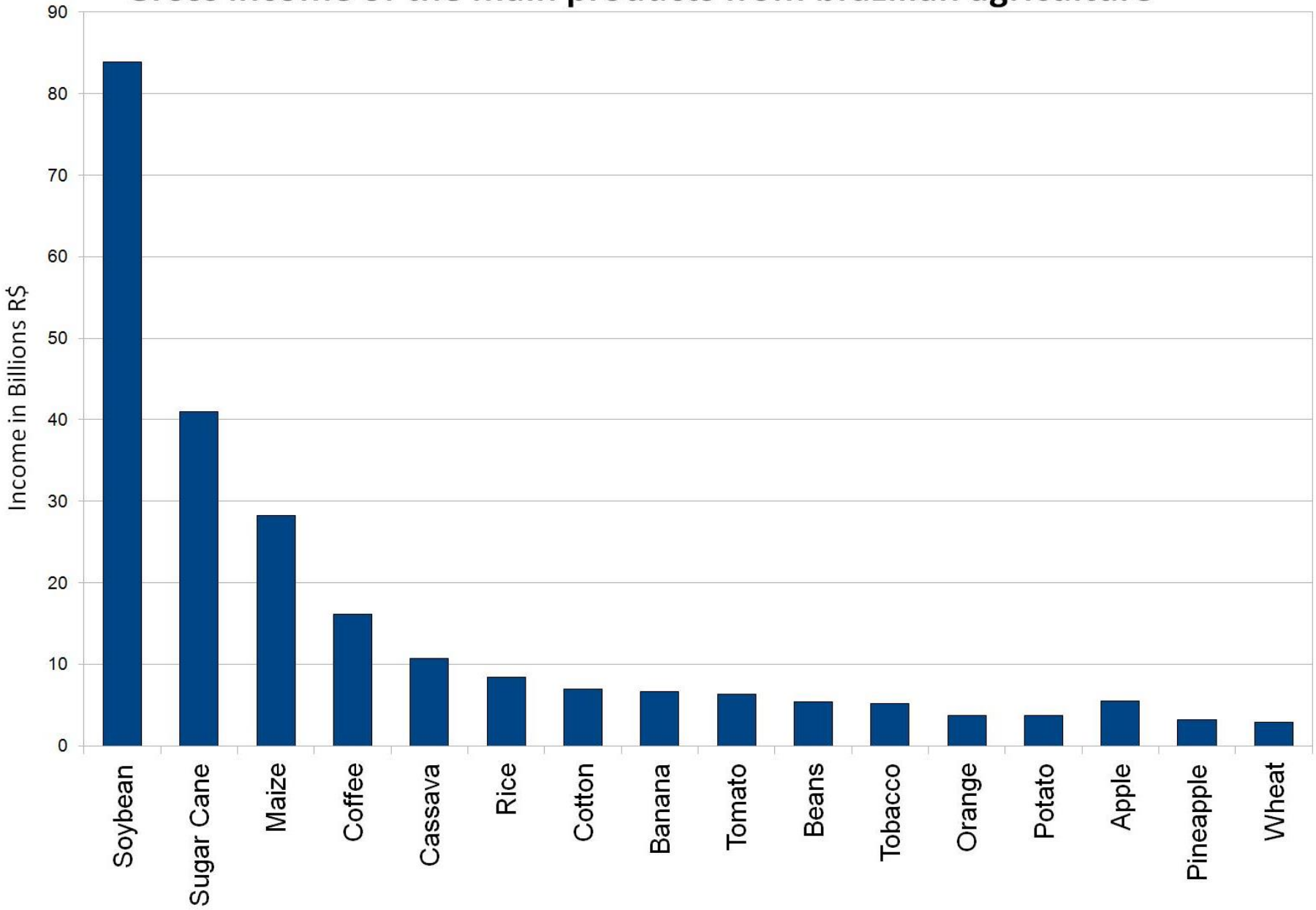
EXPOSURE

Apis mellifera X Non-*Apis* Bees

(focused on **Stingless Bees**)

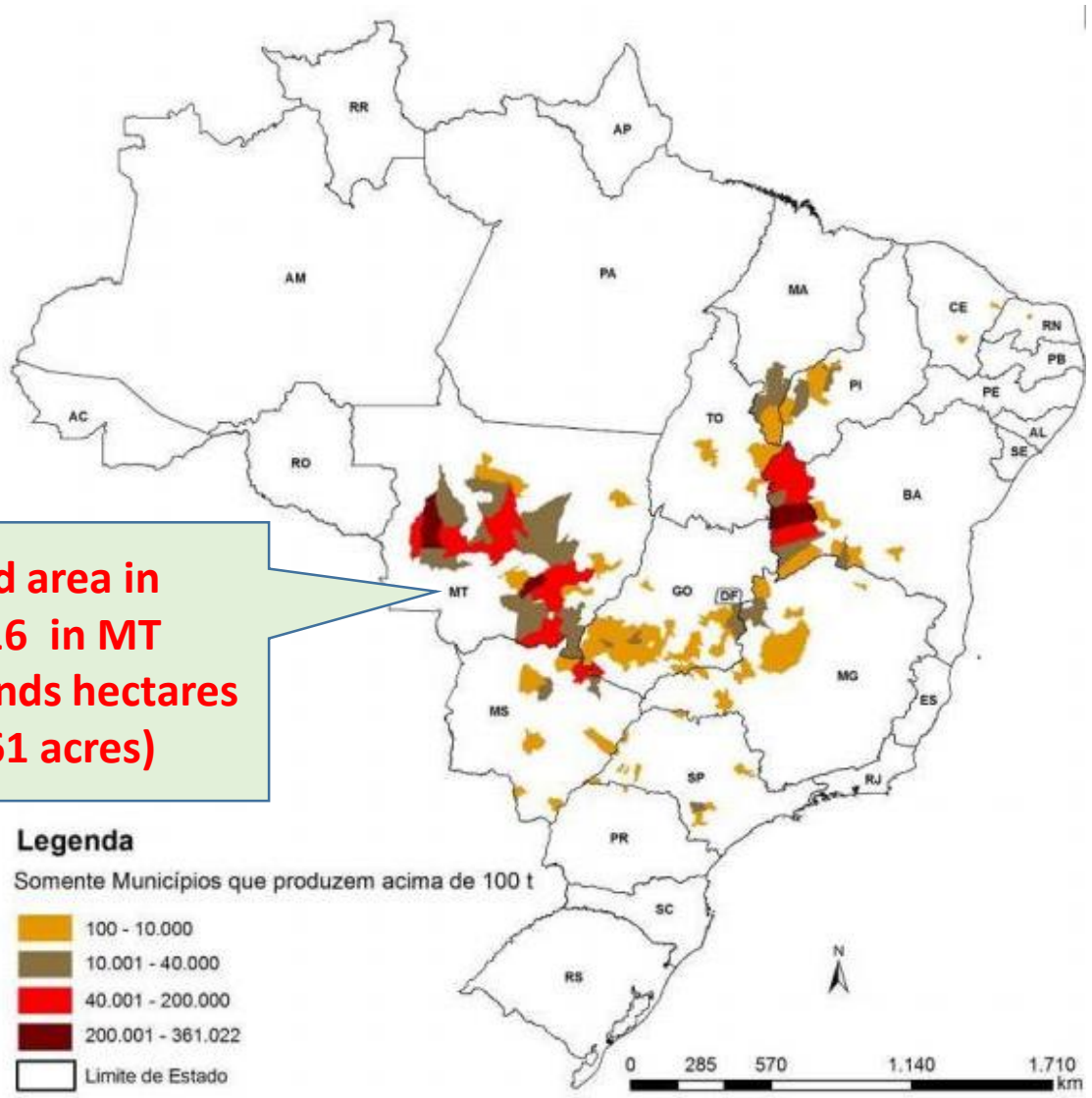
Before we start, some facts about brazilian agriculture to be considered in the exposure scenario...

Gross income of the main products from brazilian agriculture



Source: CONAB 2016 - Receita bruta dos produtores rurais brasileiros - Vol. 7 - Safra 2013/2014

One example: Map of **cotton** production - 2015



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

Fonte: Conab/IBGE.

1 Área de Preservação Permanente (APP)

Zona protegida, coberta ou não por vegetação nativa, com a função ambiental de preservar a água, a paisagem, a estabilidade geológica, a biodiversidade, o solo e assegurar o bem-estar das pessoas. Pode ocorrer em área rural ou urbana

Tipos de APPs

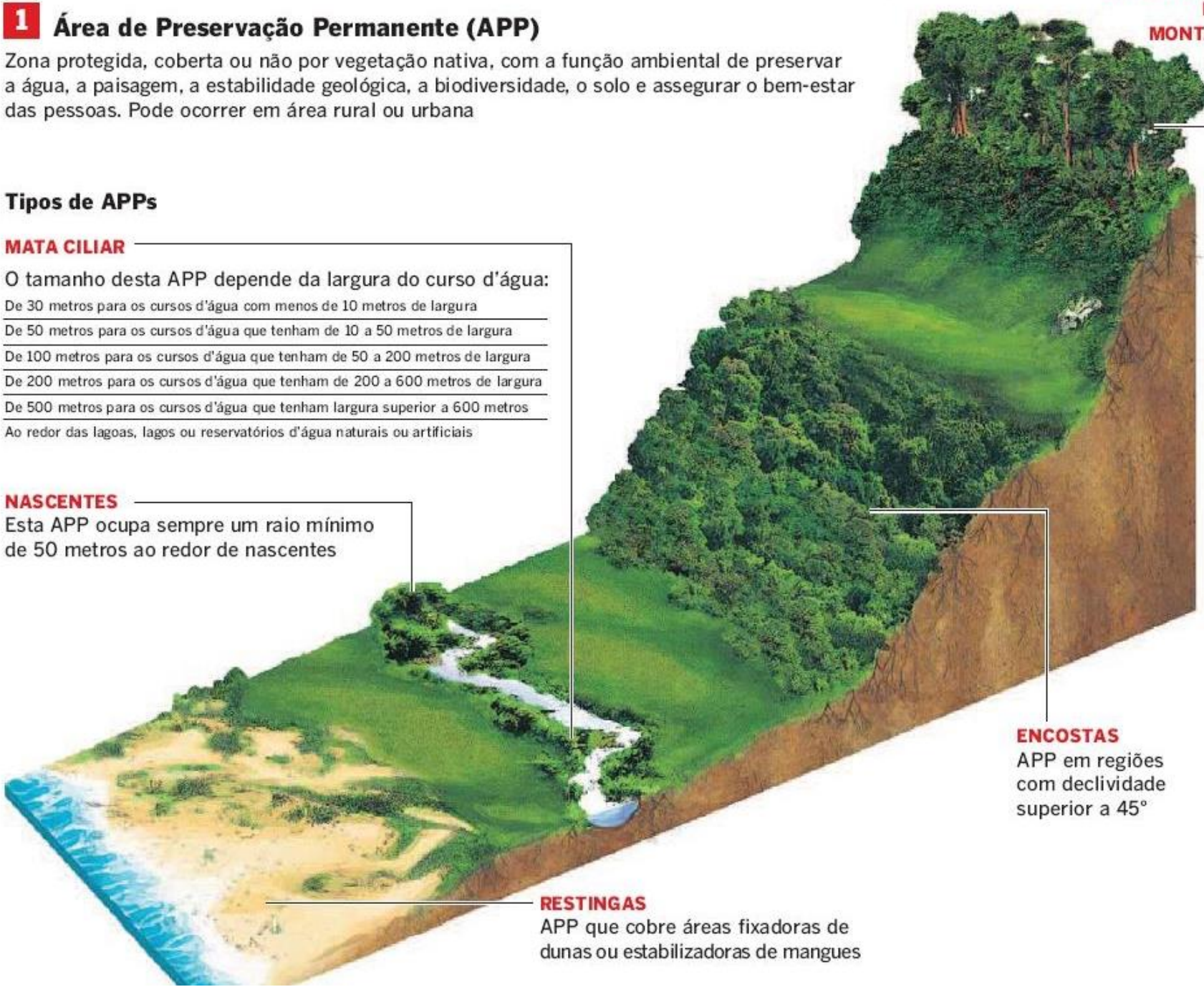
MATA CILIAR

O tamanho desta APP depende da largura do curso d'água:

De 30 metros para os cursos d'água com menos de 10 metros de largura
De 50 metros para os cursos d'água que tenham de 10 a 50 metros de largura
De 100 metros para os cursos d'água que tenham de 50 a 200 metros de largura
De 200 metros para os cursos d'água que tenham de 200 a 600 metros de largura
De 500 metros para os cursos d'água que tenham largura superior a 600 metros
Ao redor das lagoas, lagos ou reservatórios d'água naturais ou artificiais

NASCENTES

Esta APP ocupa sempre um raio mínimo de 50 metros ao redor de nascentes



TOPOS DE MORROS, MONTES, MONTANHAS E SERRAS

ENCOSTAS

APP em regiões com declividade superior a 45°

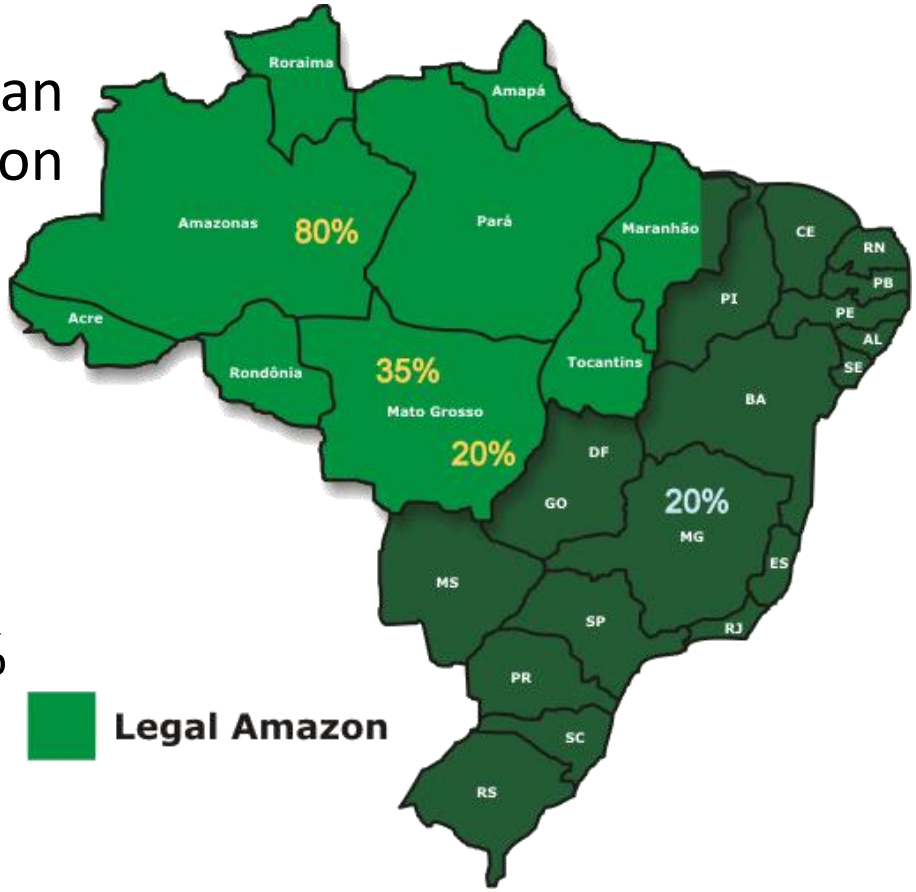
RESTINGAS

APP que cobre áreas fixadoras de dunas ou estabilizadoras de mangues

Brazilian Forestry Code - Law nº 12.651 - 25 may 2012

Article 12:

- **<u>ALL</u>** rural property must keep an area with native vegetation coverage... = **LEGAL RESERVE**
- If located in the Legal Amazon:
  - 80% if situated in forest area
  - 35% if situated in cerrado area
  - 20% in general fields
- Other regions of the country: 20%

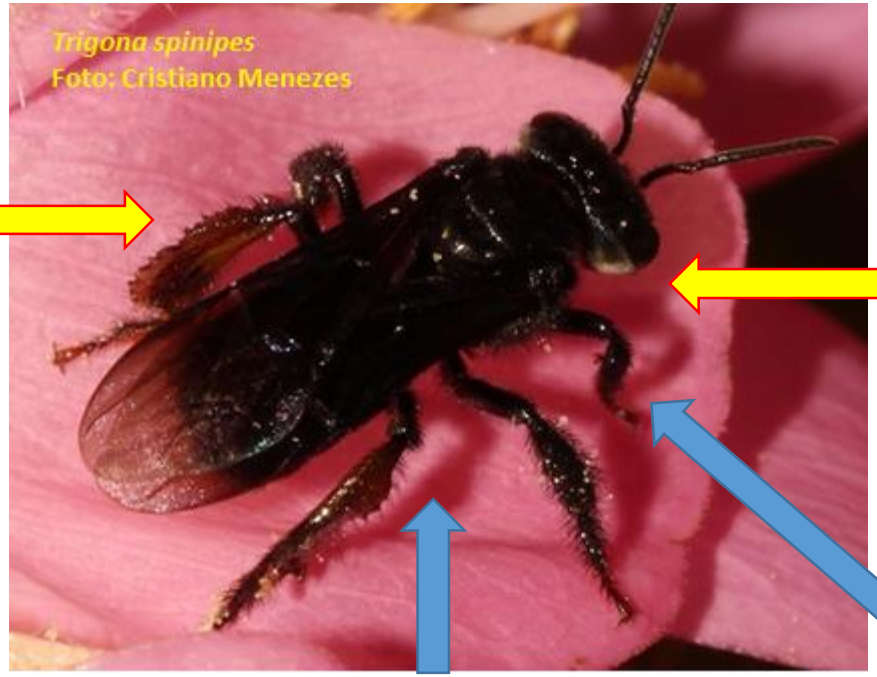


EXPOSURE OF STINGLESS BEES

Routes of exposure: workers

The routes of exposure for workers of stingless bees and *Apis mellifera* are very similar, but there are some diferences related to the type of material collected to buil the nest

Contact exposure
Direct exposure during spraying application



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

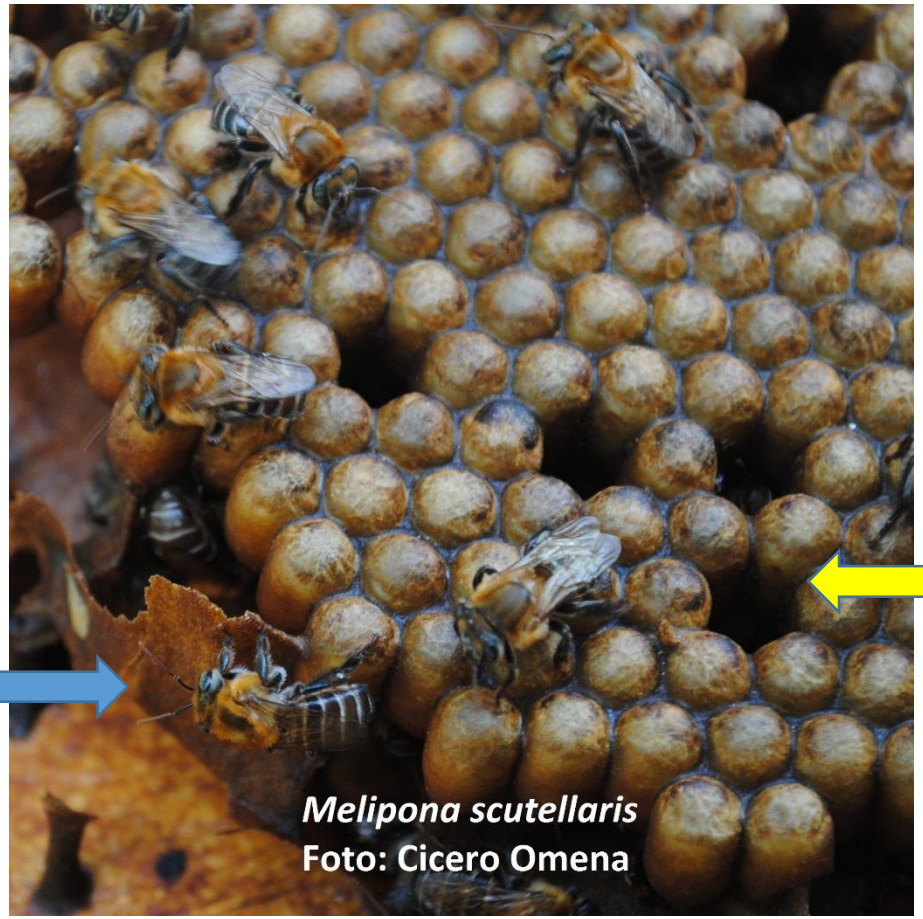
Inhalation
of droplets or gaseous phase of pesticides (TOTALLY GAP...)

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

Routes of exposure: in-hive bees

The routes of exposure for stingless bees and *Apis mellifera* are very similar, with the difference that larvae of meliponines feed directly on a relatively high amount of polen not processed

Contact exposure
Contaminated nectar, pollen, mud, resins



Oral exposure:
Consumption of nectar, pollen, water

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

...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

No data on visitors for important crops like maize, rice, wheat (no bees or lack of research?)

Crop (ranked by economic importance)	Number of species found (total)	Number of stingless bees found
Soya	11	3
Sugar Cane	5	-
Coffee	7	6
Cassava	4	3
Cotton	127	22
Tomato	64	8
Beans	37	6
Citrus	18	5
Apple	65	2

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

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


Entrance of the nest of *Melipona fuliginosa*. Photo: APACAME


Foto: Giorgio Cristino Venturieri.

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


Potes de pólen
Foto: Cristiano Menezes

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** **- Head width** **- Torax width**	11.52 3.68 4.16	5.92 2.56 2.4	3.68 1.60 1.44	4.20 1.92 1.92	9.92 3.84 4.48	10.72 4.16 4.64

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

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 flovolineata	2.32 mg	6.13 mg

Larvae	Nectar consumption bee/day (?)	Pollen consumption bee/day (?)
Scaptotrigona bipunctata	Total food consumed: 17,72 mg/bee/day 12% sugar: 2.12 mg 30% polen: 5.13 mg 56% polen: 9.92 mg	

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%

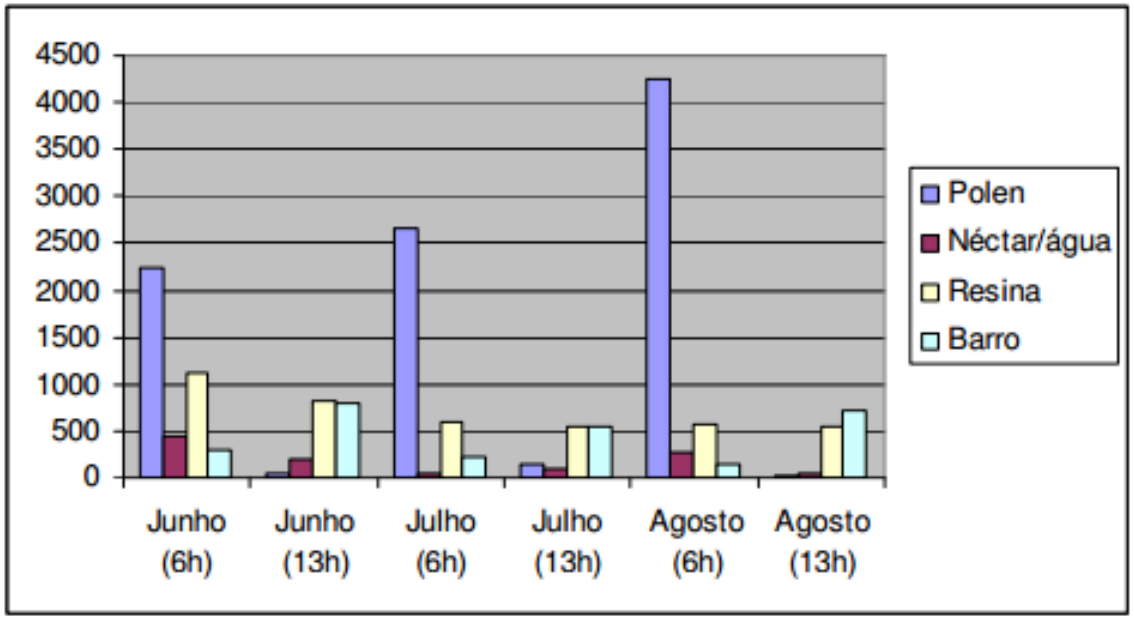
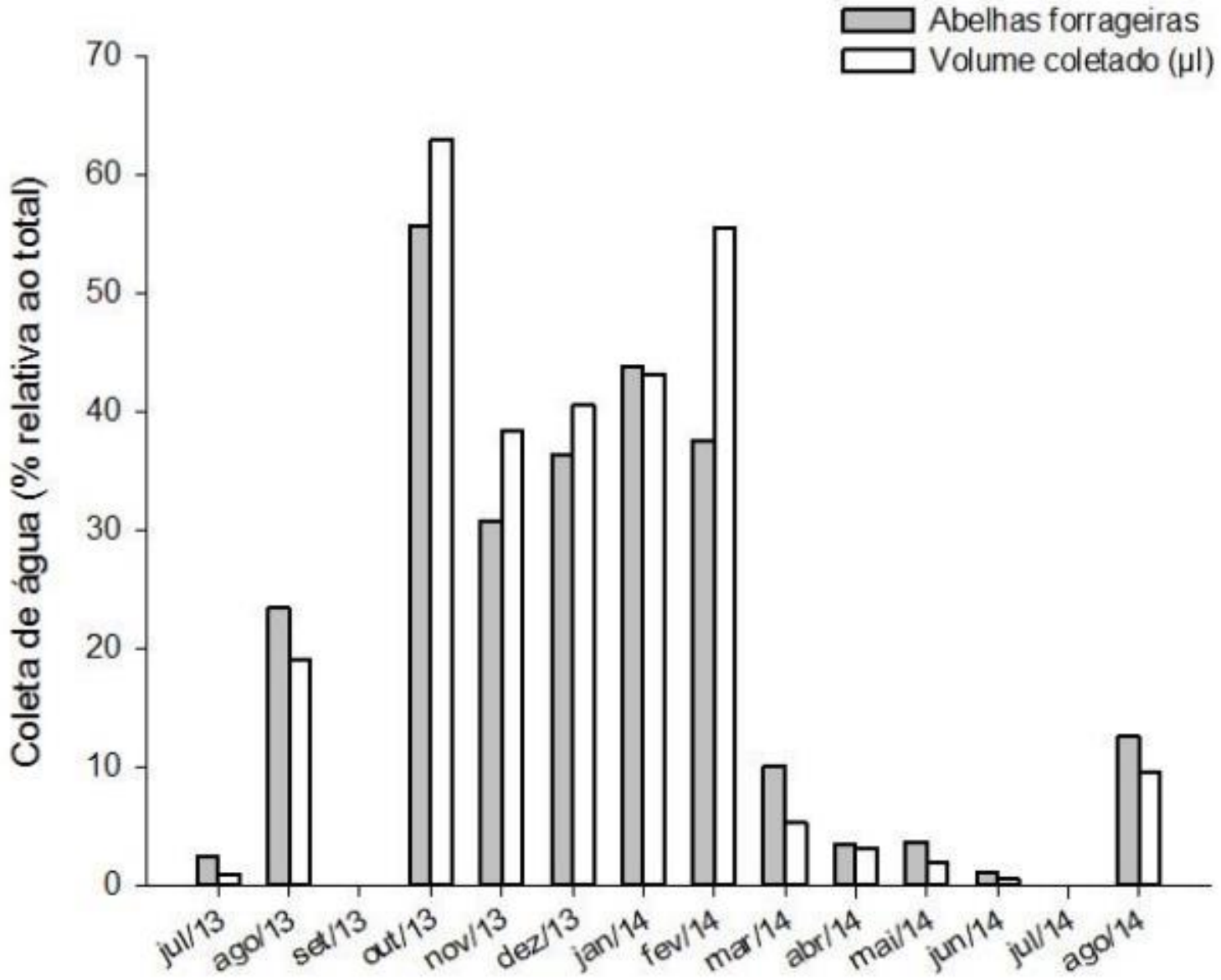


Figura 1. Cargas coletadas por operárias de duas colônias de *Melipona seminigra* e temperaturas médias (período do dia/mês) em Sistema Agroflorestal (BR-174 Km 23, Manaus – AM) no período de junho a agosto de 2005.



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

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]*

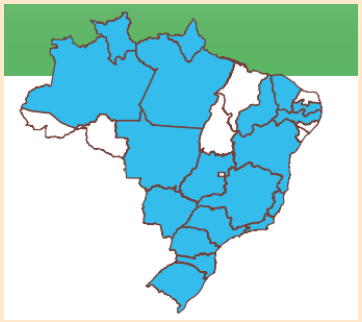
Table 1. Amount of larval food per cell and its pollen content, in different species of stingless bees.

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

Trigona spinipes

Pros	Cons
• Wide geographic distribution in the country  • 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

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

Pros	Cons
• Relatively wide geographical distribution  • small bee (more sensitive???)	• Hives not available commercially • No methods to manage colonies in laboratory conditions • No protocols for toxicity, semi-field or field tests

Melipona quadrifasciata

Pros	Cons
• Relatively wide geographical distribution  • Toxicity can be tested using standardized protocols • Hives available comercially	• Appears to be less sensitive than *Apis mellifera* (?)

Melipona scutellaris

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

SCIENTIFIC REPORTS

OPEN

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 (short / medium term):

- To publish the bibliographical survey for species selection matrix
- 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

...

Working group on risk assessment of pesticides for bees in Brazil

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- Maria Cecília de Lima e Sá de Alencar Rocha (UFBA)
- Roberta Cornélio Ferreira Nocelli (UFSCar) and
- Osmar Malaspina (Unesp Rio Claro).

THANK YOU!!!

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