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SHORT NOTE

Biological Data of Stingless Bees with Potential Application in Pesticide Risk Assessments

AS DORIGO¹, AS ROSA-FONTANA¹, IF CAMARGO², RCF NOCELLI², O MALASPINA¹

1 - Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Rio Claro, São Paulo, Brazil

2 - Universidade Federal de São Carlos (UFSCar), Araras, São Paulo, Brazil

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Corresponding author

Annelise Rosa-Fontana

Universidade Estadual Paulista Júlio de Mesquita Filho - UNESP

Centro de Estudos de Insetos Sociais

Av. 24-A, 1515 - Bela Vista

Código postal 199 - CEP: 13506900

Rio Claro-SP, Brasil.

E-Mail: annesouzar@gmail.com

Abstract

Due to the current practice of intensive pesticide use in Brazil on crops with flowers that are attractive to bees, biological information about Brazilian native bees is required in order for public authorities that are responsible for environmental safety to use them for calculations of risk assessments. Thus, the present study aimed to obtain biological data on stingless bees: *Melipona scutellaris* Latreille, *Scaptotrigona postica* Latreille and *Tetragonisca angustula* Latreille. The food consumed by larvae and by adults and the mass of forager workers were obtained. The results provide essential inputs for the risk assessment of stingless bee exposure to pesticides, which combined with information about the concentrations of these substances in crops with flowers that are attractive to bees, may be used in risk calculations.

Brazil has the greatest global richness of stingless bees (Michener, 2013) and is also the world's largest consumer of pesticides (MAPA, 2017). Therefore, it is essential that ecotoxicological information on native fauna be made available to agencies responsible for environmental assessments of pesticides, represented in the case of Brazil by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). According to IBAMA (2017), among the main limitations for pesticide risk assessments for native bees is the lack of basic data on the biology of these bees, such as food consumption at different life stages and the mass of the individuals. In addition, the safety of the current use of the exotic species *Apis mellifera* L. as a substitute for the other native species of Brazil in these assessments is questioned.

Melipona scutellaris Latreille, *Scaptotrigona postica* Latreille and *Tetragonisca angustula* Latreille are interesting species to be used as model organisms for risk assessment. The first two are considered as priorities for pesticide risk assessments, according to the recent list of species selection, which consider several criteria such as geographic distribution, association with crops, and importance as pollinators, among others (Pires & Torezani, 2018). A species of the *Scaptotrigona* genus (*S. postica*) was chosen by both its broad distribution in Brazil and its intermediate size among the other species. In addition to composing the native Brazilian fauna, there is a need for biological data on stingless bee species, which may be useful for carrying out risk assessments for native species (IBAMA, 2017). Based on the data gaps mentioned, the present study aimed to provide



data to fill the gaps in knowledge about aspects related to nutrition and body mass of stingless bees. These results will provide essential inputs for the risk assessments of exposure of stingless bees to pesticides.

To quantify the larval food consumed by brood cell, as well as the daily syrup consumption and the individual mass of worker foragers, three non-parental colonies of each species, maintained at Universidade Estadual Paulista, Rio Claro, Brazil, were used. To measure the amount of larval food consumed per brood cell, the food of 20 newly oviposited cells per colony was collected by random selection. Before food collection, the eggs were removed from cells. With an automatic micropipette, the food from each colony was homogenized in Eppendorf microtubes with volumetric graduation. They were weighed in an analytical balance before and after the food collection. The total volume and mass were divided by the number of brood cells to estimate the amount of individual food per cell.

To estimate the food consumption and individual mass of worker foragers, individuals from the three species (total number/species = 30 for each test) were collected at the entrances of the nests when they left to forage. The mass of the individuals was verified using an analytical balance. To check the food consumption, 10 individuals from the same colony were placed on plastic perforated pots for air circulation. Eppendorf microtubes syrup feeders (sugar and water 1:1,v:v), simulating nectar, were placed on the pots, and the bees fed *ad libitum*. For 4 days, every 24 hours, the feeders were weighed using an analytical balance in order to verify daily consumption.

In addition to the establishment of the data for the stingless bee species, the same data for honey bees were taken from literature, in order to compare them.

The mean values of the larval food consumed by brood cell, the daily syrup consumption by forager workers, the individual mass of the bees and the data taken from literature are shown in the Table 1.

The data on the amount of food consumed by larvae and syrup by forager workers, combined with information on the concentrations of these substances in agricultural crops with flowers that are attractive to bees, may be used for risk

assessments. Understanding this relationship is extremely relevant since Brazil currently bases its protocols on standards of the OECD (Organisation for Economic Co-operation and Development), which uses *A. mellifera* as a model organism. These protocols were adopted by the United States EPA (Environmental Protection Agency) for the schematization of risk assessment, which was used as a reference for the current Brazilian risk assessment scheme. Nevertheless, among the main uncertainties noted by IBAMA (2017), the use of *A. mellifera* stands out, since stingless bees are part of the native pollinator fauna. One way to remedy this issue would be to carry out a comparison of *A. mellifera* with native species regarding the toxicity of and exposure to pesticides, and our work provides important information that contributes to this evaluation. The differences between the groups are evident in biological parameters, such as their larval feeding systems. While *A. mellifera* nurse workers progressively deposit the food to the brood, in stingless bees, they do it all at once, depositing all the food that will be consumed (Velthuis, 1998). In addition, the volume of food consumed by the brood also differs between groups: in *A. mellifera*, each larva consumes a total of 160 μL (Aupinel, 2005), and in the stingless bee species, *M. scutellaris*, *S. postica* and *T. angustula*, each larva ingests 130.58, 25 and 5.6 μL , respectively. The nectar consumption by the forager workers and the individual mass of the bees also give rise to the uncertainty about using *A. mellifera* as a study model in Brazil. According to Cresswell et al. (2012), one forager worker of *A. mellifera* consumes a daily average of 50 mg of nectar and weighs on average of 79.1 mg, showing greater food consumption and body mass comparing to stingless bees (Table 1).

In addition to that, the mass of individuals presented herein may be a relevant factor in the sensitivity of the different species to a particular type of pesticide, for example. Comparing the sensitivity of bee species to exposure to pesticides, a meta-analysis study conducted by Arena and Sgolastra (2014) indicated the sensitivity of stingless bees was in general higher than bumblebees. In addition, Devillers et al. (2003) suggested the sensitivity of different bee species is generally inversely proportional to their mean body mass.

Table 1. Amount of larval food consumption by cell, daily syrup consumption and individual weight of forager workers. \pm indicates standard deviations values. “-” indicates absence of data.

	Volume of larval food by brood cell (μL)	Mass of larval food by brood cell (mg)	Daily syrup consumption by worker (mg)	Individual mass of forager worker (mg)
<i>Melipona scutellaris</i>	130.58 \pm 0.52	158.04 \pm 5.18	35.06 \pm 9.15	76.65 \pm 2.9
<i>Scaptotrigona postica</i>	25 \pm 1.14	32.5 \pm 0.8	9.45 \pm 2.41	17.02 \pm 0.35
<i>Tetragonisca angustula</i>	5.6 \pm 0.77	7.96 \pm 1.03	7.23 \pm 1.74	4.1 \pm 0.37
<i>Apis mellifera</i>	160 [†]	-	50 [†]	79.1 \pm 4.9 [†]

[†]The data of volume of larval food by brood cell, daily syrup consumption by worker and individual mass of forager worker were obtained, respectively, from Aupinel (2005), Cresswell et al. (2012) and Blatt and Roces (2001).

Nevertheless, it is interesting to observe that, in the workers of the species of smaller body size of this study, *T. angustula*, the amount of food consumed corresponds to twice the body mass, compared to the other species of stingless bees and also to *A. mellifera*. This is an interesting point to be considered for future investigations regarding species-sensitivity.

In this work, some information required for the characterization of pesticide exposure risks to stingless bees was presented. Although there is a high richness of stingless bee species in Brazil, little is known about their basic biological data (IBAMA, 2017). For the inclusion of native species in risk assessments of pesticides in Brazil, it is necessary that this approach continues in future studies.

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Authors' Contribution

All of the authors conceived research. Authors 1, 2 and 3 conducted experiments. Authors 1 and 2 analysed data and conducted statistical analyses. All of the authors wrote, read and approved the manuscript.

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