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Review

Special collection: Pesticide Exposure in Non-Honey Bees

Pesticide Exposure Assessment Paradigm for Stingless Bees

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Abstract

Although the importance of bees as the pollinators responsible for maintaining gene flow for many native and cultivated plants in ecosystems around the world is recognized, much of their biodiversity and behavior remains to be discovered. Stingless bees are considered key pollinators for several plant species in tropical and subtropical ecosystems and they also provide pollination services for economically important agricultural crops. Many countries are using the honey bee (*Apis mellifera* Linnaeus, 1758, Hymenoptera: Apidae) as a surrogate to evaluate the risk of pesticides to all species of bees. However, there is uncertainty regarding the extent to which honey bees can serve as surrogates for non-*Apis* bee species in the risk assessment for pesticides. This paper provides a short overview of the life history traits relevant in risk assessment of stingless bees. It summarizes what is known about stingless bee exposure to pesticides compared to that of honey bees and presents criteria for potential candidate species from Brazil for use in pesticide risk assessment in tropical environments. This paper also identifies gaps in knowledge of bee biology and pesticide exposure routes not covered by the current honey bee exposure assessment paradigm. Based on these gaps, research is needed on life history traits, estimates of nectar and pollen consumption, mud, resin, and water collection and available protocols to adequately assess toxic effects of pesticides to stingless bees. This review is part of a series of papers on the risk of exposure of non-*Apis* bees to pesticides.

Key words: Meliponini, non-*Apis* bees, risk assessment, Trigonini

Bees are the most pollinators responsible for maintaining gene flow for many native and cultivated plants in ecosystems around the world is recognized, much of their biodiversity and behavior remains to be discovered. Knowledge gaps are even more pronounced in vast regions like the Brazilian Amazon, and, due to the increasing loss of the natural systems by conversion of lands to agricultural production or by the over exploitation of the natural resources, several tropical bee species are at risk of extinction before their unique natural histories are revealed.

Stingless bees (Hymenoptera: Apidae: Tribe Meliponini) are considered key pollinators for several plant species in tropical and subtropical ecosystems, and they also provide pollination services for economically important agricultural crops (Heard 1999, Slaa et al. 2006). Some references suggest that stingless bees are responsible for 40% to 90% of the pollination of tropical native trees, depending on the biome considered (Kerr et al. 1996). Stingless bees are especially relevant for Brazil given their promising uses as commercial pollinators.

Although many countries use the honey bee (*Apis mellifera* L., Hymenoptera: Apidae) as a surrogate to evaluate the risk of pesticides to all species of bees, there is uncertainty regarding the extent to which the honey bee can serve as a surrogate for all non-*Apis* bee species in pesticide risk assessment (Arena and Sgolastra 2014, Barbosa et al. 2015). Several studies have demonstrated that there are differences to be considered in response to toxicity to pesticides between *A. mellifera* and other bee species, such as *Bombus terrestris* Linnaeus, 1758 (Hymenoptera: Apidae), *Osmia bicornis* Linnaeus, 1758 (Hymenoptera: Megachilidae) (Devillers et al. 2003, Heard et al. 2017), *Megachile rotundata* Fabricius, 1787 (Hymenoptera: Megachilidae), *Nomia melandrei* melanderi Cockerell, 1906 (Hymenoptera: Hlictidae) (Devillers et al. 2003), and some species of stingless bees (Jacob et al. 2013; Lourenço et al. 2012a,b; Costa et al. 2015; Soares et al. 2015; Lima et al. 2016). To ensure that the species *A. mellifera* is a good model, it is necessary to know and compare the biology of the species and to identify which routes are covered by the current *Apis*-based schemes and where we must move forward to reach the protection goals.

This review is part of a series of papers written from the discussions that held during the workshop on the risk of exposure of non-*Apis* bees to pesticides in January 2017 in Washington DC. In addition to stingless bees, the papers provide insight into the biology and exposure risk factors for bumble bees and different species of solitary bees such as *M. rotundata, Nomia melanderi* and *Osmia sp*.

This paper aims to provide a brief introduction to Brazilian stingless bees, a short overview of their life history traits relevant in risk assessment of stingless bees, and a summary of what is known about their exposure to pesticides compared to that of honey bees in order to identify gaps in knowledge and research needs. Once knowledge gaps are filled, then some of the uncertainty around pesticides risk and assessments can be diminished.

Stingless Bees: Occurrence and Importance as Pollinators

Stingless bees have populated Earth's tropical regions for over 65 million years, which is longer than *Apis* spp. (the stinging honey bees) have existed (Camargo and Pedro 1992, Michener 2007). Both honey bees and stingless bees make honey in perennial nests founded by a swarm of sterile female workers and a queen. Colonies maintain workers at all times and also males during certain times of the year. However, stingless bees are 50 times more diverse than the honey bees and differ from them in many biologically significant ways (Roubik 2006).

Within the Family Apidae, stingless bees belong to the Tribe Meliponini. Considered eusocial non-*Apis* bees, meliponines have extremely diverse morphology, nest building (nidification) habits, behavior, and ecology. They are small- to medium-sized bees (2–13 mm in length) with considerable variation in colony population size (from several hundred up to more than 10,000 workers per colony). They also exhibit a high rate of brood production and require substantial pollen intake (Ramalho et al. 1998).

Meliponines are distributed from Uruguay to central Mexico, and from Africa, India, Malaysia, and Indonesia to Australia (Camargo and Pedro 2013; Fig. 1). Currently, over 600 species in 56 genera have been described. They live in tropical and subtropical areas of the world (Fig. 1). There are 400 known species in Neotropical regions, and it is estimated that more than 100 species are yet to be described (Cortopassi-Laurino 2006). Approximately 244 species occur in Brazil (Pedro 2014; Fig. 2), and some of them are under threat of extinction due to the destruction of their natural habitat (Kerr 1996). Like honey bees, stingless bees also can be used for pollination services of native or cultivated plants. However, few studies have addressed the importance of these bees as pollinators.

Meliponiculture is the cultivation of stingless bees on a commercial scale. It is being proposed as a possible activity to support food production, biodiversity of native bees, and native plant conservation. Additionally, it is an alternative income source for people who practice traditional cultures and also for small crop producers, who can sell the stingless bee honey and other products and may rent colonies for pollination services. Meliponini biological and ecological characteristics make them suitable for use in sustainable agriculture activities (Venturieri 2008, Villas-Bôas 2012, Jaffé et al. 2015, Athayde et al. 2016).

The market for stingless bee honey is incipient and regionalized, which restricts it to initiatives in Brazil, Mexico, Costa Rica, and Australia (Alves 2013). Even considering that the activity constitutes the major source of income among the products and services of meliponiculture (Cortopassi-Laurino 2006) and an estimated annual production of up to 100 tons of honey, the inconsistent marketing standards and regulations limit the extent to which the market potential of this resource can be realized.

The meliponines are very promising social insects for use as commercial pollinators (Cruz and Campos 2009, Bartelli and

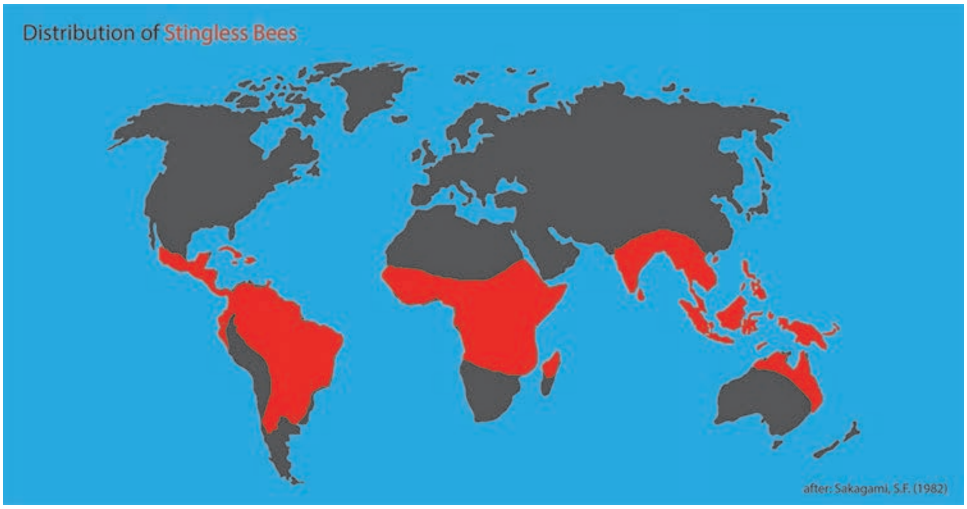

Fig. 1. Map of geographic distribution of Meliponini in the Tropical and Subtropical regions of the world. Sagakami, 1982 (in Oliveira et al., 2013—reproduction authorized).

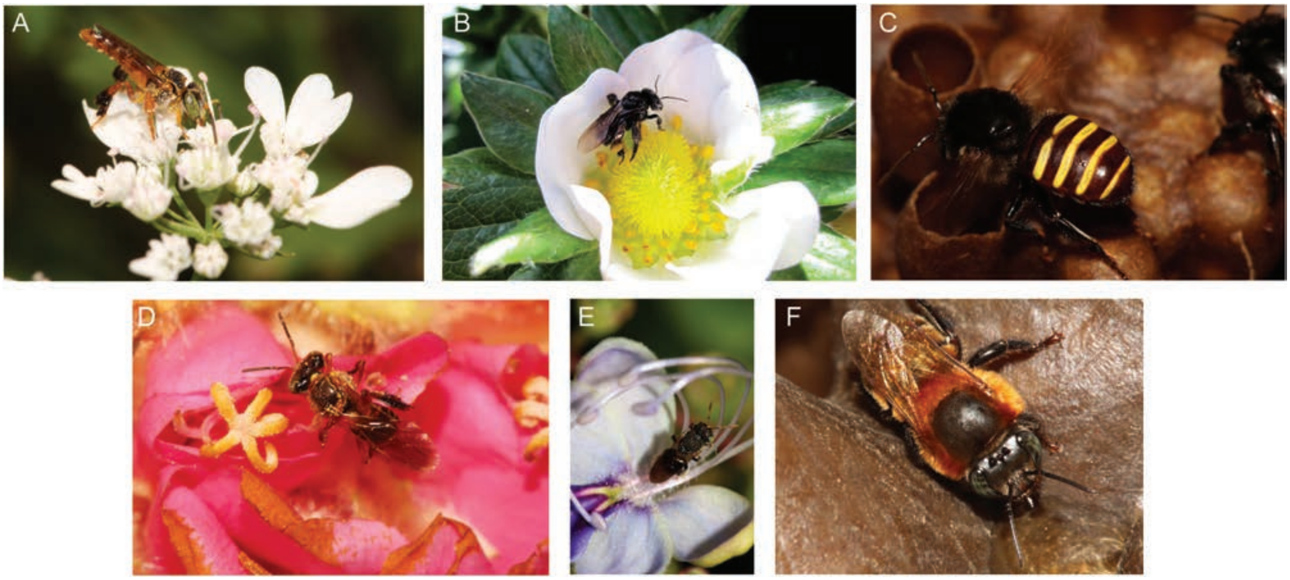

Fig. 2. Some species of stingless bees in Brazil. (A) *Tetragonisca angustula* Latreille, 1811 (Hymenoptera: Apidae) (Jataí). (B) *Trigona spinipes* Fabricius, 1793 (Hymenoptera: Apidae) (Irapuá). (C) *Melipona quadrifasciata* Lepeletier, 1836 (Hymenoptera: Apidae) (Mandaçaia). (D) *Frieseomelitta varia* Lepeletier, 1836 (Hymenopera: Apidae) (Marmelada). (E) *Nannotrigona testaceicornis* Cockrerell, 1922 (Hymenoptera: Apidae) (Iraí). (F) *Melipona bicolor* Lepeletier, 1836 (Hymenoptera: Apidae) (Guarupu). All Pictures: Cristiano Menezes.

Nogueira-Ferreira 2014). Because they do not possess a functional stinger and, therefore, pose little hazard to humans compared to honey bees, they are suitable as pollinators in greenhouses and in residential settings. Also, their flight range is shorter than that of honey bees, which promotes local foraging and may make them more efficient for enclosed or semienclosed pollination (i.e., greenhouses, hoophouses, and tunnels; Slaa et al. 2006). Although some research has been carried out to evaluate the effectiveness of pollination using stingless bees for various crops (Cruz and Campos 2009), their management for pollination is practically nonexistent in Brazil today. The lack of a supply of stingless colonies to buy or rent is one of the obstacles to the adoption of this practice (EMBRAPA 2013).

Life History Difference Between *A. mellifera* and Stingless Bees Regarding Implications for Pesticide Risk Assessment

In current classifications, stingless bees belong to the family Apidae, subfamily Apinae, and tribe Meliponini (Michener 2007). Nevertheless, some authors divide them in two large groups based on the morphology and origin of the queens: Meliponini that encompasses only the genus *Melipona* Illiger, 1806 (Hymanopetera: Apidae) with approximately 80 species, and Trigonini that includes the genus *Trigona* Jurine, 1807 (Hymenoptera: Apidae) along with all the other genera (Moure 1961).

It is important to clarify that this document acknowledges the classification proposed by Michener, i.e., considering all the meliponines grouped in only one tribe. However, for facilitating descriptions of life history traits, we use the groupings of Moure (1961) that designate tribes Meliponini and Trigonini as separate groups.

Nest Biology

Meliponini species vary considerably in the architecture of their nests with vastly different conformations for their internal and external structure. They build their nests in several substrates, such as subterranean cavities, tree trunks, branches of living trees, rock crevices, and brick walls. Occasionally, they build nests in active colonies of other social insects, such as active or abandoned termite nests, arboreal ant nests, subterranean chambers abandoned by ants, active bird nests, or empty nests attached to branches (Schwarz 1948, Camargo 1970, Wille 1983, Campos 1987, Kerr et al. 1996, Roubik 2006, Rasmussen and Camargo 2008, Carvalho et al. 2014). The architecture of the nest entrance is species-specific (Franck et al. 2004) and consists of very diverse shapes and materials (e.g., wax, resin, mud, seeds, sticks, petals, small stones; Roubik 2006; Fig. 3).

Nests are made by stingless bee workers and occupy specific locations within forests (Kerr et al. 1967, Posey and Camargo 1985, Camargo and Pedro 2013). Specific nesting habits along with characteristics of queens, workers, and males are important in helping to organize biological information that may lead to their potential application to research, economics, and conservation of both the pollinators and the floral sources upon which they depend for food and nutrients.

Because nests are apparent and observable points of bee activity and are often spectacular exhibits of animal architecture, nesting biology is a highly visible aspect of stingless bee behavior (Michener 1974). Most conspicuous are nest entrances, while less conspicuous are the internal nest components that vary in shape and arrangement of brood cells and food storage containers.

In contrast to *Apis* Linnaeus, 1758 (Hymenoptera: Apidae), meliponines generally do not use pure wax to build the nest (Roubik 2006). The building materials are usually cerumen (a mixture of wax and resins collected from plants), resins (propolis), and mud. Stingless bees also use batumen (i.e., a mixture of mud and resins) to delimit the internal nest area and coat nest surfaces (Roubik 2006). Part of the cultivated plants treated with different types of pesticides may be the source of collection of several of these materials used to build nests, which makes this an important route for exposure to adults, but also to larvae. Substances applied in soils and seed treatment may be considered low risk for *A. mellifera*, which do not collect soil material. However, for stingless bees this may be a rather important route of exposure, since some species collect large amounts of mud and should be considered in risk assessment schemes.

Brood cells are almost always enveloped by a thin membrane of cerumen, called *involucrum*, and may be organized in horizontal overlapping layers like the floors in a building, or constructed into spiral

Fig. 3. Some nest entrances of stingless bees. (A) *Plebeia minima* Gribodo, 1983 (Hymenoptera: Apidae). (B) *Partamona helleri* Friese, 1900 (Hymenoptera: Apidae). (C) *Nannotrigona* sp. (D) *Melipona quadrifasciata*. (E) *Nannotrigona testaceicornis*. (F) *Scaptotrigona bipunctata* Lepeletier, 1836 (Hymenoptera: Apidae). (G) *Paratrigona* sp. Schwarz, 1938 (Hymenoptera: Apidae). (H) *Melipona flavolineata* Friese, 1900 (Hymenopetera: Apidae). (I) *Paratrigona* sp. (J) *Leurotrigona muelleri* Friese, 1900 (Hymenoptera: Apidae). (K) *Partamona helleri*. (L) *Schwarziana quadripunctata* Lepeletier, 1836 (Hymenoptera: Apidae). (M) *Trigona pallens* Fabricius, 1798 (Hymenoptera: Apidae). (N) *Tetragonisca angustula*. All Pictures: Cristiano Menezes.

or cluster shapes (Fig. 4). The brood cells are spherical to ovoid and are constructed in an upright position, with the cell opening facing upward. Honey and pollen are usually stored separately in cerumen cells (called "pots") constructed for this purpose. Some species mix both pollen and nectar in the same pot. Pots are small-to-large spheres, egg-shaped, conical, or cylindrical. Often pots are pressed together in odd conglomerations, as are the brood cells, ranging from individual cells on pillars, to sheets of uniform cells on combs that are separated by pillars. The majority of species isolate the storage area from the brood area. Stored nectar or ripened honey are generally in nest cavity extremes (for storage during heavy flowering periods), while pollen and some honey may surround the brood area. (Figs. 4 and 5).

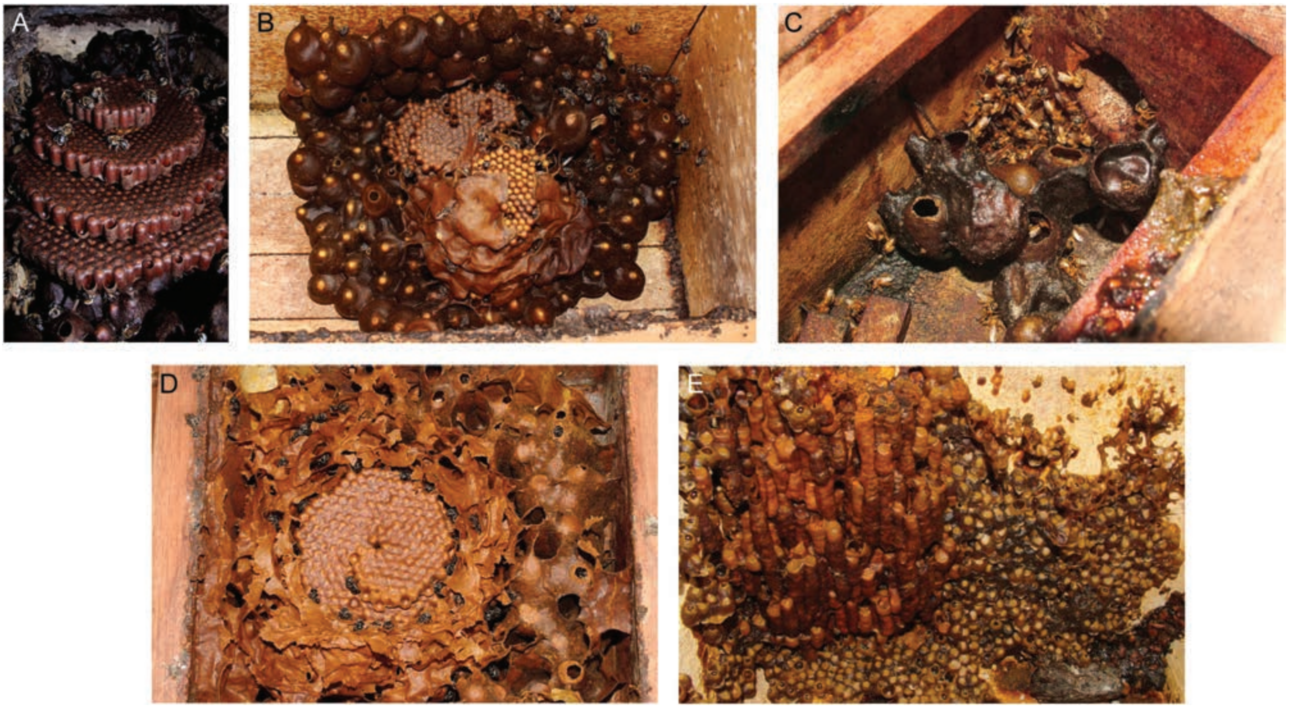

Fig. 4. Some internal structures of the nests: (A) Brood cells of *Melipona* Posey, 1983 (Hymenoptera: Apidae) compressipes organized as horizontal overlapping layers. Pictures: Cristiano Menezes. (B) nest of *Melipona fasciculata*, with brood cells in the center and food pots in the periphery. Pictures: Cristiano Menezes. (C) managed nest of *Frieseomelitta doederleini* Von Ihering, 1912 (Hymenoptera: Apidae), with brood cells on the right and food pots on the left. Pictures: Cristiano Menezes. (D) artificial nest of *Geotrigona* sp Moure, 1943 (Hymenoptera: Apidae); Pictures: Cristiano Menezes. (E) pollen pots (tube shapes) and honey pots in an artificial nest of *Frieseomelitta varia*. Pictures: Cristiano Menezes.

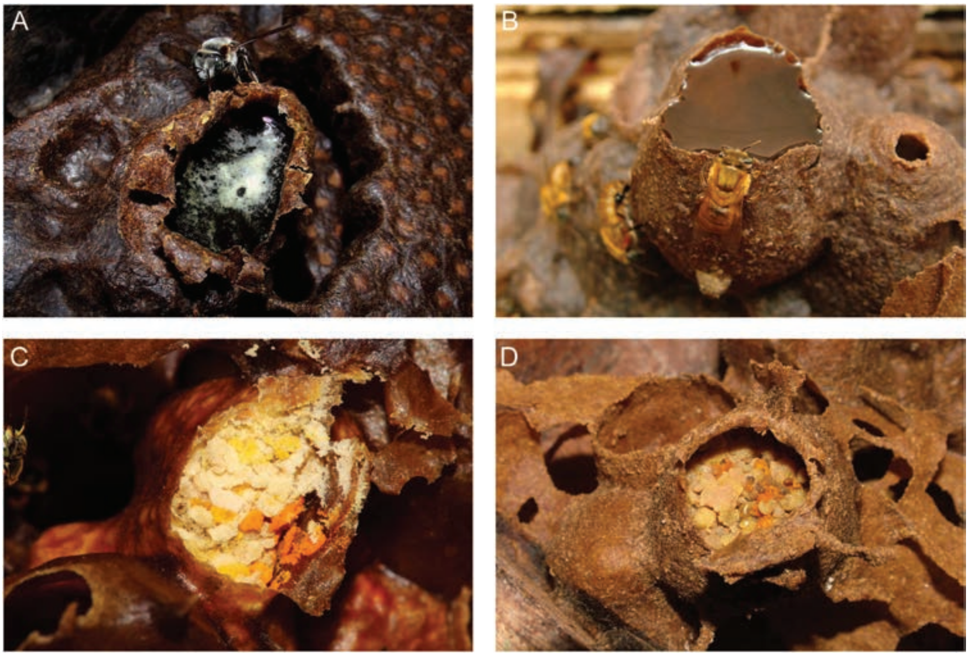

Fig. 5. Honey pot in a nest of (A) *Melipona fasciculata*. Pictures: Cristiano Menezes. (B) *Melipona rufiventris* Lepeletier, 1836 (Hymenopetera: Apidae). Pictures: Cristiano Menezes. (C) *Tetragonisca angustula*. Pictures. Cristiano Menezes. (D) *Scaptotrigona depilis* Moure, 1942 (Hymenoptera: Apidae). Pictures: Cristiano Menezes.

Brood Production, Life Cycle, and Feeding Behavior

Unlike honey bees, Meliponines produce brood in the manner of solitary bees, i.e., with an egg placed on top of a food mass in a sealed cell (Fig. 6). The oviposition of eggs follows a general pattern, although certain oviposition behavior is also unique and can be used to identify a species. The general pattern consists of the queen visiting the comb one or several times to investigate new brood cells built by workers. The queen becomes immobile in front of a cell to signal to workers her intent to oviposit. This cues the workers start to provision the cell with larval food to around 75% of its capacity (Feitas 2003). Workers prepare the larval food by mixing stored pollen with honey and glandular secretions produced by the hypopharyngeal glands. After this mixture is processed inside the digestive tract of the workers, it is regurgitated into the brood cells (Dra. Maria Augusta Lima Siqueira, personal communication).

At this moment, one of the workers lays a sterile egg in the cell (called trophic egg oviposition), and this egg is then eaten by the queen. Afterward, the queen lays her egg on the food mass, and then workers close the cell. In some species, the queen consumes part of the food in the cell before laying the egg, and in other cases workers do not offer a trophic egg before the queen begins egg-laying (Freitas 2003). The main function of the trophic egg is to feed the queen. In some species, trophic eggs are so important to the queen that she pressures workers to lay them. Encouragement of worker egg-laying is contrary to most other social hymenopteran where the queen inhibits worker oviposition through pheromones and behavioral dominance (Freitas 2003).

Because the brood cell is sealed after oviposition, the larvae will have only the previously deposited food in the cell to complete its development. Such a feeding process is called mass provisioning (compared to progressive provisioning by *Apis* and *Bombus*). Unlike honey bee larvae, meliponine larvae feed on relativity large amounts of pollen (Vollet-Neto et al. 2010). This system of mass provisioning of the larval cell involves the simultaneous exposure of the larva by contact and oral, which is quite different from what happens with *A. mellifera* and need to be considered in risk assessment schemes. The larvae stayed throughout the development period over the food, which consumes over time which, in most species, is also longer than for *A. mellifera*.

Fig. 6. A brood cell with an egg of *Melipona fasciculata*. Picture: Cristiano Menezes.

When the larva reaches its maximum size and completes its development, it spins a cocoon around itself in preparation for the metamorphosis that will transform it into a pupae and then an adult bee. The workers take an average of about 40 d to eclose as an adult after the egg has been laid. Worker adults live 50 d on average.

The production of males in meliponines occurs by parthenocarpy, like all other hymenoptera. Males can be produced by the queen or by eggs laid by the workers in the brood cells that are not consumed by the queen. Male development from egg to adult takes an average of 40 to 45 d, depending on the species (Freitas, 2003).

Queen production differs between the groups Trigonini and Meliponini. With few exceptions, the bees build larger cells in the peripheral area of the combs (called "realeiras") where queens are produced somewhat analogous to honey bee queen cells. In the Trigonini, the determining factor for the development of queen larvae is the amount of food provided for them; therefore, caste determination is defined only by feeding factors. In Meliponini, there are no realeiras. Rather, genetic and feeding factors interact to differentiate queen from worker larvae. In this case, only a percentage of larvae produced has the genetic potential to become queens, which also depends on the amount of food available. Larvae with the queen genotype that receive too little food will become workers (Freitas 2003). In stingless bees, there can be two or more egg-laying queens in the same nest. New queens are produced regularly, but most of them are killed and never allowed to produce eggs. Some queens may remain imprisoned in special cells where they are held as reserves. Replacement of the egg-laying queen does not happen every year. Although the available information is scarce for the vast majority of stingless bee species, some studies indicate that queens can live for 3–7 yr (Bradbear 2009).

Compared to the amount of time for honey bees to develop from egg to adult, meliponines take about twice as long to reach worker adulthood (Fig. 7). Therefore, use of honey bee workers for generating data on the effects of pesticides in risk assessment may not be suitable for assessing effects on stingless bee worker

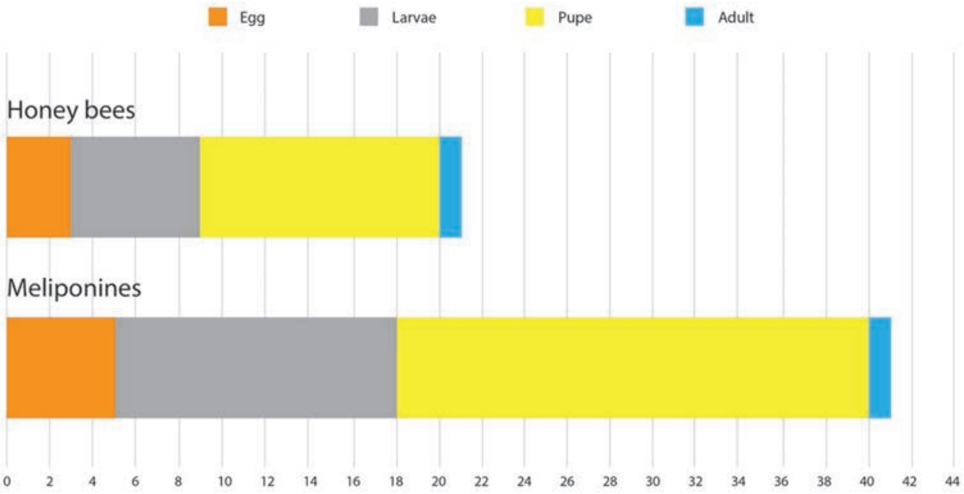

Fig. 7. Comparison between life cycle of *A. mellifera* and meliponines.

development. Similarly, compared to honey bees, the process of reproductive swarming for stingless bees to initiate a new colony is much slower and time-consuming. Therefore, the ability of a new colony to swarming and to produce adult bees to provision and protect the new colony is a more protracted process than with honey bees. Thus, considering the colony as an organism, exposure of the new colonies can have a much greater impact than observed/assessed for honey bees.

In general, when a stingless bee colony initiates new colony production, the workers search for suitable sites. When the new locality is decided upon, workers start to build the entrance of the nest. Then the workers bring wax, propolis, and cerumen from the mother colony to build the honey and pollen pots and other structures. They fill these pots with honey and pollen that are also brought from the mother colony. Only after the new nest is ready will a virgin queen move from the mother colony accompanied by a group of workers. The new queen will go out on a mating flight and then establish a new colony. In contrast, the old honey bee queen initiates a new colony, while the new queen remains in the maternal hive. For stingless bees, the contact between the mother and the daughter colony continues for approximately 40 d, and the workers of the daughter colony can take material from the nest of the mother colony during this period. This way of swarming increases the chances of success of the new colony, but on the other hand, results in slow reproduction and a dependency on the mother colony that creates a vulnerable situation where both colonies may suffer from a depletion of food resources. In addition, if the products of the mother colony contain some type of residue, the same will be transferred to the daughter colony.

The queen reproductive system enlarges such that her abdomen becomes disproportional to the rest of the body and prevents the queen from being able to fly; in this state, she is referred to as a physogastric queen (Fig. 8). Having been mated and storing enough sperm for her lifetime of egg-laying, the queen will never leave the nest. However, if the nest is disturbed by people or animals (e.g., to collect the honey) and the colony is left exposed, the colony can die quickly because it can no longer protect itself from predators and cannot escape with its queen to a safe place. If the disturbed colony is supporting a new daughter colony, the daughter colony may also perish due the lack of support.

Within the female castes, queens and workers have different diets corresponding to the activities they perform in the colony (Crailsheim et al. 1992). Queens feed on a glandular secretion produced by nurse bees that ingest pollen and trophic eggs (Sakagami and Zucchi 1963). In contrast, foraging workers need a high-energy diet, preferring nectar or honey (Zerbo and Silva de Moraes 1996). Emerged workers in the interior of the colony consume pollen to complete their development. Larvae also need a large amount of protein. The main source of protein for adults and larvae is pollen (Peruquetti and Campos 1999). The vast majority of stingless bee species feed on nectar and pollen, but some can collect honey dew (Alves et al. 2015) or are scavengers that can feed on decaying organic matter. The genus *Lestremellita* Lestrimellita Friese, 1903 (Hymenoptera: Apidae) is specialized in stealing food from other bees and does not collect food from flowers (Ribeiro 2009).

Fig. 8. Physogastric queen of *Scaptotrigona depilis*. Picture: Cristiano Menezes.

Comparisons Between Stingless Bees and Honey Bees Regarding Pesticide Exposure Risk Assessment

Differences between stingless bees and honey bee in life history traits relevant to risk assessment are outlined in Table 1. As mentioned in previous sections, the Meliponini present important life history differences compared to honey bees and, at the same time, large variability exists among the species that comprise this tribe. For this reason, a species would need to meet several requirements in order to be a good surrogate for risk assessment: 1) be commercially reared so that sufficiently large managed populations are available; 2) be easily handled in laboratory, semifield and field conditions; and 3) show behavioral and life history traits representative of other species of the same taxonomic or ecological group. Meeting such requirements is challenging and made even harder on account of the lack of relevant data needed for the risk assessment process.

In order to select some focal species for which more data could be gathered or produced, the working group on risk assessment of pesticides to bees in Brazil carried out a bibliographical survey. This working group was constituted in 2015 to discuss important topics for the risk assessment of pesticides to bees in Brazil, and was attended by representatives of the Brazilian government, academia, and industries. Over the course of three years, the group met to discuss the needs and challenges for the implementation of a risk assessment process. In January 2017, the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA) published the Normative Instruction (NI) 02/2017 that establishes the risk assessment for pollinators in Brazil (IBAMA, 2017a). However, due to the lack of information and methods established for stingless bees, NI establishes the *A. mellifera* bee as a model organism. On the other hand, IBAMA issued a technical note with a list of information deemed necessary to assess the robustness of the process in relation to stingless bees (IBAMA, 2017b).

Considering the lack of information on which stingless bee species provide pollination services to one or more crops and also the absence of toxicity data on these species, this survey aimed to identify which non-*Apis* bee species would be present within the agricultural environment (i.e., which species commonly occur in agroecosystems), and, therefore, have an increased likelihood of direct exposure to pesticides. Data were compiled in a selection matrix built following some criteria for prioritization. The main criteria included, among other factors, the geographic distribution of the species, their occurrence, and the abundance of the bees in the crops. Table 2 summarizes the pros and cons of each species selected based on the given criteria as potential surrogates for risk assessment purposes.

Data were collected from the open literature for 40 crops, and a total of 386 non-*Apis* species were identified, including social and solitary bees. Considering only the species observed in

Table 1. Differences between stingless bees and honey bee in life history traits relevant to risk assessment

Traits	*Apis mellifera*	Stingless bees	Expected implications for risk assessment
Level of Sociality	Eusocial.	Eusocial.	—
Fecundity	Up to 1000 eggs per day.	22 per day (Meliponini) 120 per day (Trigonini) (Kerr, 1948).	Bee supply to perform first tier toxicological assays is much higher for *A. mellifera*.
Trophallaxis	Present	Present	—
Nesting substrate and materials	Large cavities. Hives. The combs are made of wax secreted by bees.	The nesting substrate is highly variable. Subterranean cavities, tree trunks, branches of living trees, rock crevices, brick walls, or occasionally in active colonies of other social insects (e.g., active or abandoned termite nests, arboreal ant nests, subterranean chambers abandoned by ants, active bird nests, or empty nests attached to branches). Nests are built with cerumen (a mixture of wax + resins), batumen (wax + mud + resins), resins, mud, soil, leaves, sticks, seeds, etc.	Several environmental matrices may be highly relevant to stingless bees but less so to *A. mellifera*. E.g., pesticide exposure via soil/mud is not relevant in *A. mellifera*, but is an important exposure route in stingless bees.
Foraging range	Mean: 1.5 km. Maximum: 10 km.	Meliponini: maximum 2.1 km.[a] Trigonini: maximum 1.5 km (Roubik and Aluja, 1983)	In *A. mellifera*, distance between test hives needs to be very large to avoid overlap of control and treatment colony foraging areas.
Amenability to nest in confined conditions	Low.	Lack of data.	—
Nesting period	All year except winter.	All year.	May impact duration of stingless bee exposure to pesticides when there are multiple crop cycles per year.
Pollen transport	On hind legs. Pollen wetted with nectar and glandular secretions.	Most species carry dry pollen on hind legs or abdomen.	
Body size	~128 mg (workers).	Highly variable depending on the species, ranging from 2–100 mg (workers).	Since exposure level and sensitivity can be body-size dependent, a possible extrapolation factor from honey bees to stingless bees should consider this large body-size variability. Stingless bees also show greater intraspecific variability.
Adult food	Nectar plus small amounts of pollen.	The amounts and identity of nectar and pollen consumed may vary widely depending on body size, natural history, and physiology. Some indications that the pollen consumption is much higher than that of *A. mellifera*. Pollen ingestion by larvae of stingless bees is highly relevant.	The pollen intake is highly relevant for meliponines, but in current risk assessment schemes, pesticide residues in nectar likely account for most of the exposures to bees, and may represent most of the potential risk concerns for adult bees instead of pollen.
Larval food	Royal jelly, bee bread and honey.	Larval food (pollen mixed with nectar and glandular secretions).	*A. mellifera* larval exposure is "filtered" by nurse bees. Larvae of stingless bees consume relatively higher amounts of pollen and, therefore, are more directly exposed to pesticides, if the pollen is contaminated. However, some pesticide degradation may be possible during the storage period.
Larval food provisioning	Progressive feeding.	Mass provisioning.	Stingless bee larvae will be exposed continuously to the same food mass.
Larval feeding period	5 d.	12 to 15 d, depending on the species.	The exposure to larval food for stingless bees is continuous and longer than that of honey bees.

four or more crops, 20 social species and 28 solitary species were identified (Pires et al. 2018). The top five species of social bees identified according to the points assigned based on the selection criteria were all species of stingless bee: *Trigona spinipes*, *Tetragonisca angustula*, *Nannotrigona testaceicornis*, *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae), and *Melipona quadrifasciata*. That the most abundant bee species surveyed in agricultural environments belong to the tribe Meliponini attests to the suitability of some of these species as potential surrogate bees in risk assessments for tropical environments.

Table 2. Pros and cons of each species selected based on the given criteria as potential surrogates for risk assessment purposes

Species	Pros	Cons
Trigona spinipes	- Wide geographic distribution in Brazilian territory; - Representative and extremely abundant (found in 32 of 40 crops, *A. mellifera* found in 36 of 40 crops); - Large number of bees (can reach 180.000 individuals per colony); - Collect different types of nest materials (mud, leaves, feces, resins).	- Lack of data on life traits; - Not available commercially, very aggressive bee; - Can pollinate effectively several important crops but may also behave in a way that damages the flowers as they search for nectar, being also considered a pest in other crops; - No methods to manage colonies in laboratory conditions; - Protocols for adult acute toxicity tests available but not standardized[a]; - No protocols for semifield or field tests.
Tetragonisca angustula	- Wide geographical distribution in Brazilian territory; - Relatively representative (found in 19 of 40 crops); - Very small bee; - Commercially available; - Easy to rear and manipulate.	- Lack of data on life history traits; - No protocols for laboratory toxicity tests nor semifield and field tests.
Nannotrigona testaceicornis	- Very small bee; - Hives available commercially; - Easy to rear and manipulate.	- Geographical distribution in northeast, southeast and south, but not in the states considered part of Amazon biome (referred as "legal Amazon"); - No methods to manage colonies in laboratory conditions; - No protocols for laboratory toxicity tests, semifield nor field tests.
Melipona quadrifasciata	- Hives commercially available but not in large scale; - Easy to rear and manipulate; - Toxicity can be tested using standardized protocols available.	- Geographical distribution in northeast, southeast and south, but not in in the states considered part of Amazon biome (referred as "legal Amazon"). - Based on scarce available toxicity data, appears to be less sensitive than *A. mellifera*.
Melipona scutellaris	- Biology well known; - Hives commercially available in a large scale; - Toxicity to adults can be tested using standardized protocols available (laboratory/field); - Easy to rear and manipulate. Based on available data appears to be more sensitive than *A. mellifera*.	- Geographical distribution restricted to Northeast. - Included in 2014 in the national list of threatened species in Brazil. - Method for larvae toxicity testing available but not standardized.

[a] Macieira and Hebling-Beraldo, 1989.

Routes of Exposure Assessment

Tables 3 and 4 summarize the relative importance of different exposure routes to adult and larval honey bee and stingless bees, according data available in the literature and information from different specialists. Exposure via air particles (dust and spray) and nectar consumption are important routes of exposure in both honey bee and stingless bee adults. However, as previously stated, honey bee consumes bee bread (aged pollen mixed with nectar) while stingless bees workers consume fresh and/or unprocessed pollen, that is present in mass provisions as a larval supply of food. The time of pollen storage in honey bee colonies may contribute to the reduction of possible residues due to their natural degradation, according to the type of molecule. In the case of stingless bees, the use of fresh or poorly processed pollen may be a much more representative route than for honey bees. Stingless bees also collect resins (Fig. 9), mud, water, and honey dew (Bohart and Nye 1956; Nogueira-Neto 1997; Velthuis et al. 1997; Roubik 2006; Freitas et al. 2007; Vollet Neto 2011; Silva et al. 2013, 2015; Limão, 2015), which can be much more important as routes of exposure for this group of bees.

The relative importance of different exposure routes to stingless bee adults and larvae is presented in Tables 5 and 6. Exposure through soil is not important for honey bees, but it is very relevant for stingless bee species that nest underground or collect soil to build mud partitions. Some stingless bee species use other materials to build their nests (e.g., resins, petals, leaves, feces, small sticks, small stones, seeds, and latex) (Nogueira Neto 1997, Roubik 2006). For these species, pesticide residues in plant surfaces are especially relevant. Beyond pesticides, stingless bees that collect feces of domesticated vertebrates may be exposed to residues of hormones and antibiotics used to treated animals according to husbandry practices. Stingless bees also may be exposed orally and by contact to residues within resins and/or soil through the use of these materials to build the brood cells and food pots. Larvae may be exposed to residues in water that is incorporated into the cell through the soil matrix. If we consider that the cells where the larvae develop are constructed with wax, mud, resin, and water, and that larval food is in contact with the larvae during all development, we find that larvae of stingless bees are exposed by multiple routes at the same time. Collected mud may contain residues of pesticides applied to soil or seed treatment, and water may contain pesticides residues applied by different routes. Pollen and nectar may contain residues from systemic pesticides. All of these possibilities should be considered when assessing whether the honey bee can represent all the diversity of stingless bees, since these routes are not considered in the current assessments.

Levels of Exposure to Pesticides

Currently, the oral exposure estimation is based on the assumption that nectar and pollen may be contaminated by agrochemical residues. For this estimate, the rates of consumption of nectar and pollen by adult bees and larvae of *A. mellifera* are taken into account. Depending on the method of application, after obtaining the amount

Table 3. Relative importance (0 to 4; low to high) of different exposure routes to adult *A. mellifera* workers and stingless bees

Route of exposure	*Apis mellifera*	Stingless bees
Air particles (dust and spray) *via contact*	Foragers: 4	Foragers: 4
	In-hive bees: 0	In-hive bees: 0
	Winter bees: 1	
Nectar *via oral*	Foragers: 4	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 2	
Pollen *via oral*	Foragers: 1	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 1	
Mud/soil *via contact*	0	Foragers: 4
		In-hive bees: 4
Honey dew *via oral*	Foragers: 4	Foragers: [a]
	In-hive bees: 2	In-hive bees: [a]
	Winter bees: 0	
Wax *via contact*	Foragers: 1	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 3	
Water *via oral*	Foragers: 4	Foragers: 4
	In-hive bees: 1	In-hive bees: 4
	Winter bees: 1	
Guttation *via oral*	Foragers: 1	Foragers: 1[a]
	In-hive bees: 1	In-hive bees: 1[a]
	Winter bees: 1	
Plant surface *via contact*	Foragers: 3	Foragers: 4
	In-hive bees: 0	In-hive bees:3
	Winter bees: 0	
Propolis/resins *via contact*	Foragers: 3	Foragers: 4
	In-hive bees: 1	In-hive bees: 4
	Winter bees: 1	

Scores are based on expert opinion and reflect relative importance within columns (species), not across rows (routes of exposure).

[a]Unknown.

of residue that may be present in pollen and nectar and multiplying this value by the rate of consumption of these matrices, it is possible to estimate how much of the substance can be ingested by the bee, which is of sum importance in risk assessments.

Data to quantify the exposure of stingless bees are relatively scarce and limited. Estimates of sugar (nectar) and protein (pollen) ingestion rates for adults of stingless bees are not available. Information on energy budgets that might be used to extrapolate sugar consumption also are lacking for these species, as is information on the number of foraging trips per day, number of flowers visited per trip, and amount of energy expended during flight.

Species of *Melipona* collect nectar loads with an average of 40% to 50% sugar content, with lower concentrations being around 20% (Roubik and Buchmann 1984, Roubik et al. 1995, Biesmeijer 1997). However, preferences in relation to concentration can vary, allowing the maintenance of more than one species in the same environment. To obtain larger metabolic earnings, it has been estimated that bees should collect nectar with sugar concentrations of approximately 60% (Roubik and Buchmann 1984). According to Roubik et al. (1995), stingless bee foragers tend to use a broad range of nectar sugar concentrations, even as low as 5% or as high as 67%. Many of the nectar loads collected by *Melipona rufiventris* foragers had values between 11% and 30% (Fidalgo and Kleinert 2010), as observed for other species of the same genus in Central Panama (Roubik and Buchmann 1984) and Costa Rica (Biesmeijer et al. 1999).

Table 4. Relative importance (0 to 4; low to high) of different exposure routes to honey bee, and stingless bees larvae

Route of exposure	*Apis mellifera*	Stingless bees
Air particles (dust and spray) *via contact*	Foragers: 4	Foragers: 4
	In-hive bees: 0	In-hive bees: 0
	Winter bees: 1	
Nectar *via oral*	Foragers: 4	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 2	
Pollen *via oral*	Foragers: 1	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 1	
Mud/soil *via contact*	0	Foragers: 4
		In-hive bees: 4
Honey dew *via oral*	Foragers: 4	Foragers: [a]
	In-hive bees: 2	In-hive bees: [a]
	Winter bees: 0	
Wax *via contact*	Foragers: 1	Foragers: 4
	In-hive bees: 3	In-hive bees: 4
	Winter bees: 3	
Water *via oral*	Foragers: 4	Foragers: 4
	In-hive bees: 1	In-hive bees: 4
	Winter bees: 1	
Guttation *via oral*	Foragers: 1	Foragers: 1[a]
	In-hive bees: 1	In-hive bees: 1[a]
	Winter bees: 1	
Plant surface *via contact*	Foragers: 3	Foragers: 4
	In-hive bees: 0	In-hive bees:3
	Winter bees: 0	
Propolis/resins *via contact*	Foragers: 3	Foragers: 4
	In-hive bees: 1	In-hive bees: 4
	Winter bees: 1	

Scores are based on expert opinion and reflect relative importance within columns (species), not across rows (routes of exposure).

Fig. 9. *Melipona melanoventer* carrying resin in its hind leg.

Some studies investigating the protein content of stingless bee diets suggest that the minimum quantity of pollen necessary for suitable development of hypopharyngeal gland and oocytes is 8 mg/bee/d for *Scaptotrigona depilis* (Fernandes-da-Silva et al. 1993). The limitation of these data is that they pertain to only one species and to the minimum amount of pollen needed, not the total amount consumed. Costa (2008) observed that workers of *Melipona flavolineata* consume about 6.13 mg of pollen per day. Again, these data were obtained for one species and cannot be taken as applicable for all the Meliponini group as a whole due the high diversity within this group.

In general, the composition of larval food is 40–60% water, 5–12% sugar, 1.1–194% protein, and 0.2–1.3% free amino acids (Hartfelder and Engels 1989). Besides the water-soluble components, 15–30% of larval food is composed of pollen (Hartfelder and Engels 1989, Menezes et al. 2007, Menezes 2010) and, for some species, up to 56% of the larval food is pollen (Rensi 2006). Table 7 summarize the available data on larval food amounts for some stingless bees species from Brazil, and Table 8 summarize the available data on larval food amounts and the proportion of pollen in the total mass.

The information on stingless bees is limited to a small number of studies, but it is apparent that the amount of larval food is highly variable among species of Meliponini, making it a difficult risk assessment component due the possibility of overestimation or underestimation of the exposure through consumption of residues in various food sources. Additionally, there are some references about water collection by stingless bees (Nogueira-Neto 1997, Roubik 2006, Jones and Oldroyd 2006, Vollet-Neto et al. 2010, Limão 2015), but there are no data on quantification of the volume of water collected. The same limitation applies to the collection of mud and resins.

Table 5. Relative importance (0 to 4; low to high) of different exposure routes to adult honey bee workers and stingless bees

Route	Air particles (dust and spray)	Nectar	Pollen	Mud/soil	Honey dew	Wax	Water	Guttation	Plant surface	Propolis/ resin
Apis mellifera oral	0	4	4	0	1	0	2	1	0	1
Apis mellifera contact	4	1	1	0	0	2	0	0	3	1
Stingless bees oral	0	4	4	3	2	0	2	0	0	3
Stingless bees contact	4	0	4	4	1	0	2	0	2	4

Scores are based on expert opinion and reflect relative importance across rows (routes of exposure), not across columns (species). Columns in orange indicate exposure routes for which the worst-case scenario of exposure currently used in honey bee risk assessment is insufficient or only partially sufficient, respectively, to evaluate potential effects on other bees.

Table 6. Relative importance (0 to 4; low to high) of different exposure routes to honey bee, and stingless bees larvae

Route	Air particles (dust and spray)	Nectar	Pollen	Mud/soil	Honey dew	Wax	Water	Guttation	Plant surface	Propolis/ resin
Apis mellifera oral	0	4	4	0	1	0	2	1	0	1
Apis mellifera contact	4	1	1	0	0	2	0	0	3	1
Stingless bees oral	0	3	4	3	2	0	2	0	0	3
Stingless bees contact	4	1	4	4	1	1	2	0[a]	2	4

[a] Unknown.

Table 7. Amount of total larval food per brood cell for some stingless bees species

Species		average volume per brood cell (in µl)	Reference
Frieseomelitta varia	Worker	26.70	Baptistella et al. 2014
	Queen	59.2	
Melipona marginata	—	38.3	Rensi, 2006
Melipona scutellaris	—	119.3	Menezes et al. 2007
Scaptotrigona depilis	Worker	32.6	Cabral, 2009
	Queen	130 .0	
Tetragonisca angustula	Worker	8.0	Prato, 2010
	Queen	51.5	

Table 8. Amount of food per brood cell and its pollen content for some stingless bees species from Brazil (Rosa et al. 2015)

Species	Amount of larval food (in mg) per cell (mean)	Weight of pollen (in mg) per cell (mean)
Plebeia droryana Friese, 1900 (Hymenoptera: Apidae)	9.4	1.3
Melipona marginata obscurior Moure, 1971 (Hymenoptera: Apidae)	49.8	6.0
Scaptotrigona bipunctata	37.3	1.9
Tetragonisca fiebrigi Schwarz, 1938 (Hymenoptera: Apidae)	10.1	0.4

Conclusions

Although the most common routes of exposure such as pollen and nectar are similar between honey bees and stingless bees, it can be concluded that some stingless bee exposure routes to pesticides are not covered by current honey bee exposure assessment paradigm. Even in the face of several gaps in knowledge about the subject, some routes are very important and research is needed on life history traits of candidate species, estimates of nectar and pollen consumption, mud, resin, and water collection and available protocols to adequately assess toxic effects of pesticides on stingless bees. It is necessary to fill in these gaps in knowledge in order to assess the extent to which honey bees can be considered as suitable surrogates for stingless bee species in the risk assessment for pesticides.

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