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Oral acute toxicity and impact of neonicotinoids on *Apis mellifera* L. and *Scaptotrigona postica* Latreille (Hymenoptera: Apidae)

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Abstract

Wild and managed bees are essential for crop pollination and food production. However, the widespread use of insecticides such as neonicotinoids may affect the survival, development, behavior, and maintenance of bee colonies. Therefore, in this study we evaluated the impacts of three neonicotinoid insecticides on the survival and walking abilities of the Africanized honeybee *A. mellifera* and stingless bee *S. postica*. *A. mellifera* was more susceptible than *S. postica* to all neonicotinoids tested. The median lethal concentrations LC_{50} values estimated for acetamiprid, imidacloprid, and thiacloprid were 189.62, 22.78, and 142.31 ng μL^{-1} of diet for *A. mellifera*, and 475.94, 89.11, and 218.21 ng μL^{-1} of diet for *S. postica*, respectively. All tested neonicotinoids affected the speed, distance traveled, duration and frequency of resting, and continuous mobility of both bee species. The results showed that in spite of the different susceptibility to compounds with cyano and nitro radicals, the behavioral variables showed different levels of commitment according to the molecule insecticide and bee species. These results contribute not only to the understanding of the effects of neonicotinoid insecticides on *A. mellifera* and *S. postica*, but also to help in the development of protocols that aim to reduce the impact of these insecticides in Neotropical environments.

Keywords Africanized honeybee · Stingless bee · Susceptibility · Motor impairment · Conservation

Introduction

Pollinators play important functional roles in most terrestrial ecosystems and provide key services for the maintenance of biodiversity in wild plant communities and agricultural production (Klein et al. 2007; Garibaldi et al. 2014). About 98% of plant species rely on animal pollination to some degree to produce fruits and seeds. This service contributes with an increase of about 75% in agricultural production with human food importance (Costanza et al. 1997; Klein et al. 2007; Garibaldi et al. 2014). Among pollinators, bees are the most efficient crop pollinators (Kremen et al. 2007; Potts et al. 2010), since they possess many morphological and behavioral adaptations that allow them to exploit different types of flowers (Thorp 1979). In turn, bees are highly dependent on floral resources (pollen, nectar, oil, resin, and essences) for their survival and reproduction (Palladini and Maron 2014).

Of the nearly 20,000 bee species described worldwide (Ascher and Pickering 2018), the honeybee *Apis mellifera* L. (Hymenoptera: Apidae) and the wild stingless bees play a key role in pollination of native and cultivated plants in Neotropical ecosystems (Imperatriz-Fonseca and Nunes-Silva 2010; Kerr et al. 2010). Despite the importance of these pollinators for maintaining biodiversity and crop production in agroecosystems, the number of bee colonies has decreased in many countries (Ellis et al. 2010; Neumann and Carreck 2010; Haddad 2011), including Brazil (Barbosa et al. 2015). Multiple factors may be associated with the decline in bee populations, including poor nutrition, increased pressure from ecto- and endoparasites, increased bacterial and/or viral loads, deforestation, fragmentation of forest areas, and intensive use of chemical pesticides on crops (Ellis et al. 2010; Johnson et al. 2010; Staveley et al. 2014).

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Among chemical pesticides, the neonicotinoid insecticides have been widely used to control pests of different crops (Jeschke et al. 2011; Goulson et al. 2015), because of their systemic action in plants, long residual activity, application versatility (e.g., foliar, soil, trunk, and seed treatment) (Elbert et al. 2008; Jeschke et al. 2011), and low toxicity to mammals (Tomizawa and Casida 2003). Neonicotinoid insecticides act as nicotinic acetylcholine receptor agonists (nAChR) in post-synaptic neurons of the central nervous system, which compete for the target site of the neurotransmitter acetylcholine (Tomizawa and Casida 2003; Johnson 2015). The linkage of the insecticide molecules with nAChR is irreversible, interfering with the transmission of nerve impulses and causing paralysis and death of insects (Elbert et al. 2008; Jeschke et al. 2011). Although neonicotinoids have been successful in controlling sucking pests such as aphids, scales, whiteflies, psyllids, they have also been considered one of the main factors associated with population declines of bees and other beneficial insects (Goulson et al. 2015; Pisa et al. 2015).

The impact of neonicotinoid insecticides on bees not only from their residual activity, but mainly from their systemic activity, where residues of the insecticide are found in significant concentrations in pollen, nectar, and guttation fluids of plants. Contact with residues and ingestion of contaminated food may affect their survival rate, development, immune system, reproduction, behavior, and colony maintenance of bees (Bonmatin et al. 2003; Goulson et al. 2015; Pisa et al. 2015; Brandt et al. 2016). Previous studies have shown that neonicotinoid insecticides are highly toxic to *A. mellifera*, causing behavioral disturbances, orientation difficulties, impairment of social activities, and mortality (Decourtye et al. 2003; Lu et al. 2014; Brandt et al. 2016; Sánchez-Bayo et al. 2017; Tadei et al. 2019). Studies have also reported the impact of insecticides on stingless bees (Costa et al. 2015; Tomé et al. 2015; Jacob et al. 2019), although few have examined the effects of neonicotinoid insecticides on *S. postica* (Soares et al. 2015; Silva et al. 2016).

Considering the importance of these pollinators to ecological relationships, crop pollination, and food production, in this study, the oral acute toxicity levels of three neonicotinoid insecticides commonly used in control of pests and their effects on the walking abilities (mean speed, travelled distance, duration, and frequency of resting time, and continuous mobility) of the Africanized honeybee *A. mellifera* and wild stingless bee *S. postica* were assessed under laboratory conditions. The results of this study will help to the understanding of the impacts of these neonicotinoid insecticides on both bees species. Furthermore, these results may also contribute to the development of future management strategies that aim to reduce the impacts of these compounds on bee populations in agroecosystems.

Material and methods

Insects

In order to estimate the mean lethal concentration (LC_{50}) (concentration needed to reduce the number of surviving individuals by 50%), with individuals of similar age, in the summer, newly emerged worker (~24 h) of *A. mellifera* and *S. postica* were obtained from combs with pupae collected in three healthy colonies in an apiary in Rio Claro, São Paulo, Brazil, and in a meliponary at the Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture/ University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil, respectively. The combs were removed and transferred to a climate-controlled room at 32 ± 2 °C for *A. mellifera* and 28 ± 2 °C for *S. postica* and 70 ± 10% relative humidity (RH), and kept in darkness, simulating the same conditions as in the colonies, until the adults emerged.

Assuming that forager bees are more susceptible to pesticides exposition summer foraging worker bees of both species were used to evaluate the changes in walking ability. For this purpose, bees were collected directly from the entrance of each of three healthy colonies, placed in plastic cages (250 mL), and maintained in the same environmental conditions described above until the assays started.

Chemicals

For both bee species, the following neonicotinoid insecticides were tested: acetamiprid (Mospilan 20%, w/w soluble powder, Iharabras S.A., Sorocaba, SP, Brazil), imidacloprid (Provado 20%, w/v concentrated suspension, Bayer CropScience Ltda., Belford Roxo, RJ, Brazil), and thiacloprid (Calypso 48%, w/v concentrated suspension, Bayer CropScience Ltda.).

Estimate of the oral mean lethal concentration (LC_{50})

The oral acute toxicity levels of newly emerged worker bees of *A. mellifera* and *S. postica* to neonicotinoid insecticides were determined using the method proposed by the OECD (1998), with some modifications. To prepare the diet, the insecticides (commercial formulations) were diluted in deionized water. Considering the concentration of active ingredient of each product, a stock solution for each insecticide was prepared and known volumes were added to the food [honey: water (1:1) enriched with 30% sucrose and 4% gelatin] to prepare diets with different insecticide concentrations. Diet without insecticides and diet contaminated with dimethoate (40%, w/v Dimexion, emulsifiable concentrate, Cheminova Brasil Ltda., São Paulo, SP, Brazil) were used as a negative and positive controls, respectively. Dimethoate is an insecticide registered by the Ministry of Agriculture, Livestock and Food Supply (MAPA 2018) and used as a reference standard for toxicological studies with bees (OECD 1998). Before the assays started, newly emerged worker bees were without food for 2 h for *A. mellifera* and 1 h for *S. postica* to ensure that they would feed when the assay started. Then, ten newly emerged workers were placed in plastic cages (250 mL) lined with filter paper, totaling three replicates for each concentration. The diet contaminated and cotton soaked with water were available ad libitum. The experimental units were kept in climate-controlled chambers at a temperature of 32 ± 2 °C for *A. mellifera* and 28 ± 2 °C for *S. postica* and 70 ± 10% RH, under darkness. The number of live and dead bees in each experimental unit was recorded 24 h after exposure. Bees were considered dead when they did not react to the touch of a fine brush. Based on the mortality data for *A. mellifera* and *S. postica* obtained from different treatment levels (concentrations), the LC_{50} was estimated for each insecticide with their respective confidence intervals.

Effect of neonicotinoid insecticides on walking abilities

For the walking behavior tests, the diet was prepared as above (item 2.3) using the median lethal concentration (LC_{50}) previously estimated for each neonicotinoid insecticide. Diet without insecticide was used as a control treatment. Three different colonies were used, and groups of 10 foragers bees were randomly collected and placed in plastic cages (250 mL) lined with filter paper. Before the tests started, the honeybee and wild stingless bees were starved for 2 and 1 h, respectively. Afterward, contaminated foods were placed for 2 and 1 h to *A. mellifera* and *S. postica*, respectively. During this period, the experimental units were kept in climate-controlled chambers with a temperature of 28 ± 2 °C and 70 ± 10% RH, in darkness.

After the feeding period, the bees were anesthetized at low temperature for 5 min, to prevent escapes during transfer to a Petri dishes (150 mm diameter × 15 mm in height) lined with filter paper. After acclimatization of bees (5 min) in Petri dishes, the videos were started, with a digital camera coupled to a computer equipped with video-tracking software (EthoVision XT - Noldus Information Technology Inc., Wageningen, Netherlands). Each Petri dish was recorded for 5 min. The assays were performed at 25 ± 2 °C under artificial fluorescent light, in the morning.

The assay was performed followed a randomized block design. Each block was composed of six videos (three videos were made in treatment and three videos were performed in control). In each Petri dish, ten bees were placed. For each treatment, ten replicates (blocks) were used (n = 300). Subsequently, the videos were submitted to analysis using a video-tracking software to assessment of mean speed (cm s^{-1}), total traveled distance (cm) through the arena, duration (s) and frequency (n) of resting time (total stopped period throughout the 5 min of video recording), and continuous mobility.

Data analysis

In order to estimate the median lethal concentrations of neonicotinoid insecticides for each bees species, a binomial model with a complementary log-log link function (gompit model) using the Probit procedure of the software SAS version 9.2 (SAS Institute 2011) was used. For the analyses of the effects of neonicotinoid insecticides on walking abilities of bees, Bayesian models with 30 thousand iterations applying the Monte Carlo method and Markov chains (MCMC), with three chains for each variable and a *burn-in* of 5000 samples were used. The convergences of the chains were determined by graphical analysis (not shown). For the present study, normal models with normal priors were used. All analyses were performed using the statistical software "*R*", version 3.5.1 (R Core Team 2018) with the R2Openbugs package.

The data for mean speed, traveled distance, duration and frequency of rests did not show normality and homogeneity of variance, and therefore, they were transformed using the *box-cox* function of the "*mass*" package. Lambda values were extracted that maximized the function and were substituted in the formula: $y^T = y^\lambda - 1/\lambda$ (Box and Cox 1964). The lambda values used are provided in the tables. There was no need for transformation of the continuous mobility data.

Results

Estimate of the oral mean lethal concentration (LC_{50})

Our results showed that the neonicotinoid insecticides caused significant mortality of newly emerged workers of *A. mellifera* and *S. postica*. The toxicity levels were dependent on the bee species, insecticide, and concentration used in the assays (Table 1). *A. mellifera* was 2.51, 3.91, and 1.53-fold more susceptible than *S. postica* to acetamiprid, imidacloprid, and thiacloprid, respectively. Based on the estimated median lethal concentrations, acetamiprid was the least harmful, followed by thiacloprid, and imidacloprid for both bees species. Dimethoate insecticide used as a positive control caused high acute toxicity (Table 1).

Table 1 Estimated mean oral lethal concentration (LC_{50} in ng a.i. μL^{-1}) of insecticides for newly emerged Africanized honeybee *Apis mellifera* and stingless bee *Scaptotrigona postica*, 24 h after exposure

Insecticide	Bee species	N[a]	LC_{50} (CI 95%)[b]	Slope (±SE) (*P*-value)	χ^2 (d.f.)[c]	h[d]	SR[e]
Acetamiprid	*A. mellifera*	323	189.62 (107.38–256.58)	2.62 (±0.39) (<0.0001)	6.78 (6)	1.13	2.51
	S. postica	595	475.94 (406.65–546.03)	2.43 (±0.25) (<0.0001)	4.73 (6)	0.79	
Imidacloprid	*A. mellifera*	360	22.78 (14.58–31.15)	1.63 (±0.20) (<0.0001)	11.34 (7)	1.62	3.91
	S. postica	310	89.11 (63.14–117.74)	1.91 (±0.51) (<0.0001)	8.77 (6)	1.46	
Thiacloprid	*A. mellifera*	324	142.31 (101.31–194.05)	2.38 (±0.40) (<0.0001)	8.71 (6)	1.45	1.53
	S. postica	354	218.21 (159.07–276.21)	2.50 (±0.32) (<0.0001)	9.32 (6)	1.55	
Dimethoate	*A. mellifera*	310	0.75 (0.61–0.81)	5.94 (±1.23) (<0.0001)	4.93 (6)	0.82	90.03
	S. postica	340	67.52 (59.75–81.94)	5.70 (±0.79) (<0.0001)	9.57 (6)	1.59	

[a]N number of bees tested

[b]CI confidence interval at 95% probability of error

[c]χ^2 Pearson's chi-squared value and degrees of freedom

[d]h heterogeneity factor

[e]SR susceptibility ratio [LC_{50} *Scaptotrigona postica* (+tolerant)/LC_{50} *Apis mellifera* (−tolerant)]

Effect of neonicotinoid insecticides on walking abilities

Oral exposure of bees to the lethal concentrations (LC_{50}) of most of the neonicotinoids did not kill the bees during the experimental period. For both bee species, the mean speed decreased after exposure to all insecticides tested. Acetamiprid had the least impact on *A. mellifera*, with mean speed values similar to the group of control bees (1.39 ± 0.09 cm s^{-1}). On the other hand, imidacloprid caused the highest reduction in the mean speed (~2-fold) compared to the control group (Fig. 1a), and thiacloprid showed intermediate values to this behavioral variable. However, exposure of *S. postica* to neonicotinoid insecticides showed the greatest changes in behavioral variables than Africanized honeybee. For *S. postica*, the lowest impact (~3-fold) was observed in worker bees treated with thiacloprid, whereas imidacloprid caused the highest impairment of wild stingless bees (~5.5-fold) in relation to the control group (Fig. 2a). There was a decrease in the traveled distance from exposed bees, and there was no similarity between the results obtained for all neonicotinoids compared to the control group for both bees species (Figs 1b and 2b). Acetamiprid and thiacloprid had the lowest impacts on the locomotion of *A. mellifera* and *S. postica*, while imidacloprid was the insecticide that most affected the walking behavior of the bees.

The results showed a pronounced increase in the resting time of both bees species. For *A. mellifera*, similar results were obtained in bees treated with acetamiprid and control bees on resting time (Fig. 1c). *S. postica* demonstrated high changes in resting time after exposure to acetamiprid (7.8-fold), thiacloprid (8.5-fold), and imidacloprid (9.0-fold) neonicotinoids compared to control bees (Fig. 2c).

All neonicotinoids tested caused a decrease in the frequency that the bees stopped for resting, considering the assessment time of 5 min. Acetamiprid showed similar results to the control group, while imidacloprid was the most harmful to *A. mellifera*. The results for *S. postica* showed a divergence between neonicotinoid insecticides and control. Thiacloprid was the least harmful and imidacloprid the compound that caused the highest impact, with a decrease of ~8.0-fold in the number of resting stops compared to control.

Continuous mobility was used to quantify the movement of an insect, even if its central point remains in the same place (e.g. tremors), so any movement resulting from the neurotoxic effects of the neonicotinoids, prior to the paralysis effect, were detected by the software and considered as a behavioral variable. The results indicate that *A. mellifera* exposure to acetamiprid presented a similarity to the control bees. However, imidacloprid and thiacloprid caused the largest negative effects on *A. mellifera* (Fig. 1d). For *S. postica*, thiacloprid provided a reduced impact on continuous mobility (Fig. 2d).

Discussion

This study showed considerable effects of oral toxicity of three neonicotinoid insecticides that are commonly used to control of insect pests in many agricultural production systems on *A. mellifera* and *S. postica* newly emerged worker bees and their effects on walking abilities of worker forager bees. The results reveal the different levels of toxicity between the groups of neonicotinoids and between bee species tested. Acetamiprid and thiacloprid were less toxic to bees, they contain the N-cyano-amidine radical,

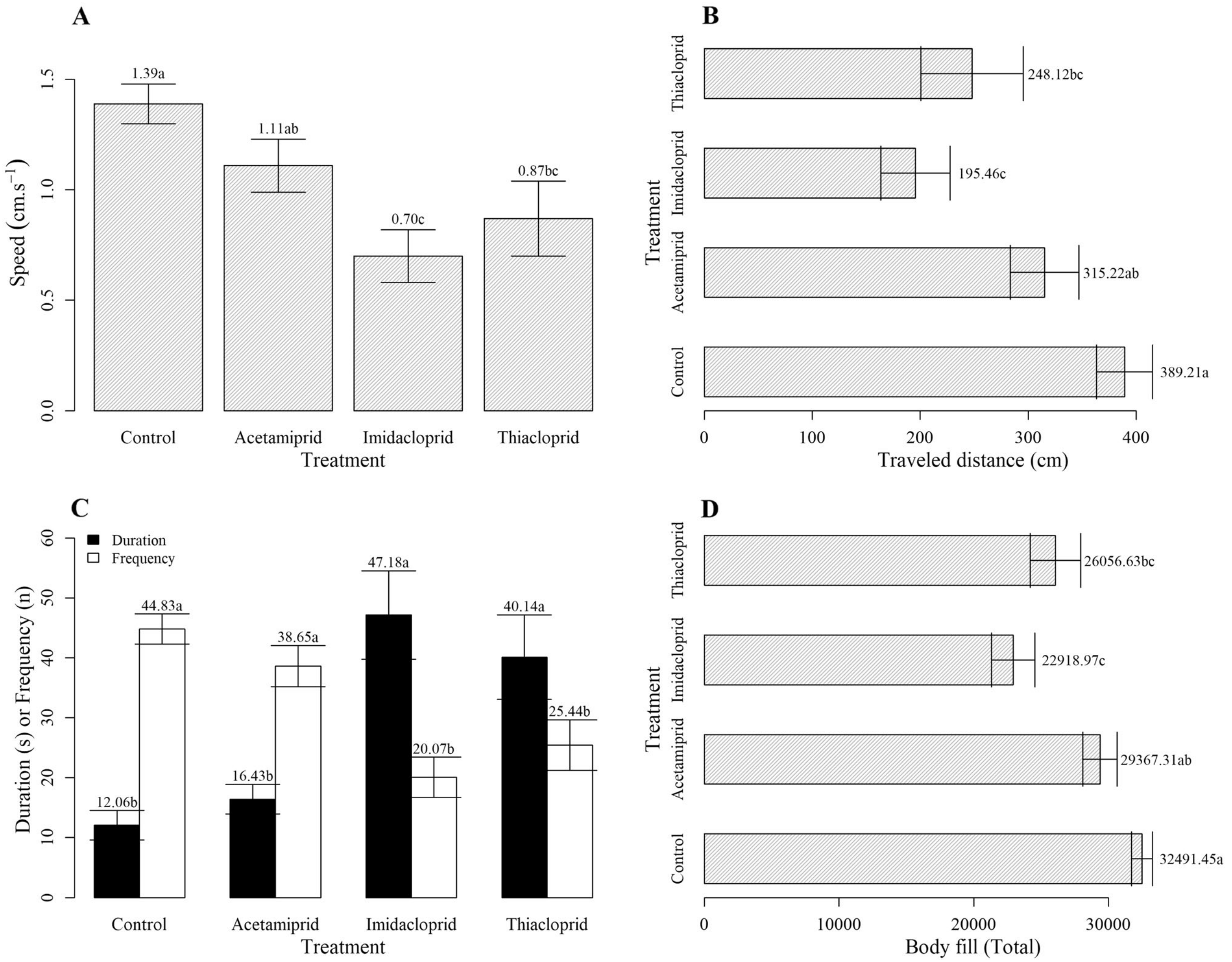

Fig. 1 Evaluation of walking abilities after oral treatment of workers foragers of Africanized honeybees *Apis mellifera* with neonicotinoid insecticides. **a** Speed, **b** Traveled distance, **c** Duration and Frequency of resting time and **d** Continuous mobility. Means followed by the same letters evidence that there is no statistically significant difference between the groups (insecticides) based on Bayesian credibility intervals at 95% (95% CrI)

which confers lower toxicity (Iwasa et al. 2004; Elbert et al. 2008; Tison et al. 2017) and those with radical N-nitroguanidine (imidacloprid), which is highly toxic to non-target insects (Elbert et al. 2008; Decourtye and Devillers 2010). Thus, acetamiprid and thiacloprid had the highest LC_{50} values for *A. mellifera* and *S. postica*, indicating that these insecticides are less harmful to these pollinators than imidacloprid, that showed the lowest LC_{50} values.

Although the information on the oral acute toxicity of neonicotinoids to bee species (especially non-*Apis* species) is sparse, the results agree with those obtained by Jacob et al. (2019) in evaluating the acute oral toxicity of the stingless bee *Tetragonisca angustula* (Latreille) to neonicotinoid insecticides. In this study, the authors found lower toxicity to acetamiprid, followed by thiacloprid and imidacloprid, as well as in topical exposure studies confirming this variation in susceptibility levels among these three neonicotinoids (Iwasa et al. 2004; Valdovinos-Núñez et al. 2009; Decourty and Devillers 2010).

In the present study, the oral toxicity levels of insecticides were higher for *A. mellifera* than *S. postica*. Differences in susceptibility levels between bee species have been reported in other studies, which were combined in a meta-analysis by Arena and Sgolastra (2014). These authors reported that of the six classes of insecticides assessed, non-*Apis* bee species were less tolerant than honeybees only to neonicotinoid insecticides. These results suggest that a difference in susceptibility may be dependent on the intrinsic sensitivity of bee species as well as on their nesting activity and foraging behavior, especially for non-*Apis* species, for which such information is sparse.

Different factors may affect variations in the susceptibility of bees to the neonicotinoid insecticides. In general,

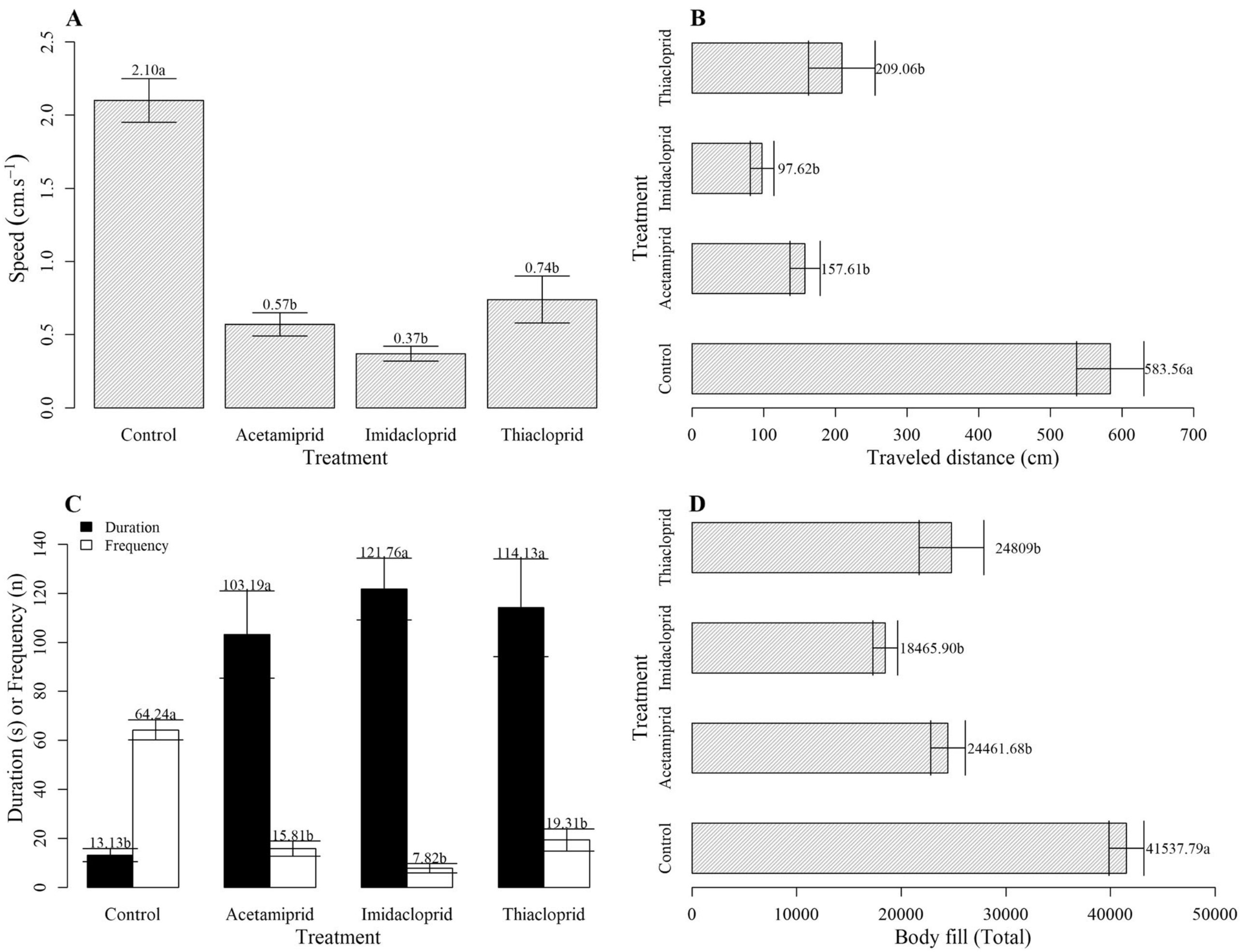

Fig. 2 Evaluation of walking abilities after oral treatment of workers foragers of stingless bees *Scaptotrigona postica* with neonicotinoid insecticides. **a** Speed, **b** Traveled distance, **c** Duration and Frequency of resting time and **d** Continuous mobility. Means followed by the same letters evidence that there is no statistically significant difference between the groups (insecticides) based on Bayesian credibility intervals at 95% (95% CrI)

bee species with a larger body size tend to tolerate higher amounts of insecticides (Devillers et al. 2003; Thompson 2016). However, in this study, the smaller *S. postica* proved to be more tolerant of the three neonicotinoid insecticides than *A. mellifera*. Similar results were reported by Yue et al. (2018) for *A. cerana*, which despite its lower body weight, showed higher tolerance than *A. mellifera* to two of the three neonicotinoids assessed. According to these authors, physiological and genetic characteristics may be responsible for the differences in sensitivity between the species. Furthermore, the time needed to metabolize compounds (Tomizawa et al. 1995; Iwasa et al. 2004; Johnson 2015) and the binding affinity between neonicotinoid molecules and/or their metabolites with the nAChR subtypes can also be involved in differential toxicity of neonicotinoids (Nauen et al. 2001; Decourtye et al. 2003; Casida 2011).

Brunet et al. (2005) observed the rapid metabolism of acetamiprid after oral exposure of *A. mellifera* bees and suggested that the relatively high tolerance of honeybees to acetamiprid was due to the metabolization of this insecticide into metabolites of low toxicity to bees. Dai et al. (2010) demonstrated that the fungus *Rhodotorula mucilaginosa* (Jörg.) Harr. converts acetamiprid into metabolites with no insecticidal activity and converts thiacloprid into metabolites with low toxicity to *Aphis craccivora* Koch (Hemiptera: Aphididae). The low toxicity of these insecticides suggests that bees have an innate ability to metabolize neonicotinoid with the N-cyano radical (Alptekin et al. 2016).

Manjon et al. (2018) reported that the large differences in toxicity levels between groups of insecticides with cyano- or nitro- radicals are related to the ability of five cytochrome P450 enzyme subfamilies to degrade acetamiprid and thiacloprid molecules more efficiently than imidacloprid and thiamethoxam. These enzymes are highly expressed during the detoxification process, ensuring rapid and efficient metabolization of acetamiprid and thiacloprid into compounds of low toxicity to *A. mellifera* and *Bombus terrestris* L. (Hymenoptera: Apidae). However, further studies are needed to assess the factors that are involved in the interspecific susceptibility levels.

In addition to oral acute toxicity, the neonicotinoid insecticides affected, in different degrees, the walking abilities of *A. mellifera* and *S. postica*. Of the insecticides tested, acetamiprid provided the lowest impact on all *A. mellifera* locomotor variables assessed. However, for *S. postica*, acetamiprid affected all behavioral variables of bees, as well as thiacloprid which caused similar effect to imidacloprid for all walking abilities of two bee species. Despite treating a stingless species, the oral exposure of *T. angustula* to the LC_{50} (173.26 ng μL^{-1}) of acetamiprid caused reduction only on the distance traveled by bees in Petri dishes, while the exposure to thiacloprid (LC_{50} = 54.09 ng μL^{-1}) had its results similar to imidacloprid in all evaluated variables (Jacob et al. 2019). No significant effect on walking abilities was observed to honeybee *A. mellifera* orally contaminated with 0.1, 0.5, and 1.0 μg bee^{-1} of acetamiprid (El Hassani et al. 2008; Aliouane et al. 2009). The detrimental effect of thiacloprid was previously reported by Fisher et al. (2014), that reported reduced flight velocity of *A. mellifera* orally exposed to thiacloprid (1.25 mg bee^{-1}). According to the authors, sublethal doses of thiacloprid, imidacloprid (11.25 ng bee^{-1}) and clothianidin (2.5 ng bee^{-1}) impaired navigation and return capacity for the colony, as well as Tison et al. (2016), where honeybees chronically exposed to sublethal concentrations of 4.5 μg mL^{-1} showed changes in foraging ability, social communication performance, navigation, and homing. Besides caused learning and memory functions disrupting (Tison et al. 2017). These results confirm that the effects of pesticides may be specific to a particular bee species (Sánchez-Bayo et al. 2017), and also related to the active ingredient of each neonicotinoid and dose used (Fischer et al. 2014; Williamson et al. 2014).

Imidacloprid caused significant impairment of walking abilities in relation to the untreated bee group, in this study. Similar results were reported for sublethal doses of imidacloprid at topical exposure (50 and 500 ng mL^{-1}) where *A. mellifera* walked shorter distances and spent more time in the feeding area. In addition, a shorter interaction time between individuals compared to the group of bees that received only sucrose was observed. In contrast, low concentrations of imidacloprid (0.05, 0.5, and 5 ng mL^{-1}) resulted in a stimulus of locomotor activity (Teeters et al. 2012). The increased locomotor activity of *A. mellifera* was also reported by Lambin et al. (2001) after topical exposure of 1.25 ng bee^{-1} imidacloprid and decrease in mobility after exposure to concentrations above 5 ng bee^{-1}. Williamson et al. (2014) found no significant behavioral changes, including walking, flying and sitting of *A. mellifera* after exposure to imidacloprid (2.56 ng mL^{-1}), thiamethoxam (2.92 ng mL^{-1}), or clothianidin (2.50 ng mL^{-1}), they reported only a loss of posture control. Intake of diets contaminated with imidacloprid in doses ranging from 0.08 to 125.0 g L^{-1} did not cause detectable changes in the rate of feed, locomotion, and longevity of *A. mellifera*, whereas for *Bombus terrestris* L. the feeding rate and locomotion were reduced when exposed to concentrations above 10 μg L^{-1}. In this study, the authors suggested that the greater tolerance of *A. mellifera* is due to the higher metabolic detoxification level of this species compared to *B. terrestris* (Cresswell et al. 2012). Oral exposure of *A. mellifera jemenitica* Ruttner to sublethal doses of imidacloprid (0.1, 0.5, and 1.0 ng bee^{-1}) caused high levels of learning impairment and memory retention, with a reduction in proboscis extension responses according to the dose used (Iqbal et al. 2018). These results agree with previous studies of exposure of *A. mellifera* to different doses of imidacloprid (Decourtye et al. 2003; Gonalons and Farina 2015; Tan et al. 2015).

The treatment of larvae of the stingless bee *Melipona quadrifasciata anthidioides* Lepeletier with different concentrations of imidacloprid compromised the development of the mushroom bodies, the brain region responsible for memory and learning, and also impaired the walking activity of adults emerged from treated larvae (Tomé et al. 2012; Çakmak et al. 2018). Similar results were verified by Tan et al. (2015) when *A. cerana* larvae were exposed to imidacloprid (0.24 ng bee^{-1}), occurring impairment of olfactory learning in adult bees, with a reduction of about 4.8-fold in the capacity of short memory acquisition compared to the control group. However, the oral exposure of adult bees (0.1 ng bee^{-1}) caused a reduction of approximately 1.6-fold in the capacity of short memory acquisition. According to Peng and Yang (2016), the exposure of bees in the larval stage harm neural development, with a decrease in the cell density of the mushroom body's region, reducing the olfactory learning capacity of adult bees.

Loss of posture control, tremors, and uncoordinated and disoriented movements were observed in the present study. Motor impairment has been commonly observed after the neonicotinoid exposure, but the intensity of changes is dependent on the dose (Lambin et al. 2001; Williamson et al. 2014; Sánchez-Bayo et al. 2017). Impairment on learning, memory, foraging, and homing performances may depend on the dose, route and exposure time of compounds of same chemical group (Karahan et al. 2015; Alkassab and Kirchner 2017; Pisa et al. 2017).

The effects of neonicotinoid insecticides are complex, since they have a different affinity to the various nAChR subtypes, and are partial agonists or superagonists of the nicotinic cholinergic receptors (Déglise et al. 2002; Brown et al. 2006). However, regardless of the degree, impairment of walking abilities can affect various activities performed by bees, including care of offspring, production of cells, and removal of debris, besides interfering with complex activities such as foraging, memory, and learning. These functions are important for the development, organization, and survival of the entire colony. Thus, the impairment of these abilities can reduce the pollination services provided by bees (Tomé et al. 2012; Tan et al. 2015; Alkassab and Kirchner 2017; Pisa et al. 2017).

In conclusion, the present study demonstrated the greater sensitivity of the Africanized honeybee *A. mellifera* in relation to the stingless bee *S. postica* to three neonicotinoid insecticides. Of the neonicotinoids tested, imidacloprid was considered highly harmful to both bee species, whereas acetamiprid and thiacloprid caused lower impact to the pollinators. Even so, they should be applied with caution, since high doses of these insecticides may affect the behavior of Africanized honeybees and stingless bees, compromising pollination services and food production in agroecosystems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The authors agree with the publication of the manuscript in this form.

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References

Aliouane Y, Hassani AKE, Gary V, Armengaud C, Lambin M, Gauthier M (2009) Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. Environ Toxicol Chem 28:113–122

Alkassab AT, Kirchner WH (2017) Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees, and solitary bees. J Plant Dis Protect 124:1–30

Alptekin S, Bass C, Nicholls C, Paine MJ, Clark SJ, Field L, Moores GD (2016) Induced thiacloprid insensitivity in honeybees (*Apis mellifera* L.) is associated with up-regulation of detoxification genes. Insect Mol Biol 25:171–180

Arena M, Sgolastra F (2014) A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology 23:324–334

Ascher JS, Pickering J (2018) Discover life: bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). http://www.discoverlife.org/mp/20q?guide=Apoidea_species&flags=HAS. Accessed 13 Feb 2018

Barbosa WF, Smagghe G, Guedes RNC (2015) Pesticides and reduced-risk insecticides, native bees and pantropical stingless bees: pitfalls and perspectives. Pest Manag Sci 71:1049–1053

Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME, Bengsch ER (2003) A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. Anal Chem 75:2027–2033

Box GEP, Cox DR (1964) An analysis of transformations. J R Soc 26:211–252

Brandt A, Gorenflo A, Siede R, Meixner M, Büchler R (2016) The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). J Insect Physiol 86:40–47

Brown LA, Ihara M, Buckingham SD, Matsuda K, Sattelle DB (2006) Neonicotinoid insecticides display partial and super agonist actions on native insect nicotinic acetylcholine receptors. J Neurochem 99:608–615

Brunet JL, Badiou A, Belzunces LP (2005) In vivo metabolic fate of [^{14}C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. Pest Manag Sci 61:742–748

Çakmak İ, Hranitz JM, Blatzheim L, Bower CD, Polk T, Levinson B, Wells H (2018) Effects of thiamethoxam on the behavior of foraging honey bees with artificial flower choices. Bee J 18:2–13

Casida JE (2011) Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. J Agric Food Chem 59:2923–2931

Costa LM, Grella TC, Barbosa RA, Malaspina O, Nocelli RCF (2015) Determination of acute lethal doses (LD_{50} and LC_{50}) of imidacloprid for the native bee *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae). Sociobiology 62:578–582

Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260

Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, Ibarra NH, Smirnoff N, Tyler CR (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). Zoology 115:365–371

Dai YJ, Ji WW, Chen T, Zhang WJ, Liu ZH, Ge F, Yuan S (2010) Metabolism of the neonicotinoid insecticides acetamiprid and thiacloprid by the yeast *Rhodotorula mucilaginosa* strain IM-2. J Agric Food Chem 58:2419–2425

Decourtye A, Devillers J (2010) In: Tany SH (ed) Ecotoxicity of neonicotinoid insecticides to bees, in Insect nicotinic acetylcholine receptors. Springer, New York, NY, pp 85–95

Decourtye A, Lacassie E, Pham-Delègue MH (2003) Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. Pest Manag Sci 59:269–278

Déglise P, Grünewald B, Gauthier M (2002) The insecticide imidacloprid is a partial agonist of the nicotinic receptor of honeybee Kenyon cells. Neurosci Lett 321:13–16

Devillers J, Decourtye A, Budzinski H, Pham-Delegue MH, Cluzeau S, Maurin G (2003) Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. SAR QSAR Environ Res 14:389–403

El Hassani AK, Dacher M, Gary V, Lambin M, Gauthier M, Armengaud C (2008) Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). Arch Environ Contam Toxicol 54:653–661

Elbert A, Haas M, Springer B, Thielert W, Nauen R (2008) Applied aspects of neonicotinoid use in crop protection. Pest Manag Sci 64:1099–1105

Ellis JD, Evans JD, Pettis J (2010) Colony losses, managed colony population decline, and colony collapse disorder in the United States. J Apic Res 49:134–136

Fischer J, Mueller T, Spatz AK, Greggers U, Gruenewald B, Menzel R (2014) Neonicotinoids interfere with specific components of navigation in honeybees. PloS ONE 9:e91364

Garibaldi LA, Carvalheiro LG, Leonhardt SD, Aizen MA, Blaauw BR, Isaacs R, Kuhlmann M, Kleijn D, Klein AM, Kremen C, Morandin L, Scheper J, Winfree R (2014) From research to action: enhancing crop yield through wild pollinators. Front Ecol Environ 12:439–447

Gonalons C, Farina WM (2015) Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. PloS ONE 10: e0140814

Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957

Haddad N (2011) Honey bee viruses, diseases and hive management in the Middle East and their relation to the colony collapse disorder and bee losses. Uludag Bee J 11:17–24

Imperatriz-Fonseca VL, Nunes-Silva P (2010) As abelhas, os serviços ecossistêmicos e o Código Florestal Brasileiro. Biota Neotrop 10:59–62

Iqbal J, Alqarni AS, Raweh HSA (2018) Effect of sub-lethal doses of imidacloprid on learning and memory formation of indigenous Arabian bee (*Apis mellifera jemenitica Ruttner*) adult foragers. Neotrop Entomol 48:1–8

Iwasa T, Motoyama N, Ambrose JT, Roe RM (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot 23:371–378

Jacob CRO, Zanardi OZ, Malaquias JB, Silva CAS, Yamamoto PT (2019) The impact of four widely used neonicotinoid insecticides on *Tetragonisca angustula* (Latreille) (Hymenoptera: Apidae). Chemosphere 224:65–70

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. J Agric Food Chem 59:2897–2908

Johnson RM (2015) Honey bee toxicology. Annu Rev Entomol 60:415–434

Johnson RM, Ellis MD, Mullin CA, Frazier M (2010) Pesticides and honey bee toxicity—USA. Apidologie 41:312–331

Karahan A, Çakmak I, Hranitz JM, Karaca I, Wells H (2015) Sublethal imidacloprid effects on honey bee flower choices when foraging. Ecotoxicology 24:2017–2025

Kerr WE, Carvalho GA, Silva AC, Assis MGP (2010) Aspectos pouco mencionados da biodiversidade amazônica. Parcerias Estratégicas 12:20–41

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc B 274:303–313

Kremen C, Williams NM, Aizen MA, Gemmill-Herren B, LeBuhn G, Minckley R, Packer L, Potts SG, Roulston T, Steffan-Dewenter I, Vázquez DP, Winfree R, Adams L, Crone EE, Greenleaf SS, Keitt TH, Klein AM, Regetz J, Ricketts TH (2007) Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol Lett 10:299–314

Lambin M, Armengaud C, Raymond S, Gauthier M (2001) Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch Insect Biochem Physiol 48:129–134

Lu C, Warchol KM, Callahan RA (2014) Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. Bull Insectol 67:125–130

Manjon C, Troczka BJ, Zaworra M, Beadle K, Randall E, Hertlein G, Singh KS, Zimmer CT, Homem RA, Lueke B, Reid R, Kor L, Kohler M, Benting J, Williamson MS, Davies TGE, Field LM, Bass C, Nauen R (2018) Unraveling the molecular determinants of bee sensitivity to neonicotinoid insecticides. Curr Biol 28:1137–1143

MAPA, Ministério da Agricultura, Pecuária e Abastecimento (2018) AGROFIT: sistema de Agrotóxicos Fitossanitários. MAPA/CGAF/DFIA/DAS, Brasília, Brazil. http://www.extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Manag Sci 57:577–586

Neumann P, Carreck NL (2010) Honey bee colony losses. J Apic Res 49:1–6

OECD (Organization for Economic Co-operation and Development). Guidelin es for testing of chemicals Number 214 (1998) Honeybees, acute oral toxicity test. Environmental health safety division, Organization for Economic Co-operation and Development, Paris, France

Palladini JD, Maron JL (2014) Reproduction and survival of a solitary bee along native and exotic floral resource gradients. Oecologia 176:789–798

Peng YC, Yang EC (2016) Sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies. Sci Rep 6:19298

Pisa L, Goulson D, Yang EC, Gibbons D, Sánchez-Bayo F, Mitchell E, Aebi A, van der Sluijs J, MacQuarrie CJK, Giorio C, Long EY, McField M, van Lexmond MB, Bonmatin JM (2017) An update of the worldwide integrated assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. Environ Sci Pollut Res 1–49. https://doi.org/10.1007/s1135

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, Morrissey CA, Noome DA, Settele J, Simon-Delso N, Stark JD, Van der Sluijs JP, Van Dyck H, Wiemers M (2015) Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res Int 22:68–102

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol 25:345–353

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Sánchez-Bayo F, Belzunces L, Bonmatin JM (2017) Lethal and sublethal effects, and incomplete clearance of ingested imidacloprid in honey bees (*Apis mellifera*). Ecotoxicology 26:1199–1206

SAS Institute (2011) Statistical analysis system: getting started with the SAS learning. Version 9.2. SAS Institute, Cary

Silva MB, Nocelli RCF, Soares HM, Malaspina O (2016) Efeitos do imidacloprido sobre o comportamento das abelhas *Scaptotrigona postica* Latreille, 1807 (Hymenoptera, Apidae). Ciênc Tecnol Ambient 3:21–28

Simon-Delso N, Stark JD, van der Sluijs JP, van Dyck H, Wiemers M (2015) Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res 22:68–102

Soares HM, Jacob CRO, Carvalho SM, Nocelli RCF, Malaspina O (2015) Toxicity of imidacloprid to the stingless bee *Scaptotrigona postica* Latreille, 1807 (Hymenoptera: Apidae). Bull Environ Contam Toxicol 94:675–680

Staveley JP, Law SA, Fairbrother A, Menzie CA (2014) A causal analysis of observed declines in managed honey bees (*Apis mellifera*). Hum Ecol Risk Assess 20:566–591

Tadei R, Domingues CEC, Malaquias JB, Camilo EV, Malaspina O, Silva-Zacarin ECM (2019) Late effect of larval co-exposure to the insecticide clothianidin and fungicide pyraclostrobin in Africanized *Apis mellifera*. Sci Rep 9:3277

Tan K, Chen W, Dong S, Liu X, Wang Y, Nieh JC (2015) A neonicotinoid impairs olfactory learning in Asian honey bees (*Apis cerana*) exposed as larvae or as adults. Sci Rep 5:10989

Teeters BS, Johnson RM, Ellis MD, Siegfried BD (2012) Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.). Environ Toxicol Chem 31:1349–1354

Thompson H (2016) Extrapolation of acute toxicity across bee species. Integr Environ Assess Manag 12:622–626

Thorp RW (1979) Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. Ann Mo Bot Gard 66:788–812

Tison L, Hahn ML, Holtz S, Rößner A, Greggers U, Bischoff G, Menzel R (2016) Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. Environ Sci Technol 50:7218–7227

Tison L, Holtz S, Adeoye A, Kalkan Ö, Irmisch NS, Lehmann N, Menzel R (2017) Effects of sublethal doses of thiacloprid and its formulation Calypso® on the learning and memory performance of honey bees. J Exp Biol 220:3695–3705

Tomé HVV, Barbosa WF, Corrêa AS, Gontijo LM, Martins GF, Guedes RNC (2015) Reduced-risk insecticides in Neotropical stingless bee species: impact on survival and activity. Ann Appl Biol 167:186–196

Tomé HVV, Martins GF, Lima MAP, Campos LAO, Guedes RNC (2012) Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata anthidioides*. PLoS ONE 7:e38406

Tomizawa M, Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol 48:339–364

Tomizawa M, Otsuka H, Miyamoto T, Eldefrawi ME, Yamamoto I (1995) Pharmacological characteristics of insect nicotinic ion channel and the comparison of the effect of nicotinoids and neonicotinoids. J Pestic Sci 20:57–64

Valdovinos-Núñez GR, Quezada-Euán JJG, Ancona-Xiu P, Moo-Valle H, Carmona A, Ruiz Sánchez E (2009) Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini). J Econ Entomol 102:1737–1742

Williamson SM, Willis SJ, Wright GA (2014) Exposure to neonicotinoids influences the motor function of adult worker honeybees. Ecotoxicology 23:1409–1418

Yue M, Luo S, Liu J, Wu J (2018) *Apis cerana* is less sensitive to most neonicotinoids, despite of their smaller body mass. J Econ Entomol 111:39–42