Effects of the Neonicotinoid Imidacloprid on the Feeding Behavior of *Melipona quadrifasciata anthidioides* Lep.

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Abstract

Even though meliponines are among the greatest pollinating agents in Neotropical ecosystems, the populations of these bees are in decline due to anthropic actions, such as the indiscriminate use of pesticides. Neonicotinoids, such as Imidacloprid, are used in many crops and have been shown to be one of the pesticides groups with the most damaging effects on the health of bee colonies. The goals of this work were (1) to evaluate the ability of *Melipona quadrifasciata*, a native Brazilian stingless bee species, to detect the presence of Imidacloprid in artificial diets, and (2) to evaluate how this insecticide may affect their behavior. For this purpose, behavioral experiments were carried out in observation arenas where artificial flowers containing different concentrations of Imidacloprid (0%, 1%, 5%, 10%, and 20%) were made available to forager bees and their choice and behavior were registered. Our results showed that bee choices were not affected by the different concentrations of Imidacloprid, even at extremely high doses in the flowers (20%). It was also found that the presence of this insecticide interfered with the communication between individuals as a result of reduced antennation events. Both findings, the apparent inability of bees to detect the presence of the insecticide and the change in behavior caused by the exposition to the volatilized product, may rapidly lead to colony losses in nature.

Keywords: Meliponini. Stingless bee. Mandaçaia. Insecticide. Antennation. Foraging.

Introduction

Seventy-five percent of all known meliponine species are found in the Neotropical region (COSTA et al., 2003). Also known as stingless bees, meliponine are considered the most important pollinators in many Neotropical ecosystems (WITTER; NUNES-SILVA, 2014). Among stingless bees, "Mandaçaia" *Melipona quadrifasciata* Lepeletier (1836) are natives to the Brazilian states of Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Rio de Janeiro, Santa Catarina and São Paulo, but they also appear in some regions of Argentina and Paraguay. This species stands out from other stingless bees due to its long daily foraging period, starting right after sunrise and stopping just before sunset (AIDAR, 2011), which allows a large number of floral visits.

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Pollination mediated by insects is considered a key factor for the balance of natural ecosystems, since most plants depend on insect pollination to produce fruits and seeds (VILLAS-BÔAS, 2012). Pollinators are also considered essential in agriculture and in environmental conservation, and they are responsible for the reproduction of 40% to 90% of native plant species (IMPERATRIZ-FONSECA et al., 2012; NOGUEIRA-NETO, 1997). Venturieri et al. (2012) suggest that the rearing of meliponine is an important alternative to the intense and constant rearing of *Apis mellifera* Linnaeus 1758, in particular, for helping with the pollination of native species and plants of economic importance, such as eggplant, pepper, pumpkin, strawberry, bell pepper, tomato, guarana, sunflower, and annatto. Because great part of food productivity is directly associated with pollination service by bees, the conservation of these insects has become even more relevant, especially when considering that the demand for food should increase as the human population size is expected to reach 9 billion by 2050 (FIGUEROA- MATA et al., 2016). Therefore, scientific studies that seek to investigate environmental effects that influence the survival and reproduction of bees are increasingly necessary.

Despite their evident ecological and economic importance, bee populations are declining around the world (NRC, 2006), mainly due to anthropic actions, including excessive use of pesticides. Their decline is largely associated to the phenomenon known as Colony Collapse Disorder (CCD), which causes a rapid loss of adult bees, compromising the colony's ability to get food, as well as the colony's ability to defend itself against attacks from other bees, arthropods, microorganisms and viruses. The fact that dead adult bees are found away from the affected hives suggests that they die while looking for food (TOMÉ et al., 2012).

Pesticides can be either lethal to insects, causing immediate death, or sublethal, causing changes in behavior or physiology, such as increased aggressiveness, slowness and disorientation during flight (SILVA et al., 2014). The use of broad-spectrum insecticides is particularly concerning as they affect a wide variety of insects (including beneficial ones). In addition, pesticides such as Fipronil and neonicotinoids can leave sublethal residues on plant surfaces, which are hard to detect (MESSAGE et al, 2012).

According to Soares (2009), even though there are several mechanisms of action in insecticides, currently the insect nervous system has been their main target, through neurotoxic substances that affect axon transmission, in either peripheral or central synapses, resulting in a fast and effective effect against unwanted insects. Among neurotoxic drugs, neonicotinoids are substances chemically similar to nicotine and that act in the propagation of nerve impulses, competing with the neurotransmitter acetylcholine and binding irreversibly to its receptor, as the insecticide is not affected by the enzyme acetylcholinesterase. The activation of acetylcholine receptors becomes abnormally prolonged, generating high excitability of the central nervous system. The most common symptoms resulting from neonicotinoid poisoning in insects are tremors, nervous system collapse and death (FARIA, 2009).

The systemic insecticide Imidacloprid is one of the most widely used neonicotinoids in the world. In Brazil, it is applied in a wide variety of crops, such as cotton, peanuts, barley, beans, corn, soybeans, wheat, pineapple, pumpkin, lettuce, garlic, almond, potato, eggplant, broccoli, onion, chicory, cabbage, tobacco, sunflower, watermelon, melon, scarlet eggplant, cucumber, cabbage, tomato, sugar cane, coffee and grapes (BOVI, 2013). Because Imidacloprid is a systemic insecticide (absorbed by different plant tissues), its residues can accumulate in pollen, nectar and wax, resulting in a high risk of poisoning for bees. Several European countries, including France and Italy, have suspended seed treatments with Imidacloprid, considering that their residues are the main factor responsible for the decline in the bee population. On the other hand, the use of Imidacloprid is extensive in tropical areas, particularly in Brazilian agricultural fields (TOMÉ et al., 2012).

Many studies have shown the detrimental effects of neonicotinoids on the biology of insects, especially bees (ARCE et al., 2018; BLACQUIÈRE et al., 2012; MANJON et al., 2018; GOÑALONS; FARINA, 2018; TOMÉ et al., 2012), but there is still a lot to be investigated, particularly regarding native bees in Brazil. Considering that the detection and possible rejection of Imidacloprid in plant parts collected by bees (nectar, pollen or resin) would prevent bees from getting toxins in their organism, this study aimed to evaluate the behavior of *M. quadrifasciata anthidioides* Lepeletier, 1836, during foraging and its ability to detect the presence of Imidacloprid in artificial diets.

Material and methods

The study was carried out in a meliponary located at the Biology Laboratory of the Instituto Federal do Sul Minas, Poços de Caldas campus (Minas Gerais, Brazil) (21°50'16.7" S and 46°33'36.1" W). The observation arena used in all experiments was composed of a plastic support with a transparent lid (15 cm high x 25 cm in diameter). Inside this support, there were 5 floral simulators built from plastic vials 7.0 cm tall and 2.5 cm diameter, with artificial flowers made from yellow cardboard paper with five 1.5 cm long, round petals, placed on top of the container, around its opening. In the opening, an acrylic straw led to the inside of the vial, at which diet was offered ("nectary"). Both the experimental model and the attractiveness of the color and shape of the petals were previously tested and approved, as reported in Costa and Teixeira (2018), who tested different floral simulators in an observation arena similar to the one used here, and it was found that Mandaçaia bees visited the round shaped and yellow colored petals more often.

For each observation, six foraging bees (25 to 40 days old) were used, randomly captured with plastic bottle at the entrance of one of the 12 colonies available in the meliponary, also randomly selected. The captured bees were carefully placed inside the arena, where they were observed continuously for one hour (60 minutes). During these observations, we recorded every time bees landed in one of the floral simulators ("number of visits"), every time they entered the floral simulator to find food contained at the bottom of the vial ("attempts of ingestion"), and any event of antennation (contact of antennae between two or more bees).

The experiment was divided into two stages. In the first stage, we sought to evaluate the behavior of *M. quadrifasciata* in relation to the preference for different artificial diets (without Imidacloprid). In the second step, we determined the preference or rejection behavior of bees when offered diets with varying concentrations of Imidacloprid. All experiments were carried out in the meliponary during the greatest foraging activity interval at this location, between 9 a.m. and 3 p.m. (personal observation - unpublished data).

Step 1: Assessing preference for different diets

All observations took place from April to June 2018. In each of the 5 floral simulators, 5 mL of different diets were added, according to the following configuration: D1- (only syrup, composed of 50% water and 50% sugar); D2 (50% syrup + 50% honey from *A. mellifera*); D3 (50% syrup + 50% lemon juice); D4 (50% syrup + 50% Aminomix®) and D5 (control): without food. Aminomix® consists of a veterinary food supplement with all essential amino acids. These diets were chosen because they were used in previous research (TEIXEIRA et al., 2017). Eight observations of 60 minutes each were made.

Step 2: Assessment of sensitivity to different concentrations of Imidacloprid

Observations occurred between the months of July and September 2018. The base diet used was D2 (50% syrup + 50% honey from *A. mellifera*), as it was determined to be the preferred diet in step 1. In each of the floral simulators (FS) 5,0 mL of diet were added, with different concentrations of the insecticide Imidacloprid (Termidil 200 SC®, 100 mL, liquid, Dipil Industria Química - lot 002.18M- manufactured on 01/18/18 - 2 years of shelf life), as following: FS 1 (syrup + 1%); FS 2 (syrup + 5%); FS 3 (syrup + 10%); FS 4 (syrup + 20%); FS 5 (control: no addition of Imidacloprid). Eight observations of 60 minutes each were made. The concentrations used here were higher than those obtained by the manufacturer recommended dilutions because we focused on the detection ability of bees. The dilution in agricultural fields, despite being variable according to the culture and substrate, is around 0.05% as reported by the manufacturer product information sheet.

Statistical analysis

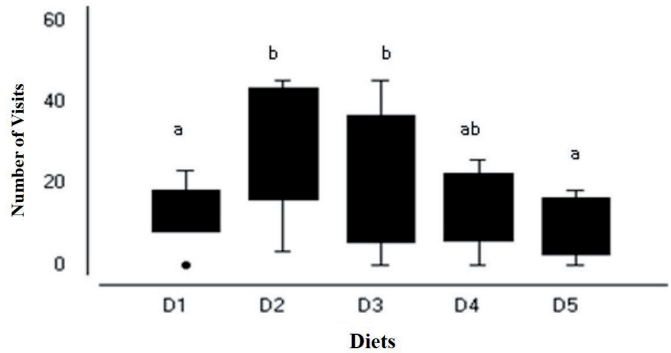
The Kruskal-Wallis test was used, considering the degree of significance $p \leq 0.05$ both for tests with multiple options and for the two-sample paired T test, when antennation behavior was compared with or without Imidacloprid. The computer program BioEstat 5.3 (AYRES et al. 2007) was used to perform these analyses.

Results and discussion

Neonicotinoids are one of the most widely used groups of insecticides in agriculture, possibly due to their low toxicity to mammals and their nature as a systemic pesticide. However, there have been growing concerns about their impact on non-target organisms (GONÑALONS; FARINA, 2018), such as bees. In this study, we found that although Mandaçaia bees have the ability to distinguish diets with different compositions, the presence of Imidacloprid does not influence their food preference behavior. The action of searching for food in the environment is called foraging, which can be divided into four phases: search, capture, handling and ingestion of food (KREBS; DAVIES, 1996). When simulating a foraging situation, among different diets, Mandaçaia bees showed preference for both D2 (50% syrup + 50% honey from *A. mellifera*) and D3 (50% syrup + 50% lemon juice) (FIGURE 1), and a greater number of attempts to ingest D2 (FIGURE 2). These diets simulate the nectar of flowers. Nectar collected by the workers during floral visits in nature presents different types of sugars, such as sucrose, glucose and/or fructose, in addition to small proportions of organic acids, lipids, minerals, vitamins and aromatic compounds (HARBORNE, 1994). The latter may have the function of chemical indicator at the time of collection (attractive/repellent). In artificial diets, the presence of honey possibly provides these other compounds that are attractive to sugar, which would explain the preference for visitation and ingestion by D2 (FIGURES 1 and 2).

In bees, it has been known for some time that taste receptors are found mainly in the antennae and distal segments of the first pair of legs (FRINGS; FRINGS, 1949) and these structures are the ones that allow bees to distinguish between edible and non-edible items, as they are very sensitive to stimulation by different sucrose concentrations (HARBORNE 1994; HAUPT, 2004). In addition, it is now known that such taste bristles also react to glucose, fructose, NaCl and KCl and these compounds are present in honey (SANCHEZ, 2011) and consequently in the diet preferred by the bees used in our experiments. Thus, we assume that such attractive compounds should be more effective in determining the direction and food intake than the possible deterrence to be exercised by the presence of a certain foreign compound, such as neonicotinoids.

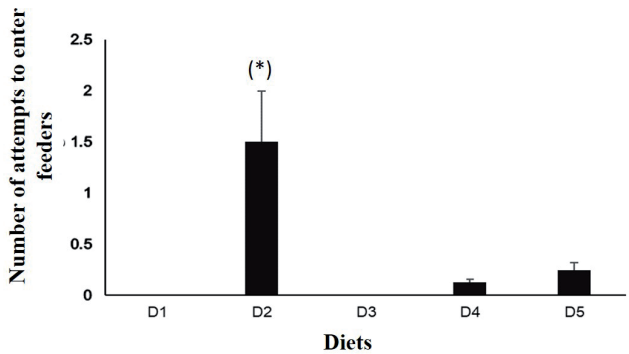
Figure 1 – Visiting preference in Floral Simulators containing different diets.



Average number of visitations in Floral Simulators with different diets. D1 – only syrup containing 40% water and 50% sugar; D2 – 50% syrup + 50% honey from *A. mellifera*; D3 – 50% syrup + 50% lemon juice; D4 – 50% syrup + 50% aminomix®; and D5 – control (without food). Eight repetitions were made. Different letters indicate statistical differences (Kruskal-Wallis $p \leq 0.02$).

Source: Elaborated by the authors (2018).

Figure 2 – Ingestion preference for different diets.

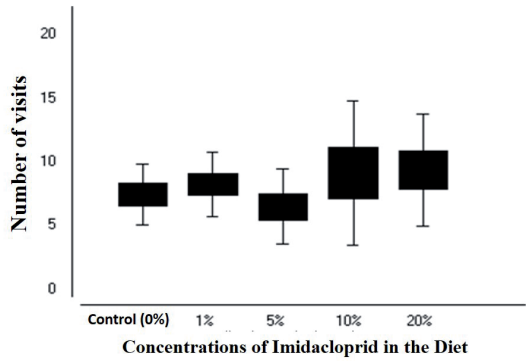


Number of attempts to enter feeders with different diets. D1 – only syrup composed of 50% water and 50% sugar; D2 – 50% syrup + 50% honey from *A. mellifera*; D3 – 50% syrup + 50% lemon juice; D4 – 50% syrup + 50% Aminomix®; and D5 – control (without food). Eight repetitions were made. (*) Diet 2 obtained the greatest number of attempts of ingestion (Kruskal-Wallis $p \leq 0.01$).

Source: Elaborated by the authors (2018).

Bees exposed to floral simulators with D2 diet and Imidacloprid did not show different food preference behavior among the concentrations of Imidacloprid, that is, there were no differences on the number of visits (FIGURE 3) nor attempts of ingestion (FIGURE 4). It is noteworthy that all bees that came into contact with diets containing insecticide had died within the floral simulator, regardless of their concentration in the diet (1%, 5%, 10% or 20%).

Figure 3 – Visitation preference in floral simulators with diets with varying concentrations of Imidacloprid.

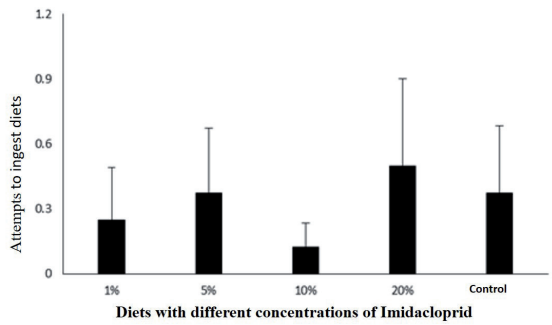


Mean and deviation number of visits in Floral Simulators containing 5.0 mL of *A. mellifera* honey syrup diet with different concentrations of Imidacloprid: FS1 (1%); FS 2 (5%); FS 3 (10%); FS 4 (20%); FS 5 (control: no addition of Imidacloprid). Eight repetitions were performed (Kruskal-Wallis $p = 0.7$).

Source: Elaborated by the authors (2018).

It is concluded, therefore, that there is no rejection by the contaminated food, even in diets with high concentration of insecticide (20%). Similarly, it is already known that *A. mellifera* not only eat foods with neonicotinoids but have acquired a propensity to visit plants with contaminated flowers (ARCE et al., 2018).

Figure 4 – Attempts to ingest diets with different concentrations of Imidacloprid.



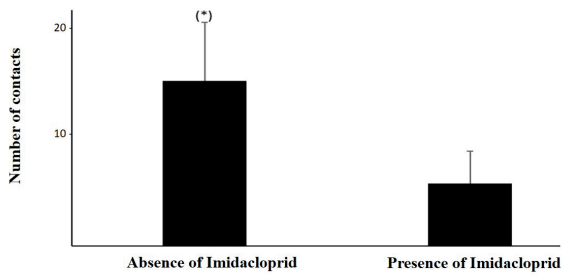
Number of attempts to ingest food treated with different concentrations of Imidacloprid. The control had no insecticide, only the diet consisting of *A. mellifera* syrup and honey. Eight repetitions were performed (Kruskal-Wallis $p = 0.8$).

Source: Elaborated by the authors (2018).

The fact that bees do not detect or reject Imidacloprid diets in nature is also supported by Mitchell et al. (2017) who found high concentrations of this compound in commercial honeys. This honey when ingested by bees of a colony can cause several disturbances in their behavior and physiology. Goñalons and Farina (2018), using lower concentrations of Imidacloprid than those found in commercial honeys, found that the association between Imidacloprid and Glyphosate in bee food impairs the olfactory and taste learning in young bees, as well as the role of these insects within colony, and also impairs the positive response to sucrose (as they have increased their perception threshold).

Among the different behaviors exhibited by social insects, communication is crucial, especially in the activity of searching for food. One of the forms of communication between insects that exhibit some degree of sociality is antennation, that is, the act of touching each other with the antennae as a tactile signal (MAGGENTI et al, 2005). Antennation is closely associated to foraging behavior, especially regarding learning and memory (TOMÉ et al., 2012). Our data demonstrate a strong relationship between the presence of Imidacloprid and the activity of antennation, since only the presence of this compound in the offered food resulted in a smaller number of contacts between antennae among bees. In experiments without Imidacloprid, 17.5 (± 6.63) antennations per hour were recorded, while in observations with Imidacloprid an average of 6.6 (± 3.42) antennations per hour was recorded (FIGURE 5). The loss of this important communication tool can greatly affect the survival of the hive.

Figure 5 – Antennation activity of bees visiting artificial food source with or without Imidacloprid.



Number of antennations among bees observed in the absence and presence of Imidacloprid. Average of 16 observations in 1 hour. (*) Groups were different (Two-sample paired t test $p \leq 0.05$).

Source: Elaborated by the authors (2018).

Conclusion

Our assays showed that *M. quadrifasciata* foragers do not reject food treated with lethal doses of the insecticide Imidacloprid, which, under natural conditions, would allow a greater chance of contamination. Furthermore, it is evident the interference of Imidacloprid in foraging behavior, specifically in bee communication, as this pesticide (volatilized from the offered food) significantly decreased the intensity of antennation activity. Both findings, the apparent inability of bees to detect the presence of the insecticide and the change in behavior caused by the exposition to the product, may rapidly lead to colony losses in nature.

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Efeitos do Neonicotinóide Imidacloprido no Comportamento Alimentar de *Melipona quadrifasciata anthidioides* Lep.

Resumo

Os meliponíneos estão entre os maiores agentes polinizadores dos ecossistemas neotropicais, porém, devido a ações antrópicas, como o uso indiscriminado de agrotóxicos, as populações destas abelhas estão em declínio. Os inseticidas do grupo dos Neonicotinóides, entre eles o Imidacloprido, usados em muitas culturas, têm sido apontados como um dos que mais interferem na saúde das colônias. O objetivo deste trabalho foi analisar a capacidade de percepção da presença de Imidacloprido em dietas artificiais e o efeito do inseticida sobre o comportamento de operárias adultas de *Melipona quadrifasciata*, uma espécie de abelha nativa brasileira sem ferrão. Para esta finalidade, foram realizados experimentos comportamentais com abelhas campeiras em arenas de observação com simulação de flores artificiais e respectivos nectários com diferentes concentrações do composto inseticida (0%, 1%, 5%, 10% e 20%) em dietas de xarope e mel. Os resultados mostraram que as abelhas não diferenciaram as diferentes concentrações de Imidacloprido, mesmo com doses elevadas na dieta (20%). Constatou-se também que a presença do produto interferiu na comunicação entre as abelhas, diminuindo a intensidade dos eventos de antenação. Ambos os fatores, a incapacidade das abelhas de perceber a presença do inseticida e os distúrbios na comunicação ocasionados pela sua ingestão, devem aumentar as chances de contaminação e desaparecimento das colônias destes insetos na natureza.
Palavras-chave: Meliponini. Abelhas sem ferrão. Mandaçaia. Inseticida. Antenação. Forrageamento.

References

AIDAR, D. S. **A mandaçaia**. 2. ed. Editora Funpec. São Paulo, SP, Brasil. 162p. 2011.

ARCE, A. N.; RODRIGUES, A. R.; YU, J.; COLGAN, T. J.; WURM, Y.; GILL, R. J. Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. **Proceedings of the Royal Society B.** Londres, v. 285, p. 1-7, ago. 2018. Disponível em: <http://rspb.royalsocietypublishing.org> Acesso em: nov. 2018.

AYRES, M.; AYRES JÚNIOR, M.; AYRES, D. L.; SANTOS, A. A. 2007. **BIOESTAT** - Aplicações estatísticas nas áreas das Ciências Biomédicas. Mamirauá. Belém, PA. 364 p.

BLACQUIÈRE, T.; SMAGGHE, G.; VAN GESTEL CA.; MOMMAERTS V. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. **Ecotoxicology, v.** 21, n. 4, p. 973-992. 2012.

BOVI, T. S. **Toxicidade de inseticidas para abelhas *Apis mellifera* L.** 2013. 69 f. Dissertação (Mestrado em Zootecnia). Programa de Pós-graduação em Zootecnia, Faculdade de Medicina Veterinária e Zootecnia, UNESP, Botucatu, 2013. p. 1-21.

COSTA, L. G. L.; TEIXEIRA, I. R. V. Comportamento de *Melipona quadrifasciata* Lep. frente a diferentes dietas, cores e formas e relação entre a intensidade de sua atividade e a temperatura ambiental. JORNADA CIENTÍFICA E TECNOLÓGICA E SIMPÓSIO DE PÓS-GRADUAÇÃO DO IFSULDEMINAS, 10., **Anais**..., Muzambinho, 2018.

COSTA, M. A.; LAMA, D. A. D.; MELO, G. A. R.; SHEPPARD, W. S. Molecular phylogeny of the stingless bees (Apidae, Apinae, Meliponini) inferred from mitochondrial 16S rDNA sequences. **Apidologie**, v. 34, p. 73–84, 2003.

FARIA, A. B. C. Revisão sobre alguns grupos de inseticidas utilizados no manejo integrado de pragas florestais. **Ambiência – Revista do setor de Ciências Agrárias e Ambientais.** Guarapuava, v. 5, n. 2, p. 345-358, maio/ago. 2009.

FIGUEROA-MATA, G.; PRENDAS-ROJAS, J.; RAMÍREZ-BOGANTES, M.; AGUILAR-MONGE, I.; HERRERA-GONZÁLEZ, E.; TRAVIESO-GONZÁLEZ, C. Identificación de abejas sin aguijón (Apidae: Meliponini) a partir de la clasificación de los descriptores SIFT de una imagen del ala derecha anterior. **Tecnología en Marcha**. Edición especial. Matemática Aplicada, Mayo 2016. p. 51-63.

FRINGS, H.; FRINGS, M. The loci of contact chemoreceptors in insects: a review with new evidence. **The American Midland Naturalist.** v. 41, n. 3, p. 602- 658, maio, 1949.

GOÑALONS, C. M.; FARINA, W. M. Impaired associative learning after chronic exposure to pesticides in young adult honey bees. **Journal of Experimental Biology**, v. 221, p. 1-7, 2018.

HARBORNE, J. B. **Introduction to ecological biochemistry**. 4. ed. London Academic Press, 1994. 317 p.

IMPERATRIZ-FONSECA, V. L.; CANHOS, D. A. L.; ALVES, D. A.; SARAIVA, A. M. **Polinizadores no Brasil:** Contribuição e Perspectivas para a Biodiversidade, Uso sustentável, Conservação e Serviços ambientais. São Paulo: Editora da USP, 2012. p. 25-45.

KREBS, J. R.; DAVIES, N. B. **Introdução à ecologia comportamental**. Atheneu Editora São Paulo, *1996*.

MAGGENTI, M. A. B.; MAGGENTI, A. R.; GARDNER S. L. (Ed). **Online Dictionary of Invertebrate Zoology.** [S.l.]. 2005. Disponível em: <http://digitalcommons.unl.edu/onlinedictinvertzoology/2/>. Acesso em: 2018.

MANJON, C.; TROCZKA, B. J.; ZAWORRA, M.; BEADLE, K.; RANDALL, E.; HERTLEIN, G.; SINGH, K. S.; ZIMMER, C. T.; HOMEM, R. A.; LUEKE, B.; REID, R.; KOR, L.; KOHLER, M.; BENTING, J.; WILLIAMSON, M. S.; DAVIES, T. G. E.; FIELD, L. M.; BASS, C.; NAUEN, R. Unravelling the Molecular Determinants of Bee Sensitivity to Neonicotinoid Insecticides. **Current Biology**, 28, 1137–1143, 2018.

MESSAGE, D.; TEIXEIRA, E. W.; DE JONG, D. Situação da Sanidade das Abelhas no Brasil. In: IMPERATRIZ-FONSECA, V. L.; CANHOS, D. A. L.; ALVES, D. A.; SARAIVA, A. M. **Polinizadores no Brasil:** Contribuição e Perspectivas para a Biodiversidade, Uso sustentável, Conservação e Serviços ambientais. São Paulo: Editora da USP, 2012. p. 237-256.

MITCHELL, E. A. D.; MULHAUSER, B.; MULOT, M.; MUTABAZI, A.; GLAUSER, G.; AEBI, A. A worldwide survey of neonicotinoids in honey. **Science,** v. 358, p. 109-111; 2017.

NRC - National Research Council, **Status of pollinators in North America.** Washigton, DC: National Academy of Sciences. 2006.

NOGUEIRA-NETO, P. **Vida e Criação de Abelhas Indígenas Sem Ferrão.** São Paulo: Nogueirapis, 1997. p. 33-38.

SANCHEZ, M. G. B. Taste Perception in Honey Bees. **Chemical Senses,** Oxford, v. 36, n. 8, p. 675-692, out. 2011.

SILVA, F. O.; BARREIRA, H. C. S.; COSTA NETO, W.; ARAUJO, R. B.; LIMA, M. C.; ROCHA, S. A.; VIANA, B. F. **Agrotóxicos e Polinizadores:** Isso combina? Rio de Janeiro: Funbio, 2014.

SOARES, H. M. **Avaliação dos efeitos do Imidaclopride sobre o sistema nervoso de *Apis mellifera* africanizada através da expressão de proteína FOS.** 2009. 46 f. Trabalho de Conclusão de Curso – Ciências Biológicas, UNESP, Rio Claro, 2009. p. 6-15.

TEIXEIRA, I. R. V.; CRUZ, F.; CARVALHO, J.; CASTRO, R. M.; BEIJO, L. A.; BARCHUK, A. R. Physicochemical Characteristics of Honey Produced by Colonies of the Stingless bee *Melipona quadrifasciata*: Seasonal Variations and Influence of Food Supplementation. **International Journal of Agriculture Innovations and Research**, v. 6, ago 2017.

TOMÉ, H. V. V.; MARTINS, G. F.; LIMA, M. A. P.; CAMPOS, L. A. O.; GUEDES, R. N. C. Imidacloprid-Induced Impairment of Mushroom Bodies and Behavior of the Native Stingless Bee *Melipona quadrifasciata anthidioides*. **PLoS ONE**, v. 7, n. 6. DOI: 10.1371/journal.pone.0038406. 2012.

VENTURIERI, G. C.; ALVES, D. A.; VILLAS-BÔAS, J. K.; CARVALHO, C. A. L.; MENEZES, C.; VOLLET-NETO, A.; CONTRERA, F. A. L.; CORTOPASSI-LAURINO, M.; NOGUEIRA-NETO, P.; IMPERATRIZ-FONSECA, V. L. Meliponicultura no Brasil: Situação atual e perspectivas futuras para o uso na polinização agrícola. In: IMPERATRIZ-FONSECA, V. L.; CANHOS, D. A. L.; ALVES, D. A.; SARAIVA, A. M. **Polinizadores no Brasil:** Contribuição e Perspectivas para a Biodiversidade, Uso sustentável, Conservação e Serviços ambientais. São Paulo: Editora da USP, 2012. p. 213-236.

VILLAS-BÔAS, J. K. **Manual tecnológico:** mel de abelhas sem ferrão. Brasília: Instituto Sociedade, População e Natureza, 2012. p. 11-25.

WITTER, S.; NUNES-SILVA, P. **Manual de boas práticas para o manejo e conservação de abelhas nativas (meliponídeos).** Porto Alegre: Fundação Zoobotânica do Rio Grande do Sul, 2014. p. 19-57.

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