Ecotoxicology and Environmental Safety 164 (2018) 548–553

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Changes in social behavior are induced by pesticide ingestion in a Neotropical stingless bee

Samuel Boff[a,b,c,*], Anna Friedel[d], Rosilda Mara Mussury[a,b,e], Patricia Roseti Lenis[a,e], Josué Raizer[a,e]

[a] Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados, Avenida Dourados-Itahum, km 12, Dourados 79804-970, Mato Grosso do Sul, Brazil
[b] Postgraduate Program in Bioprospecting and General Biology, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil
[c] The University of Milan, Department of Food, Environmental and Nutritional Sciences, via Celoria 2, 20133Milan, Italy
[d] Institute for Biology, Martin-Luther-University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany
[e] Postgraduate Program in Entomology and Biodiversity Conservation, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil

ARTICLE INFO

Keywords:
Antennation
Trophallaxis
Melipona quadrifasciata
Neonicotinoid
Pyrethroid
Social interaction

ABSTRACT

Throughout evolutionary history bees have developed complex communication systems. For social bees, communication is important for both the individual and the development of the colony. Successful communication helps bees to recognize relatives, defend the colony, and promote recruitment to optimize foraging of floral resources. Bees' contribution to pollination is of broad environmental and economic importance. However, studies have reported that anthropogenic actions, such as the use of pesticides, negatively affect bee survival and behavior. We tested the effect of a commercially available pesticide mix containing two pesticide classes, a neonicotinoid and a pyrethroid, on the social behavior of the stingless bee, *Melipona quadrifasciata* (Lepeletier, 1863). After determining a sublethal dose of the pesticides, we tested the effect of an acute dose on antennation and trophallaxis behaviors of worker bees. Our results showed a drastic reduction in the communication and social interactions of bees.

1. Introduction

Bees play a fundamental role in ecosystem services as they pollinate most flowering plants and are the most diverse pollination agents in an environment (Michener, 2007). In this way, bees increase agricultural production, especially of fruit plants (Giannini et al., 2015). According to the IPBES (2016) global assessment of pollinators, 5–8 per cent of the current global pollinator dependent crop production has an annual market value of ∼ $235 billion-$577 billion (in 2015, United States dollars) worldwide. In addition to their pollination service, some bee species, including honeybees and some species of stingless bees, are excellent honey producers.

However, such bee services and products are at risk globally, since bee populations are threatened by anthropogenic activities that cannot maintain healthy bee populations (Brown and Paxton, 2009; Potts et al., 2010). Climate change, invasive species, monocultures with less floral resources and nesting sites, and pesticides have negative effects on bees (Potts et al., 2010; Schweiger et al., 2010). Moreover, sub-lethal doses of pesticides have been linked to behavioral changes at individual and colony level (Stanley et al., 2016; Forfert and Moritz, 2017).

Some neurological effects of pesticides on bees include decreased spatial memory, homing, and foraging efficiency (see, Gill and Raine, 2014; Samuelson et al., 2016; Stanley et al., 2016), and a reduction in activities that require learning/memory (Stanley et al., 2015a). Additionally, pesticides reduce flower visitation rate, pollen collection, and sonication, which may result in a pollination deficit (Stanley et al., 2015b; Whitehorn et al., 2017) and a deficit of brood feeding inside the hive (Santos et al., 2016). Bees are usually contaminated with pesticides during foraging activities that involve collecting and ingesting treated floral resources or by fumigation through sprayed substances (Frazier et al., 2015). In eusocial bees, contamination of foraging individuals can indirectly impact the performance of the entire colony through horizontal poisoning of hundreds of nestmates, including the queen (Williams et al., 2015; Wu-Smart and Spivak, 2016). Such a phenomenon is possible since some social bees, such as honey bees and stingless bees, acquire food through horizontal liquid exchange among nestmates, which is called trophallaxis (Contrera et al., 2010), or by sharing resources within the nest (Hrncir et al., 2008; Wu-Smart and Spivak,

* Corresponding author at: The University of Milan, Department of Food, Environmental and Nutritional Sciences, via Celoria 2, 20133, Milan, Italy.
E-mail address: samuel.boff@unimi.it (S. Boff).

https://doi.org/10.1016/j.ecoenv.2018.08.061
Received 12 December 2017; Received in revised form 13 August 2018; Accepted 17 August 2018

2016).

Among the various pesticide classes, neonicotinoids and pyrethroids are both used to control a variety of pests. Pyrethroids are a common component of several commercially available pesticides (Spurlock and Lee, 2008) and target a protein (voltage-gated sodium channel) by binding to the voltage gate of the sodium channel and preventing it from closing. In insects, pyrethroids can affect nerve and muscle cells used for rapid electrical signaling and have been reported to negatively affect bee locomotion (Ingram et al., 2015). Neonicotinoids (such as acetamiprid, imidacloprid and thiacloprid) are highly toxic for pollinators. These pesticides can have a severe negative impact on the environment and on crop production (IPBES, 2016). Although efforts have been made to heavily restrict the use of neonicotinoids in the European Union, these pesticides are still widely used throughout other parts of the world. Neonicotinoids, which are effective at killing a wide range of insects, interact antagonistically with the nicotinic acetylcholine receptors in an insect's brain (Matsuda et al., 2001). Since these pesticides mainly act on bee brains, they may also interfere with bee communication, which includes an efficient communication system via the antennae (Wittwer et al., 2017) and trophallactic actions (Leonhardt et al., 2016).

Eusocial bees, where individuals are hierarchically organized into a nest, exhibit a range of behaviors to maintain cohesion amongst themselves (Leonhardt et al., 2016). Antennation, for example, is an efficient and complex form of contact communication for social bees when compared to solitary bees (Wittwer et al., 2017). Antennation, along with identification of cuticular hydrocarbons and usage of nest material, plays an important role in kin recognition (Breed et al., 1992; Nunes et al., 2011). In addition, sexual odors can be transmitted via antennation, therefore, playing an important role in reproduction (Leal, 2005). Trophallaxis, which is the means of horizontal transfer of food, molecules, and symbionts among nestmates can promote social immunization and serves as a form of communication (Farina, 1996; Leonhardt et al., 2016).

Bee antennation and trophallaxis are essential for bee communication but may be affected by pesticide contamination. Some bees can be contaminated directly through ingestion of pesticide treated floral resources and others indirectly through contact with contaminated bees. Our goal was to evaluate whether sublethal doses of pesticides affect social behaviors in a stingless bee species by changing their antennation behavior and affecting trophallaxis between pesticide contaminated and non-contaminated nestmates. Our study species, *Melipona quadrifasciata* (Lepeletier, 1863) (Apidae: Meliponini), is a common and important pollinator in Brazil and it may be exposed to a variety of pesticides (Pignati et al., 2017). Thus far, the effect of pesticides on social interactions, such as antennation and trophallaxis, has not been tested.

2. Materials and methods

2.1. Bee species

The stingless bee species *M. quadrifasciata* is originally distributed in the southeast, south, and central west of Brazil, where its range extends to the south of Paraguay (Camargo and Pedro, 2013). *Melipona* species have been reported visiting a variety of plant species including commercially used crops (Giannini et al., 2015). They mass provision their brood cells (Pech-May et al., 2012), and colonies constantly produce a relatively large number of new queens.

2.2. Bee sampling

We obtained adult workers from hives of *M. quadrifasciata* in a fragment of Atlantic Forest (22° 12′ 41″ S, 54° 55′ 01″ W), located in Dourados, Mato Grosso do Sul, Brazil. We collected bees from the nest entrance after knocking gently on the hive in the early morning (between 7 and 8 a.m.), which released bees from the interior of the hive. We put Falcon type plastic tubes [50 ml] at the hive entrance to capture individuals (max. 10 bees per tube). Tubes, with lids for ventilation, were then placed in a thermal bag for ~ 20 min during transportation to the laboratory. In the lab, bees were kept at 25–26 °C with a natural light regime.

2.3. Determination of sublethal doses

We used 145 workers, from three different hives (n = 57, 42, 46 workers per hive), of *M. quadrifasciata* to determine the sublethal doses of the pesticide Fastac® Duo. This pesticide is a mixture of a systemic (acetamiprid-neonicotinoid) and contact toxicity (alpha-cypermethrin-pyrethroid) component of BASF (Baden Aniline and Soda Factory) used to control stink bugs in crops such as barley, beans, oats, maize, millet, rye, sorghum, triticale, wheat, cotton, soybeans, and irrigated rice crops. In the field, application is achieved by spraying directly on plants either via terrestrial or aerial application from an aircraft (with spray capability), which produces droplets (see user information leaflet Fastac® Duo, Brazil). Bees may be intoxicated with the pesticide by contact during pesticide spraying, through collection and ingestion of contaminated pollen, nectar and water, or by direct ingestion of pesticide droplets in the environment (Sanchez-Bayo and Goka, 2014).

In the laboratory, bees were isolated and deprived of food for one hour in plastic pots (9.5 cm diameter and 8.0 cm height), with one bee per pot and lids that allowed ventilation. After one hour, we placed a small metal container with 15 µl of 50% sucrose solution as a control or the same 50% sucrose solution with various concentrations of pesticide solution (Fastac® Duo, 10% diluted solution in water) inside the pot. We offered 15 µl of liquid to the bees, considering a reference for feeding experiments with honeybees, another eusocial bee species (Mayack and Naug, 2009). The following pesticide doses were used: 150 and 300 ng/bee, 15 and 30 ng/bee, 1.5 and 3.0 ng/bee, 0.15 and 0.30 ng/bee, 0.015 and 0.030 ng/bee of acetamiprid and alpha-cypermethrin, respectively. Only the bees that consumed the entire amount of pesticide and control solution were used for the experiment. Bees were transferred into Petri dishes (9.5 cm diameter and 0.5 cm height) in groups ranging from 4 to 12 individuals. For each treatment (5 different concentrations + control) 3 petri dishes with bees were prepared (n = 3). Petri dishes were lined with filter paper and bees were offered an *ad libitum* supply of 50% sucrose solution.

We observed these plates for two days (48 h), removing immobile bees (considered dead) with tweezers every 24 h. We ran an ANOVA with dose, time, and plate as independent factors, where plate was included to control for its effect, interaction between dose and time, and proportion of survivors (arcsine transformed) as dependent variable. Bee survival varied regardless of the pesticide concentration (dose) or interaction with time ($df = 10$, $F = 0.450$, $p = 0.905$). Nonetheless, time ($df = 2$, $F = 3.750$, $p = 0.038$) and plate ($df = 12$, $F = 2.271$, $p = 0.042$) explained survival. None of the tested treatments (n = 5 + control) killed more than 15% of the bees (Fig. 1) and were thus considered sublethal doses. Therefore, we used 150 ng/bee and 300 ng/bee of acetamiprid and alpha-cypermethrin, respectively, to observe the effects of Fastac Duo on the social behavior of *M. quadrifasciata*.

2.4. Effect of the pesticide on social behavior

Bees were collected from five hives, as described above. We tested the effect of pesticides on social interactions (antennation and trophallaxis) between three workers from the same nest. All individuals were placed in separate plastic pots, with one bee per pot and a lid that allowed ventilation. One of the three workers received 15 µl of 50% sucrose solution with pesticide (150 ng/bee and 300 ng/bee of acetamiprid and alpha-cypermethrin, respectively) or 15 µl of 50% sucrose solution (control), while the other two workers were deprived of food

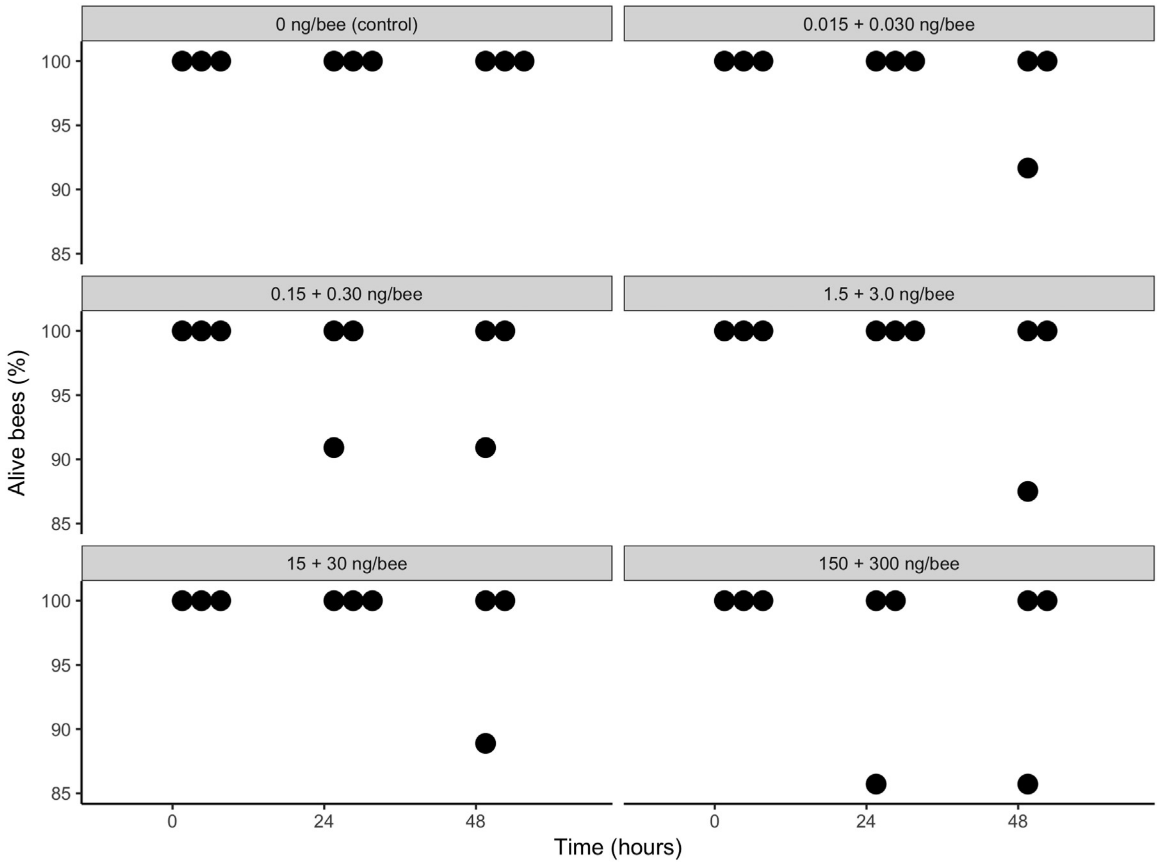

Fig. 1. Percentage of living *Melipona quadrifasciata* bees during 48 h after ingestion of five different concentrations of the pesticide Fastac® Duo and control solution (from top left to bottom right: 0 ng/bee = control; 0.015 and 0.03 ng/bee; 0.15 and 0.3 ng/bee; 1.5 and 3 ng/bee; 15 and 30 ng/bee; 150 and 300 ng/bee of acetamiprid and alpha-cypermethrin, respectively). The initial number of bee size per plate (n = 3 per treatment) at time zero was 8, 6, 10 (control), 12, 5, 6 (0.015 and 0.03 ng/bee), 11, 8, 4 (0.15 and 0.3 ng/bee), 8, 8, 8 (1.5 and 3.0 ng/bee), 8, 8, 9 (15 and 30 ng/bee), and 10, 7, 9 (150 and 300 ng/bee of acetamiprid and alpha-cypermethrin, respectively).

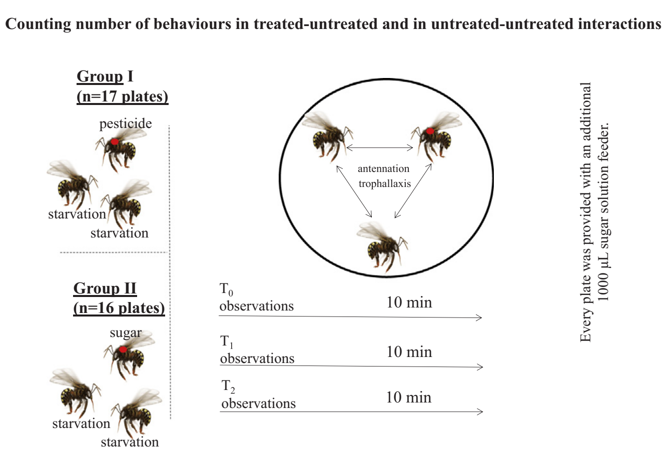

Fig. 2. Schematic of experimental set-up. We tested the effect of the pesticide on social interactions (antennation and trophallaxis) of stingless bee, *Melipona quadrifasciata.* The marked bee (red) corresponds to the bee fed pesticide or sugar solution. (Bee figure modified from https://www.google.com.br/search?q=melipona%20quadrifasciata&source=lnms&tbm=isch&sa=X&ved=0ahUKEwih3aq8qv7cAhXEIJAKHcBeAwcQ_AUICigB&biw=1094&bih=511#imgrc=LRseuUwnYuYcYM).

for one hour. We gently marked the dorsal part of the mesosoma of all the bees who received a dose of pesticide or sugar solution with a non-toxic ink for easy identification between untreated and treated bees. Afterwards the three workers were reunited in a petri dish. All plates were lined with filter paper containing a feeder in the center with 1000 µl of the sugar solution. Plates that contained a worker that received the pesticide dose are referred to as the treated group and plates where one worker received the sugar solution are referred to as the control group. We counted the number of antennations (two bees face to face with their antennae touching) and trophallaxis exchanged between two of the three workers. Thereby, interactions between two untreated workers (untreated-untreated) or interactions between a treated and an untreated worker (treated-untreated) could occur. The number of interactions between individuals were counted during three sessions of 10 min each, with a total of 30 observation minutes per plate. The interval between successive observation sessions was 1 h. In total, 51 bees were distributed among 17 plates, with one pesticide treated bee each and 48 bees were used for the control and distributed across 16 plates, with one sugar treated bee each (Fig. 2).

To account for potential differences in interaction due to treatments, the number of antennation and trophallaxis were the subtraction in two kinds of interactions: "1"- between treated and untreated bees and "2"- among untreated bees. These values indicated a standardized effect of treatment with only sucrose or sucrose with pesticide. Interaction plots were obtained using the groupwise Mean function rcompanion package (Mangiafico, 2017) in R to calculate the mean of each treatment (pesticide and sugar) along with the 95% confidence interval of each mean using the percentile method. We compared the means of these differences between treatments and time periods with repeated measures ANOVA. Tukey Honest Significant Difference (HSD) test were performed to account the effect of time within each treatment. All statistical analyses were performed in R version 3.4.2 (R Core Team, 2017).

Table 1
Total number of interactions exchanged between treated and untreated workers of *M. quadrifasciata* during three sets of 10 min (0, 1st and 2nd correspond to hour after ingestion).

Treatments Interactions	Antennation				Trophallaxis			
	0	1st	2nd	Total	0	1st	2nd	Total
Control (sugar)								
Untreated - treated	24	14	11	49	30	0	3	36
Untreated - untreated	11	12	10	33	5	1	1	9
Pesticide								
Untreated - treated	30	6	4	40	2	0	0	2
Untreated - untreated	38	20	6	64	11	3	0	17

3. Results

In absolute terms, antennation was more frequent than trophallaxis, regardless of experimental treatments (Table 1) since food exchange via trophallaxis typically occurs after antennation but is not obligatory. The number of interactions (number of antennations and trophallaxis exchanged) varied between the pesticide treated group and the untreated group (control). The pesticide used here changed the ratio of social communication by altering the number of antennations and trophallaxis events exchanged between workers of *M. quadrifasciata*. The pesticide led to a significant reduction in the number of social interactions observed. This effect was greater during the first hour after ingestion.

Interactions of bees treated with pesticides were less frequent than for bees treated with sugar (Fig. 3). On average, the antennation frequency was significantly higher for bees treated with sugar ($F = 5.432$, $df = 1$ and 93, $p = 0.022$) with no significant variation throughout time ($F = 0.831$, $df = 4$ and 93, $p = 0.589$). Similarly, the trophallaxis frequency was significantly affected by the pesticide ($F = 8.103$, $df = 1$ and 93, $p = 0.005$), but varied over time ($F = 3.767$, $df = 4$ and 93, $p = 0.007$), and was highest during the first hour for the untreated bees (Tukey HSD, $p < 0.04$).

4. Discussion

We tested how oral ingestion of the pesticide Fastac® Duo (acetamiprid + alpha-cypermethrin) affected the social behavior (antennation and trophallaxis) of the stingless bee *Melipona quadrifasciata*. We found that the pesticides reduced the number of interactions, compared to the control group, when we fed bees with a sublethal dose determined from our initial experiment. Pesticide ingestion interfered with the bee social behaviors, which are important for the homeostasis of the individual and the colony of stingless bees, including bee recruitment (Hrncir et al., 2008, 2011), regulation of individual nectar foraging (De Bruijn and Sommeijer, 1997), intranidal thermoregulation (Vollet-Neto et al., 2015), and nest defense (Shackleton et al., 2015). Herein, we found a large and significant effect on antennation and trophallaxis behaviors. Our results show that 150 and 300 ng/bee (acetamiprid and alpha-cypermethrin, respectively) of active compounds may have no effect on *M. quadrifasciata* bees ~ 2 h post ingestion. However, if the pesticide only induces a temporarily negative effect on the amount of social interactions, we emphasize that bees in natural conditions may be subject to pesticides throughout the day, every day, and not only once. Therefore, the effects of pesticides on social communication of bees could be even greater in the field, with chronic exposure that could dramatically affect their behavior.

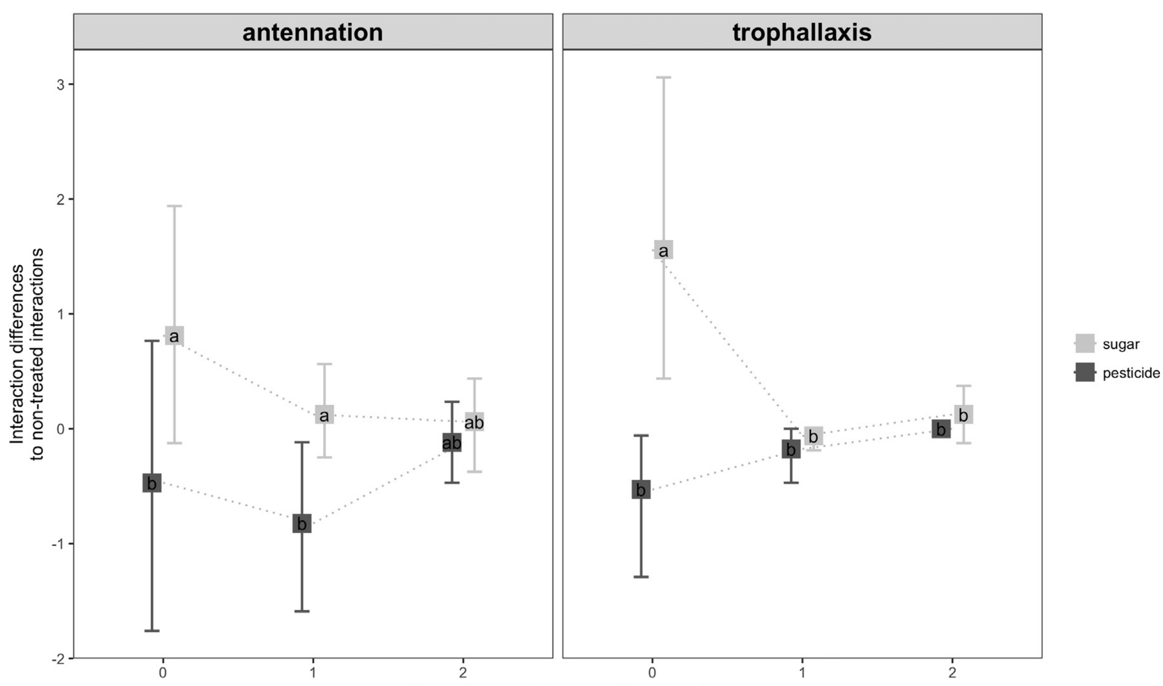

Fig. 3. The differences in social interactions (antennation and trophallaxis) between treated (light grey: sugar treatment = control; dark grey: pesticide treatment) and untreated individuals and among untreated individuals ($n_{group\ I} = 17$, $n_{group\ II} = 16$). In treated versus untreated relationships, positive values indicate that the control treatment females who received the sucrose solution interacted more actively than untreated females did. Negative values indicate that females treated with pesticides participated in fewer interactions than females who did not receive any solution, for both antennation and trophallaxis. Values equal to zero indicate absence of treatment effect, i.e. the frequency of interactions among treated and untreated workers was equal to the frequency of interactions between untreated bees. On average, bees in the control exchanged more antennation with no significant variation over time (repeated measures ANOVA, $F = 5.432$, $df = 1$ and 93, $p = 0.022$, $F = 0.831$, $df = 4$ and 93, $p = 0.589$, respectively). Similarly, the number of trophallaxis events was affected by the pesticide (repeated measures ANOVA, $F = 8.103$, $df = 1$ and 93, $p = 0.005$), although temporary (repeated measures ANOVA, $F = 3.767$, $df = 4$ and 93, $p = 0.007$), and higher in the first hour for bees treated with just sugar (Tukey HSD, $p < 0.04$). Different letters (a, b) indicate significant differences between measures. The error bars represent 95% confidence interval of each mean using the percentile method (Mangiafico, 2017).

In eusocial bee species it is expected that non-harmful social interactions among nestmates benefit directly individuals and the colony. Stressors such as pesticides have been shown to change individual behavior and may compromise social interactions. The neonicotinoid thiacloprid extended trophallaxis events for the bee *Apis mellifera carnica,* while donors that received a high dose of thiacloprid distributed more food and the pesticide did not affect the number of trophallactic events (Forfert and Moritz, 2017). Although infected honeybees managed to distribute contaminated food to other females, the pesticide treatment changed the network interaction among workers, supporting our hypothesis that there might be a cue for treated worker recognition. Our results support the hypothesis of social impairment due to pesticide ingestions. However, the effect of reduced trophallaxis seemed to have disappeared after two hours after ingestion. On the other hand, we promoted trophallaxis by depriving non-treated workers (either in the pesticide treatment or control group) of food, which differs from the former study where control bees were fed with sugar solution. Furthermore, Brodschneider et al. (2017) found similar results when they showed how trophallaxis is affected by imidacloprid in honey bees.

In our study, the non-treated bees in both groups were starved, and, therefore, likely asking for food through trophallaxis. However, the number of interactions between bees in the treated group (pesticide) was on average lower than the control group. Thus, we presume that the bees could have a mechanism to recognize the ingested pesticide. In the control group, workers deprived of food interacted more frequently with bees that received 15 µl of 50% sucrose solution than between each other. This changed in the pesticide treatment, where the non-treated workers presented a higher interaction frequency between one another and the number of interactions between non-treated and intoxicated bees (that were fed 15 µl of pesticide solution) was significantly lower. Therefore, workers fed with sugar solution probably acted as food donors for the other two workers who did not receive any food after being captured. Our results cannot fully explain that reduced trophallaxis was caused by the starving bees' ability to recognize nestmate intoxication (contact avoidance), due to direct negative effects of individual donation after pesticide treatment or pesticide treated bees refusing to interact with healthy individuals. However, it has been described that in *M. quadrifasciata* a trophallactic event is always initiated by the soliciting bee (Contrera et al., 2010), probably the one deprived of food. In addition, mechanisms of food request were initiated by the recipients (non-fed individuals) in other social insects (see, Dussutour and Simpson, 2008). Hungry bees participate in food transfer through trophallaxis but have been seen participating less often than fed individuals (Wright et al., 2012). On the other hand, an altruistic behavior or reduction in general activity caused by the pesticide may have caused the treated bee to refuse interactions with her healthy nestmates. Trophallaxis can help dilute the toxicity of pesticides by adding “clean” liquids from donor bees (Rondeau et al., 2014) and can intoxicate healthy individuals who were not exposed to pesticides beforehand. In our study, even after being separated and deprived from food for 1 h, trophallaxis exchange between worker bees who did not ingest the pesticide dose was higher than the trophallaxis exchange between untreated bees and the individuals who received 15 µl of the pesticide solution. Although females may have taken some sucrose solution from the feeder, it is evident that they interacted less with the treated females. We emphasize that the experiment (group of three bees- one treated and two untreated) was designed to double the chance a treated bee would interact with an untreated female compared to interactions between untreated individuals.

Social organisms need a refined communication system, especially when hundreds of individuals are hierarchically organized into a hive, as is the case for eusocial bees. Individuals can communicate through chemical cues (Thom et al., 2007), dances and thoracic vibrations (Hrncir et al., 2008, 2011), or direct contact (Contrera et al., 2010) as antennation, which helps recognize kin (Breed et al., 1992). Furthermore, a lack of antennation communication between nestmates can cause several problems for colony development, as it affects the olfactory memory of foraging bees (Samuelson et al., 2016; Stanley et al., 2015a), their ability to detect foreign organisms (e.g. kleptoparasites) inside the nest, and their recognition of unrelated bees (e. g. drifters) (Breed et al., 1992).

To fully understand the mechanism of trophallaxis and other social interactions that are impaired by stressors, more studies are needed to understand the other potential negative effects of agrochemicals on social insects, regarding species level and classes of pesticides. Field studies have reinforced the negative effects of pesticides found in laboratory experiments and suggest implications for social behavior, pollination and food production for other social bees. Future, semi-field and field experiments can help understand the effects of impaired communication at the colony level for stingless bees such as *M. quadrifasciata.*

Acknowledgments

We thank Eliana Ferreira, Elisabeth R. da Silva, and Nathalia Lomba, for their help with data collection in the field and in the laboratory and to Karen V. Boff and Jadson O. Silva for assistance in the experiment. We also thank the Federal University of Grande Dourados for providing material and equipment for the study.

Declarations of interest

None.

References

Breed, M.D., Smith, T.A., Torres, A., 1992. Role of guard honey bees (Hymenoptera: Apidae) in nestmate discrimination and replacement of removed guards. Ann. Entomol. Soc. Am. 85 (5), 33–37. https://doi.org/10.1093/aesa/85.5.633.

Brodschneider, R., Libor, A., Kupelwieser, V., Crailsheim, K., 2017. Food consumption and food exchange of caged honey bees using a radioactive labelled sugar solution. PLoS One 12 (3), e0174684. https://doi.org/10.1371/journal.pone.0174684.

Brown, M.J.F., Paxton, R.J., 2009. The conservation of bees: a global perspective. Apidologie 40 (3), 410–416. https://doi.org/10.1051/apido/2009019.

Camargo J.M.F., Pedro S.R.M., 2013. Meliponini Lepeletier, 1836. In: Moure, J.S., Urban, D., Melo, G.A.R. (Orgs). Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version. Available at 〈http://www.moure.cria.org.br/catalogue〉 (Accessed 25 April 2018).

Contrera, F.A., Imperatriz-Fonseca, V.L., Koedam, D., 2010. Trophallaxis and reproductive conflicts in social bees. Insect. Soc. 57 (2), 125–132. 〈http://dx.doi.org10.1007/s00040-009-0058-5〉.

De Bruijn, L.L., Sommeijer, M.J., 1997. Colony foraging in different species of stingless bees (Apidae, Meliponinae) and the regulation of individual nectar foraging. Insect. Soc. 4 (1), 35–47. https://doi.org/10.1007/s000400050028.

Dussutour, A., Simpson, S.J., 2008. Carbohydrate regulation in relation to colony growth in ants. J. Exp. Biol. 211 (14), 2224–2232. https://doi.org/10.1242/jeb.017509.

Farina, W.M., 1996. Food-exchange by foragers in the hive -a means of communication among honey bees? Behav. Ecol. Sociobiol. 38, 59–64. https://doi.org/10.1007/s002650050217.

Forfert, N., Moritz, R.F.A., 2017. Thiacloprid alters social interactions among honey bee workers *(Apis mellifera*). J. Apic. Res. 56 (4), 467–474. https://doi.org/10.1080/00218839.2017.1332542.

Frazier, M.T., Mullin, C.A., Frazier, J.L., Ashcraft, S.A., et al., 2015. Assessing honey bee (Hymenoptera: Apidae) foraging populations and the potential impact of pesticides on eight US crops. J. Econ. Entomol. 108 (5), 2141–2152. https://doi.org/10.1093/jee/tov195.

Giannini, T.C., Boff, S., Cordeiro, G.D., Cartolano, J.R., et al., 2015. Crop pollinators in Brazil: a review of reported interactions. Apidologie 46 (2), 209–223. https://doi.org/10.1007/s13592-014-0316-z.

Gill, R.J., Raine, N.E., 2014. Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Funct. Ecol. 28 (6), 1459–1471. https://doi.org/10.1111/1365-2435.12292.

Hrncir, M., Schorkopf, D.L., Schmidt, V.M., Zucchi, R., Barth, F.G., 2008. The sound field generated by tethered stingless bees (*Melipona scutellaris*): inferences on its potential as a recruitment mechanism inside the hive. J. Exp. Biol. 211 (5), 686–698. https://doi.org/10.1242/jeb.013938.

Hrncir, M., Maia-Silva, C., McCabe, S.I., Farina, W.M., 2011. The recruiter's excitement–features of thoracic vibrations during the honey bee's waggle dance related to food source profitability. J. Exp. Biol. 214 (23), 4055–4064. https://doi.org/10.1242/jeb.063149.

Ingram, E.M., Augustin, J., Ellis, M.D., Siegfried, B.D., 2015. Evaluating sub-lethal effects of orchard-applied pyrethroids using video-tracking software to quantify honey bee behaviors. Chemosphere 135, 272–277. https://doi.org/10.1016/j.chemosphere.2015.04.022.

IPBES, 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. S.G. Potts, V.L. Imperatriz-Fonseca and H.T. Ngo. Bonn, Germany, Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services: 552.

Leal, W.S., 2005. Pheromone reception. Top. Curr. Chem. 240, 1–36. https://doi.org/10.1007/b98314.

Leonhardt, S.D., Menzel, F., Nehring, V., Schmitt, T., 2016. Ecology and evolution of communication in social insects. Cell 164 (6), 1277–1287. https://doi.org/10.1016/j.cell.2016.01.035.

Mangiafico, S.S., 2017. rcompanion: Functions to Support Extension Education Program Evaluation. R package version 1.10.1. 〈https://CRAN.R-project.org/package=rcompanion〉.

Matsuda, K., Buckingham, S.D., Kleiner, D., Rauh, J.J., et al., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 22 (11), 573–580. https://doi.org/10.1016/S0165-6147(00)01820-4.

Mayack, C., Naug, D., 2009. Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection. J. Invertebr. Pathol. 100 (3), 185–188. https://doi.org/10.1016/j.jip.2008.12.001.

Michener, C.D., 2007. The Bees of the World, 2nd ed. John Hopkins University Press, Baltimore.

Nunes, T.M., Mateus, S., Turatti, I.C., Morgan, E.D., Zucchi, R., 2011. Nestmate recognition in the stingless bee *Frieseomelitta varia* (Hymenoptera, Apidae, Meliponini): sources of chemical signals. Anim. Behav. 81 (2), 463–467. https://doi.org/10.1016/j.anbehav.2010.11.020.

Pech-May, F.G., Medina-Medina, L., May-Itzá, W.D.J., Paxton, R.J., Quezada-Euán, J.J.G., 2012. Colony pollen reserves affect body size, sperm production and sexual development in males of the stingless bee *Melipona beecheii*. Insect. Soc. 59 (3), 417–424. https://doi.org/10.1007/s00040-012-0236-8.

Pignati, W.A., Souza e Lima, F.A.N.S., Lara, S.S.D., Correa, M.L.M., et al., 2017. Spatial distribution of pesticide use in Brazil: a strategy for health surveillance. Cien. Saude Coletiva 22 (10), 3281–3293. https://doi.org/10.1590/1413-812320172210.17742017.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., et al., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25 (6), 345–353. https://doi.org/10.1016/j.tree.2010.01.007.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rondeau, G., Sánchez-Bayo, F., Tennekes, H.A., Decourtye, et al., 2014. Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites. Sci. Rep. 4 (5566). https://doi.org/10.1038/srep05566.

Samuelson, E.E.W., Chen-Wishart, Z.P., Gill, R.J., Leadbeater, E., 2016. Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze. Sci. Rep. 6 (38957). https://doi.org/10.1038/srep38957.

Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees–a risk assessment. PLoS One 9 (4), e94482. https://doi.org/10.1371/journal.pone.0094482.

Santos, C.F., Acosta, A.L., Dorneles, A.L., Santos, P.D.S., et al., 2016. Queen become workers: pesticides alter caste differentiation in bees. Sci. Rep. 6 (31605). https://doi.org/10.1038/srep31605.

Shackleton, K., Toufailia, H.A., Balfour, N.J., Nascimento, F.S., Alves, D.A., Ratnieks, F.L., 2015. Appetite for self-destruction: suicidal biting as a nest defense strategy in *Trigona* stingless bees. Behav. Ecol. Sociobiol. 69 (2), 273–281. https://doi.org/10.1007/s00265-014-1840-6.

Schweiger, O., Biesmeijer, J.C., Bommarco, R., Hickler, T., et al., 2010. Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. Biol. Rev. 85 (4), 777–795. https://doi.org/10.1111/j.1469-185X.2010.00125.x.

Spurlock, F., Lee, M., 2008. Synthetic pyrethroid use patterns, properties, and environmental effects. In: Synthetic Pyrethroids Chapter 1, pp. 3–25. https://doi.org/10.1021/bk-2008-0991.ch001.

Stanley, D.A., Smith, K.E., Raine, N.E., 2015a. Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. Sci. Rep. 5 (16508). https://doi.org/10.1038/srep16508.

Stanley, D.A., Garratt, M.P.D., Wickens, J.B., Wickens, V.J., Potts, S.G., Raine, N.E., 2015b. Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. Nature 528, 548–550. https://doi.org/10.1038/nature16167.

Stanley, D.A., Russell, A.L., Morrison, S.J., Rogers, C., Raine, N.E., 2016. Investigating the impacts of field-realistic exposure to a neonicotinoid pesticide on bumblebee foraging, homing ability and colony growth. J. Appl. Ecol. 53, 1440–1449. https://doi.org/10.1111/1365-2664.12689.

Thom, C., Gilley, D.C., Hooper, J., Esch, H.E., 2007. The scent of the waggle dance. PLoS Biol. 5 (9), e228. https://doi.org/10.1371/journal.pbio.0050228.

Vollet-Neto, A., Menezes, C., Imperatriz-Fonseca, V.L., 2015. Behavioural and developmental responses of a stingless bee (*Scaptotrigona depilis*) to nest overheating. Apidologie 46 (4), 455–464. https://doi.org/10.1007/s13592-014-0338-6.

Whitehorn, P.R., Wallace, C., Vallejo-Marin, M., 2017. Neonicotinoid pesticide limits improvement in buzz pollination by bumblebees. Sci. Rep. 7 (15562). https://doi.org/10.1038/s41598-017-14660-x.

Williams, G.R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P., 2015. Neonicotinoid pesticides severely affect honey bee queens. Sci. Rep. 13 (14621). https://doi.org/10.1038/srep14621.

Wittwer, B., Hefetz, A., Simon, T., Murphy, L.E., et al., 2017. Solitary bees reduce investment in communication compared with their social relatives. Proc. Natl. Acad. Sci. USA 114 (25), 6569–6574. https://doi.org/10.1073/pnas.1620780114.

Wright, G.A., Lillvis, J.L., Bray, H.J., Mustard, J.A., 2012. Physiological state influences the social interactions of two honeybee nest mates. PLoS One 7 (3), e32677. https://doi.org/10.1371/journal.pone.0032677.

Wu-Smart, J., Spivak, M., 2016. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. Sci. Rep. 6 (32108). https://doi.org/10.1038/srep32108.