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

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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,

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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