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Short Communication

THE STINGLESS BEE SPECIES, *SCAPTOTRIGONA* AFF. *DEPILIS,* AS A POTENTIAL INDICATOR OF ENVIRONMENTAL PESTICIDE CONTAMINATION

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Abstract: Neonicotinoids have the potential to enter the diet of pollinators that collect resources from contaminated plants. The species *Scaptotrigona* aff. *depilis* (Moure, 1942) can be a useful indicator of the prevalence of these chemicals in the environment. Using high-performance liquid chromatography–mass spectrometry, the authors devised a protocol for neonicotinoid residue extraction and detected the presence of neonicotinoids in the bee bodies. Thus, the authors consider this species to be a potential indicator of environmental contamination. *Environ Toxicol Chem* 2015;34:1851–1853. © 2015 SETAC

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INTRODUCTION

Neonicotinoid pesticides are registered for use in more than 120 countries and account for 24% of the global pesticide market. This means neonicotinoids affect a wide array of plants, animals, and environments [1]. The potential harm they cause insect populations has been studied heavily [2]. As a systemic insecticide, compounds within neonicotinoids can enter plant cells and be present in substrates that are attractive to insects. As such, they may be ingested in nectar and pollen by foraging bees [3,4]. Even without robust evidence, the potential presence of neonicotinoid compounds in pollinator diets has been linked to pollinator declines [5].

In Brazil, the native stingless bee species *Scaptotrigona* aff. *depilis* (Moure, 1942) visits a wide range of floral resources [6]. This bee species is likely to come into contact with plants that have been treated with pesticides; thus, we chose to investigate the incidence of the 2 most widely used neonicotinoids using this species. Previous studies using honey bees have shown that the expression of detoxification, behavior, immunity, and nutrition genes is altered in individuals exposed to pesticides [7]. This suggests that honey bees can metabolically combat the incidence of pesticides in their bodies; indeed, the same could potentially occur in stingless bees. Against this background, the present study aimed to detect neonicotinoid chemicals in stingless bees and monitor the time it takes for detoxification of bees from imidacloprid.

MATERIAL AND METHODS

We collected *S.* aff. *depilis* foragers that were returning to the colony after foraging. These were collected at the entrance of 5 colonies at the meliponary at São Paulo University, Ribeirão Preto campus. Colonies were assigned letters: A, B, C, D, and E. One hundred bees were sampled from each colony. At the same time, we collected samples of bees from 2 natural nests (colonies F and G), approximately 3 km away from the 5 colonies. Given the flight range (903 m) of this species, we would not expect the foraging areas of our 2 groups to overlap [8]. Bee samples were frozen immediately after returning to the laboratory. Thereafter, we monitored the presence of imidacloprid and thiamethoxam in colonies of *S.* aff. *depilis* maintained in the meliponary.

We established the following protocol as the method to extract residue in workers from the *S.* aff. *depilis* species. A sample of 20 bees was put in a 50-mL Falcon tube with 10 mL of acetone. The sample was ground using a tissue homogenizer for 1 min and 30 s. It was then placed in a centrifuge for 15 min at 4000 rpm and 4° C, and the supernatant was removed. This was filtered using a 0.20-μm filter, transferred to a test tube, and set aside to evaporate in compressed air. Then, 250 μL of Mobile Phase (50% MeOH:H_2O [v/v] + 0.5% CH_3COOH_2O [v/v]) was added to the tube, and the samples were placed in the spectrometer using high-performance liquid chromatography–mass spectrometry. The liquid chromatography system consisted of an LC10AD pump and a CTO-10AS column oven from Shimadzu. The separation of the chemicals was performed on a C18 column (Agilent ZORBAX Rapid Resolution Eclipse Plus C18 Analytical 4.6 × 150 mm 5-μ; Agilent Technologies). Detection was obtained with a Quattro Micro LC triple quadrupole mass spectrometer (Micromass) fitted with an electrospray interface source and tandem mass operated in the positive ion mode.

Detection was carried out using the internal standards imidacloprid and thiamethoxam, both obtained from Sigma Aldrich (PESTANAL, analytical standard, Fluka). The instrument conditions were adapted from Kamel [9]—retention time: imidacloprid 3.89 s and thiamethoxam 3.39 s; primary transition molecules and collision energy (in electron volts; eV): imidacloprid 256>175, 23eV, and thiamethoxam 292>132, 19eV; secondary transition molecules and collision energy:

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imidacloprid 256>209, 21eV, and thiamethoxam 292>211, 31eV.

RESULTS

Imidacloprid was detected in the samples obtained from colonies A, B, and C (Figure 1). This indicated that these colonies were suitable for further study. Thiamethoxam was found in 1 sample (colony D), and 1 sample was free from contamination of both neonicotinoids (colony E). The bees collected from wild nests 3 km outside the meliponary (colonies F and G) did not show contamination with the neonicotinoids. Additional samples were collected from colonies A, B, and C. Twenty bees from the newly collected samples were tested immediately for the presence of neonicotinoids and once more showed contamination with imidacloprid. We used bees from the remainder of the sample to monitor the time before the neonicotinoid compounds were no longer present in the bees. We confined the bees in boxes of 30 individuals and kept them in a greenhouse with noncontaminated syrup (sucrose and water, 1:1). Two confinement times were used: individuals from colonies A and B were confined for 24 h, and individuals from colonies A, B, and C were confined for 96 h. At the end of these isolation periods, the bees were collected and frozen. Bee samples from colonies A and B (confined for 24 h) had peaks revealing imidacloprid was still present. Individuals from colonies A, B, and C (confined for 96 h) did not present the chemical anymore.

DISCUSSION

The present study is the first to extract and detect pesticide residues in *S.* aff. *depilis.* This procedure can be considered for application and adaptation for other species of stingless bee.

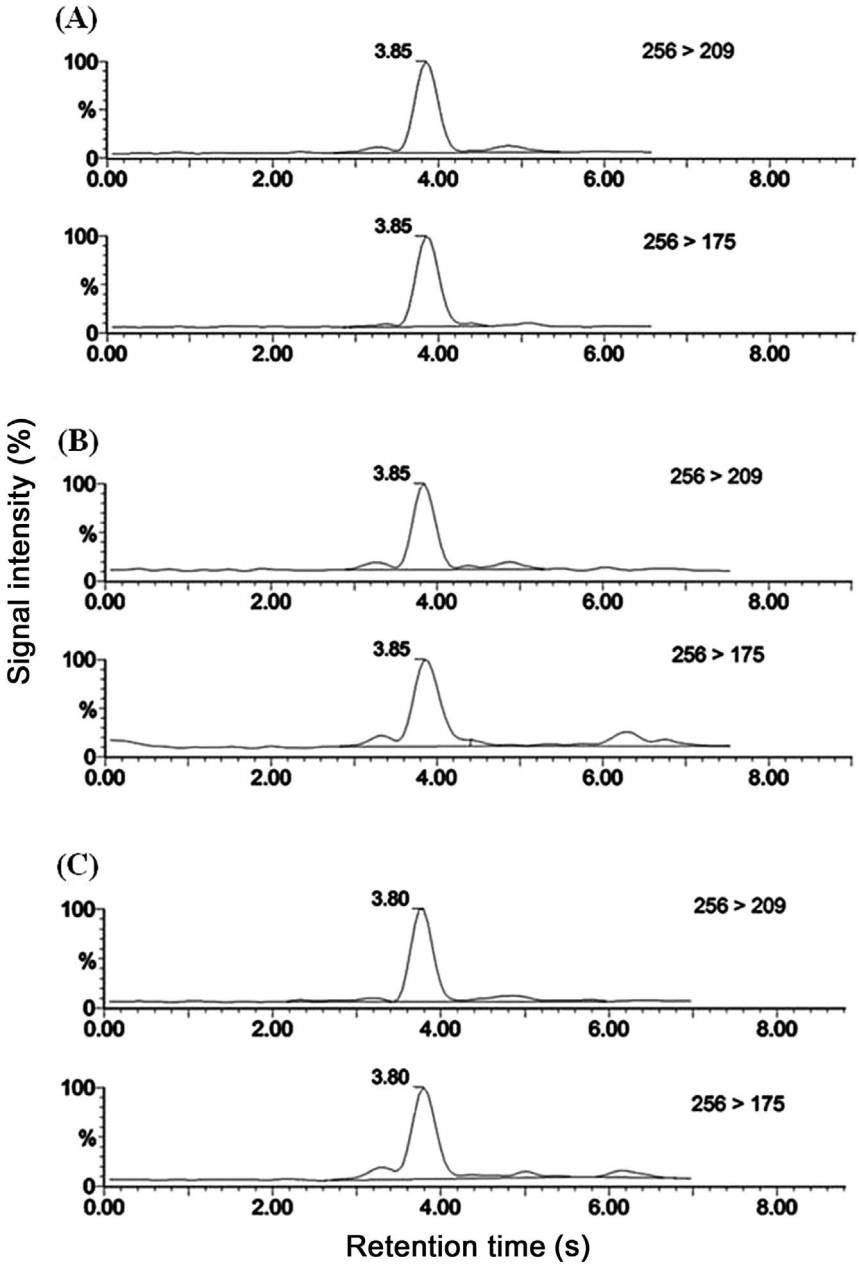

Figure 1. (**A–C**) Chromatograms showing the initial detection of imidacloprid in 3 of the 5 colonies: colony A (**A**), colony B (**B**) and colony C (**C**). Each chromatogram is followed by information on the primary (256>209) or secondary (256>175) transition molecules of imidacloprid. The *x*-axis represents the retention time of the imidacloprid molecules (in seconds) and the *y*-axis represents the signal intensity (in percentage).

Faria et al. [6] reported that this species visits a wide range of floral resources inside the São Paulo University campus by analyzing the pollen collected by *S.* aff. *depilis*. Within a 500-m radius of the meliponary, Aleixo et al. [10] surveyed 289 species of plants, among which 194 offer resources to bees. These flowers could have been contaminated and are within reach of the foraging workers of *S.* aff. *depilis*, which have an estimated flight radius of 903 m [8].

Notably, after 96 h of confinement, bees that were previously contaminated by imidacloprid showed no sign of contamination. Because the chemicals were removed in a short period of time, *S.* aff. *depilis* could be a sensitive indicator of environmental contamination. Furthermore, it is likely that the concentration at which the active substance was ingested is a key factor in the rate it is eliminated [11]. If this is the case, this species would enable researchers to quantify the current level of environmental pesticide contamination using several isolation periods. Part of our future plan is to use the stingless bee diversity on campus. It is home to at least 14 species of stingless bee in wild nests [12]; this variety offers us the opportunity to use several species when analyzing pesticide contamination in the environment. We will also continue to develop the procedures established in the present study to devise a method to quantify residue. This will enable us to deduce the concentrations of the chemicals ingested using the time to elimination. It is likely that the presence of neonicotinoids in the diet of stingless bees has nonlethal effects [13]; thus, bee mortality might not be observed initially. Sublethal effects, situations in which the insecticide does not kill individuals immediately but affects long-term colony fitness, have been demonstrated in bees [14,15]; in fact, studies have attributed the use of neonicotinoids at sublethal concentrations to colony collapse disorder [16,17]. There is a possibility that neonicotinoid chemicals are accumulated on the colonies on campus. Bees play an important role in their environment; therefore, a study that investigates the long-term effects of neonicotinoid ingestion could help us understand the environmental consequences of using neonicotinoid pesticides.

Finally, our results enable us to consider using *S.* aff. *depilis* as an indicator of environmental pesticide contamination. Further research using the techniques developed in the present study will help monitor toxicity levels in stingless bee species with the potential to create proposals that use stingless bee contamination as an environmental indicator.

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Data availability—All data are found within the present study.

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