

# A Spinosad-Based Formulation Reduces the Survival and Alters the Behavior of the Stingless Bee *Plebeia lucii*

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## Keywords

Ecotoxicology, organic farming, pollinator decline, *Plebeia*, spinosad

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## Abstract

The decline in bee populations worldwide has been associated with the use of pesticides in crop systems where these insects forage. The use of biopesticides, like spinosad, is preferred as an alternative method to control pests, because it is considered safer to non-target insects. In this study, we evaluated the lethal and sublethal effects of the spinosad-based formulation Tracer® on foragers of the stingless bee *Plebeia lucii* Moure (Apidae: Meliponini). Groups of bees were fed a pure diet (negative control) or a diet at different concentrations of spinosad. Positive control groups consisted of bees orally exposed to a diet with the neonicotinoid imidacloprid. Next, flight behavior, body mass, and respiration rate were evaluated in surviving bees. The results showed that bees' survival was reduced by all concentrations of spinosad, when compared with the negative control. Bee locomotion—walking and flight—was reduced in accordance with the increase in spinosad concentrations; however, body mass and respiration rate were not altered. Our results show that the use of Tracer® in ecosystems visited by *P. lucii* can reduce forager bee survival and reduce their locomotion, generating a negative impact on pollination services provided by these bees.

## Introduction

Pollination is an ecosystem service provided mainly by bees in the majority of terrestrial ecosystems and wild pollinators contribute to increase crop yield (Garibaldi *et al* 2013). Decline in bee populations is a worldwide concern and pollutant effects on honey bees and wild bees have been intensely debated for more than a decade (Brown & Paxton 2009). Although the effects of synthetic pesticides have been investigated on many different bee species (Arena & Sgolastra 2014, Lima *et al* 2016), the toxicity of other pesticides remains poorly explored, particularly on wild bees (Barbosa *et al* 2015). Despite their importance, wild bee populations are declining rapidly, and investigations linked to this decline are lacking when compared with research on honey bees (Powney *et al* 2019).

Bees can be exposed to agrochemical contamination during foraging or after contact with or ingestion of contaminated resources carried to the colonies and shared with nestmates (Krupke *et al* 2012). In the field, forager bees are the first to be exposed to pesticides, because they come in contact with these products during spraying or when collecting floral resources. Moreover, after the ingestion or contact with contaminated pollen and nectar, the foragers can die before they return to their colonies, thereby reducing colony fitness.

Stingless bees (Apidae, Meliponini) are pantropical wild bees with the highest diversity in the Neotropics (Kerr *et al* 1996). Although the majority of them are essential pollinators of wild and crop plants in the tropical regions, some species are in danger of extinction (Giannini *et al* 2015, Lima *et al* 2016, ICMBio 2018). Their geographic distribution, populous colonies, and species richness and abundance in

different ecosystems make stingless bees the main group of wild pollinators in tropical regions (Roubik 2006, Giannini et al 2015). Moreover, many species have morphological and behavioral adaptations that enhance their capacity to pollinate specific plants, for instance, through buzz pollination (Greenleaf & Kremen 2006, Caro et al 2017, Silva-Neto et al 2016). Therefore, the use of pesticides in crop systems can contaminate stingless bees and reduce pollination service (Lima et al 2016).

The detrimental effects of synthetic pesticides on non-target species, such as bees, prompted the development of pesticides from natural molecules, known also as biopesticides (Casida & Quistad 1998, Cisneros et al 2002, Isman 2006). Spinosad is a biopesticide formulated from a mixture of spinosins A and D, derived from the fermentation of the soil actinomycete *Saccharopolyspora spinosa* (Salgado 1998, Sparks et al 2001). Spinosins are neurotoxins that act as agonists of acetylcholine receptors in the nervous system leading to target-species death (Nauen & Bretschneider 2002). Spinosad is a non-systemic pesticide generally applied by ground spray application (MAPA 2019).

The use of spinosad to control crop pests is increasing worldwide since it is considered relatively safe on non-target species (Sarfranz et al 2005, Biondi et al 2012). However, some studies have shown lethal and sublethal toxicity on stingless bees upon exposure to spinosad (Sánchez et al 2012, Tomé et al 2015a, b, Araújo et al 2019a, b). Nevertheless, there are no investigations about the consequences of spinosad ingestion on *Plebeia* Schwarz, which is a genus of tiny stingless bees with around 40 different Neotropical species (Michener 2000, Pedro & de Carmargo 2013). *Plebeia* bees are easily found in anthropized ecosystems, such as urban and rural areas, where they visit many different plants (Ramalho et al 1990, Ribeiro & Taura 2019, Tschoeke et al 2015). In contrast with other bees, the floral visitation rates of *Plebeia* are not reduced by the use of pesticides, thus increasing their long-term exposition to these compounds in the field (Tschoeke et al 2019). *Plebeia lucii* Moure is a common species in southeast Brazil (Moure 2004) and their small colonies can be easily managed, making them suitable to conduct ecotoxicological assays. Herein, we investigated if oral contamination with spinosad reduces the survival and/or harms the physiology and behavior of *P. lucii* foragers.

## Material and Methods

### Ethics statement

In order to conduct this study in accordance with the Brazilian legislation, we acquired two licenses to bees' sampling: SISBIO (ID 71998–1) and SISGEN (ID AE230B3). Bees'

sampling was carried out without depleting the original colonies. Although some stingless bees are considered endangered species in Brazil, *P. lucii* is not an endangered or protected species.

### Bees and pesticides

We sampled *P. lucii* foragers from 10 colonies kept in a small fragment of the Brazilian rainforest (20°45'14"S, 42°52'54"W). Colonies were maintained in wooden boxes (9 cm × 12 cm × 10 cm) with a small entrance allowing the entrance of only one worker. We collected the foragers using a wooden box trap (11 cm × 11 cm × 3.5 cm), which was attached to the colony entrance, before the beginning of foraging activities. The traps had a feeder with sugarcane sucrose solution (1:1 w/w sugar syrup) to attract the foragers. The sucrose solution was prepared by dissolving commercial granulated sugar at 80°C in distilled water. After sampling, the traps were transported to a dark room, where a fluorescent lamp was placed behind plastic arenas (250 ml), which were covered with tulle. The traps were linked to arenas and the foragers were attracted to the arenas due to their phototactic behavior. Foragers were not anesthetized and the arenas were kept inside an incubator with conditions similar to that of the hive (28 ± 2°C; 75 ± 5% relative humidity (RH); 24 h of scotophase). In all experiments, bees were orally exposed to pesticides diluted in sugar syrup.

We used the commercial formulation of two pesticides to conduct the experiments: spinosad (Tracer®, 480 g a.i. L<sup>-1</sup>, concentrated suspension, Dow Agrosciences, Santo Amaro, SP, Brazil) and imidacloprid (Evidence®, 700 g a.i. L<sup>-1</sup>, water-dispersible granules, Bayer CropScience, São Paulo, SP, Brazil). Foragers were exposed to the Brazilian Ministry of Agriculture-recommended Tracer® concentration for the control of tomato leafminer (*Tuta absoluta*) (Meyrick) (81.6 ng a.i. µl<sup>-1</sup>) (MAPA 2019), as well as to decreasing dilutions of this concentration: 4.08 ng a.i. µl<sup>-1</sup>, 1.63 ng a.i. µl<sup>-1</sup>, 0.82 ng a.i. µl<sup>-1</sup>, and 0 ng a.i. µl<sup>-1</sup> spinosad. We used Evidence® in the concentration recommended for *Bemisia tabaci* (Gennadius) control in tomato crops (42 ng a.i. µl<sup>-1</sup> imidacloprid) (MAPA 2019) as the positive control and a non-contaminated sucrose solution as the negative control. We used the neonicotinoid imidacloprid as a positive control because of its high toxicity to stingless bees in comparison with other pesticides (Tomé et al 2015a).

### Survival bioassay

We transferred groups of five foragers from each colony to plastic arenas (described above) where they were food-deprived for 12 h and maintained in conditions similar to that of original colonies (24 h of scotophase; 28 ± 2°C; 75 ± 5% RH). After that, we included a plastic feeder in the arenas

with 2 ml of syrup including the tested agrochemical concentrations.

We exposed the foragers for 72 h and plastic feeders were changed every 12 h, to avoid agrochemical degradation and syrup fermentation. We used 10 arenas with 5 foragers for each treatment (one different colony for each arena). Therefore, experiments were replicated 10 times (10 arenas) and each repetition was formed by foragers collected in one colony. The survival of the foragers was checked every 12 h during the exposure period and insects were counted as dead if they were unable to walk.

### Sublethal bioassays

The same bees subjected to the survival bioassay were subsequently used in sublethal experiments, which were carried out immediately after the exposure period. Therefore, we conducted sublethal bioassays with foragers that survived for more than 72 h after exposure, and the same bees were used in all tests. After the tests, all bees were sacrificed and properly discharged. Sublethal experiments started at 8:00 am and they were conducted during the day, at constant temperature and luminosity. Firstly, we compared the locomotion of foragers exposed to different treatments to evaluate the effects of pesticides on their flight and walking behaviors. In walking bioassays, foragers were individually transferred to Petri dishes (9.0 cm of diameter), immediately after the end of exposure. In a dark room, we placed the bees inside a cage with a fluorescent lamp and we used soft plastic tweezers to transfer the bees from the arenas to the Petri dishes. These arenas were covered with filter paper and sealed with Teflon® to avoid forager escape. We maintained the foragers for 10 min in the arenas, and their walking behavior was recorded by a camera coupled to a computerized video tracking system (View Point Life Sciences, Montreal, QC, Canada). The system measured walking velocity ( $\text{cm s}^{-1}$ ), walking distance (cm), resting time (s), and number of stops per forager, following Tomé *et al* 2012. This experiment was conducted with 40, 40, and 33 foragers for treatments with 0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad, respectively.

To evaluate the foragers' capacity to recover their flight after exposure to spinosad, we subjected the foragers to a free-fall flight bioassay (adapted from Tomé *et al* 2015a, b). In a dark room, we put a 135 cm high tower, formed by three wooden boxes (45 cm  $\times$  45 cm  $\times$  45 cm), and we placed a fluorescent lamp 150 cm above the ground. We released each forager, individually, 10 cm below the lamp within the tower and recorded their site of landing. The flight was then stratified in relation to the tower base as follows: (I) absence of flight, when foragers were not able to fly and landed directly on the tower base (0 cm); (II) landing at the first

box (from 1 to 45 cm); (III) landing at the second box (from 46 to 90 cm); (IV) landing at the third box (from 91 to 135 cm); and (V) no-fall, and flight towards the lamp (from 136 to 150 cm). This experiment was conducted with 50, 43, and 34 foragers for treatments with 0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad, respectively.

In addition, we compared the body mass and respiration rate of foragers exposed to different treatments. Firstly, we weighed 20 foragers (2 foragers per colony) of each treatment (0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad) using an analytical balance (XS3DU, Mettler Toledo, Columbus, OH). Bees were placed individually in microtubes to be weighed. Then, these bees were transferred to 25 ml glass tubes hermetically closed, which were connected to a respirometer (TR3C), equipped with a  $\text{CO}_2$  analyzer (Sable Systems International, Las Vegas, NV, EUA). To transfer the bees, we transported them to a dark room, where a fluorescent lamp was placed behind plastic arenas (250 ml). The traps were linked to arenas and the foragers were attracted to the arenas due to their phototactic behavior. We used soft plastic tweezers to transfer each bee from the arenas to the glass tubes. We measured the carbon dioxide production of two foragers ( $\mu\text{l CO}_2 \text{ h}^{-1} \text{ forager}^{-1}$ ) per tube over 3 h, following Tomé *et al* (2015a, b). In both experiments, bees were evaluated in dyads.

### Statistical analyses

We considered each colony as one replicate in all models. Therefore, we analyzed the data using the mean results obtained from foragers sampled in the 10 different colonies. The effect of pesticides on survival was tested through Kaplan-Meier analysis to estimate curves and median survival times ( $\text{LT}_{50}$ ). Curves similarity was tested using  $\chi^2$  Log-Rank test and paired comparisons were carried out by Bonferroni correction ( $p < 0.05$ ). Variables of the walking behavior (distance, velocity, resting time, and number of stops) were subjected to principal component analysis (PCA). The components were defined through the correlation matrix for standardization of variances because the variables did not have the same scale. The principal components were selected based on the eigenvalues higher than the mean value of all eigenvalues. Models with component scores (from PCA), respiration rate, and body mass were submitted to analysis of variance (ANOVA) with pesticide treatments as the explanatory variable. When necessary, the data were transformed with  $\log_{10}(y + 1)$  to conform to the assumptions of ANOVA. For flight behavior data, generalized linear models (GLMs) with binomial distribution (link = logit) was applied. The proportion of individuals reaching each height was considered response variable and treatments with pesticides as explanatory variable. The comparisons between explanatory

variable levels were performed by gradual simplification of models (Crawley 2012). The residuals of all models were checked by visual inspection (residuals vs. fitted and Q-Q plot), or by Shapiro-Wilk's test and Bartlett's test. The analyses were performed using R software (version 3.4.4; R Core Team 2018).

## Results

### Survival bioassay

The ingestion of contaminated diet reduced the survival of *P. lucii* foragers ( $\chi^2 = 328$ ,  $df = 5$ ,  $p < 0.001$ , Fig 1). Bees exposed to field recommended concentrations of Tracer® (81.6 ng a.i.  $\mu\text{l}^{-1}$  spinosad) and Evidence® (42 ng a.i.  $\mu\text{l}^{-1}$  imidacloprid) showed a mortality of 92% and 100% respectively, during the first 12 h of exposure. After 72 h of exposure, foragers exposed to spinosad concentrations of 4.08, 1.63, and 0.82 ng a.i.  $\mu\text{l}^{-1}$  had a mortality of 76%, 28%, and 10% respectively (Fig 1a). The estimated  $LT_{50} \pm CL$  (median of lethal times  $\pm 95\%$  confidence limits) for Tracer® were  $12 \pm 1$  (81.6 ng a.i.  $\mu\text{l}^{-1}$  spinosad) and  $60 \pm 11.37$  h (4.08 ng a.i.  $\mu\text{l}^{-1}$  spinosad). The estimated  $LT_{50} \pm CL$  for Evidence® (42 ng a.i.  $\mu\text{l}^{-1}$  imidacloprid) was  $12 \pm 0$  h (Fig 1b). In the other treatments,  $LT_{50}$  could not be estimated because less than 50% of foragers died before the end of experiments.

### Sublethal bioassays

#### Walking behavior

The variables associated with walking behavior of foragers exposed to increasing concentrations of Tracer® (0, 0.82, and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad) were highly correlated, indicating the suitability of PCA (Fig 2). Two components that exhibited eigenvalues greater than the mean were selected. PC1 (first component of PCA) and PC2 (second component of PCA) explained, respectively, 82.7% and 14.3% of the total variance of the data (Table 1, Fig 3). The variables of PC1 had opposite signals and explained most of the variance (82.7%). PC1 compared the variables velocity and distance with resting time and number of stops between individuals walking longer and shorter distances. Negative values indicate higher velocity and distance, whereas positive values indicate greater resting time and number of stops. PC2 explained less of the total variance (14.3%), and the comparison of velocity and resting time with distance and number of stops attributable to PC2 was not interesting for interpreting the walking behavior. In PC1, a significant difference was detected among the treatments, showing that contamination with increasing concentrations of spinosad decreased locomotion activity of

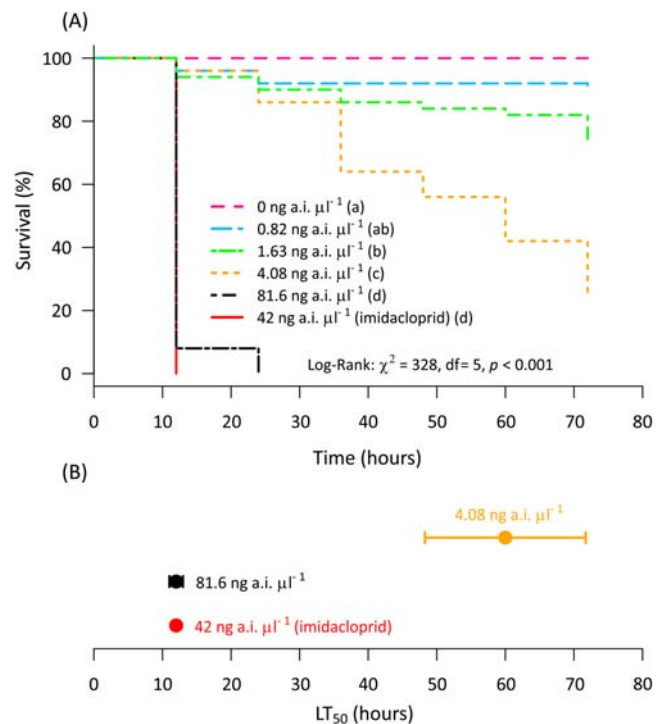


Fig 1 Survival curves (A) and  $LT_{50}$  (median of lethal times  $\pm 95\%$  confidence limits) (B) of *Plebeia lucii* foragers exposed to different concentrations of Tracer® (0, 0.82, 1.63, 4.08 and 81.6 ng a.i.  $\mu\text{l}^{-1}$  spinosad) and Evidence® (42 ng a.i.  $\mu\text{l}^{-1}$  imidacloprid). Different lower-case letters indicated significant differences by the Bonferroni method ( $p < 0.05$ ).

foragers ( $F_{2, 27} = 3.42$ ,  $p = 0.047$ , Fig 4a, b). The pesticide treatments did not elicit a significant difference in PC2 ( $F_{2, 27} = 2.4$ ,  $p = 0.1$ , Fig 4c).

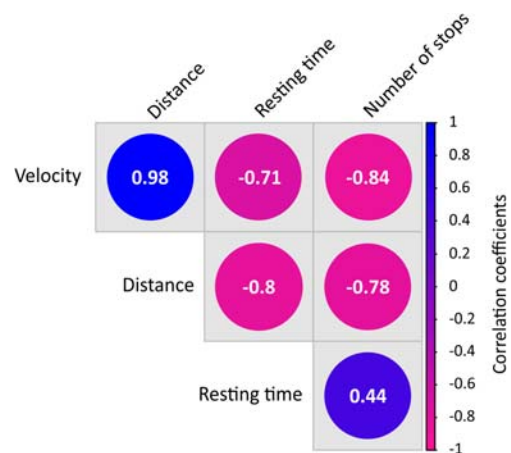


Fig 2 Upper correlation matrix of the variables associated with walking behavior of the *Plebeia lucii* foragers exposed to increasing concentrations of Tracer® (0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad). Correlation was ordered by hierarchical clustering based on the centroid. Data were obtained from mean values of experiments with foragers randomly sampled in 10 different colonies (i.e., 10 replicates).



**Table 1** Summary of the principal component analysis of the variables associated with walking behavior of *Plebeia lucii* foragers submitted to ingestion of increasing concentrations of Tracer® (0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$  and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad). Data were obtained from mean values of experiments with foragers randomly sampled in 10 different colonies (i.e., 10 replicates).

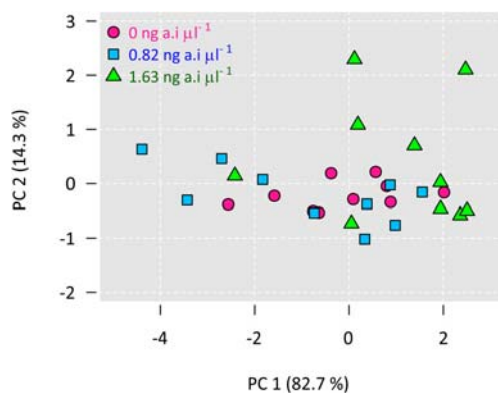
Variables	PC1	PC2
Velocity	-0.539	0.125
Distance	-0.542	-0.067
Resting time	0.445	0.749
Number of stops	0.467	-0.647
Proportion of variance (%)	82.7	14.26
Cumulative proportion (%)	82.7	96.96

### Flight behavior

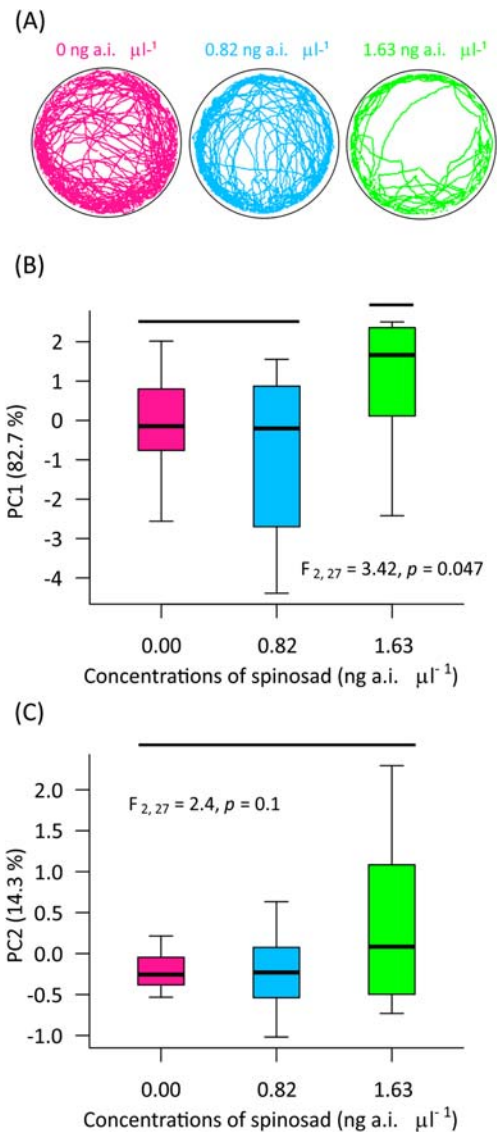
Flight behavior was also altered by Tracer® ingestion (Fig 5). Most foragers exposed to control syrup (0 ng a.i.  $\mu\text{l}^{-1}$ ) or to the lowest concentration of spinosad (0.82 ng a.i.  $\mu\text{l}^{-1}$ ) reached the lamp (150 cm) ( $\chi^2 = 26.4$ ,  $df = 2$ ,  $27$ ,  $p = 0.0045$ ). Most foragers exposed to a higher spinosad concentration (1.63 ng a.i.  $\mu\text{l}^{-1}$ ) were not able to reach the lamp and reached between 91 and 135 cm in height ( $\chi^2 = 26.92$ ,  $df = 2$ ,  $27$ ,  $p = 0.022$ ).

### Respiratory rate and body mass

Foragers exposed to the ingestion of syrup with 0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , or 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad respectively, produced  $10.16 \pm 1.54 \mu\text{l h}^{-1} \text{ bee}^{-1}$ ,  $10.4 \pm 2.16 \mu\text{l h}^{-1} \text{ bee}^{-1}$ , and  $8.34 \pm 1.19 \mu\text{l h}^{-1} \text{ bee}^{-1}$  (mean  $\pm$  SE) of  $\text{CO}_2$ . The body masses were  $5.23 \pm 0.14 \text{ mg bee}^{-1}$ ,  $5.11 \pm 0.15 \text{ mg bee}^{-1}$ , and  $5.4 \pm 0.22 \text{ mg bee}^{-1}$  (mean  $\pm$  SE) for 0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$ , and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad, respectively. Chronic



**Fig 3** Plot of principal component analysis (PCA) of variables associated with walking behavior (see Table 1) of *Plebeia lucii* foragers exposed to increasing concentrations of Tracer® (0 ng a.i.  $\mu\text{l}^{-1}$ , 0.82 ng a.i.  $\mu\text{l}^{-1}$  and 1.63 ng a.i.  $\mu\text{l}^{-1}$  spinosad). PC1 is the first and PC2 is the second principal components of PCA. Values in percentages in the axes labels indicate how much the components explain of the total variance. Data were obtained from mean values of experiments with foragers randomly sampled in 10 different colonies (i.e., 10 replicates).



**Fig 4** **A** Representative tracks showing the walking behaviour of *Plebeia lucii* foragers orally contaminated with increasing concentrations of Tracer® (0 ng a.i.  $\mu\text{l}^{-1}$ , pink; 0.82 ng a.i.  $\mu\text{l}^{-1}$ , blue; and 1.63 ng a.i.  $\mu\text{l}^{-1}$ , green; spinosad). **B**, **C** Box plot of mean and range of dispersion (lower and upper quartiles) of the computed component scores in the PCA. Scores of PC1 which represented 82.7% of variance (**B**) and scores of PC2 which represented 14.3% of variance (**C**). The treatments grouped by the same horizontal line did not differ according to gradual simplification of the model ( $p < 0.05$ ). Data were obtained from mean values of experiments with foragers randomly sampled in 10 different colonies (i.e., 10 replicates).

ingestion of increasing concentrations of Tracer® did not alter the respiration rate ( $F_{2, 27} = 0.5$ ,  $p = 0.6$ ) or body mass ( $F_{2, 27} = 0.73$ ,  $p = 0.5$ ) of foragers.

### Discussion

According to our results, concentrations of spinosad which correspond to a recent application of Tracer® were as toxic to

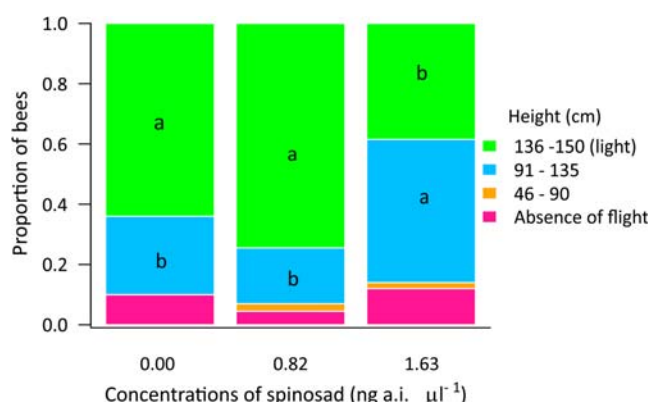


Fig 5 Flight behaviour of *Plebeia lucii* foragers orally exposed to increasing concentrations of Tracer® (0 ng a.i. µl<sup>-1</sup>, 0.82 ng a.i. µl<sup>-1</sup>, and 1.63 ng a.i. µl<sup>-1</sup> spinosad). Data were sampled from the proportion of foragers that reached different heights in a tower used in a vertical flight bioassay (for details see text). Proportions represented by the same letter are not different according to the gradual simplification of the generalized linear models ( $p < 0.05$ ). Proportions with no significant difference among the different treatments are not represented by letters. The height between 0 and 45 cm is not represented in the figure because no one forager rested on this height. Data were obtained from median values of experiments with foragers randomly sampled in 10 different colonies (i.e., 10 replicates).

a tiny stingless bee as was the neonicotinoid imidacloprid. Even field-diluted spinosad concentrations increased the mortality and impaired the motor abilities of *P. lucii*. The toxicity of biopesticides, in general, and spinosad, in particular, on stingless and other wild bees have been reported previously (Morandin et al 2005, Gómez-Escobar et al 2014; Tomé et al 2015a, b, Bernardes et al 2017, 2018, Araujo et al 2019a). This study is the first to show the effects of spinosad on a tiny wild bee, which possibly experiences greater exposure in the field when compared with other pollinators owing to its restricted foraging area and tendency of not avoiding contaminated resources (Ramalho et al 1985, Sánchez et al 2012, Tschoeke et al 2019).

The increased mortality of workers caused by pesticides can be particularly detrimental for *Plebeia* species. This is because their colonies are formed of few workers in comparison with those of other stingless bees (Roldão-Sbordoni et al 2018). Therefore, the mortality of few workers can seriously harm the entire colony, mainly during winter or periods of reduction in floral resources (Hilário et al 2012). In addition, even in the genus *Plebeia*, *P. lucii* has small workers, measuring 3 mm in total length (Moure 2004), making them more susceptible to pesticides relative to bigger bees (Valdovinos-Núñez et al 2009).

Our results showed that locomotion of *P. lucii* was impaired by chronic ingestion of Tracer®. Reduction in walking behavior induced by increasing concentrations of Tracer® was probably due to the neurotoxic activity of spinosad. Neurotoxicity of synthetic pesticides impeded walking and reduced the volume of mushroom bodies in the stingless

bee *Melipona quadrifasciata* Lepeletier (Tomé et al 2012). Mushroom bodies—areas in the bee's brain associated with navigation—of *Scaptotrigona postica* (Latreille), another stingless bee, was also impaired by the pesticide fipronil (Jacob et al 2013). Another hypothesis is that spinosad acts on different target-organs in *P. lucii*, causing indirect behavioral changes. In the stingless bee *Partamona helleri* Friese, spinosad harmed the midgut and walking behavior of adult workers, but changes in the brain were not detected (Araujo et al 2019b). Midgut modifications in *P. helleri* were also caused by spinosad oral contamination during larval development (Araujo et al 2019a).

The reduction in flight capacity of *P. lucii* after Tracer® ingestion, as reported in this study, can be highly detrimental to their colonies in the field and may reduce their pollination service. *Plebeia* have a small foraging range, and inside this area, they forage on few floral sources, when compared with other eusocial bees (Ramalho et al 1985). Therefore, owing to their small size, which is directly proportional to their foraging range, they are forced to collect restricted floral resources (Van Nieuwstadt & Ruano Iraheta 1996, Kuhn-Neto et al 2009). Maybe this is the reason they are not repelled by flowers contaminated with synthetic and organic pesticides, keeping their visitation rate in treated fields intact, unlike the case with honey bees and Halictid bees (Tschoeke et al 2019). It can also be a non-adaptive behavior of stingless bees, because other species of this group were not repelled by the biopesticide azadirachtin (Bernardes et al 2017). Even in choice experiments, *Plebeia* workers ingest solutions contaminated with spinosad (Sánchez et al 2012), probably enhancing their long-term exposure to this pesticide in a realistic scenario. In contrast, *Plebeia droryana* Friese larvae ate less larval food due to contamination with the synthetic pesticide chlorpyrifos (Dos Santos et al 2016). These differences in *Plebeia* feeding behavior mediated by different pesticides can be an effect of the molecule's mode of action or a result of the specific physiology of each *Plebeia* species. Because the monophyly of *Plebeia* is controversial (Drumond et al 2000, Rasmussen & Cameron 2009, Melo 2016), physiological differences among *Plebeia* and their susceptibility to pesticides should be further investigated.

The lack of effects on respiration rate and body mass of workers here reported is in accordance with the results of previous studies that analyzed these parameters as indicators of toxicity of different pesticides on stingless bees (Bernardes et al 2017, 2018). However, in *M. quadrifasciata*, modifications on respiration rate mediated by spinosad contamination were detected after 24 h of exposure, in contrast with the lack of this effect 3 h after exposure (Tomé et al 2015a, b). In our experiments with *P. lucii*, we tested the respiration rate, a physiological measure of stress, soon after contamination. We found less modifications in the respiration rate which may be due to the

small time gap between the end of exposure and estimation of the respiration rate. The absence of effects on body mass was not surprising, because experiments were conducted with adult bees.

Although biopesticides are supposedly safer for non-target organisms, owing to their biological origin, this study shows that they can harm the survival and locomotion of *P. lucii*, thus reinforcing the argument that the natural origin of these compounds is not necessarily a determinant of their toxicity to stingless bees (Barbosa *et al* 2015). The high toxicity of spinosad to stingless bees in relation to synthetic pesticides, such as neonicotinoids, deserves attention, because, in general, there is a public perception that natural products are safer for pollinators and therefore, they can be extensively applied (Barbosa *et al* 2015). Because it is considered a low-risk biopesticide, spinosad's use in organic farming is approved in many countries (Biondi *et al* 2012). We suggest that the ecotoxicological profile of Tracer® and other formulations of spinosad should be further investigated, because its safety to pollinators such as stingless bees is not well established.

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**Compliance with Ethical Standards**

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

## References

- Araujo R d S, Bernardes RC, Fernandes KM *et al* (2019a) Spinosad-mediated effects in the post-embryonic development of *Partamona helleri* (Hymenoptera: Apidae: Meliponini). *Environ Pollut* 253:11–18. <https://doi.org/10.1016/j.envpol.2019.06.087>
- Araujo R d S, Lopes MP, Barbosa WF *et al* (2019b) Spinosad-mediated effects on survival, overall group activity and the midgut of workers of *Partamona helleri* (Hymenoptera: Apidae). *Ecotoxicol Environ Saf* 175: 148–154. <https://doi.org/10.1016/j.ecoenv.2019.03.050>
- Arena M, Sgolastra F (2014) A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology* 23:324–334. <https://doi.org/10.1007/s10646-014-1190-1>
- 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. <https://doi.org/10.1002/ps.4025>
- Bernardes RC, Tomé HVV, Barbosa WF, Guedes RNC, Lima MAP (2017) Azadirachtin-induced antifeeding in Neotropical stingless bees. *Apidologie* 48:275–285. <https://doi.org/10.1007/s13592-016-0473-3>
- Bernardes RC, Barbosa WF, Martins GF, Lima MAP (2018) The reduced-risk insecticide azadirachtin poses a toxicological hazard to stingless bee *Partamona helleri* (Friese, 1900) queens. *Chemosphere* 201:550–556. <https://doi.org/10.1016/j.chemosphere.2018.03.030>
- Biondi A, Mommaerts V, Smagghe G *et al* (2012) The non-target impact of spinosyns on beneficial arthropods. *Pest Manag Sci* 68:1523–1536. <https://doi.org/10.1002/ps.3396>
- Brown MJF, Paxton RJ (2009) The conservation of bees: a global perspective. *Apidologie* 40:410–416. <https://doi.org/10.1051/apido/2009019>
- Caro A, Moo-Valle H, Alfaro R, Quezada-Euán JJG (2017) Pollination services of Africanized honey bees and native *Melipona beecheii* to buzz-pollinated annatto (*Bixa orellana* L.) in the neotropics. *Agric For Entomol* 19:274–280. <https://doi.org/10.1111/afe.12206>
- Casida JE, Quistad GB (1998) Golden age of insecticide research: past, present, or future? *Annu Rev Entomol* 43:1–16. <https://doi.org/10.1146/annurev.ento.43.1.1>
- Cisneros D, Derwent LC *et al* (2002) Toxic effects of Spinosad on predatory insects. *Biol Control* 23:156–163. <https://doi.org/10.1006/BCON.2001.1000>
- Crawley MJ (2012) *The R book*, 2nd edn. Wiley, Chichester
- dos Santos CF, Acosta AL, Dorneles AL, dos Santos PDS, Blochtein B (2016) Queens become workers: pesticides alter caste differentiation in bees. *Sci Rep* 6:31605–31609. <https://doi.org/10.1038/srep31605>
- Drumond PM, Zucchi R, Oldroyd BP (2000) Description of the cell provisioning and oviposition process of seven species of *Plebeia* Schwarz (Apidae, Meliponini), with notes on their phylogeny and taxonomy. *Insect Soc* 47:99–112. <https://doi.org/10.1007/PL00001703>
- Garibaldi LA, Steffan-Dewenter I, Winfree R *et al* (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* (80-) 339:1608–1611. <https://doi.org/10.1126/science.1230200>
- Giannini TC, Boff S, Cordeiro GD *et al* (2015) Crop pollinators in Brazil: a review of reported interactions. *Apidologie* 46:209–223. <https://doi.org/10.1007/s13592-014-0316-z>
- Gómez-Escobar E, Liedo P, Montoya P, Vandame R, Sánchez D (2014) Behavioral response of two species of stingless bees and the honey bee (Hymenoptera: Apidae) to GF-120. *J Econ Entomol* 107:1447–1449. <https://doi.org/10.1603/EC13490>
- Greenleaf SS, Kremen C (2006) Wild bees enhance honey bees' pollination of hybrid sunflower. *Proc Natl Acad Sci* 103:13890–13895. <https://doi.org/10.1073/pnas.0600929103>
- Hilário SD, Ribeiro M de F, Imperatriz-Fonseca VL (2012) Can climate shape flight activity patterns of *Plebeia remota* Hymenoptera, Apidae? *Iheringia Série Zool* 102:269–276. <https://doi.org/10.1590/S0073-47212012000300004>
- ICMBio. Instituto Chico Mendes de Conservação da Biodiversidade (2018) *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção*. Volume I. Brasília, Brasil
- Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* 51:45–66. <https://doi.org/10.1146/annurev.ento.51.10104.151146>

- Jacob CRO, Soares HM, Carvalho SM, Nocelli RC, Malaspina O (2013) Acute toxicity of Fipronil to the stingless bee *Scaptotrigona postica* Latreille. *Bull Environ Contam Toxicol* 90:69–72. <https://doi.org/10.1007/s00128-012-0892-4>
- Kerr WE, Carvalho GA, Nascimento VA et al (1996) Abelha urucu : biologia, manejo e conservacao. Fundacao Acangau
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7:e29268. <https://doi.org/10.1371/journal.pone.0029268>
- Kuhn-Neto B, Contrera FAL, Castro MS, Nieh JC (2009) Long distance foraging and recruitment by a stingless bee, *Melipona mandacaia*. *Apidologie* 40:472–480. <https://doi.org/10.1051/apido/2009007>
- Lima MAP, Martins GF, Oliveira EE, Guedes RNC (2016) Agrochemical-induced stress in stingless bees: peculiarities, underlying basis, and challenges. *J Comp Physiol A* 202:733–747. <https://doi.org/10.1007/s00359-016-1110-3>
- MAPA (2019) Ministério da Agricultura, Pecuária e Abastecimento. [http://extranet.agricultura.gov.br/agrofit\\_cons/principal\\_agrofit\\_cons](http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons). Accessed 7 Nov 2019
- Melo GAR (2016) *Plectoplebeia*, a new Neotropical genus of stingless bees (Hymenoptera: Apidae). *Zool* 33. <https://doi.org/10.1590/S1984-4689zool-20150153>
- Michener CD (2000). The bees of the world. University of Kansas Natural History Museum and Department of Entomology, USA.
- Morandin LA, Winston ML, Franklin MT, Abbott VA (2005) Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Manag Sci* 61:619–626. <https://doi.org/10.1002/ps.1058>
- Moure JS (2004) Duas espécies novas de *Plebeia* Schwarz do Brasil (Hymenoptera, Apidae, Meliponinae). *Rev Bras Entomol* 48:199–202. <https://doi.org/10.1590/S0085-56262004000200007>
- Nauen R, Bretschneider T (2002) New modes of action of insecticides. *Pestic Outlook* 13:241–245. <https://doi.org/10.1039/b21171n>
- Pedro SRM, de Camargo JMF (2013) Stingless Bees from Venezuela. In: Pot-honey. Springer New York, New York, pp 73–86. [https://doi.org/10.1007/978-1-4614-4960-7\\_4](https://doi.org/10.1007/978-1-4614-4960-7_4)
- Powney GD, Carvell C, Edwards M, Morris RKA, Roy HE, Woodcock BA, Isaac NJB (2019) Widespread losses of pollinating insects in Britain. *Nat Commun* 10:1018. <https://doi.org/10.1038/s41467-019-08974-9>
- R Core Team (2018). R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <https://www.R-project.org/>
- Ramvalho M, Imperatriz-Fonseca VL, Kleinekt-Giovannini A, Cortopassi-Laurino M (1985) Exploitation of floral resources by *Plebeia remota* Holmberg (Apidae, Meliponinae). *Apidologie* 16:307–330
- Ramvalho M, Kleinert-Giovannini A, Imperatriz-Fonseca VL (1990) Important bee plants for stingless bees (*Melipona* and *Trigonini*) and Africanized honeybees (*Apis mellifera*) in neotropical habitats: a review. *Apidologie* 21:469–488. <https://doi.org/10.1051/apido:19900508>
- Rasmussen C, Cameron SA (2009) Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biol J Linn Soc* 99:206–232. <https://doi.org/10.1111/j.1095-8312.2009.01341.x>
- Ribeiro MDF, Taura TA (2019) Presence of *Plebeia* aff. *flavocincta* nests in urban areas. *Sociobiology* 66:66. <https://doi.org/10.13102/sociobiology.v66i1.3474>
- Roldão-Sbordoni YS, Nascimento FS, Mateus S (2018) Estimating colonies of *Plebeia droryana* (Friese, 1900) (Hymenoptera, Apidae, Meliponini): adults, brood and nest structure. *Sociobiology* 65:280. <https://doi.org/10.13102/sociobiology.v65i2.2345>
- Roubik DW (2006) Stingless bee nesting biology. *Apidologie* 37:124–143. <https://doi.org/10.1051/apido:2006026>
- Salgado VL (1998) Studies on the mode of action of Spinosad: insect symptoms and physiological correlates. *Pestic Biochem Physiol* 60:91–102. <https://doi.org/10.1006/PEST.1998.2332>
- Sánchez D, De J, Solórzano E, Liedo P, Vandame R (2012) Effect of the natural pesticide spinosad (GF-120 formulation) on the foraging behavior of *Plebeia moureana* (Hymenoptera: Apidae). *J Econ Entomol* 105:1234–1237. <https://doi.org/10.1603/ec12047>
- Sarfraz M, Dosdall LM, Keddie BA (2005) Spinosad: a promising tool for integrated Pest management. *Outlooks Pest Manag* 16:78–84. <https://doi.org/10.1564/16aplog>
- Silva-Neto CM, Bergamini LL, Elias MAS et al (2016) High species richness of native pollinators in Brazilian tomato crops. *Brazilian J Biol* 77:506–513. <https://doi.org/10.1590/1519-6984.17515>
- Sparks TC, Crouse GD, Durst G (2001) Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. *Pest Manag Sci* 57:896–905. <https://doi.org/10.1002/ps.358>
- Tomé HVV, Martins GF, Lima MAP et al (2012) Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata* anthidioides. *PLoS One* 7:e38406. <https://doi.org/10.1371/journal.pone.0038406>
- Tomé HVV, Barbosa WF, Corrêa AS et al (2015a) Reduced-risk insecticides in Neotropical stingless bee species: impact on survival and activity. *Ann Appl Biol* 167:186–196. <https://doi.org/10.1111/aab.12217>
- Tomé HVV, Barbosa WF, Martins GF, Guedes RNC (2015b) Spinosad in the native stingless bee *Melipona quadrifasciata*: regrettable non-target toxicity of a bioinsecticide. *Chemosphere* 124:103–109. <https://doi.org/10.1016/j.chemosphere.2014.11.038>
- Tschoeke PH, Oliveira EE, Dalcin MS et al (2015) Diversity and flower-visiting rates of bee species as potential pollinators of melon (*Cucumis melo* L.) in the Brazilian Cerrado. *Sci Hortic (Amsterdam)* 186:207–216. <https://doi.org/10.1016/J.SCIH.2015.02.027>
- Tschoeke PH, Oliveira EE, Dalcin MS, Silveira-Tschoeke MCAC, Sarmento RA, Santos GR (2019) Botanical and synthetic pesticides alter the flower visitation rates of pollinator bees in Neotropical melon fields. *Environ Pollut* 251:591–599. <https://doi.org/10.1016/J.ENVPOL.2019.04.133>
- Valdovinos-Núñez GR, Quezada-Euán JJG, Ancona-Xiu P et al (2009) Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini). *J Econ Entomol* 102:1737–1742. <https://doi.org/10.1603/029.102.0502>
- Van Nieuwstadt MGL, Ruano Iraheta CE (1996) Relation between size and foraging range in stingless bees (Apidae, Meliponinae). *Apidologie* 27:219–228. <https://doi.org/10.1051/apido:19960404>

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