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Sublethal agrochemical exposures can alter honey bees' and Neotropical stingless bees' color preferences, respiration rates, and locomotory responses

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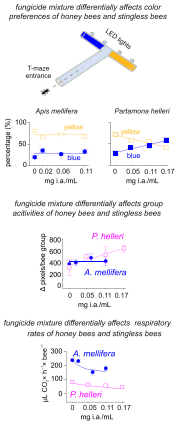
HIGHLIGHTS

- *Apis mellifera* and *Partamona helleri* exhibited different color preference patterns.
- *Partamona helleri* color preference was altered by insecticides and fungicides.
- Fungicide mixture-exposed *P. helleri* showed altered locomotion and respiration rate.
- Honeybees may not always be a proxy for pesticide risks imposed on stingless bees.

GRAPHICAL ABSTRACT



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ABSTRACT

Stingless bees such as *Partamona helleri* Friese play important roles in pollination of native plants and agricultural crops in the Neotropics. Global concerns about declining bee populations due to agrochemical pollutants have, however, been biased towards the honey bee, *Apis mellifera* Linnaeus. Here, we analysed the unintended effects of commercial formulations of a neonicotinoid insecticide, imidacloprid, and a fungicide mixture of thiophanate-methyl and chlorothalonil on color preference, respiration rates and group locomotory activities of both *P. helleri* and *A. mellifera*. Our results revealed that *P. helleri* foragers that were not exposed to pesticides changed their color preference during the course of a year. By contrast, we found that pesticide exposure altered the color preference of stingless bees in a concentration-dependent manner. In addition, imidacloprid decreased the overall locomotion of both bee species, whereas the fungicide mixture increased locomotion of only stingless bees. The fungicide mixture also reduced respiration rates of forager bees of both species. Forager bees of both species altered their color preference, but not their locomotory and respiration rates, when exposed to commercial formulations of each fungicidal mixture component (i.e., chlorothalonil and thiophanate-methyl). Our findings emphasize the importance of *P. helleri* as a model for Neotropical wild pollinator species in pesticide risk assessments, and also the critical importance of including groups of agrochemicals that are often considered to have minimal impact on pollinators, such as fungicides.

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1. Introduction

Many insect pollinator species contribute significantly to agricultural production, with honey bees, bumble bees, and stingless bees playing a prominent role in pollination services (Gill et al., 2012; Potts et al., 2016). Bee and other pollinator species are, however, in decline, which increases the need to better understand and mitigate the underlying causes of these declines. When visiting flowers, forager bees are exposed to a wide range of pesticides (hereafter agrochemicals) applied to control various agricultural pests. Residues of many insecticides, acaricides, fungicides, or herbicides are frequently found in bees, honey, pollen, and wax (Desneux et al., 2007; Mullin et al., 2010; Hladik et al., 2016; Colwell et al., 2017; Niell et al., 2017; Main et al., 2020; Tomé et al., 2020). Studies addressing lethal and sublethal effects of pesticides have focused primarily on the European honey bees (*Apis mellifera* L. (Hymenoptera: Apidae: Apini)) and bumblebees (*Bombus* sp.). Despite recent studies showing that bumblebees have a higher susceptibility to insecticides than honey bees (Gradish et al., 2019), other ecologically important native wild pollinators such as the stingless bees found in Neotropical regions, have received little attention despite their key role in pollination in those areas (Del Sarto et al., 2014; Heard et al., 2017; Lima et al., 2016; Tschoeke et al., 2019).

Stingless bees are highly eusocial and share many characteristics with honey bees such as polylecty, floral constancy, domesticability, and colony perennialism, and are also tolerant to some honey bee parasites (Heard, 1999; Michener, 2007). Despite their phylogenetic affinities (Cardinal and Danforth, 2011), recent studies have highlighted the risks of extrapolating ecotoxicological results based on European populations of honey bee as a surrogate and model for environmental regulatory risk assessment of pesticides, especially in the Neotropics (Artz and Pitts-Singer, 2015; Rodrigues et al., 2016; Tomé et al., 2017), where we have limited knowledge of the impact of pesticides on the physiology, behavior, and health of stingless bees (Del Sarto et al., 2014; Lima et al., 2016; Rodrigues et al., 2016).

Pollinators, such as the stingless bee species *Partamona helleri* Friese (Hymenoptera: Apidae: Meliponini), use mainly visual and olfactory floral cues to navigate and to communicate accurately to their nestmates the distance and direction of food sources (Andrione et al., 2016; Flaig et al., 2016; Hrncir et al., 2016). Studies on honey bees has shown that neonicotinoid insecticides can impair the coding of odors in the brain, which can compromises olfactory learning (Andrione et al., 2016). Much less is known, however, regarding the effects of neonicotinoids on bee vision (Ludicke and Nieh, 2020), despite its importance in food source localization and selection. Exposure to sublethal levels of pesticides that cause changes in color discrimination of bees can, therefore, have severe adverse implications for both individual bees and colony health (Ludicke and Nieh, 2020), and may also compromise pollination services. Furthermore, the majority of evidence for the sublethal effects of pesticides on bees comes from studies of European populations of honey bees, *A. mellifera* Linnaeus, but the extent to which sublethal exposure to pesticides affects life traits such as sensory detection (e.g., color preference), mobility and consequently the pollination efficiency of Neotropical stingless bees remains to be understood in detail.

As intra- and intercolonial heterogeneity in time and space are important aspects to consider when sampling bees, by using colonies of the stingless bee *P. helleri* and the honey bee *A. mellifera*, we firstly characterized changes in their color preference over 12 months, a period of time sufficiently long to offset potential influences from colony heterogeneity in time and space. Furthermore, we analysed the unintended effects of commercial formulations of two pesticides commonly used in Neotropical agricultural fields, the neonicotinoid imidacloprid and a fungicide mixture of thiophanate-methyl and chlorothalonil, on color preference, group locomotory activity, and respiration rate of both bee species.

2. Materials and methods

2.1. Bees and agrochemicals

Forager bees of *A. mellifera* (Africanized) and *P. helleri* were collected from four healthy colonies never exposed to pesticide residues in the experimental apiary of the Federal University of Viçosa (UFV, Viçosa, MG, Brazil, 20° 45′ S, 42° 52′ W). For each bee species, worker bees were kept for 24 h in 500 mL non-toxic, polypropylene, transparent plastic containers with perforated lids to allow gas exchange. The colonies were maintained under controlled conditions similar to those found in their respective field colonies (28 ± 2 °C for *P. helleri* and 34 ± 2 °C for *A. mellifera*, 70 ± 10% relative humidity), fed with sucrose solution (50% w/v) ad libitum and kept in the dark until experiments were carried out. Four colonies of each bee species were used in experiments, and to offset potential intercolonial variations in responses (see Pirk et al., 2013) we ensured that all treatments were evenly represented for each colony. Forager bees from each colony were tested for each treatment at least once within the replicates.

The agrochemicals tested were commercial formulations widely used as plant protectants on commercial crops pollinated by bees (e.g., melon, *Cucumis melo* L.) in Brazil. We used the neonicotinoid insecticide imidacloprid [Evidence® water-dispersible granules at 700 g active ingredient (a.i.)/L; Bayer CropScience, São Paulo, Brazil] and a fungicide mixture of chlorothalonil (500 g a.i./L) and thiophanate-methyl (200 g a.i./L) [Cerconil WP, wettable powder; Iharabras S.A. Chemical Industries, Sorocaba, SP, Brazil]. We also tested commercial formulations that contained only chlorothalonil [Daconil BR®, 750 g (a.i.)/kg, wettable powder; Iharabras S.A. Chemical Industries, Sorocaba, SP, Brazil] or thiophanate-methyl [Cercobin®, 700 g (a.i.)/kg, wettable powder; Iharabras S.A. Chemical Industries, Sorocaba, SP, Brazil].

2.2. Feeding exposure to pesticides

For each bee species, 20 forager bees caged in plastic containers were deprived food for 1 h and then allowed to feed for 5 h on a sucrose solution mixed with selected doses of each pesticide. Unless stated otherwise, bees were fed pesticides diluted in 1.8 mL honey-based syrup solution (50%, v/v) and offered to bees in 2 mL Eppendorf tubes inserted into plastic cages. We considered each plastic container as an experimental unit with individuals of the same colony and used four units or replicates (one for each colony) for each treatment. After 5 h exposure period, the feeding solution was replaced with a new one that was pesticide-free. Untreated controls consisted of a sucrose solution only. All subsequent bioassays were performed after 24 h from the beginning of exposure to treatments. During experimental periods, bees were maintained under controlled conditions, as described above.

The concentrations used for imidacloprid and the fungicide mixture corresponded to LC_1, LC_5, and LC_{10}, as previously established elsewhere for the same bee species (Table 1) using similar procedures (Tomé et al., 2017). The label recommendations for imidacloprid (for the control of phytosuccivorous insects such as whiteflies, aphids and trips) and fungicide mixture (for fungi control, including *Colletotrichum orbicular*, *Alternaria cucumerina*, *Cercospora citrulline* and *Sphaerotheca fuliginea*) in melon crops in Brazil (AGROFIT, 2019) are also given in Table 1. The concentrations used of chlorothalonil (1.5 g a.i./L of water) and thiophanate-methyl (0.49 g a.i./L of water) formulations corresponded to their recommended field rates for the control of fungi *Septoria lycopersici* Speg. and *Colletotrichum orbicular* (Berk.) in tomato and melon crops in Brazil, respectively (AGROFIT, 2019).

2.3. Color preference bioassay

Two different bioassays of color preference were carried out. The first was conducted six times to investigate seasonal variation in bee color preference during a period spanning one year (i.e., from January

Table 1
Concentrations of imidacloprid and the fungicide mixture containing chlorothalonil and thiophanate-methyl used in exposure bioassays with *Apis mellifera* and *Partamona helleri*.

Agrochemicals	Activie ingredients	*A. mellifera*			*P. helleri*		
		LC_1[a]	LC_5	LC_{10}	LC_1	LC_5	LC_{10}
Evidence	Imidacloprid	0.105	0.236	0.364	0.002	0.009	0.022
	(Label recommendation proportion)[b]	(0.075)	(0.169)	(0.260)	(0.001)	(0.006)	(0.016)
Cerconil	Thiophanate-methyl + chlorothalonil	0.018	0.059	0.114	0.053	0.112	0.168
	(LABEL recommendation proportion)[b]	(0.009)	(0.030)	(0.057)	(0.027)	(0.056)	(0.084)

[a] *LC* (mg a.i./ mL): lethal concentrations for each compound according to previous investigations with the same bee species and applying similar procedures from Tomé et al., 2017.
[b] Relates to how much these lethal concentration refers to the label recommendation of Evidence and Cerconil to protect melon field crops in Brazil (AGROFIT, 2019).

to November 2015) and without previous exposure to pesticides. For each bee species, batches of 20 forager bees from each of the four colonies were kept in 500 mL transparent plastic pots with perforated lids and maintained for 24 h under controlled conditions (28 ± 2 °C for *P. helleri* and 34 ± 2 °C for *A. mellifera*, 70 ± 10% relative humidity). The bees were fed with sucrose solution (50% w/v) ad libitum until the trials. Based on the results of the color preference bioassay, we conducted a second bioassay in November 2016 in which color preference was determined following exposure to selected concentrations of pesticides (as described in Section 2.2). We used four replicates (i.e., colonies) for each treatment and each replicate consisted of 20 forager bees from a single bee colony.

In both bioassays, color preference of the two bee species was studied using a glass T-tube method (Fig. 1A) (Han et al., 2010). Briefly, the tube had a 20 cm long 1.6 cm diameter entry arm ending with two 12 cm lateral arms to form a T-like shape. The two lateral arms were completely covered with cellophane paper (567526, Cromus Embalagens Industria e Comercio LTDA, Sao Paulo, Brazil) capable of filtering yellow or blue light. These colors were selected as they are spectrally distinct for bees (Zhang et al., 1996). Analyses of the colors used were done on the CIE L*a*b scale (L: lightness; a: green–red and b: blue-yellow color components) using a colorimeter (Color Reader CR-10 Minolta). Yellow corresponded to L = 83.10; a = −3.80; b = 90.30 (with RGB color values of red: 231, green: 207 and blue: 0) and blue corresponded to L = 32.40; a = −6.80; b = −18.90 (with RGB color values of red: 46, green: 80 and blue: 106). Analyses were performed in a dark room where the tubes were placed on a black base containing light-emitting diodes (LED, K1432, Dualcom Comercio de Maquinas Equipamentos e Serviços LTDA, Curitiba, PR, Brazil) spaced equidistantly on the two lateral arms (yellow or blue). The bees were released individually at the T-tube main arm entrance, and the choices made by the bees (yellow or blue) recorded. Bees that remained at the T-tube entrance for a period longer than 1 min were disregarded. To avoid interference by any potential pheromone traces left by the bees, tubes were used only once and then sanitized with 96% ethanol before re-use. The position (right or left) of the lateral arms was alternated between tests to avoid any position effect in the choices.

2.4. Overall group activity bioassays

Groups of five newly emerged bees, minimizing prior confounding exposure to pesticides, were taken from a single colony of either *A. mellifera* or *P. helleri* and exposed to the various concentrations of imidacloprid and fungicide mixture as described above. Newly-emerged bees (less than 3 days old) were collected following procedures described by Ferreira et al. (2017). Four replicates of five newly-emerged bees were subjected to walking activity bioassays in arenas made of Petri dishes lined at the bottom with filter paper discs (9 cm in diameter with 80 g/m^2 density; Nalgon Equip. Científicos, Itupeva, SP, Brazil) and had their inner walls coated with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent insect escape (Del Sarto et al., 2014).

Overall insect activity, including walking behavior, insect interactions, and movement of body parts was recorded for 10 min using a video tracking system equipped with a digital charge-coupled device (CCD) camera (ViewPoint Life Sciences, Montreal, QC, Canada). Overall insect activity was recorded as spatial movement of bees over time (i.e., change (Δ) in registered pixels/1×10^{-2} s). We used four replicates (i.e., groups of five newly emerged bees, where individuals of each replicate were obtained from the same colony) for each combination of bee species and pesticide concentration (i.e., corresponding LC_1, LC_5, and LC_{10} for imidacloprid and fungicide mixture) or control (i.e., pesticide unexposed bees) treatments.

2.5. Respiration rate bioassay

Respiration rate was determined for batches of bees collected from the same colony of either three insects in the case of *A. mellifera* or four insects in the case of *P. helleri*. CO_2 production was recorded using a TR3C respirometer equipped with a CO_2 analyzer (Sable Systems International, Las Vegas, NV, USA). Each bee batch was introduced into a 25 mL glass chamber in a completely closed system. CO_2 production (μmol CO_2/h/batch) was determined after a 3 h period by injecting CO_2-free air into the chamber for 2 min at a flow rate of 600 mL min^{-1}. The air current directed the CO_2 produced by the bees to an infrared reader connected to the system. CO_2 production was also determined in a control chamber without any insects (Tomé et al., 2015). A total of 72 forager bees per treatment were used for *A. mellifera* (18 bees per colony), and 96 forager bees per treatment were used for *P. helleri* (24 bees per colony). Bees were exposed to the various concentrations of imidacloprid and fungicide mixture following the same procedures described above.

2.6. Statistical analyses

A Chi-square test was used to compare potential seasonal variation in bees' color preference using SAS software (PROC GLM; SAS Institute, Cary, NC, USA). Analysis of covariance (ANCOVA) was used to test the potential effects of imidacloprid and fungicide mixture on bees' color preference, activity, and respiratory rate using SAS software (PROC GLM; SAS). Species and agrochemicals were the independent variables tested using concentration as a covariate. Analysis of variance (ANOVA) followed by Tukey's HSD test ($P < 0.05$), when appropriate, were used to test the effect of the fungicides chlorothalonil and thiophanate-methyl alone on color preference, walking activity, and respiratory rate (PROC GLM, SAS).

Regression analyses were used to test the relationship between pesticide concentrations and color preference, locomotion and respiration rates using the curve-fitting procedure of TableCurve 2D (Systat, Jan Jose, CA, USA). Models, from the simplest (e.g., linear, quadratic, exponential growth, or exponential decay) to the more complex were tested to leverage the level of significance and R^2 values. Model selection was based on parsimony (i.e., simplest model with highest Adj. R^2 value), high *F*-values and steep (relative) increases in R^2 with model complexity. Adjusted R^2 values were estimated and used for model selection to minimize problems of overfitting, as it provided the level of explanation associated with only the independent variable. Relative adjusted R^2 was

also calculated as a ratio between the Adj R^2 value of the selected model to that of the Adj R^2 of the best fitted model to indicate how good the fit was to the best possible one, securing a minimum threshold of Rel. Adj. $R^2 > 0.90$.

3. Results

3.1. Seasonal changes in color preference

The color preference of forager honey bees remained unchanged throughout the year with a significant preference for yellow over blue (χ^2 tests, $df = 1$, Fig. 1B). By contrast, the color preference of *P. helleri* changed significantly over the year (χ^2 tests, $df = 1$, Fig. 1B), with bees showing significant preferences for yellow in November and January, and for blue in June, and no color preference in February and May (χ^2 tests, $df = 1$, Fig. 1B).

Based on the color preferences of unexposed bees, November was selected for subsequent bioassays to analyze the effects of exposure to pesticides on color preference. There were no significant differences in color preference between species in the absence of exposure to pesticides, with the average preference for yellow being 71% for both species ($n = 430$ bees/species), in concordance with values previously reported for *A. mellifera* (Han et al., 2010).

3.2. Effects of sublethal exposure on color preference

Analysis of covariance of color preference for both *A mellifera* and *P. helleri*, after pesticide exposure, showed that species and concentration, as well as the interaction between species and concentration, significantly affected color preference (Table 2). Changes in color preference were not found at any of the imidacloprid or fungicide mixture concentrations tested for *A. mellifera* (Fig. 2A, B). Exposure to the lowest sublethal concentration ($LC_1 = 0.002$ mg a.i./mL) tested for imidacloprid significantly reduced the yellow color preference of *P. helleri* (Fig. 2A). Similarly, sublethal fungicide exposure gradually reduced preference for yellow by *P. helleri*.

At doses equivalent to field recommendation rates, the two main components of the fungicide mixture, chlorothalonil (1.5 mg a.i./mL) and thiophanate-methyl (0.49 mg i.a./mL), differentially affected color preference of the two species (Supplementary Fig. 1). Chlorothalonil altered only the preference of the exposed *P. helleri* bees (Tukey's HSD test; $q = 9.68$; $P < 0.001$) compared to controls, while thiophanate-methyl altered only the preference of the exposed *A. mellifera* (Tukey's HSD test; $q = 5.81$; $P = 0.007$) compared to control (Supplementary Fig. 1A).

3.3. Effects of sublethal exposure on group activity

Analysis of covariance for group activity indicated that the effect of sublethal exposure depended not only on pesticide, but also on species-pesticide interaction, and the pesticide-concentration interaction (Table 2). The group activity of *A. mellifera* worker bees (expressed as changes [Δ] in registered pixels over time) reduced following exposure to imidacloprid in a concentration-dependent manner (Fig. 3), while it was not affected by the fungicide mixture (Fig. 3). By contrast, in *P. helleri* exposure to the lowest concentration of imidacloprid ($LC_1 = 0.002$ mg a.i./mL) resulted in a sharp decrease in group activity (Fig. 3). The opposite trend was found when *P. helleri* foragers were exposed to the fungicide mixture, as group activity linearly increased with an increase in concentration of the fungicide (Fig. 3).

No effects on the group activity of worker bees of either species were observed when they were exposed to field rates of the two main components alone of Cerconil, chlorothalonil and thiophanate-methyl (*A. mellifera*: $F_{2,14} = 0.91$; $P = 0.43$. *P. helleri*: $F_{2,14} = 0.80$; $P = 0.48$) (Supplementary Fig. 1B).

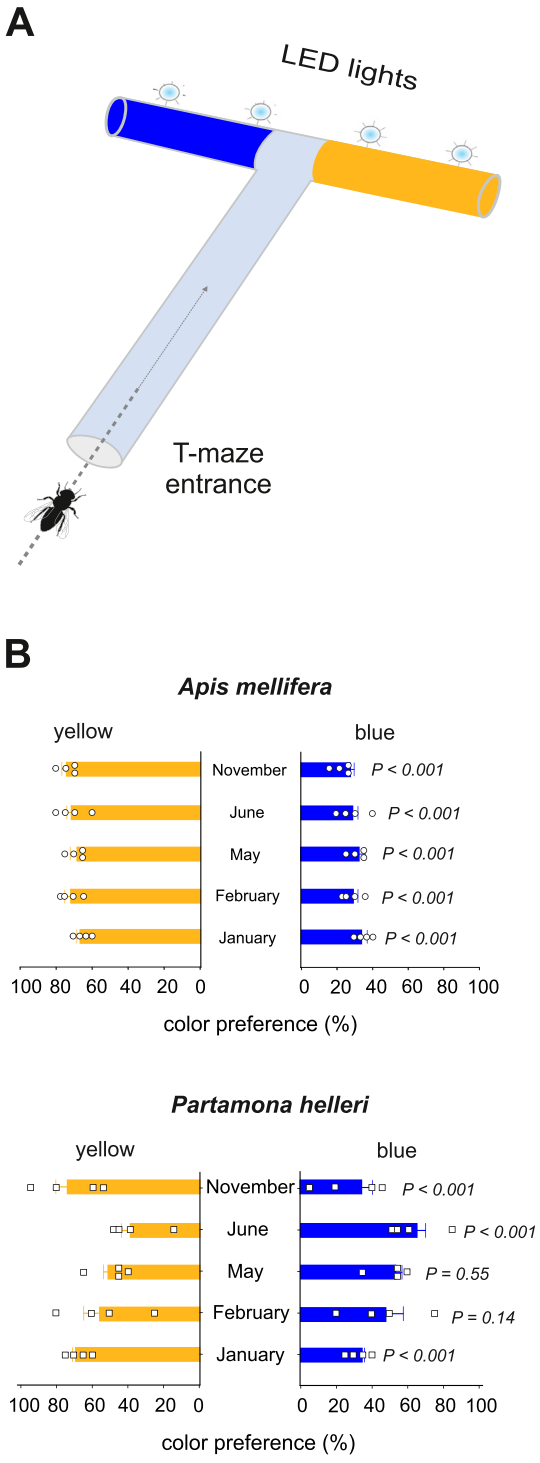

Fig. 1. Schematic representation of the T-maze (***A***) and color preference (***B***) of *Apis mellifera* and *Partamona helleri* foragers not exposed to pesticides from January to November 2015 ($N = 160$). (***B***) Each bar represents the result of four replicates (i.e., colonies) for each bee species. Circles (*A. mellifera*) and squares (*P. helleri*) represent the responses of individual replicates. *P* values less than 0.05 indicate significant differences for one color type following Chi-square analyses.

3.4. Effects of sublethal exposure on respiration rate

The respiration rate of forager bees was significantly different between species, pesticides, and concentrations used, but without significant interactions between these three factors (Table 2). Forager bees of both species exposed to either imidacloprid (Fig. 4, Supplementary

Table 2
Summary of analysis of covariance (ANCOVA) of the color preference, group activity, and respiration rate of *Apis mellifera* and *Partamona helleri* exposed to sublethal concentrations of the insecticide imidacloprid and the fungicide Cerconil.

Source of variation	*Df*	F	*P*	*Df*	F	*P*	*Df*	F	*P*
	Color preference			Group activity			Respiration rate		
Model	7	6.49	<0.0001*	7	14.65	<0.0001*	7	85.36	<0.0001*
Residual	56	–	–	72	–	–	376	–	–
Specie (E)	1	8.42	0.0053*	1	0.60	0.4421	1	547.08	<0.0001*
Pesticide (P)	1	0.74	0.3934	1	62.51	<0.0001*	1	12.95	0.0004*
Concentration (C)	1	25.48	<0.0001*	1	0.87	0.3539	1	28.58	<0.0001*
E × P	1	2.27	0.1379	1	8.33	0.0051*	1	1.49	0.2223
E × C	1	8.05	0.0063*	1	0.92	0.3410	1	1.39	0.2399
P × C	1	0.00	1.0000	1	26.66	<0.0001*	1	4.15	0.0424*
E × P × C	1	0.45	0.5037	1	2.70	0.1048	1	1.89	0.1695

* Significant at $P < 0.05$.

Fig. 2) or to the fungicide mixture (Fig. 4, Supplementary Fig. 2) exhibited reductions in respiration rates with increasing concentration. The fungicides chlorothalonil and thiophanate-methyl alone did not, however, show any significant effect on the respiration rates of *A. mellifera* ($F_{2,71} = 0.83$; $P = 0.56$) and *P. helleri* ($F_{2,95} = 0.83$; $P = 0.44$) (Supplementary Fig. 1C).

4. Discussion

To our knowledge, this is the first study of color preferences of both *A. mellifera* and *P. helleri* over a 12-month period. Our results clearly show that the yellow/blue color preferences of *P. helleri* vary during the course of a year, while those of *A. mellifera* remain constant. We also found that exposure to sublethal doses of imidacloprid and the fungicide mixture of thiophanate-methyl and chlorothalonil changed the abilities of bees to discriminate between yellow and blue in *P. helleri*, but not in *A. mellifera*. In addition, sublethal exposure to imidacloprid altered the locomotory activity and respiration rates in groups of forager bees of both *P. helleri* and *A. mellifera*. Interestingly, the fungicide mixture alone altered both locomotory activity and respiration rates of *P. helleri* bees.

Our results with *A. mellifera* showed that they had a constant preference for yellow over time. This contrasts with previous studies that reported preferences for color stimuli with a dominant blue wavelength in the European *A. mellifera* and bumblebee, *Bombus terrestris* (Hudon and Plowright, 2011; Ings et al., 2009; Morawetz et al., 2013; Raine and Chittka, 2011). These different results may have been influenced by some degree of pre-existing color preference acquired as an adaptive response to flower sources where these forager bees were collected (Maharaj et al., 2019; Schiestl and Johnson, 2013). Interestingly, a shift in the color preference between blue and yellow throughout the year was found for the stingless bee *P. helleri*, but not in the honey bee *A. mellifera*, suggesting that *P. helleri* may change its foraging patterns during some periods of the year in Neotropical regions.

Despite recent studies indicating that Brazilian stingless bee species can exhibit disparate color preferences (Koethe et al., 2018) dependent on spectral purity, intensity, and dominant wavelength, as found here for Africanized *A. mellifera* and *P. helleri*, few studies have analysed color preferences in the Meliponini tribe (Hrncir et al., 2016; Koethe et al., 2016; Spaethe et al., 2014) and none analysed the potential harm of pesticides on such relevant behavioral traits. Stingless bees are true generalist foragers that are able to collect nectar and pollen from a wide range of plants, which results in their essential role as pollinators in the Pantropical and Pansubtropical regions. Individuals, however, show flower constancy and tend to specialize in a single floral species for specific periods (Slaa et al., 2006), which may explain the shift in color preferences when those plants stop flowering. The presence or absence of alterations in color preference over seasons may, therefore, be related to ecosystem conditions in which these species are evolving.

Color preference changes for stingless bees occurred during the transition between the humid-summer and dry-winter (from February to June) seasons in the Neotropical region, which coincides with a gradual decrease in luminosity, temperature, rainfall rates, and can result in lower flower availability. Such reduced flower availability may favor a more generalist search pattern on forager stingless bees (Slaa et al., 2006). This change in color preference is likely to be an evolutionary ecological adaptation to seasonal variations (Chittka and Menzel, 1992; Dyer et al., 2012).

Alternatively, the change in color preference found in *P. helleri*, but not in *A. mellifera*, may be based on the foraging behavior of stingless bees and their smaller foraging range compared to honey bees (Slaa et al., 2006). Smaller foraging ranges could force stingless bees to modify their color preference to better use the available spectrum of potential food sources over time. Further studies could reveal how such behavior can interfere with color preference in pollinating bees in different environments.

In addition to naturally occurring environmental conditions, the foraging activity of bees can face challenges imposed by anthropogenic interference, including agrochemical-induced stresses in both honey bees and stingless bees (Goulson et al., 2015; Lima et al., 2016; Rodrigues et al., 2016; Tomé et al., 2017). Moreover, available evidence suggests that sublethal doses of agrochemicals can impair the ability of bees to function at the individual and colony levels (Barbosa et al., 2015; Del Sarto et al., 2014; Goulson, 2013; Tomé et al., 2015). Here our results suggest species-related differences in coping with agrochemical-induced stresses.

These species-specific differences are likely to derive from differences in body size and related metabolic rate (Brittain and Potts, 2011; Chown and Gaston, 1999) since stingless bees are generally smaller compared to *A. mellifera* (Thompson, 2016; Tomé et al., 2017). The effects of other life-history traits should not, however, be disregarded (Brittain and Potts, 2011). Such results indicate the risks of extrapolating the results of toxicity bioassays based only on the honey bee *A. mellifera* for environmental regulatory risk assessment of agrochemicals, especially in Neotropical regions (Del Sarto et al., 2014; Lima et al., 2016; Tomé et al., 2017).

Among other factors that can be perceived differently by foraging individuals after pesticide exposure (Ludicke and Nieh, 2020), color preference exerts a major influence on the choice behavior of bees during foraging activity (Dyer and Chittka, 2004). To date, color preference experiments have revealed the existence of key distinct traits of color stimuli including the contrast between colors, dominant wavelength, spectral purity and color intensity that interact in a complex way to influence a bees' decision-making (Dyer and Chittka, 2004; Koethe et al., 2018, 2016; Lunau and Maier, 1995; Papiorek et al., 2013). When bees are exposed sublethally to agrochemicals, their flower source preferences may become compromised as these xenobiotics can alter bee color preference by impairing a bee's ability to discriminate wavelengths or by impairing their adaptive learning. Thus, considering that

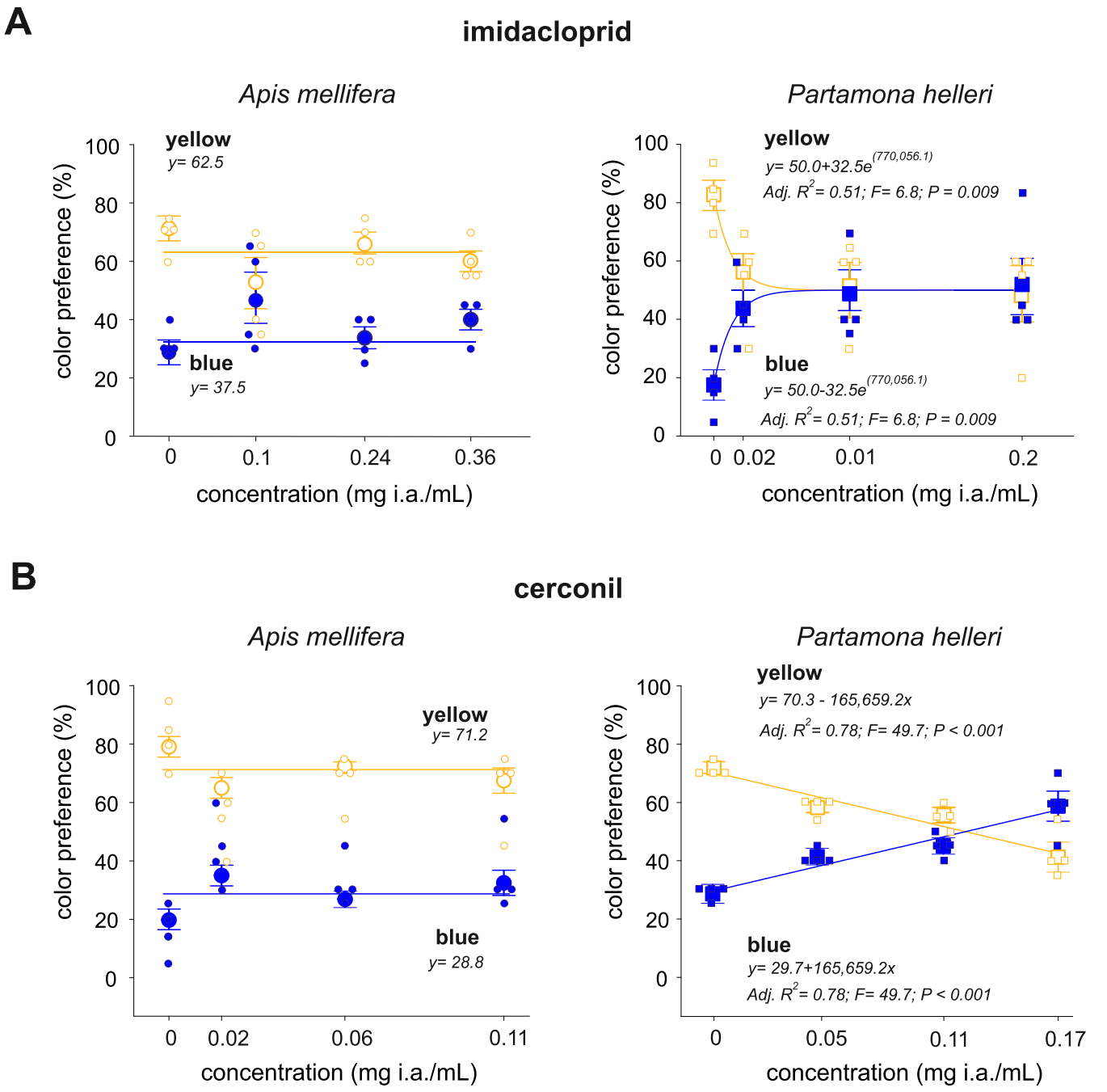

Fig. 2. Percentage of bees of *Apis mellifera* and *Partamona helleri* foragers that chose yellow or blue after being exposed to sublethal concentrations of imidacloprid (***A***) and Cerconil (***B***) ($N = 80$ bees/pesticide treatment/species). Circles (*A. mellifera*) and squares (*P. helleri*) represent the responses of individual replicates. Vertical bars represent the standard error (SE) of the mean. The equations represent the best models selected based on parsimony and highest level of significance.

bees can adjust color preferences in response to concurrent social information from conspecifics and heterospecifics (Romero-Gonzalez et al., 2020), the potential agrochemical-mediated changes in color preferences can affect not only individual bees but also the social communication/interaction, flow of food resource and colony health among pollinator bees that coexist in the same environment.

Sublethal exposure to imidacloprid impacted negatively the locomotion and respiration activity of both species in a dose-dependent manner. The physiological and, consequently, behavioral abnormalities caused by imidacloprid and other neonicotinoid insecticides (e.g., thiametoxam, Ludicke and Nieh, 2020) on individual bees have been widely reported (Cabirol and Haase, 2019). Here, we not only reinforce those findings that low concentrations of agrochemicals can affect individual bees but also highlight that group activity may also be compromised and many alterations (e.g., locomotion and respiratory activity) can co-occur and possibly affect overall colony health.

Despite the widespread assumption that fungicides are safer to bees than other agrochemicals, an increasing body of evidence suggests that this is often not the case (Mao et al., 2017; Tomé et al., 2017), and that native pollinators such as *P. helleri* are even more susceptible to these chemicals than *A. mellifera* (Heard et al., 2017; Tomé et al., 2020; Tison et al., 2017). Furthermore, formulation composition of both active

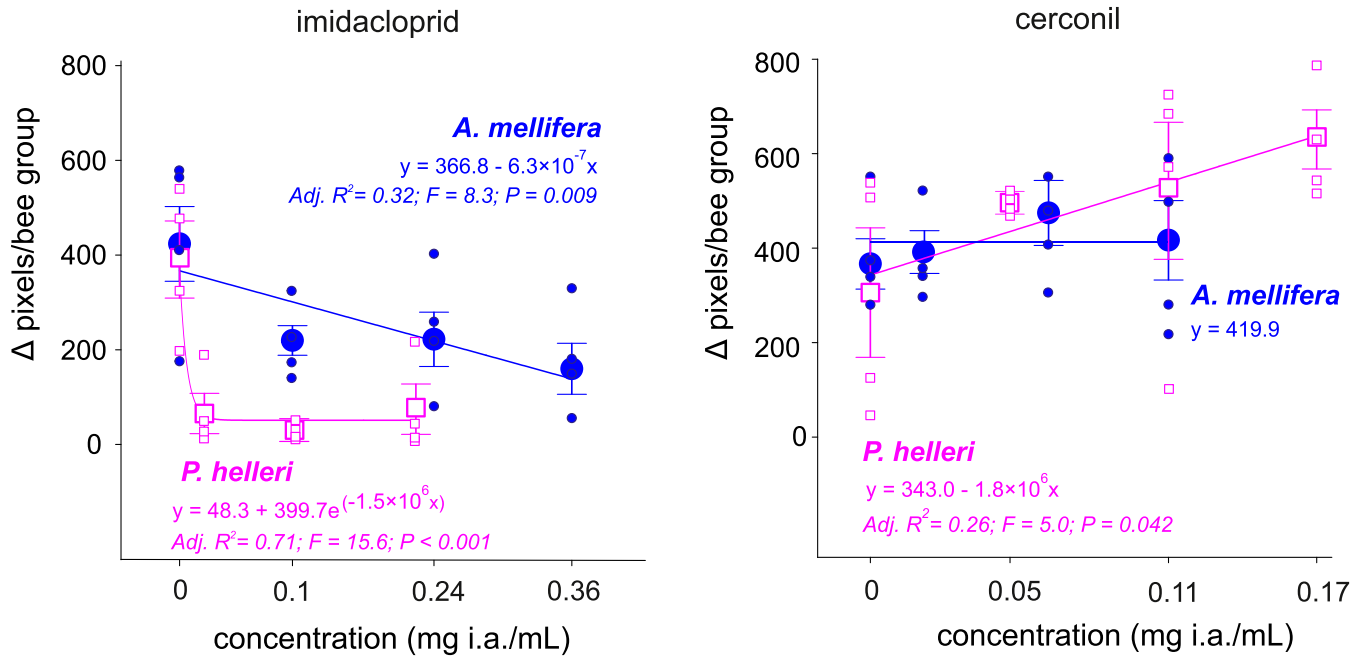

Fig. 3. Effects of sublethal exposure to imidacloprid and Cerconil on locomotory group activities of *Apis mellifera* and *Partamona helleri*. (***A***) N = 80 bees/pesticide treatment/species. Circles (*A. mellifera*) and squares (*P. helleri*) represent the responses of individual replicates. Vertical bars associated with the symbols represent the standard error (SE) of the mean. The equations represent the best models selected based on parsimony and highest level of significance.

ingredients and additional components (e.g., adjuvants and surfactants) do vary and may also lead to significant effects on different bee species (Chen et al., 2018; Mullin, 2015).

The assumption that honey bees are generally more tolerant to pesticides compared to stingless bees and other wild pollinator bees may result from their domestication process, aiming their use for crop pollination and production of honey and other by-products. Under such conditions, honey bees experience frequent exposure to agrochemicals applied to protect agricultural crops or to keep the bee colonies in healthy conditions (e.g., insecticides applied for killing bee parasites or insect that attack the bee hives) ultimately leading to selecting bees with lower susceptibility to these xenobiotics (Chakrabarti et al., 2018). It is clear, therefore, that risk assessment studies that consider only mortality as a toxicological endpoint will overlook important toxicity outcomes that may prove detrimental to native pollinators.

Collectively, our findings suggest that under Neotropical conditions, methods for assessing the risks of agrochemical exposure to bees, especially those often considered safe such as fungicides, need to be adjusted and should take into consideration native pollinators, sublethal effects and interactions among active chemical ingredients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146432.

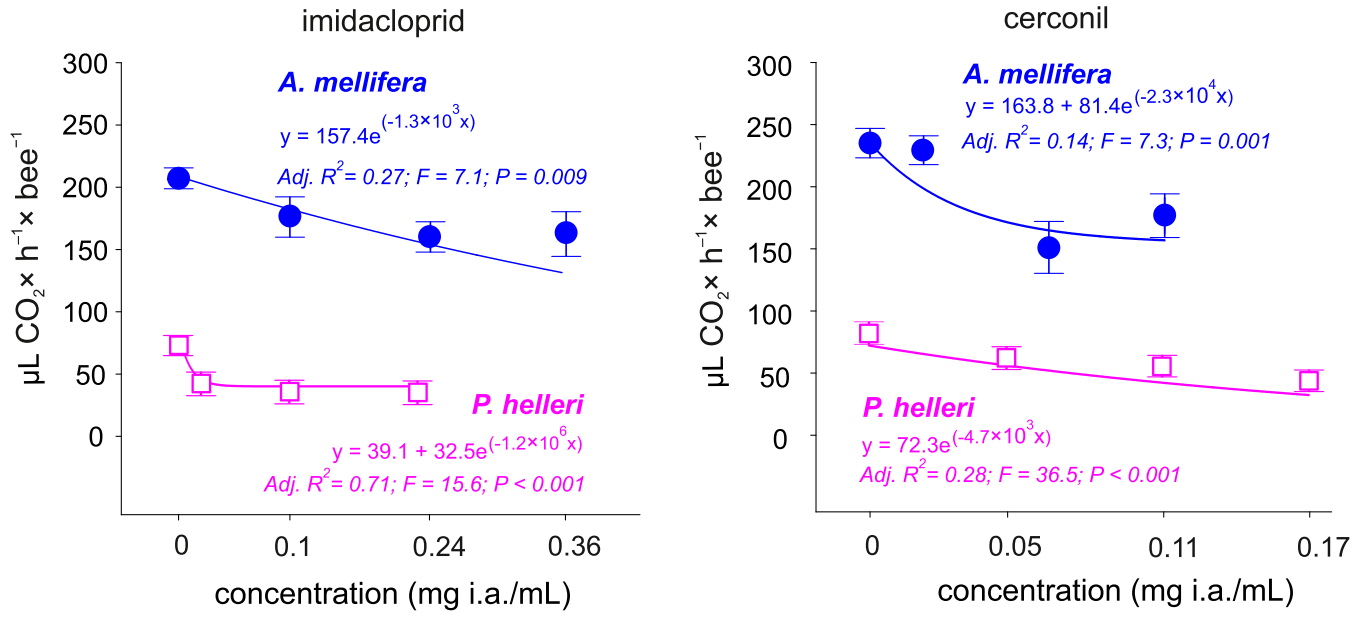

Fig. 4. Effects of sublethal exposure to imidacloprid and Cerconil on the respiration rates of *Apis mellifera* and *Partamona helleri*. *N* = 96 bees/pesticide treatment/species. Vertical bars represent the standard error (SE) of the mean. The equations represent the best models selected based on parsimony and highest level of significance.

Author contribution statement

CHSA, WCS, PLN, KH, and EEO conceived/designed the research. CHSA, PFST and SMR conducted the experiments. WCS, RNCG and EEO contributed new reagents and/or analytical tools. CHSA, KH, and EEO analysed the data. KH, PLN, and EEO wrote the manuscript. All authors read, corrected, and approved the manuscript.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation. As all bees used were reared at the Apiary Facilities of the UFV and their culture and use adhered to Brazilian laws and no permissions were required.

Informed consent

All authors of this manuscript accepted that the paper is submitted for publication in the *Science of the Total Environment* journal, and report that this paper has not been published or accepted for publication in another journal, nor is it under consideration at another journal.

CRediT authorship contribution statement

Carlos H.S. Almeida: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Khalid Haddi:** Funding acquisition, Software, Supervision. **Pedro F.S. Toledo:** Investigation, Formal analysis, Writing – review & editing. **Sarah M. Rezende:** Investigation, Formal analysis. **Weyder C. Santana:** Conceptualization, Formal analysis, Software, Supervision. **Raul Narciso C. Guedes:** Conceptualization, Funding acquisition, Software, Supervision, Writing – review & editing. **Philip L. Newland:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing. **Eugenio E. Oliveira:** Conceptualization, Investigation, Funding acquisition, Software, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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