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Exposure to field-realistic concentrations of imidacloprid at different ambient temperatures disrupts non-flight metabolic rate in honey bee (*Apis mellifera*) foragers

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Abstract

Pollinators facilitate the reproduction of the majority of the world's plants but some of the most economically important pollinating insects (Hymenoptera Apoidea) have undergone declines, possibly due to neonicotinoid agricultural pesticides. Sublethal exposure to neonicotinoids can affect insects' behavioral and physiological performance, and the detoxification of these pesticides likely affects insects' metabolism. We measured honey bee (*Apis mellifera* L.) foragers' CO_2 production rates at different temperatures (25, 30, or 35 °C) after they consumed syrup dosed with a field realistic (5 µg/L) or high (20 µg/L) concentration of a neonicotinoid insecticide (i.e. imidacloprid) for 48 hours. We found that imidacloprid exposure significantly disrupted honey bees' non-flight metabolic rates and there was a significant interaction between imidacloprid dosage and ambient temperature. Honey bee foragers dosed with 5 µg/L imidacloprid displayed higher average metabolic rates and those dosed with 20 µg/L imidacloprid displayed similar average metabolic rates compared to the corresponding control group across all temperatures. Exposure to field realistic concentrations of neonicotinoid may have a higher energetic cost for honey bees at 25 °C than at higher ambient temperatures. Disrupted energy costs in honey bees fed imidacloprid might be due to the thermoregulation, nerve excitation, or detoxification processes. Metabolic rate changes caused by pesticide exposure could result in less available energy for honey bees to perform hive duties and forage, which could negatively affect colony health.

Key words: CO_2, metabolic rate, neonicotinoid, temperature, honey bee.

Introduction

Insects have limited energy budgets that can be distributed into different physiological functions to maximize individual fitness (Maryanski *et al*., 2002). This energy allocation, also known as energy trade-offs, has been extensively studied at the physiological level (within individuals) as well as the evolutionary level (within generations) (Schwenke *et al*., 2016). Insect's physiological processes are energetically costly, and their limited internal energy is utilized for many purposes, including reproduction, growth, metabolism, immune response, and lipid storage (Zera and Harshman, 2001; Schwenke *et al*., 2016). Metabolic rate is a direct measure of the energy use of living insects, which can be affected by temperatures (Dingha *et al*., 2009), body mass (Niven and Scharlemann, 2005), gender (Rogowitz and Chappell, 2000), activities (Oertli and Oertli, 1990), reproduction (Coquillaud *et al*., 1990), and chemical stressors such as exposure to heavy metals (Bednarska and Stachowicz, 2013) and insecticides (Maliszewska and Tegowska, 2016). Measurements of insects' CO_2 production after exposure to different external stressors have been conducted on German cockroaches *Blattella germanica* (L.) (Dingha *et al*., 2009), American cockroaches *Periplaneta americana* (L.) (Ardia *et al*., 2012), mealworms *Tenebrio molitor* L. (Olszewska *et al*., 2010), house crickets *Acheta domesticus* (L.) (Ardia *et al*., 2012), and carpenter ants *Camponotus maculatus* (F.) (Duncan and Newton, 2000). However, only recently have studies utilized this method to investigate the effect of pesticides on bees' metabolic rates and respiration patterns (Campbell *et al*., 2016; Nicholls *et al*., 2017; Cook, 2019).

Honey bees (*Apis mellifera* L.) can achieve some of the highest mass-specific metabolic rates of all animals (Suarez, 2000): about threefold greater than hovering hummingbirds (Suarez *et al*., 1990) and 30 times greater than human athletes during maximal aerobic exercise (Blomstrand *et al*., 1986). Metabolic rates of flying honey bees vary widely (Roberts and Harrison, 1998; Harrison and Fewell, 2002) depending on flight speed (Dudley and Ellington, 1990), load carriage (Coelho, 1991), individual activity levels (Lighton and Lovegrove, 1990), and genetics (Hepburn *et al*., 1998), while the metabolic rates of resting honey bees vary depending on age, activity, and ambient temperature (Stabentheiner and Crailshein, 1999; Stabentheiner *et al*., 2003). Honey bee workers (5-13 days old) and foragers (>18 days old) have similar resting metabolisms, while young adults (<7 hours old) have lower values (Stabentheiner *et al*., 2003; 2010) due to incomplete development of flight muscles and enzymatic systems (Kovac *et al*., 2007). Energy turnover in honey bees is complicated because they are heterothermic insects; they are ectothermic at rest and while walking (no flight muscle shivering) but change to endothermic when foraging or preparing to fly (Stabentheiner *et al*., 2002; 2003). During the ectothermic states, the resting metabolic rates of honey bees are positively related to the ambient temperature at a range of 14-35 °C (Rothe and Nachtigall, 1989; Schmolz *et al*., 2002) even though some honey bees will dissipate heat through their mouthparts when the ambient temperature is above 33 °C (Kovac *et al*., 2007). When honey bees are actively regulating thorax and body temperatures (endothermic states), their metabolic rates decrease as the ambient

temperature increases (Rothe and Nachtigall, 1989; Moffatt, 2001; Woods *et al*., 2005). In addition to flying outside the hive, forager honey bees also need to walk through the inside of the hive to store collected nectar and pollen. Foragers that are less efficient due to poor physiological condition may adversely impact colony-level net gain, energy balance, and survival (Harrison and Roberts, 2000; Karise and Mänd, 2015; du Rand *et al*., 2015), eventually leading to colony failure (Feltham and Goulson, 2014; Tosi *et al*., 2017a). However, no study has investigated honey bees' non-flight metabolic rate changes due to external factors such as ambient temperature variation or pesticides exposure.

Neonicotinoid insecticides are commonly applied as seed coating or foliar spray to agricultural crops and orchards for systemic uptake by plants and are suspected causes of honey bee colony declines in the USA (Grossman, 2013; Cutler *et al*., 2014; Kulhanek *et al*., 2017). Europe has recently banned the outdoor application of these compounds (European Food Safety Authority, 2018). Imidacloprid, a neonicotinoid insecticide used worldwide (Imran, 2020), has been detected in nectar (concentrations range from 0.13 to 11.2 µg/kg) (Pohorecka *et al*., 2012; Dively and Kamel, 2012), pollen (1.61 to 206 µg/kg) (Mullin *et al*., 2010; Jiang *et al*., 2018), and beebread (4.68 to 11.5 µg/kg) (Gooley *et al*., 2018), where it may adversely affect honey bees' larval development (Gregorc *et al*., 2012; Cook, 2019), adult foraging behaviors (Tan *et al*., 2015), social activities (Laurino *et al*., 2011), colony growth (Laycock *et al*., 2012; Whitehorn *et al*., 2012), and queen production (Tsvetkov *et al*., 2017). Study of the sublethal effects of neonicotinoid insecticide exposure on metabolism has primarily focused on newly emerged bees in the laboratory. Field-realistic concentrations of clothianidin (another neonicotinoid insecticide) exposure during larval development had no effect on metabolic rates in solitary bees, *Osmia bicornis* (L.), (0-10 µg/L; Nicholls *et al*., 2017) or honey bees (5-50 µg/L; Cook, 2019). However, newly emerged honey bees that had been treated with a sublethal concentration (5 µg/L) of imidacloprid for 14 days during their larval development had suppressed metabolic rates and decreased body weights (Cook, 2019). Neonicotinoids can negatively affect honey bee larval development (Hatjina *et al*., 2013; Dai *et al*., 2019) and nutritional balance (Mogren and Lundgren, 2016; Tosi *et al*., 2017b), so suppressed metabolic rates of young bees chronically exposed to neonicotinoids may have been an indirect result of exposure instead of a direct result of the sublethal effects. In the field, honey bees are exposed to neonicotinoids as foragers if they collect pollen and nectar from treated crops or contaminated wild flowers (Stewart *et al*., 2014), while nurse bees usually do not forage but work within their hive (Fairbrother *et al*., 2014). Studies on the effects of neonicotinoid exposure on honey bees' non-flight physiology performance are important yet lacking.

The detoxification of chemical substance is an enzymatic breakdown process and insects may increase their energy use as detoxification enzymes (e.g. cytochrome P450, glutathione transferases, and carboxylesterases) are synthesized after ingesting toxins (Hemingway, 2000; Claudianos *et al*., 2006; Li *et al*., 2007; Azevedo-Pereira *et al*., 2011; du Rand *et al*., 2015), which likely affects insects' metabolism and is reflected in their physiological performance (i.e. respiratory regulation). Maliszewska and Tegowska (2016) found that exposure to neonicotinoid insecticide caused increasing CO_2 production in mealworms. Similar results were also found in dor beetles, *Anoplotrupes stercorosus* (Scriba), after being exposed to indoxacarb and beta-cyfluthrine (Piechowicz *et al*., 2015). However, these studies were not able to distinguish if the increased CO_2 production was caused by the energy expenditure for detoxification processes or hyperactivity of the neuromuscular system, because CO_2 measurements were taken during the poisoning process.

We investigated the impact of neonicotinoid exposure on honey bee foragers' non-flight metabolic rates at a range of temperatures (i.e. 25 °C, 30 °C, and 35 °C) after they were fed *ad libitum* designated concentrations of imidacloprid for 48 hours. Honey bees are largely ectothermic but can remain active and forage under a wide range of ambient temperatures (14 to 45 °C) (Belzunces *et al*., 1996; Harrison and Fewell, 2002) through endothermic heat production individually and when working cooperatively within the hive (Stabentheiner *et al*., 2002). Thus, there may be strong interactive effects of ambient temperature and neonicotinoid exposure on honey bees' metabolic rates, especially when ambient temperature is less than their ideal foraging body temperature (30 to 35 °C; Cooper *et al*., 1985) or core hive temperature (32 to 36 °C; Southwick and Heldmaier, 1987). Our specific objectives were to assess: (1) if honey bee foragers' non-flight metabolic rates would increase after being fed with field-realistic concentrations of neonicotinoid insecticide, and (2) if any increase in honey bee metabolic rates in response to imidacloprid exposure is affected by ambient temperature. Results from this study will improve the basic understanding of the metabolic cost of pesticide exposure in honey bees.

Materials and methods

Honey bee collection and maintenance

We collected honey bee foragers in October 2018 from a hummingbird feeder within a residential area located 2.6 km NW of Southern Illinois University Carbondale (SIUC), Illinois, USA. Daily ambient temperature during sample collection ranged from 14 to 32 °C. The hummingbird feeders contained untreated commercial liquid bee feed (henceforth syrup; sucrose solution with spearmint and lemongrass oils) (Harvest Lane Honey, Utah, USA) as an attractant. We captured honey bee foragers and kept them individually in a clear cup-shaped cage (approximately 55 mm in diameter and 40 mm in height) with removable lid, ventilation holes, and one feeding device (figure 1; Williams *et al*., 2013). Each individual honey bee was provided with untreated syrup immediately after being caged and transported to SIUC in a dark box. We maintained honey bees in a temperature-controlled environmental chamber (Geneva

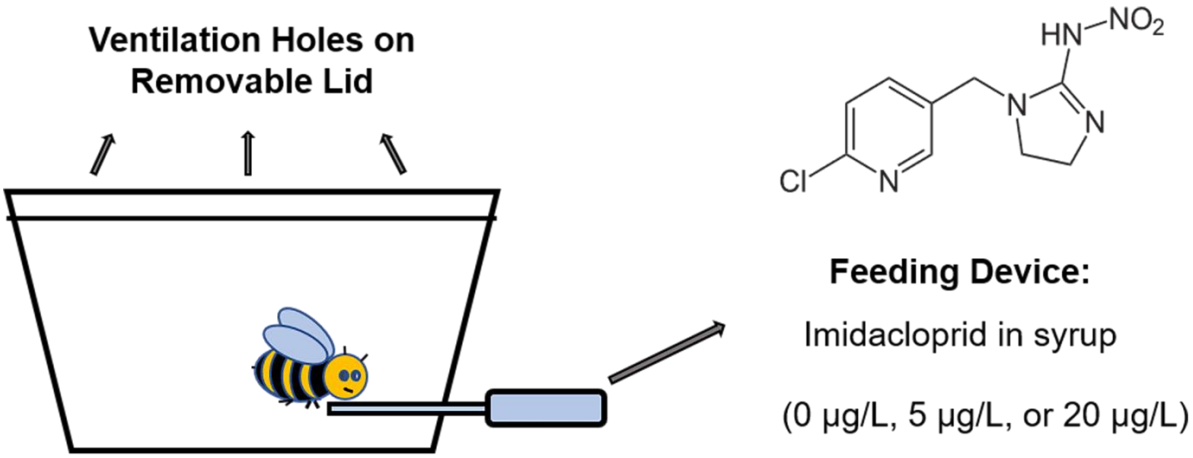

Figure 1. Individual honey bee that was maintained in a clear cup-shaped cage with designated diet for 48 hours.

Scientific, Fontana, WI, USA) with light cycles (12h:12h, light:darkness) for 48 hours at their respective treatment temperatures (i.e. 25 °C, 30 °C, and 35 °C) to allow acclimation prior to the dosing. An open pan (20 × 25 cm) of water was placed in the incubator to maintain relative humidity > 45% (maximum value ≤ 50%).

Experimental design

A combination of one of two concentrations of imidacloprid (i.e. 5 µg/L and 20 µg/L) and one of three ambient temperatures (i.e. 25 °C, 30 °C, and 35 °C) were applied on individual honey bees as different treatments. Imidacloprid standard was obtained as a solution in acetonitrile (10 µg/mL, AccuStandards, CT, USA) and added into the syrup to produce the treatment concentrations. In each treatment group, we provided each individual honey bee with a continuous supply of either syrup (control) or syrup dosed with 5 µg/L (3.53 µg/kg, w/w) or 20 µg/L (14.1 µg/kg, w/w) imidacloprid. The final concentration of acetonitrile in the syrup solutions for the control and treatment groups was 0.1% (v/v). The 5 µg/L dose represented a field-realistic concentration of imidacloprid detected in nectar (reported concentrations range 0.3-11.2 µg/kg) (Dively and Kamel, 2012; Sanchez-Bayo and Goka, 2014), and the higher dose of imidacloprid (20 µg/L) was chosen for comparison but was less than the reported 24 hours oral LD50 in honey bees (25.3-540 µg/kg; Fairbrother *et al*., 2014). We included 7 replicates (n = 7) for each treatment for a total of 63 honey bees (N = 63). After being fed with their respective treatment diets for 48 hours *ad libitum*, we weighed the honey bees using an analytical balance (Mettler Toledo XS64; accurate to 0.01 mg) and then measured each individual honey bee's carbon dioxide production ($\dot{V}_{CO2}$, mL/min) while they were walking.

Respirometry measurements

We estimated honey bees' non-flight metabolic rates from their CO_2 production using standard open-flow respirometry techniques (Carey and Boyles, 2015). All honey bees were measured under a dim light inside an environmental chamber (Binder Inc. BD series, Bohemia, NY, USA), equipped with an internal circulating fan that regulated the ambient temperature within the incubator at ± 0.1 °C accuracy. A pan of water was placed in the incubator to maintain the relative humidity > 45% (maximum value ≤ 50%). We transferred each honey bee individually from the cup-shaped cage into a 5 mL clear plastic respirometry chamber (figure 2), where honey bee was only allowed to walk but not giving enough clearance to fly. Honey bees were allowed at least half an hour of acclimation in the environmental chamber at their respective treatment temperatures (i.e. 25 °C, 30 °C, and 35 °C) prior to recording the $\dot{V}_{CO2}$ data. Air was drawn through the respirometry chamber at 50 mL/min (subsample SS-3; Sable Systems Int.). The excurrent air from the chamber passed through a magnesium perchlorate drying column and then to a carbon dioxide analyzer (CA-10a; Sable Systems Int., Las Vegas, NV, USA). Three respirometry chambers with an individual honey bee in each were placed in the environmental chamber prior to $\dot{V}_{CO2}$ measurements (figure 3). We waited until honey bees were walking constantly (circling along the sidewall of the respirometry chamber) and then recorded $\dot{V}_{CO2}$ for 10 minutes. Before switching to the next honey bee respirometry chamber, baseline air was subsampled from an empty chamber for 2 minutes. Each honey bee was measured at least three times (i.e. 10 minutes per honey bee per each measurement for a total of ≥ 30 minutes of measurement). All $\dot{V}_{CO2}$ data was collected from a walking but non-flying honey bee within 2 hours after being transferred into the respirometry chamber. A stream of air was directed into chambers that were not being measured (Flowbar-8, Sable Systems Int.) to prevent CO_2 build up.

Prior to the experiment, the carbon dioxide analyzer was zeroed using ultra high-purity nitrogen gas (Airgas, Benton, IL, USA) and spanned with trace gas of carbon dioxide (0.985% CO_2 in N_2; Airgas, Benton, IL, USA) per manufacturer specifications. We baseline corrected CO_2 and calculated the $\dot{V}_{CO2}$ (equation 10.5, Lighton, 2008) using the most level 3 minutes samples (i.e. honey bee walking calmly) from each 10 minutes recording period and averaged the closest three measurements (figure 4) (Cary and Boyles, 2015). Honey bees only use carbohydrates to fuel metabolism (Suarez *et al*., 1999) so we assumed a respiratory quotient of 1 (Lighton, 2008).

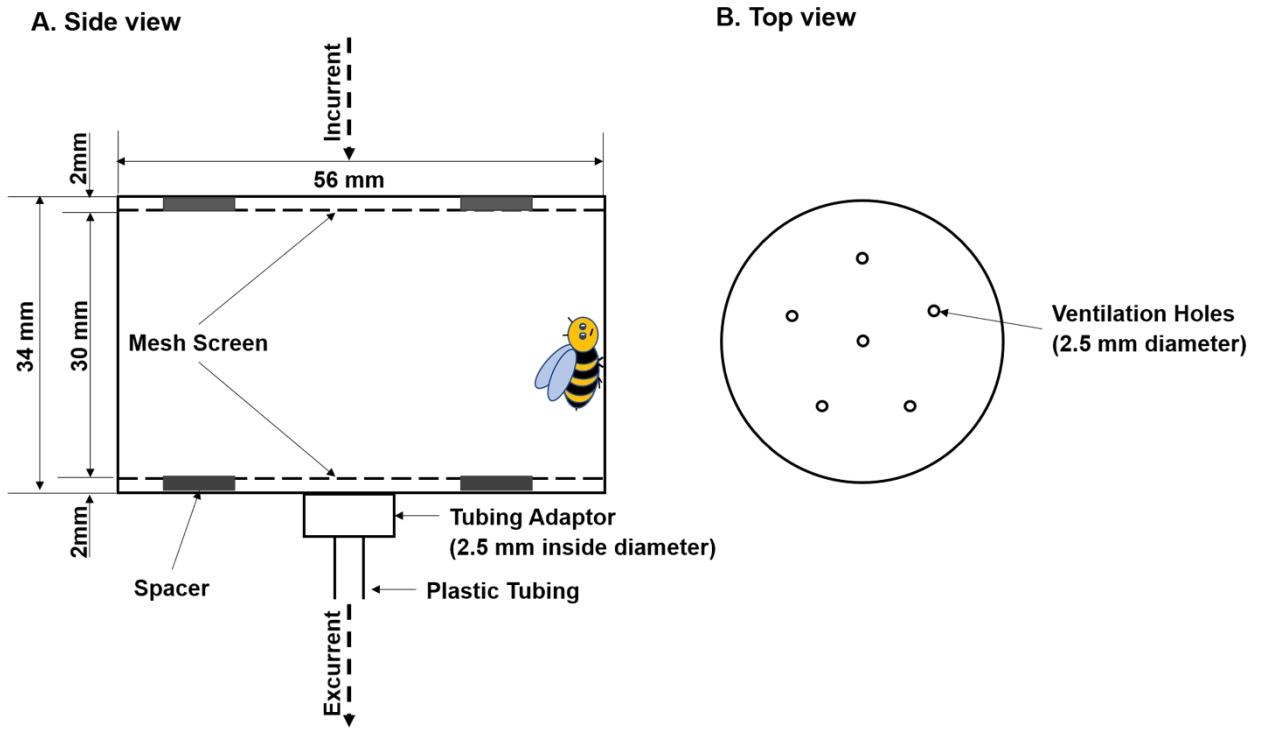

Figure 2. (A) Side and (B) top view of the respirometry chamber. Airflow through the chamber is controlled by the inside diameter of the tubing adapter and ventilation holes. Air flow through the ventilation holes cannot be restrictive and air flow into the plastic tubing must prevent CO_2 buildup in the chamber or escape through the ventilation holes.

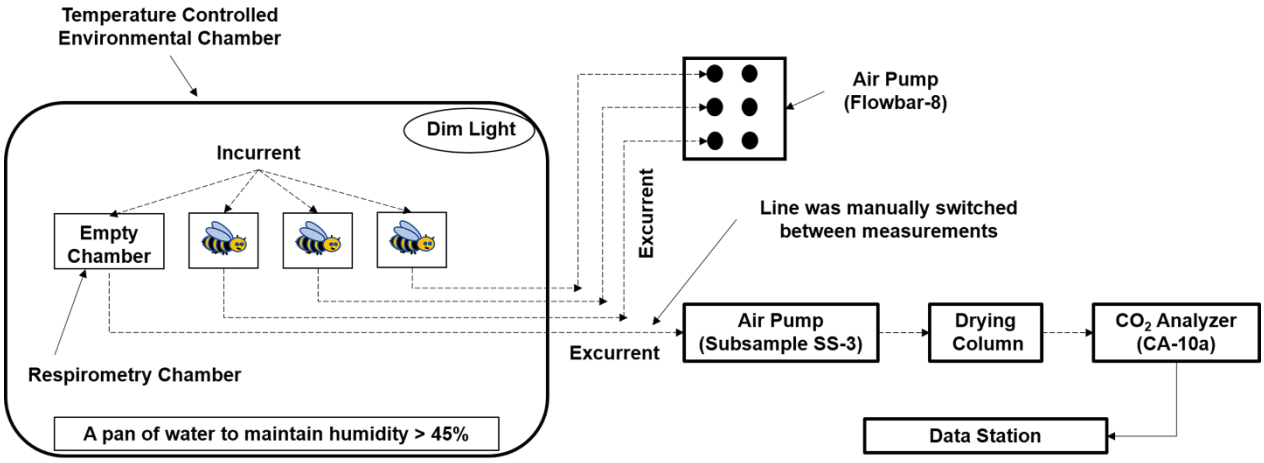

Figure 3. Flow chart of honey bee CO_2 production measurements. Baseline air was subsampled from the empty chamber for 2 minutes between measurements. A constant stream of air was drawn through the respirometry chambers to prevent CO_2 buildup while honey bees were not being measured.

Statistical analysis

We used two-way analysis of covariance to evaluate the effect of imidacloprid and temperature on honey bees' non-flight CO_2 production ($\dot{V}_{CO2}$), where the ambient temperature T_a and imidacloprid treatment (i.e. control, low dose, or high dose) were fixed effects. We included honey bees' initial body mass as a covariate because flying insects' body masses are linearly related to their resting metabolic rate (Reinhold, 1999). An analysis of simple main effects (i.e. the effect of one independent variable within one level of the other independent variable) for T_a and imidacloprid was performed with statistical significance receiving a Bonferroni adjustment and being accepted at $P < 0.0167$ (IBM SPSS statistic 25, IBM Corporation, Armonk, NY, USA).

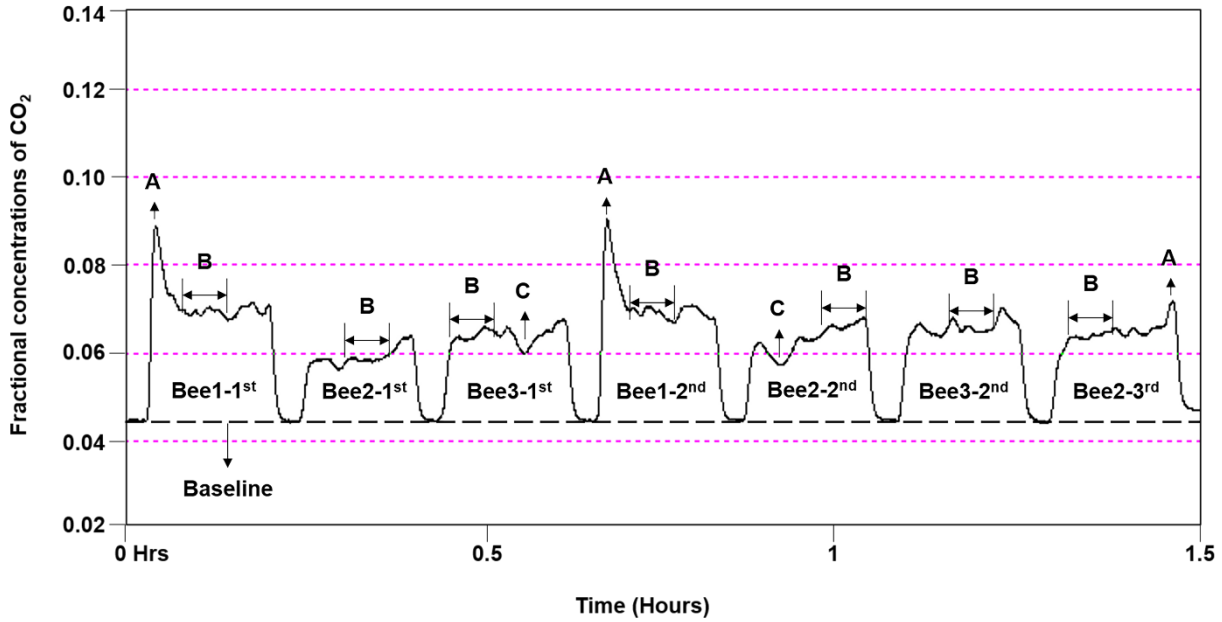

Figure 4. Example of ExpeData analysis (Sable Systems Int.) window while honey bees were measured in the respiratory cup. (A**)** CO_2 build up due to honey bees' hyperactivity; (B) honey bees walked constant and calm; (C) honey bee stopped walking for a very short period.

Results

We found a significant effect of imidacloprid treatment ($F_{2, 53}$ = 4.194, P = 0.020) (table 1, figure 5) and a significant T_a × imidacloprid treatment interaction ($F_{4, 53}$ = 2.896, P = 0.031) on honey bees $\dot{V}_{CO2}$ (table 1). However, we did not find any significant effect of T_a on honey bees $\dot{V}_{CO2}$. Honey bees that received 5 µg/L imidacloprid treatment group under 25 °C had significantly higher $\dot{V}_{CO2}$ compared to the control group (P < 0.0005) with a mean difference of 2.144 (95% CI: 0.898 to 3.390) mL/minutes (302% higher). The $\dot{V}_{CO2}$ of honey bees that received 20 µg/L imidacloprid treatment under 25 °C was 0.776 (95% CI: −0.474 to 2.025) mL/min higher compared to the control group but was not statistically significant (P = 0.392 < 0.0167) (172% higher). The effect of imidacloprid on honey bees' $\dot{V}_{CO2}$ under higher ambient temperatures (i.e. 30 °C and 35 °C) was not statistically significant.

Discussion

While there have been growing international concerns about the impacts of neonicotinoid insecticides on pollinators (Lu *et al*., 2014; Kiljanek *et al*., 2016; Woodcock *et al*., 2017), only recently have physiological studies begun to address the effects of sublethal doses of neonicotinoids on bees (e.g. Karise and Mänd, 2015; Campbell *et al*., 2016; Cook, 2019). Little is known about adult honey bees' respiratory response to sublethal neonicotinoid exposure. We collected a mix of actively foraging bees in the wild to test if honey bee foragers' CO_2 production rate increase after exposure to field-realistic concentrations of neonicotinoid insecticide under different environmental conditions. We found that imidacloprid exposure significantly disrupted honey bees' non-flight metabolic rates after receiving dosed syrup at field-realistic concentration for 48 hours and there was a significant interaction effect between imidacloprid dosage and ambient temperature. Exposure to

Table 1. Effect of ambient temperature and imidacloprid treatment on CO_2 production of non-flight honey bees evaluated by two-way ANCOVA.

Effect	df	Mean Square	F	P	Partial Eta Squared
Body mass (covariate)	1	0.043	0.049	0.826	0.001
Temperature	2	1.013	1.144	0.326	0.041
Imidacloprid	2	3.711	4.194	0.020	0.137
Temperature*Imidacloprid	4	2.562	2.896	0.031	0.179
Residuals	53	0.885			
Significant at P < 0.05					

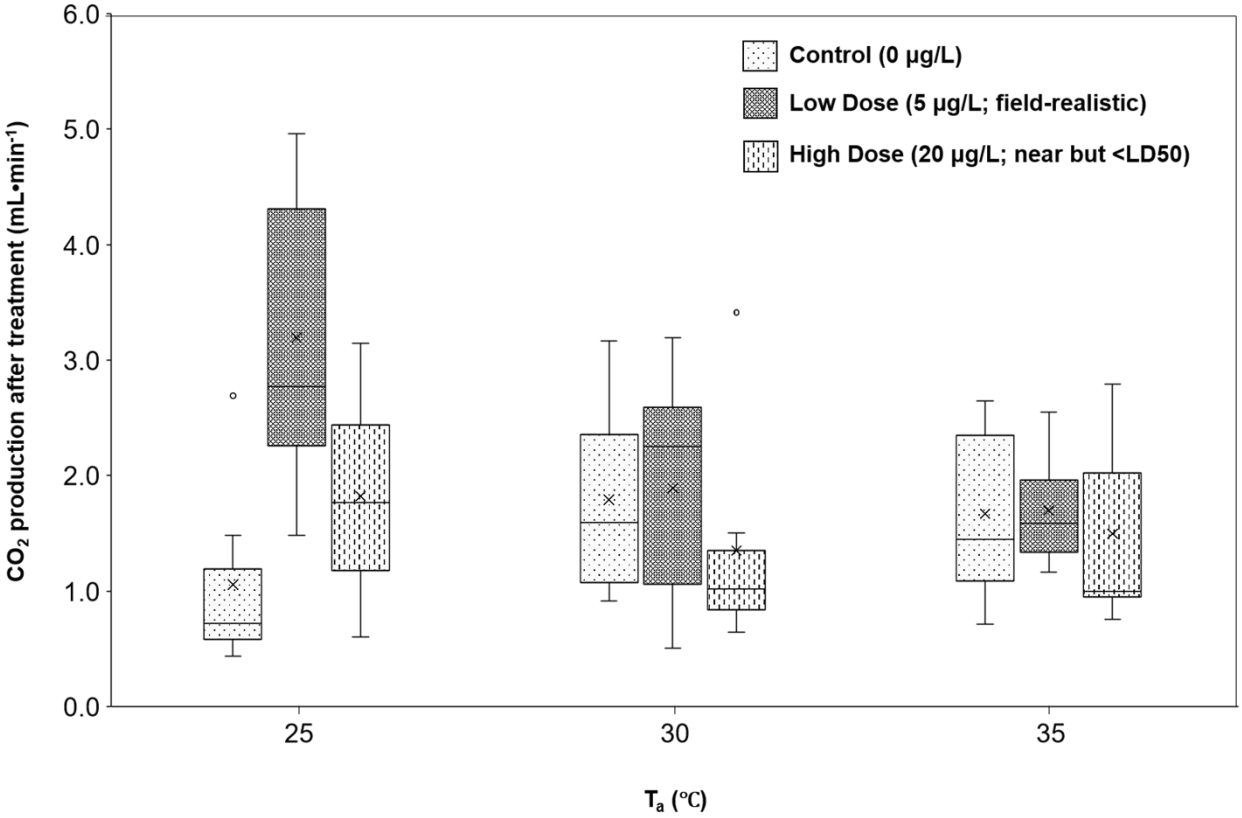

Figure 5. $\dot{V}_{CO2}$ distribution of honey bees under different ambient temperatures (T_a) after consuming low (5 µg/L; field-realistic) and high (20 µg/L; near but <LD50) concentrations of imidacloprid dosed syrup for 48 hours ($\dot{V}_{CO2}$) by using box plot. Imidacloprid significantly affected the $\dot{V}_{CO2}$ ($F_{2,53}$ = 4.194, P = 0.020), and there was a significant T_a × imidacloprid interaction effect on honey bees' $\dot{V}_{CO2}$ ($F_{4,53}$ = 2.896, P = 0.031).

sublethal concentrations of neonicotinoid may have a higher energetic cost for honey bees at 25 °C compared to the higher ambient temperatures.

While ambient temperature alone had no significant effect on captive honey bees' walking metabolic rates in our study, we do not think this result indicates that ambient temperature has no influence on honey bee metabolic rates, regardless of the presence of pesticides. Bees are heterothermic insects that can regulate their thoracic temperatures in the absence of solar heat gain (Roberts and Harrison, 1999; Kovac *et al*., 2007). When the ambient temperature is above chill coma (> 10 °C) (Kovac *et al*., 2007), active honey bees can shiver their flight muscles to increase their thoracic temperature to prepare for flight and will not take off until their thoracic temperature is raised slightly above the ambient temperature (Heinrich and Esch, 1994; Woods *et al*., 2005; Kovac *et al*., 2007). Honey bees either maintained thoracic temperatures slightly above ambient temperatures (25 or 35 °C) at low activity levels or increased their temperatures approximately 9-14 °C (at 25 °C) and 4-6 °C (at 35 °C) corresponding to activity level (Stabentheiner and Crailshein, 1999). Our results indicated that honey bees may have adjusted to captivity after 96 hours and may not have been attempting to maintain heightened metabolic rates to be ready for any escape opportunities (Z. Gooley and A. Gooley, unpublished data). Although flight muscles are the primary source of metabolic heat generation in honey bees and walking activity may not contribute much to heat generation (Stabentheiner and Crailshein, 1999), we cannot distinguish the amount of CO_2 output that resulted from walking instead of metabolic thermoregulation or detoxification in our study. The low metabolic rate observed in the control group at 25 °C could reflect acclimation to captivity and/or slow walking honey bees under relatively low temperature. Future studies on how duration and maintenance in captivity affect honey bees' metabolic rates are needed to help identify the best methods for testing captive honey bees' physiological responses to stressors.

We found that the field-realistic concentration of imidacloprid greatly affected honey bees' metabolic rate. Dietary neonicotinoids were found to disrupt thermoregulation in non-flying worker bumble bees (Potts *et al*., 2018), African honey bees (Tosi *et al*., 2016), and solitary bees (Azpiazu *et al*., 2019). Bumble bees maintained lower thorax temperatures after they were fed with sublethal to lethal doses (ranged from 0.08 µg/L to 125 µg/L) of imidacloprid (Potts *et al*., 2018). We assumed that reduced thorax temperature caused by imidacloprid exposure might reflect insufficient heat production through metabolic processes in dosed bees. However, our results showed that honey bees fed a low concentration (5 µg/L) of imidacloprid appeared to have higher average metabolic rates compared to corresponding individuals in the control group across all tempera-

tures and those fed a high concentration (20 µg/L) of imidacloprid appeared to have similar metabolic rates compared to the control group. Honey bee thermogenesis is mainly achieved by shivering the flight muscles (Azpiazu *et al*., 2019) that cannot be seen during heat production (Belzunces *et al*., 1996). Whether honey bees would increase their metabolic rate to compensate for lowered thorax temperature after exposure to pesticides is complicated due to the interchangeable thermoregulation ability of honey bees. We were not able to distinguish if honey bees had any indirect flight muscles contraction during the measurement unless they were in sleep (Schmolz *et al*., 2002).

Neonicotinoids bind irreversibly to the nicotinic acetylcholine receptors (nAChRs) in insects' brains causing uncontrollable muscle movement (Kimura-Kuroda *et al*., 2012; Kiljianek et al., 2016) At low doses, neonicotinoid can act as nicotine and cause behavioral stimulation and nerve excitation in honey bees that increase their activity level (Picciotto *et al*., 2002; Tosi and Nieh, 2017). At high doses, the neurotoxic disruption from imidacloprid exposure can cause fundamental physiological problems in honey bees that suppress their activity level (Medrzycki *et al*., 2003; Potts *et al*., 2018; Z. Gooley, A. Gooley, and J. Reeve, unpublished data). The elevation in honey bee foragers' metabolic rates in treatment groups fed 5 µg/L imidacloprid (field-realistic concentration) could be due to neonicotinoid-induced hyperactive behavior. Hypoactive behavior in honey bees foragers fed 20 µg/L (high concentration but less than the lowest reported LD50) could have been counteracted by physiological weakness.

Insects utilize metabolic energy for heat production, fueling muscle movement, detoxification enzyme synthesis, and other cellular functions (Rantala and Roff, 2006; Li *et al*., 2007; Arrese and Soulages, 2010; du Rand *et al*., 2015). In our study, we found that honey bee metabolic rates were affected by an interaction between ambient temperature and dosage of imidacloprid in their diet. Honey bees exposed to 5 µg/L imidacloprid while being maintained at 25 °C showed the highest metabolic rates among all treatment groups. However, the differences in metabolic rates were not significant when the ambient temperatures were more favorable for honey bees (30 and 35 °C). This could have been because the energetic investment needed for honey bees to raise and regulate their thoracic temperatures was much lower at higher ambient temperatures (Stabentheiner and Crailshein, 1999). Campbell *et al*. (2016) reported that while honey bees' mitochondrial function was inhibited after consuming the contaminated pollen and syrup (fungicide Pristine®, up to 10 mg/L) up to 24 days, flight metabolic rates and thorax temperatures were not affected. This suggested that metabolic detoxification in insects occurred after exposure to high levels of pesticides, but the resulting consequences were not reflected in measures of insects'CO_2 production rates. It is possible that in our study, honey bees fed imidacloprid at 25 °C increased their metabolic rate to both increase body temperature to a range that was more ideal for detoxification enzymes to function and provide increased energy to support the production of enzymes (du Rand *et al*., 2015). However, it is difficult to attribute significant differences in honey bees' metabolic rates after exposure to neonicotinoids to detoxification unless the activity responses at different sublethal doses are better understood (Karise and Mänd, 2015).

Sublethal doses of neonicotinoids have been found to greatly reduce honey bees' flight duration, distance, and average velocity after two days exposure (Tosi *et al*., 2017a). Increased energy demands for the detoxification of neonicotinoids may disrupt the amount of energy available for flight muscles and lower their foraging efficiency. Bees foraging on contaminated floral resources continuously at cool temperatures (T_a < 30 °C) are potentially at higher risk of physiological hindrance from neonicotinoids exposure. We suggest that studies of the energy costs to honey bees from exposure to field-realistic neonicotinoids during flight in low ambient temperatures (i.e. from 14 °C to 25 °C) are needed to assess the effect of neonicotinoids on honey bees' physiological performance and flight ability. Future studies of the relationship between detoxification process and physiological performance (i.e. metabolism) in honey bees and other pollinators after being exposed to pesticide should combine measures of CO_2 output, infrared photography, mitochondrial function, and metabolic fingerprinting using mass spectrometry to attain a more complete understanding of energy usage.

Conclusions

Our study is the first to describe the metabolic rates in non-flight honey bee foragers after being fed field-realistic concentrations of neonicotinoid insecticide under different ambient temperatures. We found that honey bee foragers exposed to low dose of imidacloprid insecticide at relatively low ambient temperature had higher metabolic rates and those exposed to a higher dose of imidacloprid insecticide had similar metabolic rates (except at 25 °C) compared to the control honey bees. This disrupted energy cost might be due to several causes, including thermoregulation, neonicotinoid-induced abnormal behavior, or detoxification process. Changes in energy use could potentially affect the amount available for flight and performance of hive duties, such as maintaining hive temperature for brood rearing. Further studies are needed to determine the metabolic costs of detoxification for honey bees during flight. Understanding how honey bees utilize their metabolic energy after exposure to neonicotinoid insecticides in nectar and pollen during and after foraging is essential for estimating the true effects of pesticides on both individuals and colony health.

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