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Influence of honey bee seasonal phenotype and emerging conditions on diet behavior and susceptibility to imidacloprid

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Abstract – Honey bee *Apis mellifera* L. colonies produce two distinct phenotypes of workers during summer and winter to cope with drastic seasonal variations in climate and food resources. Imidacloprid (IMP) is a neonicotinoid insecticide widely used in agriculture for pest management control. In this study, we investigate the influence of seasonal phenotype and emerging conditions on the diet behavior of bees fed ad libitum two concentrations of IMP. We performed three independent two-choice feeding experiments using summer bees either emerged in the laboratory or in-hive and winter bees. Diet behavior post-ingestive aversion responses to IMP were investigated as well as potential affinity to the physical location and contents of the diets. Caged bees were challenged with a physical rotation of the diet's location and their susceptibility to 5 and 20 PPB of IMP was tested. From a behavioral standpoint, our results show that winter bees expressed no affinity to the physical location of the diet but rather to its content and strongly favored IMP-tainted syrup at both 5 and 20 PPB. The opposite was recorded for naïve summer bees that emerged in the laboratory, which avoided the tainted syrup at both concentrations, particularly at 20 PPB. Summer bees emerged in-hive, expected to have developed a mature intestinal microbiota through trophallaxis from older bee-mates, were mainly neutral, and showed no affinity to the diet location nor its contents. Our results indicate that the physiological changes associated with seasonal phenotype and initial exposure to older mates have important consequences on the bee diet behavior toward pesticides.

Apis mellifera / Imidacloprid toxicity / Honey bee phenotypes / Diet behavior

1. INTRODUCTION

The domestic honey bee *Apis mellifera* L. and other non-*Apis* bees play a crucial worldwide role in crop and plant pollination (Aizen et al. 2009; Lorenzo-Felipe et al. 2020; Sampson and Cane 2000). Despite being required for pest management control, excessive and off-label use of agriculture pesticides, particularly highly toxic insecticides such as neonicotinoids, has become a consequential threat to bees and other pollinators (Alburaki et al.

2016, 2017; Mullin et al. 2010; Rundlof et al. 2015; Traynor et al. 2016; Whitehorn et al. 2012). Honey bees are eusocial insects that exhibit highly sophisticated social behaviors (Seeley 2010). Honey bee colonies survive extreme seasonal differences in temperature and forage by producing two seasonal phenotypes of workers: summer and winter bees. These two worker groups show significant differences in their physiological traits and lifespan (Bresnahan et al. 2022). For instance, summer bees display a lifespan of a few weeks (3–6) during the peak of the summer season (Winston 1987), while winter bees, which emerge from the late brood reared by the colony (Smedal et al. 2009), have a much higher life expectancy reaching 5 to 8 months (Fluri et al. 1982; Page and Peng 2001). Recent

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data show that colony losses overwhelmingly occur during the winter (Kulhanek et al. 2017), suggesting the involvement of various pathological and immunological factors mainly occurring in *winter bees* (Schafer et al. 2010; Steinmann et al. 2015).

Many honey bee behavioral and toxicological experiments are conducted in cages under laboratory conditions, which offer researchers better control over complex variables involved in their experiments (Evans et al. 2009; Gregorc et al. 2018b; Huang et al. 2014). In cage experiments, particularly when diets are administered *ad libitum*, it is important to consider how the administered treatments affect the bee diet behaviors. Bee preference, dislike, or avoidance of particular treatments may induce significant bias in the experiment outcomes, due to dietary behaviors and not necessarily to the effect of the treatment itself. Nonetheless, both toxicodynamic and toxicokinetic aspects of a given treatment or pesticide should be considered for a complete understanding of its effects (Poquet et al. 2016). From a toxicological point of view, honey bees under field conditions can cope with the effect of pesticides by two main measures: first, by avoiding the exposure or intake of toxic food sources. Second, by alleviating the molecules' harmful effects through detoxification processes (Mao et al. 2013). In this study, we addressed the former scenario by testing whether bees would make a safe choice if offered multiple food sources, one being laced with imidacloprid (IMP). Imidacloprid is a broadly used neonicotinoid in agriculture, which can, even at sublethal doses, impair honey bee performance, immunocompetence, memory, and brain metabolism (Brandt et al. 2016; Chakrabarti et al. 2015; Decourtye et al. 2004; Williamson et al. 2013). IMP was also described to weaken honey bees through a synergistic effect with the microsporidia *Nosema* (Alaux et al. 2010). Aside from its well-documented detrimental effects on bees, this study focuses on IMP to build on our previous effort to understand honey bee diet behavior *vis-à-vis* this particular molecule (Alburaki et al. 2019a). The post-ingestive aversion response (Behmer et al. 2005) is a deterrent mechanism that can be categorized under the first measure

mentioned above, as it averts bees from consuming diets that have caused them potential malaise based on their previous experience.

Winter and summer bee phenotypes undergo physiological changes to cope with drastic seasonal changes in temperature and the availability of nutritional resources (Steinmann et al. 2015). However, most studies analyzing diet behavior response to pesticide exposure have been conducted using summer bees (Démarets et al. 2016; Liao et al. 2017). Moreover, exposure routes to pesticides may vary between summer and winter bees. While summer bees, particularly foragers, are more likely to encounter pesticides through direct foliar exposure, guttation, seed-coated dust, winter bees in a cluster would mainly be exposed through contaminated stored food such as pollen and honey (Krupke et al. 2012; Mullin et al. 2010). The field-relevant concentrations of IMP that foragers may encounter vary significantly based on the type of crops, landscape compositions, and pesticide applications. For instance, IMP residues were identified in cotton pollen and nectar at 64 and 2 PPB, respectively, and recorded at 3 and 152 PPB in trapped pollen collected from hives located in crop growing areas in the USA (Alburaki et al. 2018; Jiang et al. 2018; Mullin et al. 2010). The first goal of the present study was to investigate whether summer and winter bees show differences in diet behavior and susceptibility to IMP. Another factor that can play an essential role in honey bee susceptibility to stressors is bee gut microbiota (Li et al. 2019; Wang et al. 2021; Wu et al. 2020). At emergence, bees have very low gut bacterial levels and initiate the development of their core microbiota soon after. These newly emerged bees build and acquire their gut microbiota through consumption of bee bread, honeycomb, and oral trophallaxis with older bee-mates (Powell et al. 2014). Recent advances in molecular toxicology have shown that a mature core of intestinal microbiota is vital in protecting honey bees against pesticides (Wu et al. 2020). Exposure to neonicotinoids such as imidacloprid, nitenpyram, and thiacloprid has been shown to exert adverse effects on bee gut microbiota's homeostasis (Daisley et al.

2017; Liu et al. 2020; Zhu et al. 2020). It is, however, still unclear how disturbed or nonoptimal microbiota may affect or shape honey bee diet behavior. Since most toxicology studies using caged bees in the laboratory have been performed using bees born in the laboratory lacking a mature microbiota, the second goal of our study was to address the potential influence of initial postemergence trophallaxis with older hive mates on bee diet behavior vis-à-vis IMP.

Herein, we investigated the effects of seasonal phenotype and postemergence conditions on dietary behavior toward IMP in three 18-day trials using summer bees that emerged in-hive or the laboratory as well as overwintering bees. We tested the potential manifestation of post-ingestive aversion responses to IMP at both 5 and 20 PPB, as well as the toxicological effect of IMP on both seasonal and differently emerged bee groups.

2. MATERIAL AND METHODS

This diet behavioral and toxicological study was conducted in cages and under a controlled environment. Honey bees used in this study were obtained from our current stock at the Bee Research Laboratory at Beltsville, MD, USA, composed mainly of Carniolan and Italian bees. Caged bees were administered two concentrations (5 and 20 PPB) of IMP (MilliporeSigma Corporation, MO, USA) through a 1:1 sugar syrup solution ad libitum.

2.1. Summer honey bees

2.1.1. Emerged in the lab

In April, 4–5 capped brood frames were transferred from healthy colonies and placed in an incubator at 35 °C and ~55% RH to hatch newly emerged bees. The next day (24 h), 1-day-old bees were distributed into nine cages, averaging 100 bees per cage, and provided with 5 g of Global Patty, which consisted of 15% pollen,

sugar, soy flour, brewer's yeast, water (BetterBee Co., NY, USA), and 1:1 untreated sugar syrup. Cages were placed in an incubator at 32 °C and ~55% RH, and bees were allowed a 2 day acclimatization period prior to administering any treatment (Figure 1).

2.1.2. Emerged in-hive

Five capped brood frames were collected from colonies in the previously mentioned apiary during the summer season. They were placed in a new upper chamber of a strong colony separated by a wired screen from the bees of the lower chamber. Brood frames were kept 24 h to emerge in the new hive, and newly emerged bees were shaken to the bottom screen, and frames were removed. This procedure enhances oral trophallaxis through the screen separator between newly emerged bees and older hive mates. Bees were kept in this stage for ~2 h and then collected to be caged following the same procedures of summer bees that emerged in the lab (Figure 1).

2.2. Winter honey bees

In December, winter bees were obtained from a healthy wintering colony from the same stock. Approximately a thousand bees were collected from this colony and immediately transferred to the laboratory in a plastic box. The age of the winter bees used in this experiment is estimated to range between 2 and 3 months, assuming they resulted from the last brood produced in mid to late autumn. Winter bees were subjected to the same experimental procedure and conditions as summer bees.

2.3. Experimental design and timeline

This study was conducted at cage level, on three biological replicates, and on a total of 2700 summer and winter bees, which were equally distributed in 27 cages (100/cage). Cages were

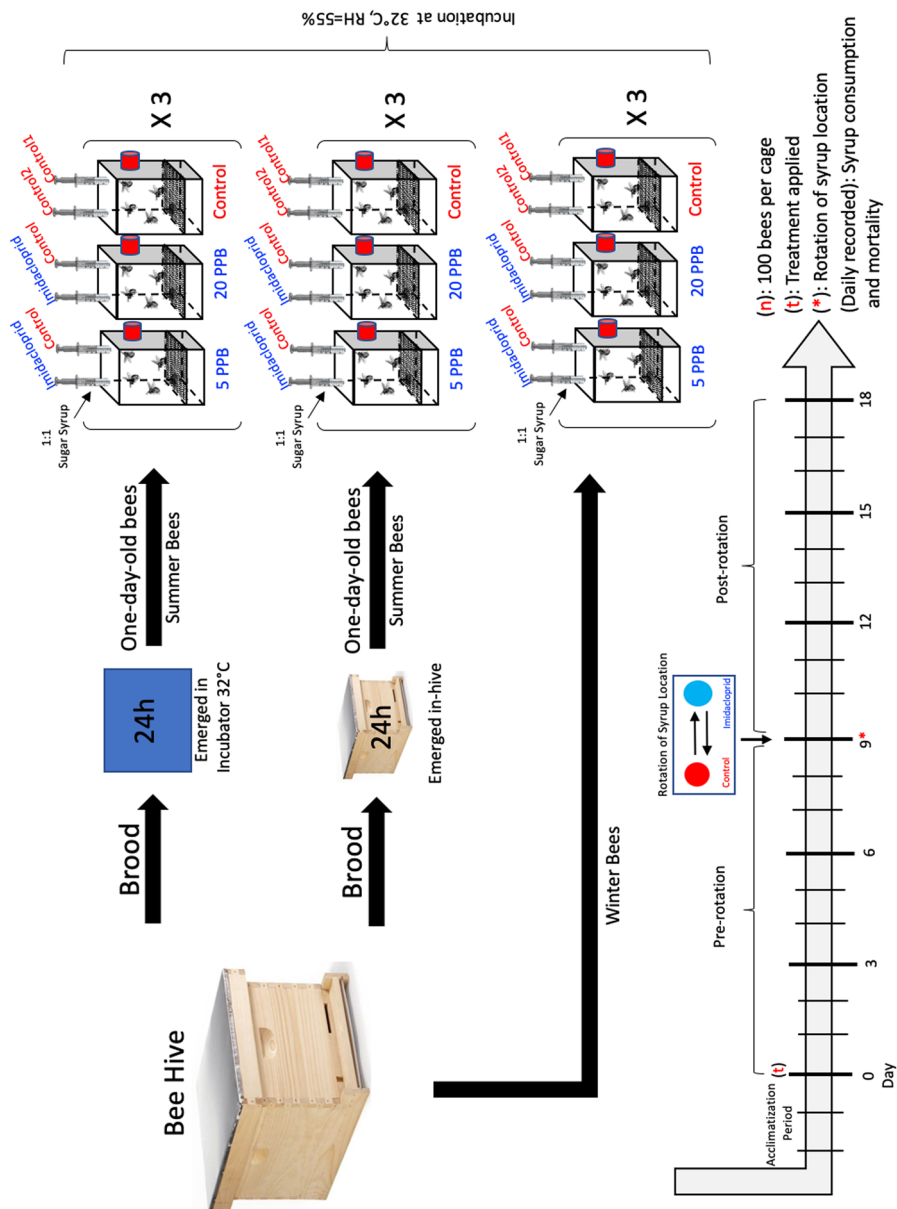


Figure 1. Cage experimental design and treatment timeline. The study was conducted on 1-day-old summer and over-winter bees, three biological replicates for each treatment and 100 bees per cage. A 2-day acclimatization period was given prior to administering the treatment. The experiment lasted 18 days, rotating the control and treatment syrup location at day 9. IMP was administered through 1:1 sugar syrup at 5 and 20 PPB. Syrup consumption and bee mortality were recorded daily

maintained at 32 °C and ~55% RH in an incubator. Each experiment lasted 18 days, starting from day 0 to 18 (Figure 1). As stated previously, bees were given an acclimatization period of 2 days to familiarize themselves with the cage conditions prior to applying the treatment. Specifications of the cages used in this experiment were detailed in a previous study (Gregorc et al. 2018a), and the same cages were used in previous bee transcriptional and toxicological studies (Alburaki et al. 2019a, b). Bees were constantly provided with two syrup sources in each cage, as shown in Figure 1. In the treatment groups, one source contained the treatment (5 or 20 PPB IMP) and the other the untreated syrup (control). Bees of the control cages were provided with two untreated sugar syrup sources (control 1 and control 2) (Figure 1). To uncouple the treatment effect from the bees' affinity to a specific source of syrup, the locations of both syrup sources were rotated on day 9 of the experiment (Figure 1).

2.4. Diet behavior and toxicity

Two main parameters were recorded daily for each cage; bee syrup consumption and mortality. The syrup was administered using 20 mL syringes, and its intake was measured using a sensitive scale (± 0.01 g) by weighing both syrup sources of each cage: IMP vs. control for the treatment and control 1 vs. control 2 for the control. Dead bees were counted and removed from cages. The potential toxicological effect of IMP on summer and winter bees was evaluated within each treatment group independently using two different methods: (1) Kaplan–Meier survival probability analysis and (2) overall bee mortality percentage for each bee group.

2.5. Statistical analysis

All statistical analyses of this study were carried out in the R environment (R Core Team 2016) using RStudio version 1.3.1073. This study was conducted at the cage level with three biological replicates and three variables: syrup

consumption, bee mortality, and type of bees. To evaluate bees' affinity to specific types and locations of diets, average consumption was reported in two categories: prerotation and post-rotation. Each dataset was tested for normality using the Shapiro test. ANOVA was conducted at a 95% confidential interval with three levels of significance ($P < 0.05$, < 0.001 , < 0.001) on data normally distributed. Wilcoxon signed-rank test and Kruskal–Wallis rank test, both nonparametric tests, were used on data that failed the normality test. Figures were generated in the same environment utilizing four main libraries: “ggplot2,” “doby,” “plyr,” and “beeswarm.” All error bars of this study represent the standard error (SE) except for the boxplots (box and whisker plots), which display the median, first and third quartiles, and both maximum and minimum values of variables. Bee mortality was evaluated within each of the three studied bee groups (summer bees emerged in the lab, summer bees emerged in the hive, and winter bees) by the Kaplan–Meier survival probability model in R using three packages: “dplyr,” “survival,” and “survminer.”

3. RESULTS

3.1. Summer bees emerged in the laboratory

3.1.1. Diet preference

The summer bee daily syrup consumption in the three studied treatments (5 PPB, 20 PPB, and control) shows distinct patterns (Figure 2). In the control group, bees showed no preference for specific syrup sources (control 1 and control 2), and the overall average consumption showed no significant differences between both provided syrup sources ($W = 1550$, $P = 0.6$) (Figure 2). However, summer bees expressed more affinity and preference to the control syrup in the IMP treatment groups at both concentrations. Significantly higher overall consumption of the control syrup occurred in 5 PPB and 20 PPB treatment groups ($P < 0.01$, $P < 0.001$), respectively (Figure 2).

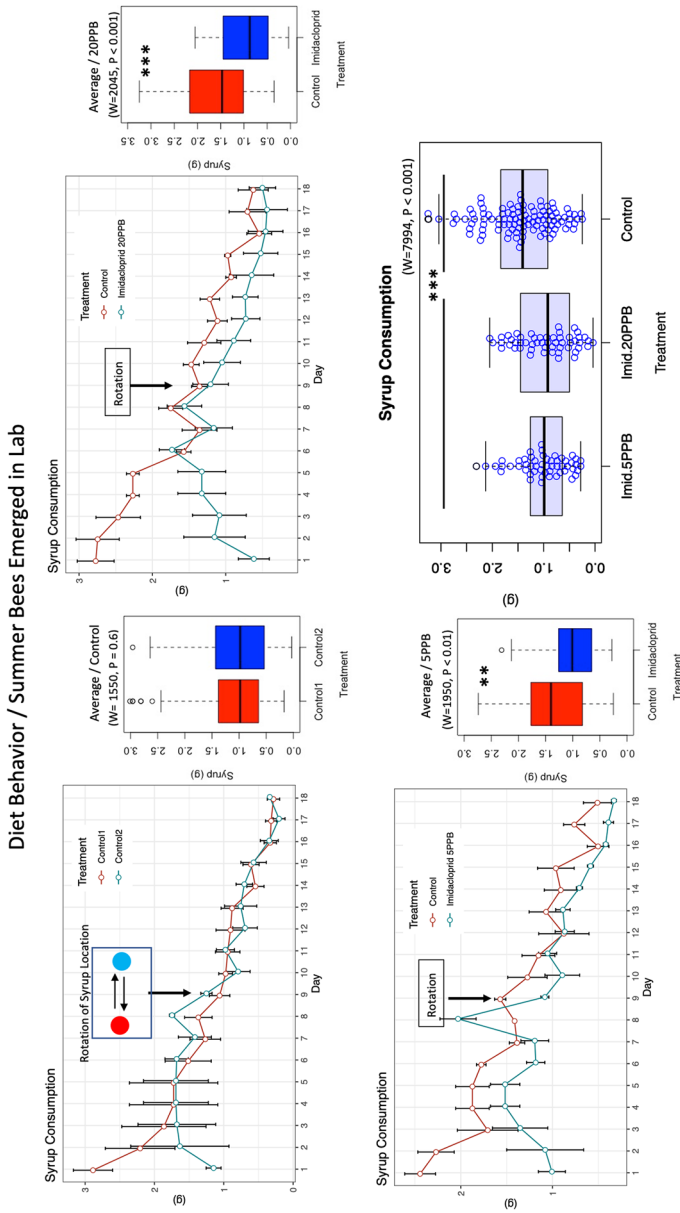


Figure 2. The diet behavior of summer bees emerged in the laboratory and exposed to IMP. The line graphs show daily bee consumption of both control and IMP-tainted sugar syrups. Error bars represent the SE. Box and whisker plots represent the average of the overall syrup intake for each treatment (control, 5 and 20 PPB). Rotation of the syrup location occurred on day 9 for all treatments and the control. In the control category, cages were provided with two sources (control 1, control 2) of untreated sugar syrup. The boxplots show overall averages of syrup intake for both treatments (5 and 20 PPB) and their controls. The Wilcoxon signed-rank test was conducted at three levels of significance ($P < 0.05$ *, < 0.001 ***, < 0.0001 ****)

The overall syrup consumption data shows no differences in bee consumption of 5 and 20 PPB syrup but significantly greater ($W=7994$, $P<0.001$) overall consumption of the control syrup compared to both IMP concentrations (Figure 2). Note that the “control” treatment of Figure 2’s boxplot is the average of the “control” syrup consumed within 5 and 20 PPB IMP categories only.

3.1.2. Diet location and toxicity

In the control category, where bees were provided with two sources of untreated syrup (control 1 and control 2), bees expressed no preferences to any of the syrup sources or their locations with no pre- and post-rotation significant differences in the syrup intake ($P=0.7$ and 0.5), respectively (Figure 3). In contrast, bees in both IMP groups (5 and 20 PPB) constantly avoided the treated syrup and consumed significantly higher amounts of the control syrup pre- and post-rotation (Figure 3). In terms of toxicity and mortality within the summer bee group that emerged in the laboratory, IMP at both concentrations showed no significant effect compared to the control (Figure S1a; $P=0.4$) using a simple mortality percentage comparison. However, Kaplan–Meier assigned a significantly lower survival probability in the control group compared to the treatments and a survival median (50%) between days 15 and 16 of the experiment for all groups (Figure 8).

3.2. Summer bees emerged in-hive

This summer cohort of emerged in-hive bees is expected to have developed a typical core of intestinal microbiota through oral trophallaxis from older hive mates. These bees differed in their diet behavior compared to summer bees that emerged in the laboratory and showed no preference to the nature of the proposed diets except at 5 PPB IMP ($W=1116$, $P<0.05$) where they initially consumed a higher overall amount of tainted syrup compared to the control

(Figure 4). Moreover, bees that emerged in-hive showed no affinity to the physical location of the diet and did not respond to diet rotation, even for the 5 PPB tainted syrup (Figure 5). While the Kruskal–Wallis test conducted on the average mortalities among treatments (control, 5, and 20 PPB) showed no significant differences ($P=0.8$) (Figure S1b), the Kaplan–Meier model assigned significantly lower survival probability for the control compared to IMP treatments (Figure 8). Similar to the summer bees that emerged in the lab, the Kaplan–Meier survival median was estimated to occur between days 15 and 16 of the experiment (Figure 8).

3.3. Winter bees

3.3.1. Diet behavior

Similar to summer bees, the daily consumption patterns for the winter bees of both untreated syrup sources (control 1 and control 2) overlapped and showed no significant ($F_{(1,108)}=0.1$, $P=0.7$) preference to either source (Figure 6). However, regardless of the IMP concentrations, whether 5 or 20 PPB, winter bees overwhelmingly preferred to consume from the treated syrup compared to the control syrup, showing a stark contrast compared to the summer bees that emerged in the lab (Figure 6). Within both treated categories (5 PPB and 20 PPB), winter bees consumed significantly ($W=3493$, $P<0.001$) more treated syrups (5 PPB and 20 PPB) than the control (Figure 6).

3.3.2. Effect of the syrup location and toxicity

The rotation of the syrup locations in the control category showed no effect on bee behavior and affinity to a specific syrup type or location. There were no significant differences pre- or post-rotation ($P=0.5$ and 0.6) in the control group between both syrup sources (control 1 and control 2), respectively (Figure 7). This result was not the case for the IMP treatment groups (5

Diet Behavior / Summer Bees Emerged in Lab

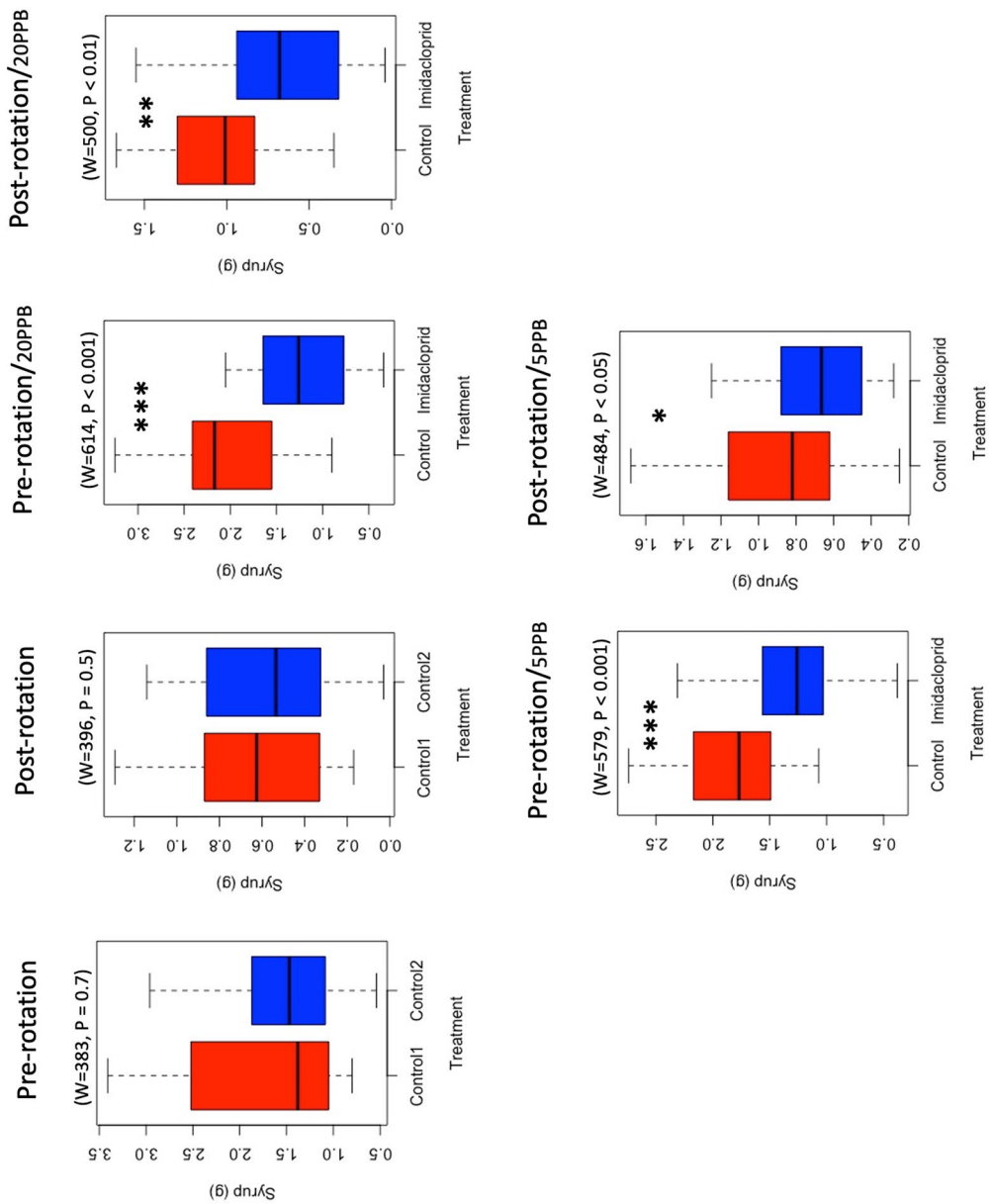


Figure 3. Diet behavior of summer bees emerged in the lab. Average syrup consumption for each treatment category was grouped into two phases: pre- and post-rotation of the syrup location. The Wilcoxon signed-rank test was reported at three levels of significance ($P < 0.05^*$, $< 0.001^{**}$, $< 0.001^{***}$)

Diet Behavior / Summer Bees Emerged in-Hive

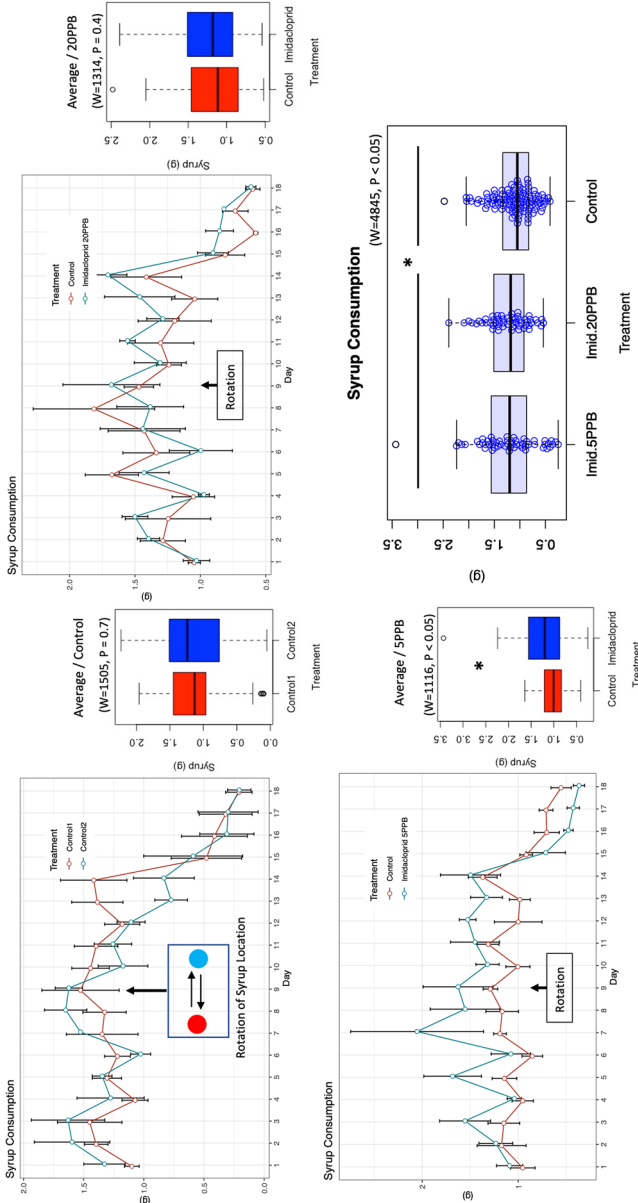


Figure 4. Diet behavior of summer bees emerged in the laboratory and exposed to IMP. Daily bee syrup consumption of control, 5 and 20 PPB IMP-tainted sugar syrups. In the control category, cages were provided with two sources (control 1, control 2) of untreated sugar syrup. Rotation of the syrup location was carried out on day 9 for all treatments. Boxplots represent the average of the overall syrup intake for each treatment (control, 5, and 20 PPB). The boxplots show overall averages of syrup intake for both treatments (5 and 20 PPB) and their controls. The Wilcoxon signed-rank test was conducted at three levels of significance ($P < 0.05^*$, $< 0.001^{**}$, $< 0.0001^{***}$)

Diet Behavior / Summer Bees Emerged in-Hive

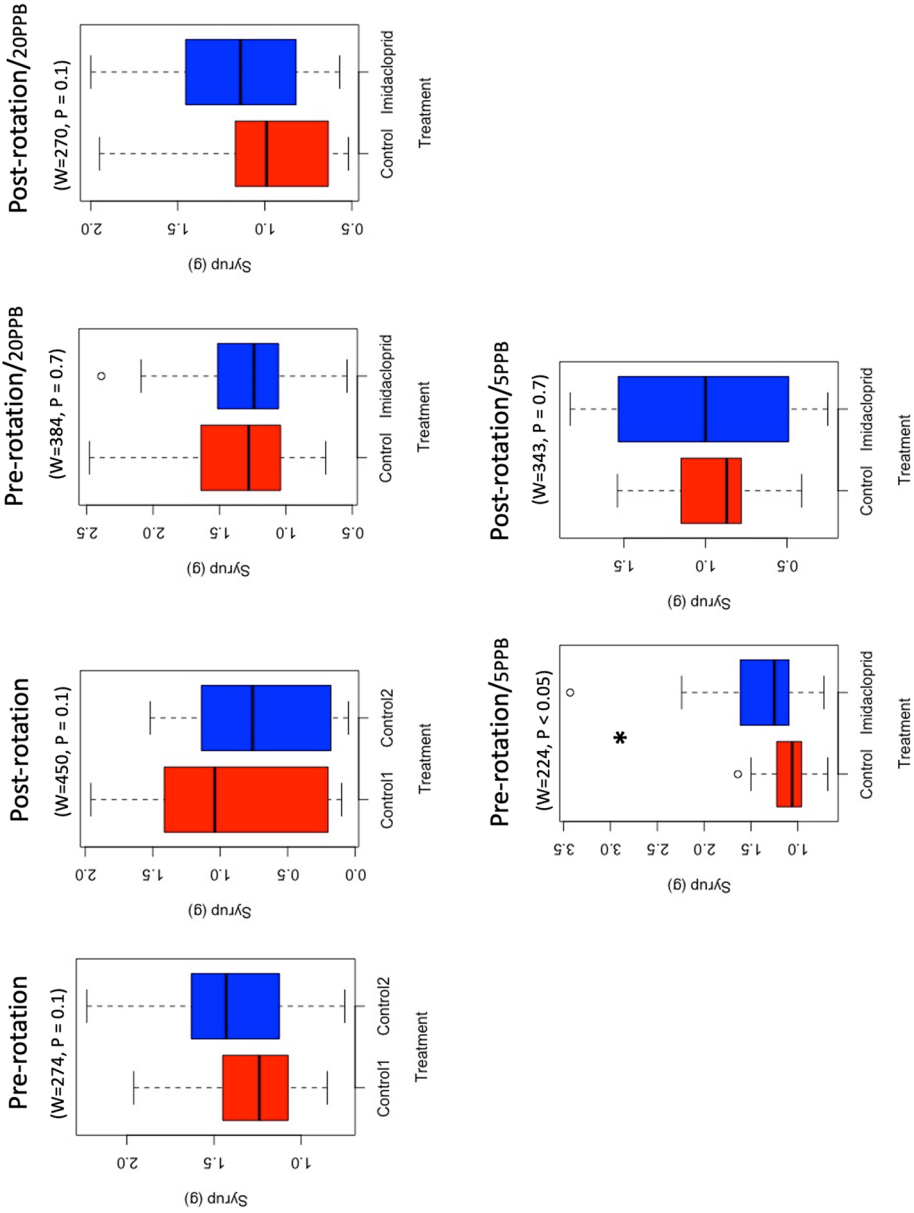


Figure 5. Diet behavior of summer bees emerged in-hive vis-à-vis IMP. Graphs represent the average syrup consumption for each treatment categories pre- and post-rotation of the syrup location. The nonparametric Wilcoxon test shows a single significant ($P < 0.05$) value between the consumption of the control and IMP syrups in the 5 PPB treatment group

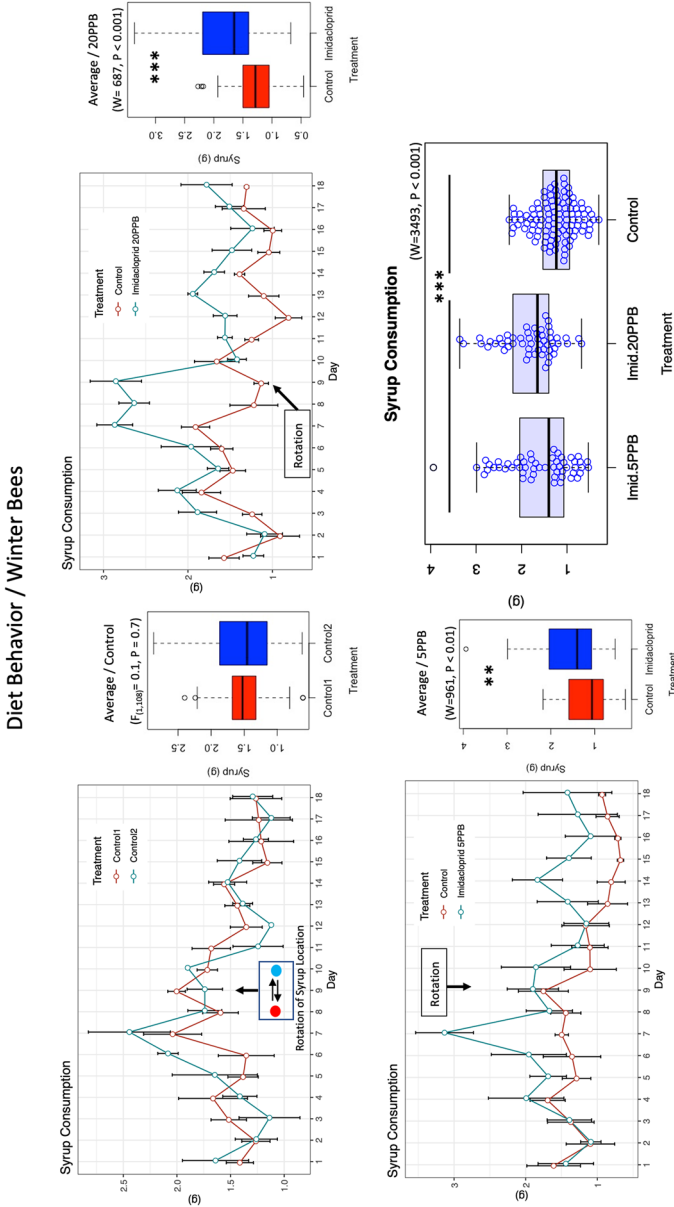


Figure 6. Daily average syrup consumption for winter bees. Syrup syringes were rotated on day 9 of the experiment. Overall average consumptions of each diet source are also given for each treatment. Control of the boxplot graph is the average consumption of the untreated syrup in both 5 and 20 PPB categories. ANOVA and Wilcoxon signed-rank test were conducted at three levels of significance ($P < 0.05$ *, < 0.001 **, < 0.001 ***).

Diet Behavior / Winter Bees

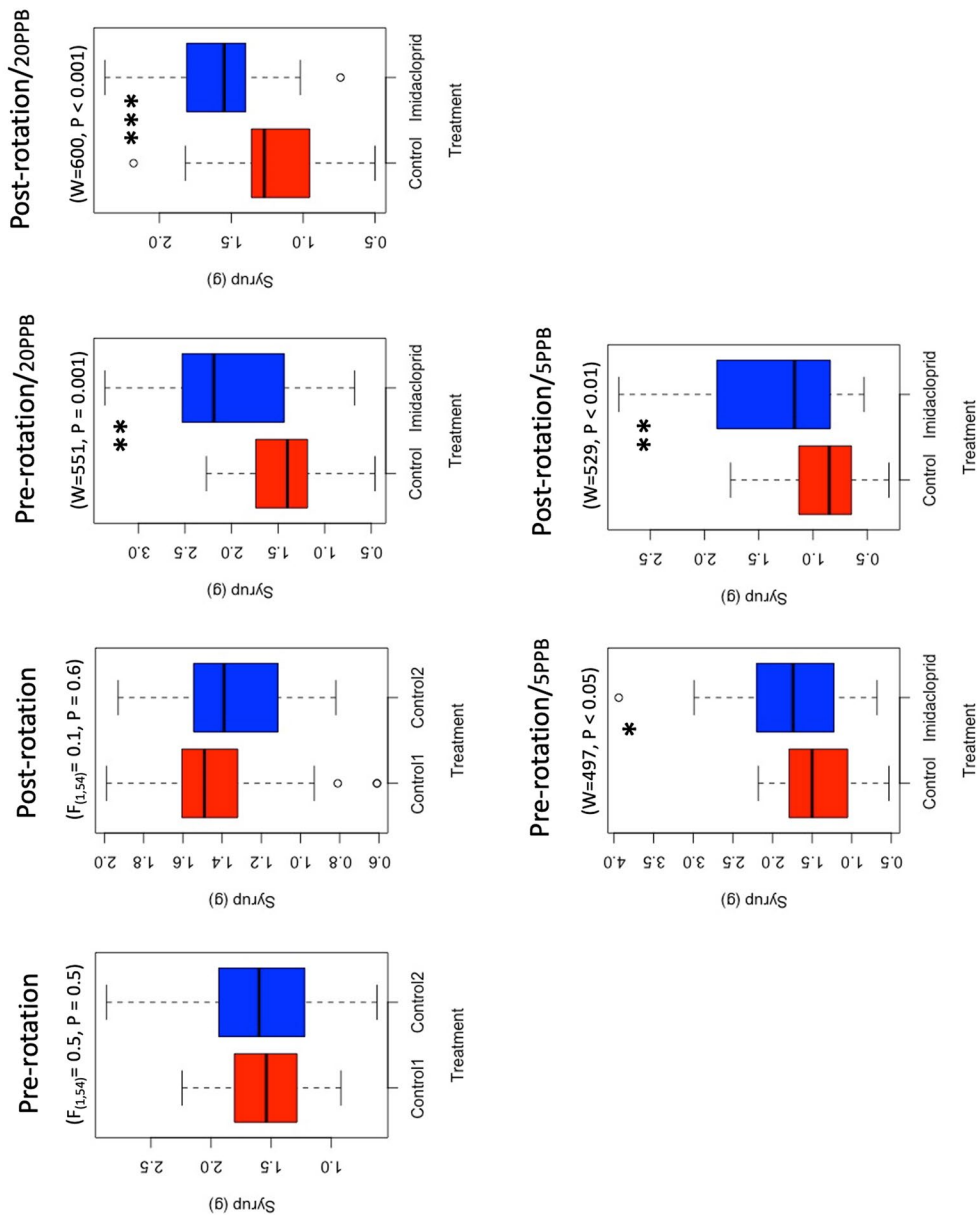


Figure 7. Winter bee diet behavior exposed to 5 and 20 PPB IMP-fainted sugar syrup and a control group containing two similar control syrup sources (control 1 and control 2). Average syrup consumptions are displayed by pre- and post-rotation of the syrup syringes for each of the three categories (control, 5 PPB, and 20 PPB). ANOVA and Wilcoxon signed-rank test were conducted at three levels of significance ($P < 0.05^*$, $< 0.001^{**}$, $< 0.001^{***}$)

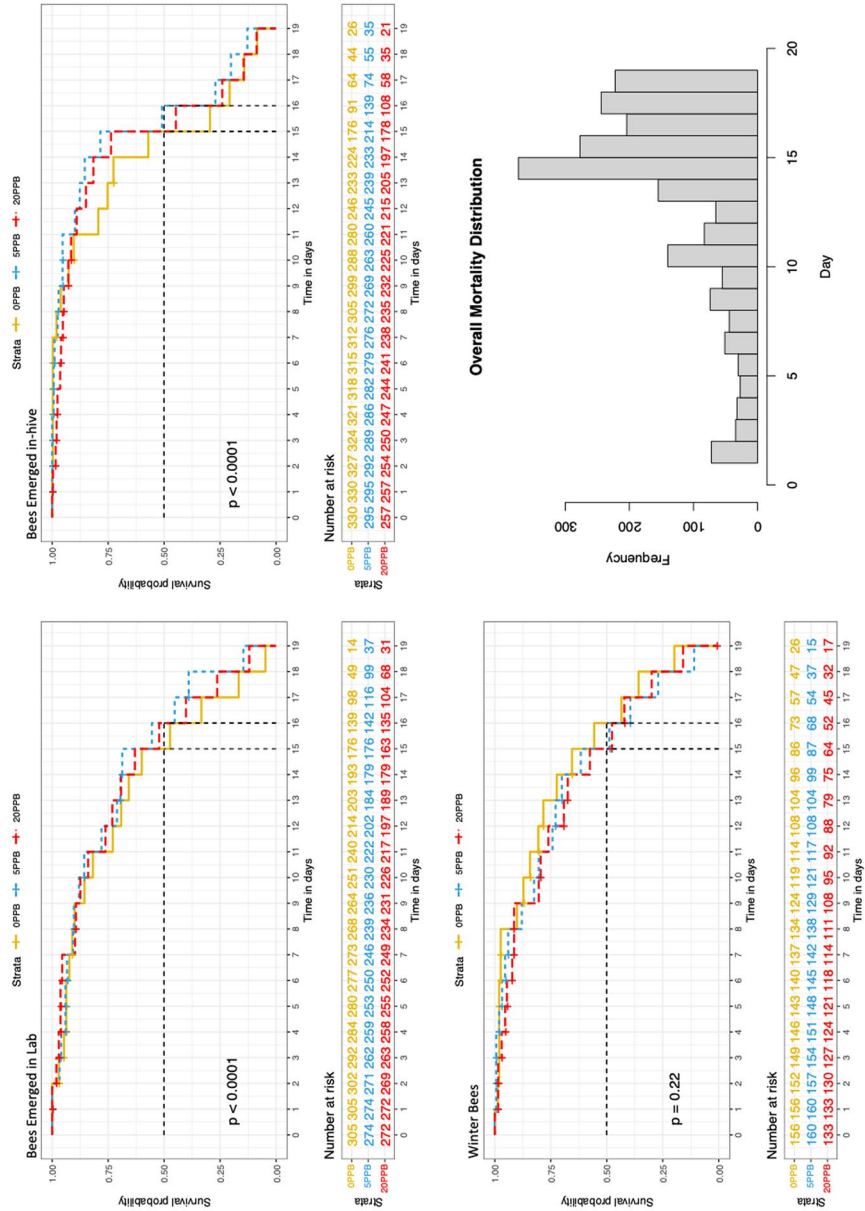


Figure 8. Kaplan–Meier survival probability model conducted on each bee group (emerged in Lab, emerged in-hive and winter bees) independently. The number of bees at risk is given through the same model for each treatment category (control, 5 PPB, and 20 PPB). The fourth graph represents the overall mortality frequency distributed throughout the experiment for all bee groups and treatment together

and 20 PPB). Winter bees showed higher affinity to the IMP-tainted syrup at both concentrations before and after the syrup source rotation (5 PPB = $P < 0.05$; 20 PPB = $P < 0.001$). When the syrup sources were rotated (post-rotation) at day 9, bees followed their preferred source of tainted syrup, demonstrating awareness of the syrup content and no attachment to its location (Figure 7). This behavior is reflected by significantly ($P < 0.01, 0.001$) higher intake of treated syrup (5 and 20 PPB) despite the rotation of the diet location compared to the control (Figure 7). As seen in the summer bee results, none of the IMP concentrations increased bee mortality compared to the control treatment ($P = 0.7$), Figure S1c. Unlike summer bees, the Kaplan–Meier model is in full agreement with the outcome of the Kruskal–Wallis test showing no significant difference ($P = 0.22$) in the survival probability between treatment groups (Figure 8). Similar to summer bees, the Kaplan–Meier survival median is between days 15 and 16 of the experiment (Figure 8).

4. DISCUSSION

IMP is a highly toxic neonicotinoid insecticide for honey bees with an acute oral LD_{50} of ~5 ng/bee (European Food Safety Authority 2012), which causes multiple impairments to bee olfactory memory and learning capacity at sublethal concentrations (Aliouane et al. 2009; Williamson et al. 2014). No observed effect level (NOEL) was described at doses smaller than 3.7 ng/bee (European Food Safety Authority 2012). In previous toxicological studies conducted on caged bees, signs of post-ingestive aversion responses in bees fed IMP-tainted syrup were documented (Alburaki et al. 2019a; Gregorc et al. 2018a). This behavior is a defense mechanism in insects to alleviate the effect of potentially toxic or undesired diets (Behmer et al. 2005, 1999; Simões et al. 2016).

In one instance, when 1-day-old summer bees were provided with lethal concentration (100 PPB) of IMP-tainted sugar syrup and water, bees refrained from consuming the

syrup and increased their water intake instead compared to the control treatment (Alburaki et al. 2019a). This observation, however, was exclusively recorded at high concentrations (100 PPB) and was absent at (5 PPB) and (20 PPB). Under relatively similar conditions, other authors found that older bees tend to consume a higher amount of (5 PPB) IMP-tainted syrup compared to the control, with often no pronounced differences in bee mortality rates between (5–20) PPB IMP versus control (Alburaki et al. 2019a; Gregorc et al. 2018b; Meikle et al. 2016). The post-ingestive aversion responses identified in this study on summer bees are not exclusive to IMP and were recorded in the case of selenium (Alburaki et al. 2019b), a nonmetal element toxic to bees at high concentrations (Hladun et al. 2012).

This current study was conducted to shed more light on this subject by trying to uncouple bee diet behavior vis-à-vis IMP consumption and how seasonal bee phenotypes shape this behavior. Such behavior is critical to discern, as the assumption of equal consumption of treated syrup in cage experiments (ad libitum) may not be accurate in the case of IMP or other substances and molecules. This study provides new insights on this particular aspect, showing that summer and winter bees exhibit opposite diet behavior regarding IMP-tainted sugar syrup. It is well known that winter and summer bees differ significantly in their physiological characteristics. Winter bees exhibit a significantly longer life span compared to summer bees (Fluri et al. 1982) and express higher vitellogenin (Vg) levels and lower juvenile hormone (JH) compared to the summer bees (Steinmann et al. 2015). This expression pattern has been associated with extended longevity (Corona et al. 2007; Seehuus et al. 2006). Our results showed that there seems to be a significant divergence in diet behavior attributed to laboratory versus in-hive emergence within the summer bee category. Unlike bees that emerged in an incubator, bees that emerged in the hive would presumably have more opportunity of exposure and acquisition of microbiota from older hive mates and hive products such as honey and pollen (Powell et al. 2014). This study

reported stark contrast in their diet behavior vis-à-vis IMP.

The microbiota has a key function in protecting honey bees against pesticides by upregulating the expression of genes involved in pesticide detoxification in the intestine, including a member of the cytochrome P450 (CYP450) (Wu et al. 2020). Recent studies indicated that dietary phytochemicals in honey bees, mainly acquired through honey and pollen consumption, could enhance bee tolerance to IMP and other pesticides (Ardalani et al. 2021; Wong et al. 2018). Moreover, honey compounds were described to upregulate the expression of detoxification genes, including twelve xenobiotic-metabolizing enzymes (Mao et al. 2013), which could explain the similar mortality rates among treatments found in the winter bee category (Figure. 8).

From a behavioral standpoint, summer bees that emerged in the laboratory consistently preferred untreated syrup, avoiding IMP-laced syrup at both concentrations (5 PPB and 20 PPB), as opposed to winter bees, which relentlessly opted for the treated syrup over the control. Despite the rotation of the syrup's physical locations conducted in the middle of the experiment, both bee cohorts persisted in their preference trends, excluding the theory of bees' affinity to the diet's physical location but rather to its content (Figures. 3 and 5). This finding reveals both bees' ability to identify as low as 5 PPB of IMP in their diets and complete divergence in diet choice between summer and winter bees. It is conceivable to hypothesize that such diet behavior and preference to IMP-treated syrup would likely subject winter bees to a greater toxicity risk under field conditions compared to summer bees. The reason behind a winter bee's attraction to treated syrup is not apparent. However, this phenomenon could be attributed to the pharmacological action of the neonicotinoids on bees' nicotinic acetylcholine receptors (nAChRs), experienced previously by winter bees and not naïve newly emerged ones, or simply slight differences in the flavor of the treated syrup, more appealing to winter bees.

A previous study demonstrated that both bee species, *Apis mellifera* (foragers) and *Bombus*

terrestris (Bumblebee), preferred sucrose solutions laced with IMP and thiamethoxam over sucrose alone. Nevertheless, stimulation with IMP elicited no spiking responses from gustatory neurons in the bees' mouthparts, nor inhibited the responses of sucrose-sensitive neurons (Kessler et al. 2016). Our data aligns with Kessler et al.'s finding conducted on foragers and newly emerged bees. However, we provided further evidence that winter bees exhibited the opposite diet behavior seen in newly emerged summer bees, and that exposure to older mates could have contributed to shaping the summer bee diet behavior regarding IMP. The diet behavior of winter bees and their pre- and post-rotation persistence ($P < 0.001$) in consuming IMP-laced syrup could be driven by previously acquired experience, which intertwines with bee age; older bees showing preference to IMP-laced solution compared to newly emerged bees, such as foragers (Kessler et al. 2016) and winter bees in the current study.

The absence of visible toxicological effect of IMP at 20 PPB within each bee group (Figure S1) was not surprising to us, as similar findings were reported on 1-day-old bees exposed to 5, 20, and 100 PPB of IMP (Alburaki et al. 2019a). Although it was not intended to determine the ingested dose of IMP per bee in this study, it is safe to conclude that the IMP LD₅₀ was not reached under our experimental conditions. In addition to that, honey bees are equipped with a robust and complex detoxification mechanism capable of quickly alleviating oxidative stress induced by abiotic stressors (Corona and Robinson 2006; Mao et al. 2013). This argument seems to clearly apply in the case of winter bees, in which the Kaplan–Meier model predicted similar survival probability ($P = 0.22$) among treatments (Figure. 8), even though winter bees had effectively consumed significantly ($P < 0.001$) higher IMP-laced syrup than control syrup (Figure. 6). In the case of summer bees, Kaplan–Meier results diverged from those of the simple mortality comparison showing lower survival probability in the control groups compared to the treatments (Figure. 8). Despite the precision of the Kaplan–Meier's outcomes, its results should be read in the context of our experimental design, which primarily addressed the diet

behavior of the bees and not the direct toxicological effect of IMP. The latter is usually evaluated by administering precise and similar doses of IMP and control syrup across treatments.

In conclusion, our study demonstrates that summer and winter bees differ in their affinity to IMP, a diet behavior that is not affected by the physical location of the food source but rather by its content. Furthermore, both summer and winter bees can sense minuscule concentrations of IMP in their diet. Newly emerged summer bees, never exposed to older hive mates, refrain from consuming food sources containing IMP, mainly due to post-ingestive aversion responses or nonfamiliarity with the molecule's taste. Winter bees, however, show preference to IMP-laced syrup over the control and seem resilient to 5 and 20 PPB of IMP for the duration of our experiment. This study provides new insights on honey bee diet behaviors and responses to IMP, which should be accounted for in toxicological studies, particularly when treatment is administered ad libitum.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

Study conception and design: MA and MC; data collection: MA, SM, and PV; data analysis: MA; wrote the manuscript: MA; revised it: MC, SM, and PV. All authors revised and approved the final version of this manuscript.

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DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article and its supplementary information.

DECLARATIONS

Ethics approval Not applicable.

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REFERENCES

- Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2009) How much does agriculture depend on pollinators? Lessons from long-term trends in crop production *Annals of Botany* 103:1579–1588. <https://doi.org/10.1093/aob/mcp076>
- Alaux C et al. (2010) Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*) *Environ Microbiol* 12:774–782. <https://doi.org/10.1111/j.1462-2920.2009.02123.x>
- Alburaki M, Cheaib B, Quesnel L, Mercier PL, Chagnon M, Derome N (2016) Performance of honeybee colonies located in neonicotinoid-treated and untreated cornfields in Quebec *J of Appl Entomol*. <https://doi.org/10.1111/jen.12336>

- Alburaki M, Gregorc A, Adamczyk J, Stewart S (2018) Insights on pollen diversity of honey bee *Apis mellifera* L. colonies located in various agricultural landscapes The Southeastern Naturalist in press
- Alburaki M, Karim S, Lamour K, Adamczyk J, Stewart SD (2019a) RNA-seq reveals disruption of gene regulation when honey bees are caged and deprived of hive conditions Journal of Experimental Biology 222. <https://doi.org/10.1242/jeb.207761>
- Alburaki M, Smith KD, Adamczyk J, Karim S (2019b) Interplay between Selenium, selenoprotein genes, and oxidative stress in honey bee *Apis mellifera* L J Insect Physiol 117:103891. <https://doi.org/10.1016/j.jinsphys.2019b.103891>
- Alburaki M et al. (2017) Agricultural Landscape and pesticide effects on honey bee (Hymenoptera: Apidae) Biological Traits J Econ Entomol 110:835–847. <https://doi.org/10.1093/jee/110>
- Aliouane Y, El Hassani AK, Gary V, Armengaud C, Lambin M, Gauthier M (2009) Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior Environ Toxicol Chem 28:113–122. <https://doi.org/10.1897/08-110.1>
- Ardalani H, Vidkjaer NH, Kryger P, Fiehn O, Fomsgaard IS (2021) Metabolomics unveils the influence of dietary phytochemicals on residual pesticide concentrations in honey bees Environ Int 152:106503. <https://doi.org/10.1016/j.envint.2021.106503>
- Behmer ST, Belt CE, Shapiro MS (2005) Variable rewards and discrimination ability in an insect herbivore: what and how does a hungry locust learn? J Exp Biol 208:3463–3473. <https://doi.org/10.1242/jeb.01767>
- Behmer ST, Elias DO, Bernays EA (1999) Post-ingestive feedbacks and associative learning regulate the intake of unsuitable sterols in a generalist grasshopper Journal of Experimental Biology 202:739–748. <https://doi.org/10.1242/jeb.202.6.739>
- Brandt A, Gorenflo A, Siede R, Meixner M, Buchler R (2016) The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.) J Insect Physiol 86:40–47. <https://doi.org/10.1016/j.jinsphys.2016.01.001>
- Bresnahan ST, Doke MA, Giray T, Grozinger CM (2022) Tissue-specific transcriptional patterns underlie seasonal phenotypes in honey bees (*Apis mellifera*) Mol Ecol 31:174–184. <https://doi.org/10.1111/mec.16220>
- Chakrabarti P, Rana S, Sarkar S, Smith B, Basu P (2015) Pesticide-induced oxidative stress in laboratory and field populations of native honey bees along intensive agricultural landscapes in two Eastern Indian states Apidologie 46:107–129. <https://doi.org/10.1007/s13592-014-0308-z>
- Corona M, Robinson GE (2006) Genes of the antioxidant system of the honey bee: annotation and phylogeny Insect Mol Biol 15:687–701. <https://doi.org/10.1111/j.1365-2583.2006.00695.x>
- Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, Hughes KA, Robinson GE (2007) Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity Proceedings of the National Academy of Sciences of the United States of America 104:7128–7133. <https://doi.org/10.1073/pnas.0701909104>
- Daisley BA et al. (2017) Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum* immune stimulation in a *Drosophila melanogaster* model Scientific Reports 7. <https://doi.org/10.1038/s41598-017-02806-w>
- Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, Gauthier M, Pham-Delegue MH (2004) Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.) Pesticide Biochemistry and Physiology 78:83–92. <https://doi.org/10.1016/j.pestbp.2003.10.001>
- Démare FJ, Crous KL, Pirk CWW, Nicolson SW, Human H (2016) Sucrose sensitivity of honey bees is differently affected by dietary protein and a neonicotinoid pesticide PLoS One 11. <https://doi.org/10.1371/journal.pone.0156584>
- European Food Safety Authority (2012) Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe EFSA J 10:2752. <https://doi.org/10.2903/j.efsa.2012.2752>
- Evans JD, Chen YP, Prisco Gd, Pettis J, Williams V (2009) Bee cups: single-use cages for honey bee experiments Journal of Apicultural Research 48:300–302. <https://doi.org/10.1080/00218839.2009.11101548>
- Fluri P, Lüscher M, Wille H, Gerig L (1982) Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees Journal of Insect Physiology 28:61–68. [https://doi.org/10.1016/0022-1910\(82\)90023-3](https://doi.org/10.1016/0022-1910(82)90023-3)
- Gregorc A, Alburaki M, Rinderer N, Sampson B, Knight PR, Karim S, Adamczyk J (2018a) Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments Sci Rep 8:15003. <https://doi.org/10.1038/s41598-018-33348-4>
- Gregorc A, Alburaki M, Sampson B, Knight PR, Adamczyk J (2018b) Toxicity of selected acaricides to honey bees (*Apis mellifera*) and varroa (*Varroa destructor* Anderson and Trueman) and their use in controlling varroa within honey bee colonies Insects 9. <https://doi.org/10.3390/insects9020055>
- Hladun KR, Smith BH, Mustard JA, Morton RR, Trumble JT (2012) Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival PLoS One 7:e34137. <https://doi.org/10.1371/journal.pone.0034137>
- Huang SK et al. (2014) Evaluation of cage designs and feeding regimes for honey bee (Hymenoptera: Apidae) Laboratory Experiments Journal of Economic Entomology 107:54–62. <https://doi.org/10.1603/EC13213>
- Jiang J, Ma D, Zou N, Yu X, Zhang Z, Liu F, Mu W (2018) Concentrations of imidacloprid and thiamethoxam in

- pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (*Apis mellifera* L.) *Chemosphere* 201:159–167. <https://doi.org/10.1016/j.chemosphere.2018.02.168>
- Kessler SC et al. (2016) Corrigendum: Bees prefer foods containing neonicotinoid pesticides *Nature* 533:278. <https://doi.org/10.1038/nature17177>
- 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>
- Kulhanek K et al. (2017) A national survey of managed honey bee 2015–2016 annual colony losses in the USA *Journal of Apicultural Research* 56:328–340. <https://doi.org/10.1080/00218839.2017.1344496>
- Li JH et al. (2019) New evidence showing that the destruction of gut bacteria by antibiotic treatment could increase the honey bee's vulnerability to nosema infection *PLoS One* 12. <https://doi.org/10.1371/journal.pone.0187505>
- Liao LH, Wu WY, Berenbaum MR (2017) Behavioral responses of honey bees (*Apis mellifera*) to natural and synthetic xenobiotics in food *Scientific Reports* 7. <https://doi.org/10.1038/s41598-017-15066-5>
- Liu YJ, Qiao NH, Diao QY, Jing Z, Vukanti R, Dai PL, Ge Y (2020) Thiacloprid exposure perturbs the gut microbiota and reduces the survival status in honeybees *J Hazard Mater* 389:121818. <https://doi.org/10.1016/j.jhazmat.2019.121818>
- Lorenzo-Felipe I, Blanco C, Corona M (2020) Impact of Apoidea (Hymenoptera) on the World's Food Production and Diets *Annals of the Entomological Society of America*:1–18. <https://doi.org/10.1093/aesa/saaa016>
- Mao W, Schuler MA, Berenbaum MR (2013) Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera* *Proc Natl Acad Sci U S A* 110:8842–8846. <https://doi.org/10.1073/pnas.1303884110>
- Meikle WG et al. (2016) Sublethal Effects of Imidacloprid on Honey Bee Colony Growth and Activity at Three Sites in the U.S *PLoS One* 11:e0168603. <https://doi.org/10.1371/journal.pone.0168603>
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health *PLoS One* 5:e9754. <https://doi.org/10.1371/journal.pone.0009754>
- Page RE, Jr., Peng CY (2001) Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L *Exp Gerontol* 36:695–711. [https://doi.org/10.1016/s0531-5565\(00\)00236-9](https://doi.org/10.1016/s0531-5565(00)00236-9)
- Poquet Y, Vidau C, Alaux C (2016) Modulation of pesticide response in honeybees *Apidologie* 47:412–426. <https://doi.org/10.1007/s13592-016-0429-7>
- Powell JE, Martinson VG, Urban-Mead K, Moran NA (2014) Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera* *Applied and Environmental Microbiology* 80:7378–7387
- R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rundlof M et al. (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees *Nature* 521:77–80. <https://doi.org/10.1038/nature14420>
- Sampson BJ, Cane JH (2000) Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry *J Econ Entomol* 93:1726–1731
- Schafer MO, Ritter W, Pettis JS, Neumann P (2010) Winter losses of honeybee colonies (Hymenoptera: Apidae): the role of infestations with *Aethina tumida* (Coleoptera: Nitidulidae) and *Varroa destructor* (Parasitiformes: Varroidae) *J Econ Entomol* 103:10–16
- Seehuus SC, Norberg K, Gimsa U, Kreckling T, Amdam GV (2006) Reproductive protein protects functionally sterile honey bee workers from oxidative stress *Proceedings of the National Academy of Sciences of the United States of America* 103:962–967
- Seeley TD (2010) Honeybee democracy. Princeton University Press, Princeton
- Simões PMV, Ott SR, Niven JE (2016) Environmental adaptation, phenotypic plasticity, and associative learning in insects: the desert locust as a case study *Integrative and Comparative Biology* 56:914–924. <https://doi.org/10.1093/icb/icw100>
- Smedal B, Brynne M, Kreibich CD, Amdam GV (2009) Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*) *Journal of Experimental Biology* 212:3795. <https://doi.org/10.1242/jeb.035063>
- Steinmann N, Corona M, Neumann P, Dainat B (2015) Overwintering is associated with reduced expression of immune genes and higher susceptibility to virus infection in honey bees *PLoS One* 10:e0129956
- Traynor KS, Pettis JS, Tarpy DR, Mullin CA, Frazier JL, Frazier M, VanEngelsdorp D (2016) In-hive Pesticide Exposure: Assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States *Scientific Reports* 6. <https://doi.org/10.1038/srep33207>
- Wang K et al. (2021) Gut microbiota protects honey bees (*Apis mellifera* L.) against polystyrene microplastics exposure risks *Journal of Hazardous Materials* 402. <https://doi.org/10.1016/j.jhazmat.2020.123828>
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production *Science* 336:351–352. <https://doi.org/10.1126/science.1215025>
- Williamson SM, Moffat C, Gomersall M, Saranzewa N, Connolly C, Wright GA (2013) Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees *Frontiers*

in Physiology 4. <https://doi.org/10.3389/fphys.2013.00013>

Williamson SM, Willis SJ, Wright GA (2014) Exposure to neonicotinoids influences the motor function of adult worker honeybees *Ecotoxicology* 23:1409–1418. <https://doi.org/10.1007/s10646-014-1283-x>

Winston ML (1987) *The biology of the honey bee*. Massachusetts: Harvard University Press, Cambridge,

Wong MJ, Liao LH, Berenbaum MR (2018) Biphasic concentration-dependent interaction between imidacloprid and dietary phytochemicals in honey bees (*Apis mellifera*) *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0206625>

Wu Y, Zheng Y, Chen Y, Wang S, Chen Y, Hu F, Zheng H (2020) Honey bee (*Apis mellifera*) gut

microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract *Microbial Biotechnology* 13:1201–1212. <https://doi.org/10.1111/1751-7915.13579>

Zhu L, Qi S, Xue X, Niu X, Wu L (2020) Nitenpyram disturbs gut microbiota and influences metabolic homeostasis and immunity in honey bee (*Apis mellifera* L.) *Environmental Pollution* 258. <https://doi.org/10.1016/j.envpol.2019.113671>

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