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Fabien J. Démares, Christian W.W. Pirk, Susan W. Nicolson, Hannelie Human

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RESEARCH ARTICLE

# NEONICOTINOIDS DECREASE SUCROSE RESPONSIVENESS OF HONEY BEES AT FIRST CONTACT

AUTHORS: FABIEN J. DÉMARES* [a,1], CHRISTIAN W.W. PIRK [a], SUSAN W. NICOLSON [a] AND HANNELIE HUMAN [a]

Affiliation: [a] *Social Insects Research Group, Department of Zoology & Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa.*

[1] Present Address: *Emerging Pathogens Institute, Department of Entomology & Nematology, University of Florida, 2055 Mowry Road, Gainesville, Florida 32610, USA.*

*** Corresponding author:** F.Démares

fabien.demares@ufl.edu or fabien.demares@gmail.com

**Abstract**

For two decades, neonicotinoid insecticides have been extensively used worldwide. Targeting neuronal receptors, they have deleterious effects on the behaviour and physiology of many of many beneficial as well as harmful insects. Bees are exposed to these insecticides in pollen and nectar while providing pollination services to agricultural crops, and neonicotinoids have been shown to impair navigation by bees and to decrease their foraging activity. We have previously reported the effect of dietary thiamethoxam on sucrose responsiveness of young worker bees. Here, we exposed caged foragers to sublethal acute doses of clothianidin, imidacloprid, and thiamethoxam, then tested them individually for sucrose responsiveness using standard methods. In addition, we tested the response to a range of sucrose solutions laced with neonicotinoids on bees previously unexposed to neonicotinoids. This paradigm mimics the situation where foragers would first encounter poisoned nectars varying in sugar concentration. Bees were exposed to the insecticides in the feeding solution for 24 hours before testing, or in the test solutions, or both. The three compounds had a detrimental effect on responses to mid-to-high sucrose concentrations under all experimental conditions, and unexposed bees tested with laced sucrose displayed unexpected low responses to the higher sucrose concentrations tested. This attenuation of sucrose response is further evidence that neonicotinoids are multisensory disruptors, with potent actions against pollinators and other beneficial insects at first contact.

## Highlights

- Classically, honey bee sugar responsiveness is tested with pure sucrose solutions
- Here, we laced both feeding and test solutions with neonicotinoids
- Bees fed before testing with laced sucrose solutions have reduced sucrose responses
- Bees first exposed to pesticide in test solutions also have reduced responses
- This is further evidence for neonicotinoids acting as multisensory disruptors

## Keywords

*Apis mellifera scutellata*, Sucrose threshold, Neonicotinoid, Taste attenuation, Honey bee foraging.

## Abbreviations

CLO, clothianidin; CTRL, control; IMI, imidacloprid; nAChR, nicotinic acetylcholine receptor; PER, proboscis extension reflex; THX, thiamethoxam.

## 1. Introduction.

Since their commercialisation, neonicotinoids have been used globally in pest management (Jeschke et al 2011; Godfray et al 2014). The first-generation compound imidacloprid (IMI) was the most widely used during the 1990s (Jeschke et al 2011), until the second generation was synthesised, namely thiamethoxam (THX) and clothianidin (CLO) (Maienfisch et al, 2001; Nauen et al 2003). Recently, under the European Food and Security Agency (EFSA) investigation, the EU decided to promote a partial 2-year ban on these three compounds (Fryday et al 2015). This ban was prompted by extensive research highlighting the various deleterious effects of neonicotinoids on non-target species and especially beneficial invertebrates such as pollinators (extensively reviewed by Blacquière et al 2012; Godfray et al 2014, 2015).

Among pollinators, honey bees (*Apis mellifera* L.) in particular have been observed and tested for their sensitivity to neonicotinoids. At sublethal and field-realistic doses (Table 1; Henry et al 2015; Stoner and Eitzer, 2012), several studies have reported several behavioural and physiological effects. For instance, neonicotinoid pesticides impair navigation and decrease foraging activity, both of which reduce pollination efficiency (Henry et al 2012; Schneider et al 2012; Stanley et al 2015). They also affect physiological processes such as olfactory learning and memory and odour differentiation, and they can reduce the thermoregulation ability of individual honey bees (Tison et al 2017; Tosi et al 2016; Williamson and Wright 2013).

Another physiological process affected by neonicotinoid pesticides is the sensitivity to sucrose (Aliouane et al 2009; Démares et al 2016). The response to sugar is an ideal proxy for assessing the response of foragers to nectar, and it has been examined under conditions of acute and chronic exposure to sublethal doses of neonicotinoids. Honey bees fed single sublethal doses of IMI showed higher sucrose response thresholds one hour later (Eiri and Nieh 2012). Chronic oral exposure to THX also decreased the response of restrained honey bees to high concentrations of sucrose (Aliouane et al 2009; Démares et al 2016). Recently, neonicotinoids have also been included in the solutions used for testing sucrose responsiveness of honey bees. Tison et al. (2017) found no effect of sublethal doses of another neonicotinoid, thiacloprid, while Kessler et al (2015) showed that the proboscis extension response of honey bees was not inhibited when various concentrations of IMI,

THX and CLO were included in the 1 M sucrose solution touched to the antenna. In addition, freely-moving honey bee and bumblebee foragers preferred to consume sucrose solutions laced with IMI and THX over 24 hours, compared to control solutions, seemingly without being able to taste these compounds (Kessler et al 2015).

Here we compared both methods for testing the effect of neonicotinoids on honey bee sucrose responsiveness. While the classical method involves testing control or poisoned bees with a series of pure sucrose solutions, testing the response to neonicotinoid-laced sucrose solutions is appropriate to the situation where foragers would first encounter poisoned nectar, but within a more controlled environment. We tested the response of restrained foragers to a range of sucrose concentrations, with different types of exposure to sublethal doses of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam): either in the solution fed for 24 hours prior to testing sucrose responsiveness; or during testing with a range of laced sucrose solutions. The former would be similar to a forager being exposed to a neonicotinoid and then foraging again the next day, while the latter represents the sucrose response of a forager while being exposed. We expected to observe reduced sucrose responsiveness in pre-exposed foragers, and hypothesised no significant effect of exposure during testing.

## 2. Materials and Methods.

### 2.1. Animal collection.

Returning adult foragers (*Apis mellifera scutellata*) were caught at the University of Pretoria apiary, during September and October 2016. They were predominantly composed of pollen foragers, recognizable by pollen loads on their corbiculae. In total, we used 876 honey bees from five colonies. In the laboratory, for each colony replicate, foragers were randomly placed in standard hoarding cages (Köhler et al 2013). Each cage was provided with a small hanging piece of wax foundation, a 20-ml water tube and two 2-ml Eppendorf food tubes filled with 50% w/w sucrose solution. Three empty cages were set up in the same way to assess evaporation of water and food. Cages were kept in incubators at 35°C and 50% RH for 24 hours.

### 2.2. Pesticides and diet.

For each colony replicate (N = 5), cages containing 30-35 foragers were randomly given either a pure sucrose solution (control group, CTRL), or a sucrose solution laced with of one of three different neonicotinoids (CLO, IMI, THX) (see also Fig. S1). According to standard practice, pesticides were dissolved in acetone and the final concentration in feeding solutions was 7.5 nM for each; we chose this value to be within the range of concentrations used in previous experiments (Kessler et al 2015; Démares et al 2016; 10 nM and 5 nM respectively). The proportion of acetone in feeding solutions, including CTRL, was < 0.05% (Aliouane et al 2009; Medrzycki et al 2013). After 24 hours, survival was monitored and sucrose and water consumption were measured; evaporation controls were accounted for.

**2.3. Sucrose responsiveness.**

We carried out the sucrose responsiveness tests 24 hours after setting up the cages and diets. We removed foragers from cages, briefly immobilised them on ice, and then fixed them in individual Plexiglas holders. The procedure was the same as described previously (Démares et al 2016), with the following modification: each group exposed to a neonicotinoid in the cage was divided into two subgroups, one being tested on a range of pure sucrose solutions, the other with a range of sucrose solutions laced with the same compound at the same concentration as the feeding solution, *i.e.* 7.5 nM. We divided the control group into four subgroups: bees tested on pure sucrose solutions and bees tested on sucrose solutions laced with each of the three neonicotinoids. For each sucrose concentration (from 0.03% to 30% w/w sucrose), the proboscis extension reflex (PER; Bitterman et al 1983) was recorded. The inter-trial time between each presentation of ascending concentration of sugar was 4 minutes minimum. For analyses, we used bees responding to 50% sucrose 1 hour before and 30 minutes after the sucrose responsiveness test; this also allowed for a control of locomotor response. To avoid false positives, bees responding to water before testing and to all sucrose concentrations during testing were discarded from analysis; to avoid false negatives, bees not responding to water or to any test concentrations were also discarded (Aliouane et al 2009).

**2.4. Statistics.**

Survival, consumption of water and sucrose, and the ingested dose of pesticide were analysed with one-way ANOVA and Tukey HSD post-*hoc* comparisons. To investigate potential

interactions of the pesticides between diet and treatment and the sucrose concentration range, the sucrose responsiveness data were analysed using Generalized Estimating Equations / Generalized Linear model (GEE/GLM) for repeated-measures logistic regression since each bee was exposed to increasing sucrose concentrations, with or without a sublethal dose of one of the three neonicotinoids. Pairwise comparisons were calculated within the model. Estimate marginal means have been calculated and are reported here and in Supplementary Materials 2 (Tables S3 to S6). The α-level of pairwise comparisons was Bonferroni-corrected accordingly. All statistical analyses were performed with SPSS23, and all statistical values are reported in Supplementary Tables.

## 3. Results.

### 3.1. Survival and consumption.

After 24 hours of exposure, survival did not differ significantly between groups (ANOVA, $F_{3,19} = 0.271$, $p = 0.846$). Similarly, there were no significant differences in water and sucrose consumption between control bees and those exposed to pesticides (ANOVA; Water, $F_{3,16} = 0.845$, $p = 0.493$; Sucrose, $F_{3,19} = 2.582$, $p = 0.090$) (Table 2).

The dose of pesticide ingested differed between the neonicotinoid groups (ANOVA, $F_{2,12} = 7.929$, $p = 0.006$): foragers exposed to THX ingested more of it than those exposed to CLO and IMI. However, when this was related to the $LD_{50}$ of each pesticide, there was no difference between groups (ANOVA, $F_{2,12} = 1.250$, $p = 0.321$), with doses ranging from 2.66% to 2.96% of reported $LD_{50}$ values (Table 2).

### 3.2. Effect of neonicotinoid pesticides depending on the type of exposure.

#### 3.2.1. General effect of the pesticides.

The GEE/GLM model showed that the different factors affected the sucrose responsiveness differently (Table 3): while there was a significant effect of the neonicotinoid compounds when presented to the bees during the test phase ($\chi^2 = 15.146$, df = 3, $p = 0.002$), they did not

interfere with sucrose responsiveness when presented during the feeding phase ($\chi^2$ = 1. 412, df = 3, $p$ = 0.703). Not surprisingly, there was a significant difference in responses between sucrose concentrations; the higher the concentration the higher the response rates ($\chi^2$ = 481.270, df = 6, $p$ < 0.001). Interestingly, all the 2-way interactions were significant: there was a significant difference between groups depending on the type of exposure during the feeding phase and testing phase ($\chi^2$ = 10.111, df = 3, $p$ = 0.018), and a significant effect of the pesticide exposure - either during the feeding phase or the test phase - on the sucrose responsiveness depending on the sucrose concentration (Feeding*Sucrose Concentration, $\chi^2$ = 38.836, df = 18, $p$ = 0.003; Test*Sucrose Concentration, $\chi^2$ = 25.589, df = 18, $p$ = 0.011). These two interactions are detailed in the next paragraphs below. Finally, there was no significant effect of the interaction between the factors Feeding*Test*Sucrose Concentration ($\chi^2$ = 12.519, df = 18, $p$ = 0.254).

**3.2.2. Pesticides in the feeding solution.**

3.2.2.1. Responsiveness within groups.

As described above, there was a significant interaction between the neonicotinoid applied in the feeding phase and the increasing sucrose concentrations used during the test phase. For the CTRL groups fed with no pesticide in the feeding phase, the pairwise comparisons revealed that almost all the sucrose concentrations were significantly different from each other (see Tables S4), except 0.03% and 0.10% sucrose ($p$ = 0.412), and 0.30% and 1.0% sucrose ($p$ = 0.238). When fed clothianidin, the responses to sucrose concentrations from 0.03% to 1.0% were not significantly different (cf. $p$-values in Tables S4). When fed imidacloprid, sucrose concentrations 0.10% and 0.30% were not significantly different ($p$ = 0.789); nor were sucrose concentrations 1.0% and 3.0% ($p$ = 0.546) and 10.0% and 30.0% ($p$ = 0.260). When fed thiamethoxam, only sucrose concentrations 0.03% and 0.10% ($p$ = 0.261), and 3.0% and 10.0% ($p$ = 0.101) were not significantly different.

3.2.2.2. Responsiveness between groups at same sucrose concentrations.

When we compared the responses of each group within the same sucrose concentrations, only a few pairwise comparisons showed significant differences: at 10.0% sucrose, bees fed with no pesticide responded significantly more than those fed with CLO ($p$ = 0.026) and those fed

with THX ($p$ = 0.040); similarly at 30.0% sucrose, CTRL bees responded significantly more than those fed with IMI ($p$ < 0.001) and those fed with THX ($p$ = 0.013), as shown in Fig.1. The pairwise comparisons between bees fed with any of the three neonicotinoids, for each sucrose concentration, revealed no significant differences (cf. Tables S4)

**3.2.3. Pesticides in the test solutions.**

3.2.3.1. Responsiveness within groups.

As described in paragraph 3.2.1, there was also a significant interaction between the neonicotinoid applied in the test phase and the increasing sucrose concentrations used during that same phase. For the CTRL groups exposed to no pesticide in the test phase, the pairwise comparisons revealed that within the sucrose range, all the responses were significantly different from each other (see Tables S5). For bees tested with clothianidin, the responses to sucrose concentrations 0.03% and 0.10% were not significantly different ($p$ = 0.790), as well as the responses to 0.30% and 1.0% sucrose ($p$ = 0.058). When tested with imidacloprid, sucrose concentrations from 0.03% to 3.0% were not significantly different from the direct previous concentration (0.03% and 0.10%, 0.10% and 0.30%, 0.30% and 1%, and 1.0% and 3.0%, cf. $p$-values in Tables S5). Bees tested with thiamethoxam showed no significant difference in response to sucrose concentrations 0.03% to 1.0% (cf. $p$-values in Tables S5).

3.2.3.2. Responsiveness between groups at same sucrose concentrations.

Several pairwise comparisons showed significant differences when responses of each group within the same sucrose concentration were compared: CTRL bees responded significantly more to 0.10% and 1.0% sucrose compared to bees tested with CLO (0.10%, $p$ = 0.050; 1.0%, $p$ = 0.014); CTRL bees tested with no pesticides responded more to sucrose than those tested with IMI, from 1.0% to 30%, and more than those tested with THX, from 0.30% to 30% (cf. all the $p$-values of these interactions in Tables S5), as shown in Fig.2. The pairwise comparisons between bees tested with CLO revealed a significant difference with IMI at 3.0% sucrose ($p$ = 0.039) and with THX at 1.0% ($p$ = 0.027).

**3.3. Effect of the type of exposure depending on the neonicotinoid pesticide.**

The GEE model revealed a significant interaction between the pesticides fed and the pesticides tested on the honey bees' sucrose responsiveness. As shown in Fig. 3 and Tables S6, all pairwise comparisons demonstrated that the bees that were fed and/or tested with any neonicotinoid had significantly lower response rates than the CTRL/CTRL group, *i.e.* the bees that did not receive any pesticide in any phase (Pairwise comparisons with CTRL/CTRL: CTRL/CLO, $p = 0.003$; CTRL/IMI, $p < 0.001$; CTRL/THX, $p < 0.001$; CLO/CTRL, $p = 0.020$; IMI/CTRL, $p = 0.007$; THX/CTRL, $p < 0.001$; CLO/CLO, $p = 0.003$; IMI/IMI, $p = 0.003$; THX/THX, $p = 0.001$). Interestingly, pairwise comparisons between any groups fed and/or tested with any neonicotinoid were not significantly different from each other (see Fig. 3).

## 4. Discussion

In this experiment, foragers were exposed to one of the three major neonicotinoids either in food or test solutions or both. Independent of the pesticide, exposed foragers responded significantly less than control bees when presented with test solutions containing 10% to 30% sucrose in the case of feeding exposure or containing 1% sucrose or higher in the case of test exposure (Figs. 1 to 3). However, these decreasing responses to sucrose resulting from the different types of exposure require different interpretations.

Forager honey bees, due to their foraging experience, respond more to lower sucrose concentrations than do newly-emerged bees (Pankiw and Page 1999; Pankiw et al 2001), but THX chronic oral exposure affects them in the same way by decreasing the PER rate at higher sucrose concentrations, as previously reported in young bees (Aliouane et al 2009; Démares et al 2016). Interestingly, caged foragers do not consume more of laced sucrose solutions than pure sucrose (Table 2) which differs from results reported for freely-moving bumblebees (Kessler et al 2015). Indeed, Kessler and colleagues showed a preference for THX-laced solutions within the same range of concentrations (10 nM in Kessler et al 2015 and 7.5 nM in this report). They also reported a mild effect of clothianidin, from 1 nM to 100 nM, which does not elicit preference in freely-moving bees (Kessler et al 2015); in this report, CLO does not reduce the response to high sucrose concentrations as much as THX and IMI (Fig. 1). Hypothetically, an explanation for this difference between THX and CLO

could lie in the by-products resulting from the conversion of the first into the second – even though these metabolites have not been identified (Maienfisch et al 2001; Nauen et al 2003). It is possible that the by-products of this conversion might affect honeybee physiology, but this needs further exploration.

In contrast with prior results stating that bees cannot taste neonicotinoids (Kessler et al 2015), we observed a reduced response to neonicotinoid-laced solutions in previously unexposed bees, suggesting that they can taste neonicotinoids. The experimental procedures were different: in Kessler et al. (2015), bees were presented with different concentrations of neonicotinoid (from 0.1 nM to 10 µM) in a sucrose solution at constant concentration (1 M); in this report, we tested one concentration of each neonicotinoid (7.5 nM) in an ascending range of sucrose concentrations (from 0.98 mM to 0.98 M). In the first case, bees showed no response to the presence of neonicotinoids, and this was confirmed by electrophysiological recordings (Kessler et al 2015); in the second, bees tended to reduce their response to the neonicotinoids in high-sucrose concentration solutions. This apparent contradiction could be easily explained if neonicotinoids attenuate taste as we go through the ascending sucrose series, as hypothesised previously (Démares et al 2016). In fact, the effect of the insecticides here is probably two-fold, with different mechanisms applicable to short exposure and long exposure. In acute exposure such as that reported here, the neonicotinoids might quickly desensitize the nicotinic acetylcholine receptors (nAChR) leading to transient sucrose taste impairment: bees cannot taste sugar but can still detect the pesticide in the solution. In chronic exposure situations such as used by Kessler et al (2015), and the 24-hour feeding in this report, we postulate a general knockdown of the gustatory pathway, coupled with an additional metabolic effect of detoxification (du Rand et al 2016). Bees would then be unable to assess the proper sucrose concentrations, but those feeding on the higher laced concentrations might be more able to cope with the toxicity of the pesticides. This is supported by the responses to lower sucrose concentrations when bees are exposed to THX, and even IMI, in the test solutions, which are not as impaired in the feeding exposure (Figs 1 & 2, and Tables S4 & S5)

Food aversions can be explained by post-ingestive effects as well as unpalatable taste. The physiological consequences of ingesting insecticides might lead to a non-specific malaise sensation in bees, as shown by the effects of toxins such as quinine and amygdalin on harnessed honey bees (Ayestaran et al 2010; De Brito-Sanchez et al 2005; Wright et al.

2010). Malaise-like behaviour in response to toxins is also exhibited by freely-moving bees, which spend less time walking and more time grooming (Hurst et al 2014). Such a malaise will affect the motivation state of bees and was postulated to underly the reduced responsiveness to sucrose of bees fed quinine, amygdalin or LiCl (Ayesteran et al 2010). While the malaise effect may apply to bees fed neonicotinoids chronically before testing, it cannot explain the reduced response to neonicotinoid-laced solutions when bees encounter these toxins for the first time. The nAChR, target of the neonicotinoid insecticides, are expressed in the gustation pathway in the dorsal lobe of the honey bee brain (Haupt 2007; Kreissl & Bicker 1989; Thany et al 2005). It is therefore possible that these insecticides desensitize the nAChR, eventually attenuating (and impairing) the activation of the gustation pathway and leading to toxic metabolic effects. In this situation, the malaise sensation and the sensory attenuation as a consequence of the neonicotinoid effect on bees would be intertwined.

Foragers are specialised in sucrose detection and neonicotinoid-exposed foragers responded less to mid-concentrations of sucrose; it is worrying to observe how a brief exposure (during the sucrose range test) is sufficient to affect sucrose threshold at a low field-realistic dose (Figs. 2 & 3) (Henry et al 2015; Stoner and Eitzer, 2012; also see references in Démares et al 2016). This means that unexposed bees encountering neonicotinoid-treated crops for the first time (like our first exposure in the test solution) may be biased regarding the actual quality of the nectar and this might eventually affect foraging efficiency and overall pollination services (Henry et al 2012; Stanley et al 2015). Nonetheless, the dual exposure does not show additive effect, *i.e.* the exposure through food and test solutions is not different from either single exposure (Fig.3). In addition, the effect of pre-exposure to neonicotinoid insecticides before testing the response to pure sucrose highlights the role of these compounds in the decreased responsiveness.

Although nicotine is present in nectar, acting as a deterrent for honey bees except at low concentrations (Köhler et al 2012; Singaravelan et al 2005), nicotine-derived insecticides do not seem to hinder sugar consumption (Démares et al 2016; Kessler et al 2015). Due to the nature of their molecular targets, neonicotinoids act as multisensory disruptors in bees, from larvae to foragers and queens (Derecka et al 2013; Williams et al 2015). Therefore, there is an urgent need to enforce the global regulation of these pesticides for better worldwide

pollinator conservation (Potts et al 2016). Investigating the effects of substances disrupting sensory abilities in related species, like honey bees and bumblebees, will not only help to protect crucial pollinators but also deepen our understanding of the evolution of multisensory pathways.

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**Competing Interests.** We declare no competing interest.

**Author Contributions.** FD, CWWP, SWN and HH designed the study; FD and HH collected the bees and performed the experiments; FD analysed the data; all authors revised the manuscript and approved of its final version.

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**Tables and Figure Legends.**

**Table 1. Molar mass and $LD_{50}$ values of neonicotinoid compounds.** The $LD_{50}$ value is the oral dose on which 50% of the exposed animals will die after 24 hours.

| | Clothianidin | Imidacloprid | Thiamethoxam |
|---|---|---|---|
| **Molar Mass (g/mol)** | 249.7 | 255.7 | 291.7 |
| **Oral $LD_{50}$ (ng/bee)** | 3.0 to 3.8 | 3.7 to 4.5 | 4.0 to 5.0 |
| **References** | Godfray et al. 2014<br>Kessler et al. 2015 | Godfray et al. 2014<br>Henry et al. 2015 | Godfray et al. 2014<br>Démares et al. 2016 |

**Table 2. Number of bees used and parameters observed during the experiment.** Values are mean ± S.E.M. (N=5 colonies). Each parameter was analysed through one-way ANOVA, and only the "Dose ingested" was significant ($p$=0.006). Tukey HSD post-hoc tests revealed that the ingested dose of thiamethoxam is significantly higher than that of the two other compounds (* $p$ <0.05).

| | | Control | Clothianidin | Imidacloprid | Thiamethoxam |
|---|---|---|---|---|---|
| **Number of bees** | | 349 | 177 | 177 | 173 |
| **Survival after 24h (%)** | | 95.04% ± 1.28 | 94.93% ± 2.61 | 93.14% ± 1.59 | 95.14% ± 1.54 |
| **Consumption (µl/bee)** | **Water** | 11.33 ± 5.30 | 5.02 ± 2.28 | 11.78 ± 4.08 | 6.58 ± 2.52 |
| | **Sucrose** | 48.90 ± 3.00 | 53.72 ± 3.49 | 56.80 ± 1.35 | 59.25 ± 2.79 |
| **Dose ingested (ng/bee)** | | NA | 0.101 ± 0.006 | 0.109 ± 0.003 | ***0.130 ± 0.006 **** |
| **Percentage of $LD_{50}$** | | NA | 2.96% ± 0.19 | 2.66% ± 0.06 | 2.88% ± 0.14 |

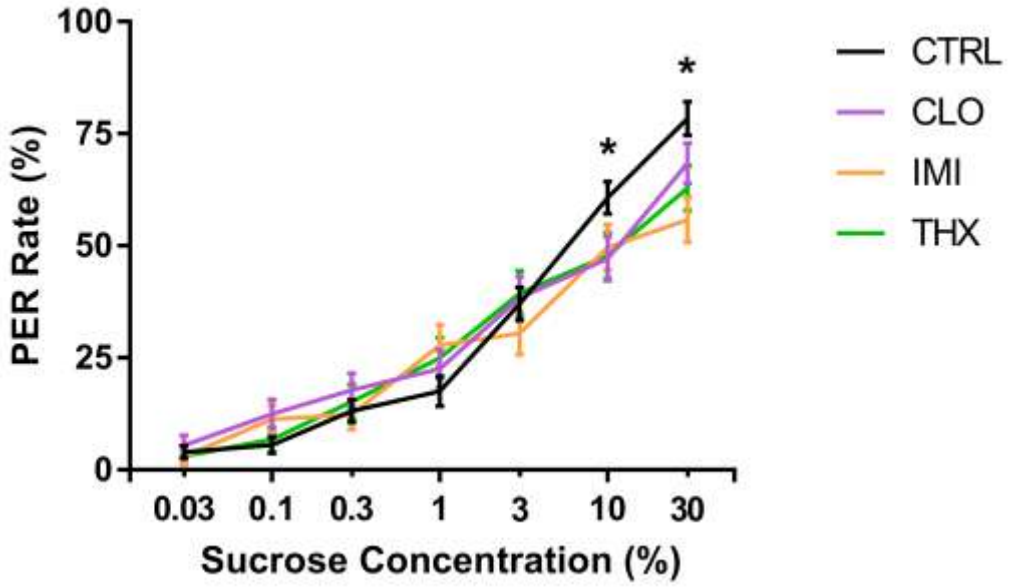
**Table 3: Results of the GEE/GLM regarding sucrose responsiveness.**

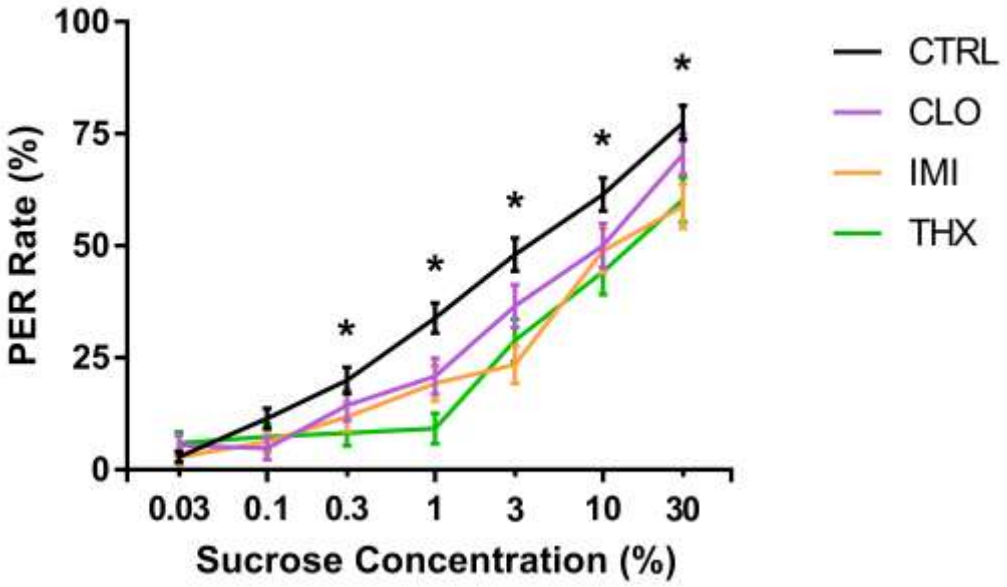
| Factors | Wald Chi-Square | Degree of Freedom | *p*-value |
|---|---|---|---|
| (Intercept) | 257.233 | 1 | 0.000 |
| Feeding | 1.412 | 3 | 0.703 |
| Test | 15.146 | 3 | 0.002 |
| Sucrose Concentration | 481.270 | 6 | 0.000 |
| Feeding * Test | 10.111 | 3 | 0.018 |
| Feeding * Sucrose Concentration | 38.836 | 18 | 0.003 |
| Test * Sucrose Concentration | 25.589 | 18 | 0.011 |
| Feeding * Test * Sucrose Concentration | 12.519 | 18 | 0.254 |

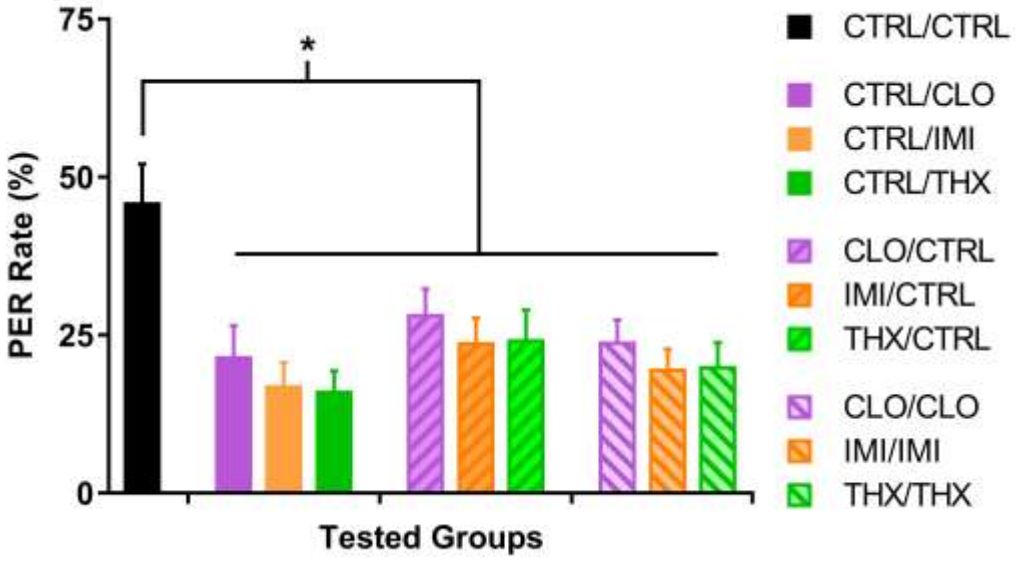
**Figure 1. Sucrose responsiveness is affected by neonicotinoids in the feeding solution.** Estimated marginal means of PER rates of foragers exposed to dietary neonicotinoids for 24 hours prior to testing with the pure sucrose concentration range. At 10% and 30% sucrose, foragers exposed to neonicotinoids responded significantly less than CTRL foragers (* $p<0.05$ LSD pairwise comparison; CTRL vs CLO and CTRL vs THX at 10% sucrose, CTRL vs IMI and CTRL vs THX at 30% sucrose). PER rates for each group are displayed in Tables S4.

**Figure 2. Sucrose responsiveness is affected by neonicotinoids in the sucrose test solutions.** Estimated marginal means of PER rates of foragers fed with pure sucrose solution for 24 hours prior to testing with the sucrose concentration range laced with neonicotinoids. From 0.30% sucrose and higher, foragers exposed to neonicotinoids respond significantly less than CTRL foragers (* $p<0.05$ LSD pairwise comparison; cf. main text and supplementary for *p*-values). PER rates for each group are displayed in Tables S5.

**Figure 3. Sucrose responsiveness is decreased by neonicotinoid exposure**. Estimated marginal means (EMM) of averaged PER rates of foragers exposed to clothianidin (CLO), imidacloprid (IMI), thiamethoxam (THX) or none (CTRL). Honey bees have been either exposed through the feeding solution (X/CTRL) or through the test solutions (CTRL/X), or both (X/X), with X being any of the three aforementioned neonicotinoids. For each exposure, foragers exposed to neonicotinoids respond significantly less than CTRL/CTRL foragers, on average 20% to 25% less (* $p<0.05$ LSD pairwise comparison; cf. main text and supplementary Tables S6 for *p*-values and EMM).







**Tables.**

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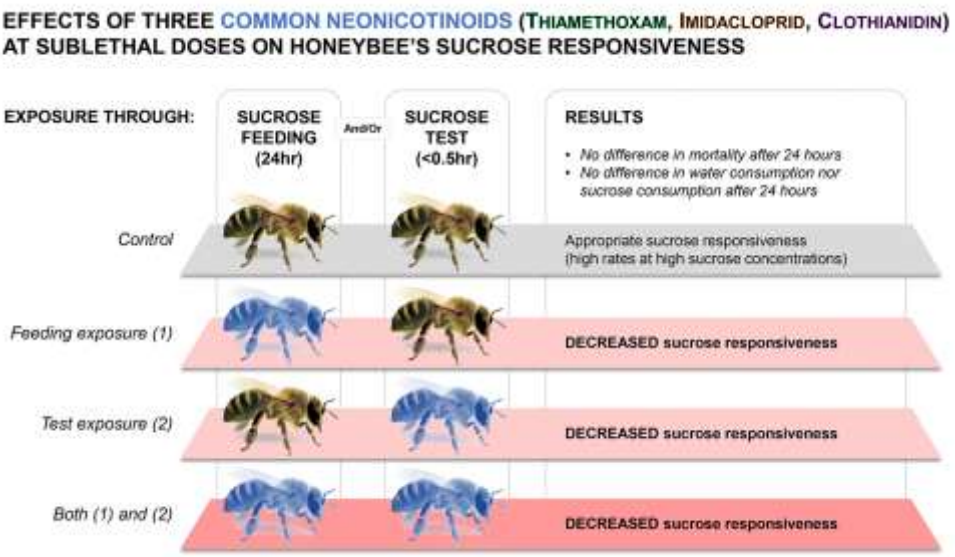

**Highlights**

- Classically, honey bee sugar responsiveness is tested with pure sucrose solutions
- Here, we laced both feeding and test solutions with neonicotinoids
- Bees fed before testing with laced sucrose solutions have reduced sucrose responses
- Bees first exposed to pesticide in test solutions also have reduced responses
- This is further evidence for neonicotinoids acting as multisensory disruptors