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Nadège Forfert & Robin F A Moritz

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## ORIGINAL RESEARCH ARTICLE

### Thiacloprid alters social interactions among honey bee workers (*Apis mellifera*)

Nadège Forfert<sup>a\*</sup>  and Robin F A Moritz<sup>a,b</sup>

<sup>a</sup>Institute of Biology, Martin-Luther-University, Halle-Wittenberg, Germany; <sup>b</sup>Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

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Experiments have shown that sublethal doses of neonicotinoids can interfere with honey bee (*Apis mellifera*) performance, yet sublethal effects on an individual level may be either enhanced or buffered against at the colony level, and this response to pesticide exposure depends on how it affects worker-worker interactions. We quantified worker interactions in experimental groups to assess the effects of thiacloprid on social network structure established by a group of worker individuals. We also quantified the amount of food exchanged via trophallaxis among worker individuals. Bees were force-fed a “low” dose of 0.17 µg or a “high” dose of 0.80 µg thiacloprid in 20 µl 2.7 M sucrose solution. Bees fed with thiacloprid significantly reduced their network centrality, but they nevertheless exchanged more food to other group members, which resulted in a dilution of the contaminated food. Hence, although thiacloprid may act as a general perturbator of social network structure, it still may play a role in the dynamics of disease transmission in the colony if pathogens are transmitted via food exchange.

#### El thiacloprid altera las interacciones sociales entre las obreras de la abeja de la miel (*Apis mellifera*)

Diversos experimentos han demostrado que las dosis subletales de neonicotinoides pueden interferir con el rendimiento de la abeja melífera (*Apis mellifera*), pero los efectos subletales al nivel individual pueden ser mejorados o amortiguados al nivel de la colonia, y esta respuesta a la exposición ante pesticidas depende de cómo afecta a las interacciones obrera-obrera. Hemos cuantificado las interacciones entre obreras en grupos experimentales para evaluar los efectos del thiacloprid sobre la estructura de la red social establecida por un grupo de obreras. También hemos cuantificado la cantidad de alimentos intercambiados a través de trofalaxia entre las obreras. Las abejas recibieron una dosis “baja” de 0,17 µg o una dosis “alta” de 0,80 µg de thiacloprid en 20 µl de solución de sacarosa 2,7 M. Las abejas alimentadas con thiacloprid redujeron significativamente la centralidad de su red, pero sin embargo intercambiaron más alimento con otros miembros del grupo, lo que dio como resultado una dilución de los alimentos contaminados. Por lo tanto, aunque el thiacloprid puede actuar como un perturbador general de la estructura de la red social, también puede jugar un papel en la dinámica de la transmisión de enfermedades en la colonia si los patógenos se transmiten a través del intercambio de alimentos.

**Keywords:** neonicotinoid; social network; social interaction; *Apis mellifera*; trophallaxis

#### Introduction

The western honey bee *Apis mellifera* L. is of essential economic and ecological importance for the pollination of many crops and wild plants worldwide (Klein et al., 2007) and colony losses are therefore of major concern. Several causal mechanisms for colony losses have been proposed, ranging from pests and diseases, to pesticides, nutrition and habitat deficiencies, and management challenges (van der Sluijs et al., 2013). Honey bees can be exposed to neonicotinoids by foraging and storing contaminated plant products such as nectar and pollen in the hive (Krupke et al., 2012). Three neonicotinoid compounds (imidacloprid, clothianidin and thiamethoxam) have received particular attention, because of their systemic properties and high toxicity to honey bees in the laboratory (oral LD<sub>50</sub> = 0.004, 0.003 and 0.005 µg/bee, respectively; Decourtye & Devillers, 2010). The seed treatment of pollinator attractive crops with those three neonicotinoids is subject to a moratorium by the

European Commission (2013). Another neonicotinoid, thiacloprid is much less toxic to honey bees unless they are starved (oral LD<sub>50</sub> = 17.32 µg/bee; Decourtye & Devillers, 2010; Laurino et al., 2011), but can be found at high levels in hives (Mullin et al., 2010) because it is commonly used as a spray rather than as a seed dressing. Nevertheless, it is still not well documented whether the use of neonicotinoids poses a critical risk for apiculture at a global scale (Moritz & Erler, 2016).

Several laboratory based studies have shown that neonicotinoids can cause a wide range of adverse effects for individual bees including a reduction of memory, learning ability, orientation, foraging success, brood care, social activities and enhanced susceptibility to diseases (reviewed in Blacquière et al., 2012; Desneux et al., 2007; Fairbrother et al., 2014). Yet, all of these studies focus on the effects of the most toxic neonicotinoids (more particularly imidacloprid) on traits of individual workers, and it is unclear how these effects have consequences at the

\*Corresponding author. Email: [nadege.forfert@zoologie.uni-halle.de](mailto:nadege.forfert@zoologie.uni-halle.de)

social or even the colony level. However, it is well known that the social organization of the colony is a key factor in dictating honey bee success (Moritz & Southwick, 1992). Self-organized group dynamics are essential for maintaining colony fitness, and negative effects that are barely detectable at the individual level can be amplified through positive feedback loops and have grave effects at the colony level (Fewell, 2003). Therefore, based on the social interactions of a colony, potentially harmful effects could be either buffered or enhanced by these interactions. For example, often a few keystone individuals distribute information (connect) to many nestmates and act as central hubs for information flow within a colony (Krause et al., 2007). The loss of such individuals or any reduction in their frequency can therefore severely disrupt the social organization and this can have important fitness consequences at the colony level (Fewell, 2003). In addition, social interactions also provide opportunities for pathogen transmissions. A few key individuals in a group can play a major role for infection dynamics (Naug, 2008). Hence, the structure of the interaction network within the colony is not just important for information exchange, because it can also influence the transmission dynamics of contagious diseases.

In this study we go beyond testing the impact of pesticides on individual workers' behavior and health, and analyze how social contact networks might change amongst workers exposed to one of the less toxic neonicotinoids, thiacloprid. First, the change in the patterns of antennation and trophallaxis of a single worker bee treated with thiacloprid was measured. Then the entire social network was assessed and not just single bee. Secondly, the amount of food transferred via trophallaxis interaction of worker bee treated with thiacloprid was quantified.

## Materials and methods

Two different experimental approaches were used to assess the response of a group of untreated workers towards a treated worker fed with either a "low" or "high" dose of thiacloprid and compared this with a sugar solution control. In experiment 1 the effect of thiacloprid on the structure of social network was compared by quantifying antennal or trophallactic contacts (TC). In experiment 2 the amount of food transferred by trophallaxis within the group was quantified.

### Experiment 1: Antennal and trophallactic interactions

#### Treatment groups and mortality

Sealed worker brood was taken from a colony of *Apis mellifera carnica* and kept in an incubator at  $34.0 \pm 2.0$  °C, 60% RH until hatching. Emerging worker bees were kept in groups of ~100 and fed with sucrose solution (2.7 M), candy and pollen, *ad libitum*. After five days, the bees were starved for 2 h and individually transferred into microcentrifuge tubes with cut-off

bottoms at the ends to allow for proboscis extension, such that they could be individually fed with a micropipette. A stock solution of 1 mg/ml thiacloprid was made by dissolving 1 mg (99.9% purity Sigma-Aldrich) of thiacloprid into 20 µl of dimethylsulfoxid (DSMO, Rotipuran), this was then mixed into 1 ml of sugar solution (2.7 M). Bees were force-fed a "low" dose of 0.17 µg or a "high" dose of 0.80 µg thiacloprid in 20 µl 2.7 M sucrose solution with 2% of DSMO. Control bees received 20 µl of the 2.7 M sucrose and 2% solvent solution to account for potential solvent effects on the bees. Only those bees which had consumed the full volume and had not regurgitated within 20 min after feeding were transferred into a glass Petri-dish arena (diameter = 70 mm) in groups of ten. Comb wax spacers at the rim of the Petri-dish allowed for air circulation to avoid condensation of water. During the experimental period all bees were fed with sucrose solution (2.7 M), candy and ground pollen, *ad libitum*, and kept at 25 °C in the incubator. Mortality was recorded 24 and 48 h after initial exposure.

#### Treated bees in social network

**Video analysis.** Experimental groups of five individually labelled workers kept in Petri-dishes (as above) were used to analyze the social network using standard statistical practices (Wasserman & Faust, 1994). Contacts (antennation and trophallaxis) among all bees were assessed, including the non-treated bees (fed with sugar control) and the treated bee (one per group), which was either fed with a control, low or high dose of thiacloprid on day five as described above. The experiments were conducted in a dark room (25 °C, red light illumination: 850 nm), where the groups were aligned according to the time when they had been fed. A continuously recording CCD camera (1392 × 1040 pixels, Basler scA1400-30 fm) was attached to a metal rail above the aligned Petri-dishes and moved by a software controlled step motor (LinearV, Point Electronics, Halle Saale) from one position to the next (i.e., from one group to another) in 1 min intervals. VirtualDub (distributed under the GNU General Public License) was used to capture, process, and analyze the recordings. Video clips were recorded during 5 h the day before the treatment to obtain a base line activity measure of all bees in the group, including the bee that will be treated in the experiment. The next day, video clips were recorded starting 1 h and ending 6 h after the feeding of the treated bee. All recordings were performed at the same time of the day to reduce any bias due to potential variation in circadian activity rhythms.

**Behavioral analysis.** All antennal contacts (AC) among all group members were evaluated. If a contact included the extension of a proboscis of one bee, then it was considered as a "trophallactic contact", with the recipient extending its proboscis, and the donor opening its

mandibles. All contacts were analyzed “blind” to the observer that had no prior knowledge about the identity of the treated and the control bee. The individual identities of the bees were revealed only at the end of the experiment for the final statistical analysis. Bees were kept for an additional 24 h to screen for differential mortality between the treated and control bees.

**Social network analysis.** A weighted and undirected network was constructed (Newman, 2004) for antennal interactions. Each individual participating in an interaction was considered as a “node” in the network, and the weight of connection between two nodes was given by the total number of interactions between them. The *degree centrality* (Freeman, 1979) was determined as a centrality index for every individual in the group based on their number of antennal interactions. A worker with the highest *degree centrality* is the most central and the one with the lowest *degree centrality* is the most peripheral individual. To evaluate the network position of each worker in a group based on their number and direction of trophallactic interactions, the *outdegree centrality* and *indegree centrality* were used as centrality indexes for each individual in the group (Freeman, 1979). A worker with a high *outdegree centrality* mostly serves as a donor in trophallactic contacts, while a worker with a high *indegree centrality* is most often a recipient. All network analyses were performed using the software Visone version 2.8 (Brandes & Wagner, 2004).

### Experiment 2: Quantitative food exchange

In this experiment, the experimental groups were composed of a “donor” bee fed with thiacloprid and non-treated recipient bees. These donor bees were 5 day old workers that were labelled and fed as described above, but now fuchsin (3.5%) tracer dye was added in the sucrose solution and then a control, low, and high dose treatments were employed. The fuchsin dye allowed for the quantification of the amount of food transferred via trophallaxis by the end of the experiment. The donor bees were introduced into a group of nine same aged “recipient” bees which had been fed with 2.7 M sugar solution *ad libitum* to ensure they were not starved prior the experiment. The recipient bees were not starved so that they would not artificially enhance any food transfer. The group of bees were kept in Petri-dish arenas as described above in an incubator  $34.0 \pm 2.0$  °C, 60% RH in constant dark for 30 min and were then immediately freeze killed on dry ice. We conducted the same experiments with 15 day old bees to test for a potential age effect. A filter paper was placed at the bottom of all arenas to control for regurgitation during the experiment. Petri-dishes that contained dye traces on the filter paper by the end of the experiment were discarded. The amount of transferred food was quantified for each bee by homogenizing the honey stomach and gut in 150  $\mu$ l ethanol (70%) and

centrifuging for 10 min at 10,000g. The absorption of the supernatant was measured in a microplate reader (Synergy Mx, BioTek) at 548 nm to quantify the amount of food transferred.

### Statistical analysis

The effects of thiacloprid on honey bee mortality were analyzed with survival analyses using a Cox-Mantel test (Life Tables). In experiment 1, for each treatment group of bees, interactions and centrality indexes for both post-treatment phases were compared to before the treatment using either *t*-tests (normally distributed datasets) or permutational *t*-tests (non-normally distributed datasets). Changes in interactions and centrality indexes were then analyzed using linear mixed model (LMM) or Kruskal-Wallis test. In experiment 2, data were analyzed using general linear models (GLM). All statistical analysis were conducted using the software R (R core team, 2013).

## Results

### Experiment 1: Antennal and trophallactic interactions

#### Mortality

Exposures to both the high ( $n = 34$  bees) (Cox-Mantel test,  $p = 0.06$ ) and the low dose ( $n = 30$  bees) (Cox-Mantel test,  $p = 0.30$ ) had no significant effect on the mortality compared to untreated control group ( $n = 55$  bees) over 48 h (number of dead bees:  $n = 6$  for the high dose,  $n = 4$  for the low dose and  $n = 5$  for the control). Only bees treated with the high dose displayed signs of intoxication that included agitation, circular movement and occasional tremors, immediately after feeding. These symptoms were never observed on the second day of the experiment. There was also no mortality in all treatment and control groups ( $n = 37$ ) during the social network experiments.

#### Antennal contacts

There was no significant difference of antennal contacts during the early post treatment phase (1–3.5 h after treatment) for any of the treated bees in comparison with the antennal contacts before the treatment (*t*-test,  $p = 0.06$  for the control;  $p = 0.86$  for the low dose treatment;  $p = 0.32$  for the high dose treatment; Table 1). During the late post treatment phase (3.5–6 h after treatment), the low dose treated bees decreased significantly their antennal contacts in comparison with before the treatment (*t*-test,  $p = 0.04$ ) whereas there was no significant difference of antennal contacts for the controls (*t*-test,  $p = 0.59$ ) and the high dose treated bees (*t*-test,  $p = 0.35$ ) during this time window (Table 1).

Overall, there was a stronger reduction of antennal contacts ( $\Delta AC = 0.48 \pm 0.82$  for the early post-treatment phase,  $\Delta AC = -1.22 \pm 0.69$  for the late post-treat-

Table 1. Social interactions before and after honey bee (*A. mellifera*) pesticide exposure. Bees were administered doses of a neonicotinoid insecticide, thiacloprid, of 0  $\mu\text{g}$  (control,  $n = 14$ ), 0.17  $\mu\text{g}$  ( $n = 14$ ) and 0.80  $\mu\text{g}$  ( $n = 9$ ) and placed in groups of five worker bees. Antennal and trophallactic interactions were recorded before treatment, and 1–3.5 h (early phase) and 3.5 h–6 h (late phase) after the pesticide treatment. AC corresponds to the number of antennal contact,  $\text{TC}_{\text{out}}$  and  $\text{TC}_{\text{in}}$  correspond to the number of given and received trophallactic contact respectively. In a social network context, three measures of *degree centrality* were calculated as centrality indexes:  $\text{DC}_{\text{AC}}$ , based on the antennal contacts,  $\text{DC}_{\text{TCout}}$  and  $\text{DC}_{\text{TCin}}$ , based on the given and received trophallactic contacts. Means are given with their standard error.

Time	Treatment ( $\mu\text{g}$ )	AC	$\text{DC}_{\text{AC}}$	$\text{TC}_{\text{out}}$	$\text{DC}_{\text{TCout}}$	$\text{TC}_{\text{in}}$	$\text{DC}_{\text{TCin}}$
Before treatment	0	$3.29 \pm 0.76$	$14.07 \pm 2.82$	$0.29 \pm 0.29$	$4.76 \pm 4.76$	$0.07 \pm 0.07$	$7.14 \pm 7.14$
	0.17	$5.50 \pm 0.98$	$21.70 \pm 2.83$	$0.14 \pm 0.14$	$5.36 \pm 5.36$	$0.14 \pm 0.14$	$7.14 \pm 7.14$
	0.80	$4.56 \pm 0.94$	$21.39 \pm 3.35$	$0.33 \pm 0.25$	$22.22 \pm 15.43$	$0 \pm 0$	$0 \pm 0$
Early (1–3.5 h)	0	$5.79 \pm 0.88$	$21.60 \pm 2.15$	$0.36 \pm 0.17$	$19.04 \pm 9.70$	$0.43 \pm 0.14$	$32.14 \pm 12.38$
	0.17	$5.14 \pm 1.09$	$20.03 \pm 2.84$	$0.07 \pm 0.07$	$7.14 \pm 7.14$	$0.21 \pm 0.11$	$21.43 \pm 11.38$
	0.80	$3.22 \pm 0.88$	$12.46 \pm 2.45$	$0.33 \pm 0.35$	$8.33 \pm 8.83$	$0 \pm 0$	$0 \pm 0$
Late (3.5–6 h)	0	$3.79 \pm 0.68$	$19.58 \pm 3.70$	$0.07 \pm 0.07$	$7.14 \pm 7.14$	$0.29 \pm 0.29$	$7.14 \pm 7.14$
	0.17	$2.64 \pm 0.46$	$21.44 \pm 4.14$	$0.07 \pm 0.07$	$7.14 \pm 7.14$	$0.21 \pm 0.11$	$21.43 \pm 11.38$
	0.80	$3.22 \pm 1.10$	$12.39 \pm 3.19$	$0.22 \pm 0.12$	$16.67 \pm 5.89$	$0 \pm 0$	$0 \pm 0$

ment phase) in the late phase of the experiment (LMM,  $p = 0.001$ ). There was however neither a significant effect of the thiacloprid treatment (LMM,  $p = 0.09$ ) nor of the interaction between the thiacloprid treatment and the post treatment phase on the  $\Delta\text{AC}$  (LMM,  $p = 0.10$ ).

#### Degree centrality based on antennal contact

The analyses above reflect the individual based behavioral responses of the treated bees, but do not address the reactions of the other group members and the overall group structure. It was therefore determined a *degree centrality* (DC) based on antennal interactions to

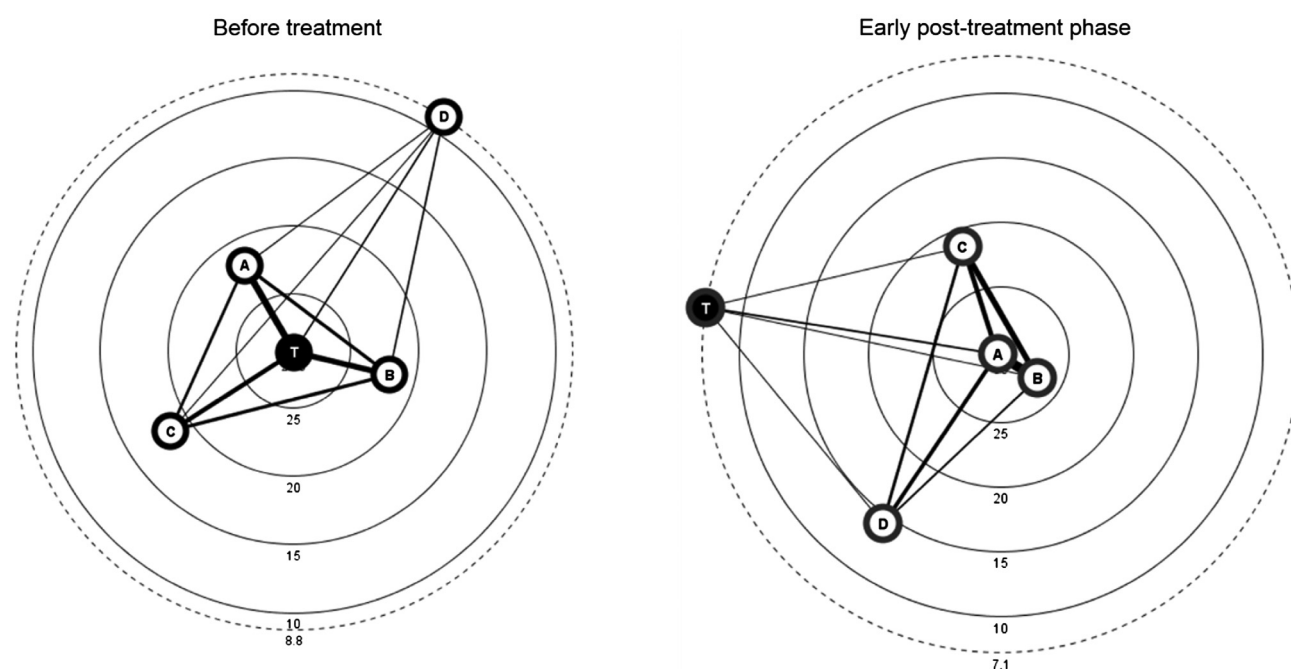


Figure 1. Undirected network of a thiacloprid treated honey bee (*A. mellifera*) in group of five worker bees illustrating the derivation of the difference in *degree centrality* ( $\Delta\text{DC}$ ). This example shows the network position of a focal bee ( $T$ ) treated with 0.80  $\mu\text{g}$  of thiacloprid within its social group ( $n = 5$ ) before (left network) and after treatment (1–3.5 h after exposure) (right network). The individuals are presented as nodes according to their *degree centrality* (concentric circles with values decreasing from the middle to the periphery). The most central is the individual with the highest *degree centrality* of all group mates. In this undirected weighted network the thickness of the connecting lines (edges) reflects the number of connections (contacts) between two nodes.  $T$  = treated bee,  $A$  to  $D$  = untreated bees. In the right network before the treatment, the subsequently treated bee had been in a central position of the network ( $\text{DC} = 28.95$ ), functioning as a “hub”. After thiacloprid exposure,  $T$  moved to the periphery ( $\text{DC} = 7.14$ ) thereby not only changing its own network position but completely transforming the entire network structure. In this case the difference of *degree centrality* for the treated bee equals to  $\Delta\text{DC} = -21.81$ .

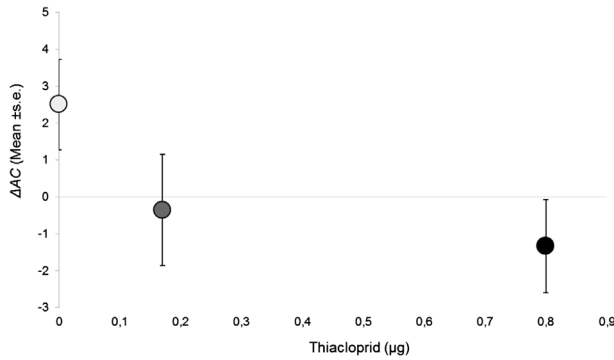


Figure 2. Difference of degree centrality ( $\Delta DC$ ) in the early phase after (1–3.5 h) honey bees (*A. mellifera*) pesticide exposure. The degree centrality of a treated individual bee placed in group of five bees was measured based on its antennal contacts occurring with and among its other group members. Treated bee of each groups received sugar solution only (i.e., the controls) ( $n = 14$ ) (in white), or received acute dose of thiacloprid,  $0.17 \mu\text{g}$  ( $n = 14$ ) (in grey) and  $0.80 \mu\text{g}$  ( $n = 9$ ) (in black). Means are given with their standard error.

evaluate the response of the social network to the introduction of the various treated bees.

During the early post treatment phase, the high dose treated bees decreased significantly their DC in comparison with before the treatment ( $t$ -test,  $p = 0.02$ ; Table 1), whereas there was no significant difference of

DC for the controls ( $t$ -test,  $p = 0.07$ ; Table 1) and the low dose treated bees ( $t$ -test,  $p = 0.67$ ; Table 1). During the late post treatment phase, there was no significant difference of DC in comparison with before the treatment for any of the treatment groups ( $t$ -test,  $p = 0.29$  for the controls;  $p = 0.93$  for the low dose treatment;  $p = 0.10$  for the high dose treatment; Table 1).

Figure 1 shows the change in degree centrality  $\Delta DC$  of a bee before treatment and in the early phase after a high thiacloprid dose exposure. Overall, there was a significant thiacloprid dose dependent decrease of  $\Delta DC$  (LMM,  $p = 0.03$ ; Figure 2) whereas there was neither a significant effect of the post-treatment phase (LMM,  $p = 0.91$ ) nor the interaction between the thiacloprid dose and the post treatment phase on the  $\Delta DC$  (LMM,  $p = 0.83$ ).

#### Trophallactic contacts

We determined the number of received ( $TC_{in}$ ) and given contacts ( $TC_{out}$ ) for every bee. There was no significant difference in  $TC_{in}$  and  $TC_{out}$  between before the treatment and both post-treatment phases for any of the treated bees (Permutational  $t$ -test, all  $p > 0.05$ ; Table 1). In addition, there was neither an effect of thiacloprid dose nor of post-treatment phases on  $\Delta TC_{in}$  and  $\Delta TC_{out}$  (Kruskal–Wallis test, all  $p > 0.05$ ).

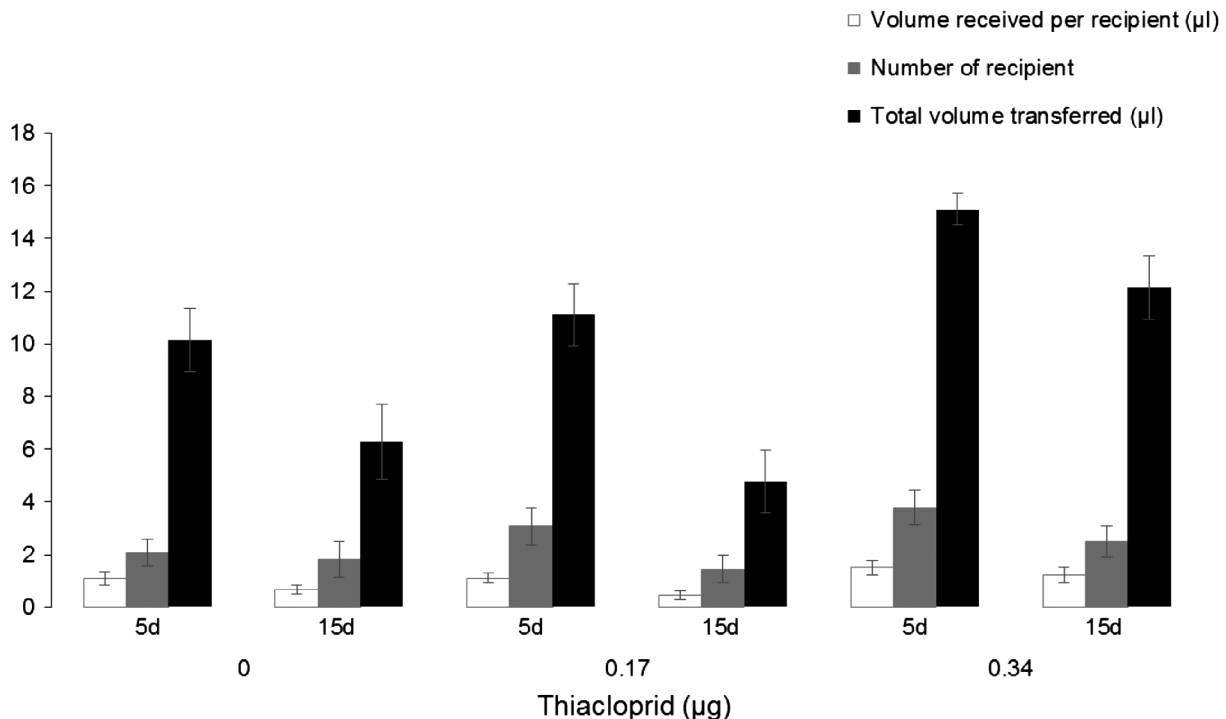


Figure 3. Effect of thiacloprid on food transfer within 5 and 15 day old groups of honey bees (*A. mellifera*). Donors were previously fed an initial volume of  $20 \mu\text{l}$  sucrose solution mixed with 0, 0.17 or  $0.34 \mu\text{g}$  of thiacloprid, a neonicotinoid insecticide, and placed in a group of ten bees of either 5 or 15 days-old bees. Trophallactic interactions between the single donor bee and its group members at 5 and 15 days-old after the treatment. The volume of food received per recipient bee (in white), the number of recipient bees (in grey) and the total volume of food transferred per donor bee (in black) are shown as mean  $\pm$  se.



### Degree centrality based on trophallactic contact

The analyses of the trophallactic contacts only reflect individual behavior but not the overall group response. We therefore determined a *degree centrality* (DC) based on trophallactic contacts to evaluate the response of the social network to the introduction of the various treated bees.

There was no significant differences in  $DC_{in}$  and  $DC_{out}$  between before the treatment and both post-treatment phases for any of the treated bees (Permutational *t*-test, all  $p > 0.05$ ; Table 1). In addition, there was neither an effect of thiacloprid dose nor of post-treatment phases on  $\Delta DC_{in}$  and  $\Delta DC_{out}$  (Kruskal–Wallis test, all  $p > 0.05$ ).

### Experiment 2: Quantitative food transfer

Donor bees within the 5 day old group of bees ( $n = 43$ ) reached on average one recipient more ( $2.95 \pm 0.40$ ) than within the 15 day old group of bees ( $n = 32$ ,  $1.91 \pm 0.34$ ) (GLM,  $p = 0.005$ ). The number of recipients also significantly increased with the thiacloprid dose (GLM,  $p = 0.005$ , Figure 3). There was no significant interaction between the thiacloprid dose and the age of the group of bees on the number of recipient bees (GLM,  $p = 0.53$ ). The 5 day donor bees transferred more food ( $n = 43$ ,  $12.07 \pm 1.07 \mu\text{l}$ ) to recipient worker bees than 15 day old donor bees ( $n = 32$ ,  $7.59 \pm 1.36 \mu\text{l}$ ) (GLM,  $p = 0.008$ ). Moreover, the total volume of transferred food also increased with the dose (GLM,  $p = 0.01$ , Figure 3). There was no significant interaction between the thiacloprid dose and the age of the group of bees on the total volume of transferred food (GLM,  $p = 0.85$ ). In addition, the 5 d old group of bees received more food per recipient than the 15 day old group of bees, with a volume of  $1.24 \pm 0.14 \mu\text{l}$  being received per recipient for the 5 day old groups ( $n = 430$ ), and  $0.77 \pm 0.13 \mu\text{l}$  being received per recipient for the 15 day old groups ( $n = 320$ ) (GLM,  $p = 0.02$ ; Figure 3). The volume of food received per recipient significantly increased with the thiacloprid dose (GLM,  $p = 0.048$ ; Figure 3). There was no significant interaction between the thiacloprid dose and the age of the group of bees on the volume of food received per recipient bees (GLM,  $p = 0.79$ ). The food was more evenly distributed in the thiacloprid treated groups with a variance of  $s^2 = 20.63$  for the controls ( $n = 286$ ),  $s^2 = 19.31$  for the low dose thiacloprid treated bees ( $n = 275$ ) and  $s^2 = 13.23$  ( $n = 264$ ) (Bartlett test,  $p < 10^{-3}$ ).

### Discussion

The highest concentration of thiacloprid that we used was four times lower than that recommended for use in the field (i.e., 144 mg/l) which has been found to be harmless to honey bee workers (Laurino et al., 2011). However, the concentrations we used were substantially higher than those that have been reported in hive

products (e.g., 0.13 mg/kg in honey, Laaniste et al., 2016; 0.9 mg/kg in pollen loads, Škerl et al., 2009; 0.014 mg/kg in pollen loads, 0.0059 mg/kg in beeswax, Mullin et al., 2010; and 0.2 mg/kg in bee bread, Genersch et al., 2010). The concentrations tested are therefore particularly relevant for returning foragers that have encountered high field doses. Upon return to the colony, such workers may experience a reduction in social interactions with other bees, which also may result in a reduced recruitment to these nectar sources to the benefit of the colony.

In this study, we investigated the effect of this level of thiacloprid on the social network interactions of an individual treated bee within a group of worker bees. In the first experiment, we recorded and made a distinction between antennal and trophallactic interactions. Our results showed that after the immediate toxic symptoms had disappeared, there were no significant pesticide effects on individual level behavior of bees. However, when considering social behavior using network analyses, the impact of thiacloprid became visible, and had a significant effect on altering antennation events. Individuals that had a central position within the social network became more peripheral once they had been exposed to thiacloprid in comparison to controls. In contrast, we did not find any significant effect of thiacloprid on the frequency and the direction of trophallactic contacts. However, the mean number of actual trophallactic interactions was very small ( $0.18 \pm 0.04$  se trophallactic contact per treated bee) rendering the lack of significance to be interpreted with caution due to low statistical power. Because only less than 5% of observed trophallactic interactions result in actual food transfer (Korst & Velthuis, 1982), we increased the sample size and quantified the amount of transferred food using the fuchsin dye instead of just relying on behavioral observations in the second experiment (Moritz & Hallmen, 1986).

With a much larger sample size, we found a pronounced dose-dependent increase of the number of food recipients. In addition, it seems that this effect was age dependent, with younger bees spreading food more readily than older ones. In spite of a more peripheral position in the group based on the antennation data, the thiacloprid treated workers spread more of the received food resulting in a more even distribution of the food among the group members. Regardless of bee age, donors that received the high thiacloprid dose distributed more food to more individuals than those which received the low dose or the controls despite less frequent interactions with others. The mechanism for this enhanced trophallaxis may be very simple. For example thiacloprid might not taste particularly good, and the bees might simply try to get rid of their contaminated food as soon as possible. Other, less direct mechanisms are also clearly possible, but the actual biological mechanism does not change the fact that this pattern was observed at the group level. Although the

treated workers are avoided by the others, they nevertheless manage to spread the contaminated food in the group resulting in a dilution of the pesticide in the colony. They accomplish this by increasing the food volume transferred per bee without increasing the frequency of trophallactic interactions.

In our experiments, bees were kept in glass Petri dishes to allow video-recording of their interactions. These experimental conditions and the use of 2% DSMO in the food solution may explain the high mortality rate found in both control and treatment groups.

Although the experimental groups of bees may seem artificial, the observed interference with the social network structure may have grave consequences. Within-group communication and social interactions are essential for the regulation of the colony to function as adaptive unit (Bortolotti & Costa, 2014). The decentralized control of colonial decision-making is based on the integrity and functioning of local social networks (Seeley et al., 1991). Organization through local control eliminates the need of a time-consuming communication between the peripheral sensor/effector individuals and the actual central decision-making. Thus, impairing the very local information exchange in the social network may very well affect colony performance as a whole. Although we found the effects of thiacloprid disappear after few hours, this exposure may be still very relevant in a natural setting, where bees are consistently exposed to pesticide. Because even the low dose treatment interfered with the social network structure, one cannot exclude that there may be more profound effects at the colony level. Vidau et al. (2011) showed that *Nosema ceranae* infected bees that had been exposed to thiacloprid doses similar to the low dose treatment suffered higher mortality than control bees. More recently, similar interactions between pathogen and thiacloprid have been reported by Doublet et al. (2015). In this study chronic exposure to the low dose of thiacloprid resulted in both additive interactions between a honey bee pathogen and increased larval and adult mortality.

Social interactions do not only regulate colony functions but they also influence intra-colony pathogen transmission dynamics (Naug, 2008). Physical contact, communication interactions, or trophallactic food exchange all provide plenty of opportunities for pathogen transmission in a colony (Fries & Camazine, 2001). Thus, central individuals that interact more frequently than peripheral individuals are not only more likely to get infected but also enhance the spread of pathogens within a colony. On the one hand, thiacloprid did not cause the treated bees to increase the centrality coefficient in our study, yet on the other hand, thiacloprid increased the volume of contaminated food with a more even spread among the recipient bees. Because interactions with pathogens can influence this effect, it may be premature to draw any general conclusions for

intra-colony epidemiology effects at this point. Parasites can alter host behavior to enhance their own transmission to new susceptible hosts (e.g., Forfert et al., 2015). In contrast, the microsporidian gut parasite *Nosema* spp. has been shown to reduce trophallaxis among workers (Naug & Gibbs, 2009). *Nosema* spp. infections can thus turn workers into trophallactic sinks and decrease the connectivity of social networks within the colony. This potential strategy to reduce the transmission of the parasite may be compromised if honey bees become momentarily poisoned by pesticides. Therefore, the role of neonicotinoids on the transmission dynamics of pests and pathogens within the colony can be expected to be highly pathogen specific.

Unanswered questions remain in regards to what we can conclude about pesticide effects on the impact on entire colonies. We are aware that honey bees in the colony are connected by many more edges and nodes in much more complex networks than tested here. Nevertheless, groups of individuals provide a simple social structure that is more accessible to experimental manipulation than other more complex social systems. When studying a group, one cannot only address the behavior of a treated bee, but also must look at the behavior of the non-intoxicated bees and their interactions towards the treated bee. Therefore, studies on pesticide effects on honey bees should not just focus on individual worker bees but also address how it alters the complexity of social organization as well. Our study shows that we can detect significant pesticide effects which became only visible on a social level. As the number of individuals in a group can either amplify or dampen the pesticide effects by self-organized processes, the quantification of worker interactions in larger and eventually colony level groups may be highly relevant to understand the impact of pesticides on honey bee colonies.

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## ORCID

Nadège Forfert  <http://orcid.org/0000-0001-6908-4407>



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