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Supplementary Material for

A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

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Materials and Methods

RFID monitoring system and study design

We simulated intoxication events on free ranging honey bee foragers using homing experiments. Individual honey bees were monitored using RFID tags (mic3®-TAG 64-bit RO, iID2000, 13.56 MHz system, 1.0×1.6×0.5mm; Micro-sensys GmbH, Erfurt, Germany) and RFID readers and custom-made data-loggers (Tag Tracing Solutions Inc., Valence, France) placed at the entrance of ca. 30,000-individuals colonies (20). Tags were pasted using dental cement (Temposil®2, Coltène Whaledent®) and did not impair honey bees' flight (20).

We carried out four separate homing experiments where we varied either the degree to which honey bees were familiar with the release site (experiment 1 vs. 2), the distance from release site to colonies (1 vs. 3) or the type of landscape (2 vs. 4). We used three distinct colonies, namely one for experiments 1 and 2 combined, and one for each of experiments 3 and 4. To avoid colony drift, all colonies were isolated from any apiary.

Study areas

Experiments 1 to 3 were conducted in an intensive cereal farming system of western France (Zone Atelier *Plaine et Val de Sèvre*, French département des Deux-Sèvres, 46°15'N, 0°30'W). This study area is a long-term research facility managed by the CEBC research unit. It covers about 450 km², with land use exhaustively georeferenced and updated annually. Agricultural practices are dominated by cereal and maize crops in rotation with oilseed rape and sunflower crops. All experiments in this area took place in mid-May, after oilseed rape crops and before maize and sunflower blooming, to avoid any concomitant intoxication event.

Experiment 4 took place during the same season in a suburban area in southern France (Avignon, French département de Vaucluse, 43°54'N, 4°52'E) with mixed farming fields and orchards of moderate size. In both areas, colonies were placed ten days before experiments for habituation.

Capture and experimental intoxication

Adult foragers were captured at the entrance of the hive in the morning. To avoid selecting young honey bees performing simple orientation flights, we only captured foragers returning to the colony with pollen loads. Before processing the honey bees, we synchronized their dietary state. They were first offered a professional beekeeping candy ad libitum for 60 min, and then were fasted for 90 min before individually receiving an experimental (*treated* vs. *control*) sucrose solution. Honey bees were then tagged and kept for an additional 40-min time lapse before final release to ensure that assimilation was complete.

We used experimental doses of 1ng of thiamethoxam per honey bee to match values found in realistic environmental conditions. For instance, honey bees foraging for nectar in treated winter oilseed rape would be exposed to thiamethoxam doses ranging from 0.17 to 2.3ng/individual/day [calculated from (7)]. To ensure each tagged honey bee would receive this dose, they were kept individually and offered 20µl of a 50% (weight/weight) sucrose solution with a thiamethoxam content of 50µg/l – or no thiametoxham for *control* groups. The sucrose solution was offered in a truncated pipette spike. Honey bees that did not consume their individual 20µl reward were not kept in the experiment. Our experimental solution was further sent to an independent biological analysis laboratory for validation of thiamethoxam content. The real content was measured to be 67µg/l, i.e. slightly above the expected 50µg/l, leading to an effective dose of 1.34 ng per honey bee (Fig. S1). This dose remained nearly four times smaller than the LD50 (50% lethal dose) measured for honey bees and reported by the French AFSSA food agency in its official thiamethoxam authorization report (file n°2009 – 1235 – CRUISER 350).

We further confirmed the non-lethal nature of our experimental dose by maintaining 240 captive honey bees (from colonies of experiments 1,2 and 4) split into six *treated* and six *control* groups of equal size. No abnormal mortality was observed. Survival at 4 h was 100% in all groups. After 24h, only three *treated* and

two *control* individuals died, out of the 240 captive honey bees (survival = 97.5% and 98.3% for *treated* and *control* honey bees, respectively). After 48h, 12 *treated* and 10 *control* individuals died (survival = 90.0% and 91.7%, respectively).

Homing experiments

Tagged honey bees were released up to 1 km away from the colony, i.e. at a distance usually covered by foragers during normal foraging flights (23). Most released foragers returned to their colony the day of release, usually in within few minutes. A smaller proportion (about 5% to 20%) returned the second day of release, and up to the third day on very rare occasions. To ensure our monitoring had covered all returns, recordings at colonies lasted for five to seven days after release. Releases within a given experiment were split into two or three consecutive days.

In experiment 1, foragers were released 1 km away from their colony, in a site they were familiar with, i.e. from which they have returned to the colony at least once. To ascertain they had a prior knowledge of the pathway back to the colony, we selectively captured foragers that came back to the colony with bright blue pollen loads from a known phacelia field (*Phacelia tanacetifolia*). Phacelia was planted in a 1-ha field specifically for the need of the experiment, and the colony subsequently placed 1 km away (Fig. 2).

In experiment 2, foragers were released 1 km away as well, but at random sites regarding past foraging experience. For that purpose, we used the non-phacelia pollen foragers and released them in equal groups at six sites equally spaced along the 1-km release boundary (Fig. 2).

In experiment 3, foragers were released nearby their colony (70 m) in a site they were familiar with. To do so, experiment 1 was repeated using a second colony placed in the phacelia field margin. Phacelia foragers were released from inside the field.

In experiment 4, foragers were released 1 km away from their colony, within a more complex landscape compared to the previous cereal farming system experiments. We chose a suburban landscape, including a matrix of smaller, more diversified, agricultural fields and orchards. Release site locations conformed to experiment 2.

Data analysis

Homing probabilities of *treated* and *control* foragers were compared using exact binomial tests. When the difference was significant, we measured the mortality resulting from post-exposition homing failure, m_{hf} , as the proportion of non-returning *treated* foragers relative to proportion of returning foragers we would expect from *control*:

$$m_{hf} = [\text{control homing probability} - \text{treated homing probability}] / \text{control homing probability}$$

Under this form, m_{hf} estimates the proportion of exposed foragers that might disappear due solely to post-exposure homing failure, all other sources of mortality or homing failure set apart (natural mortality, predation, manipulation stress). Experiments 1 and 2, involving familiar and unfamiliar foragers, respectively, were intended to return the lower and upper bounds for m_{hf} .

Honey bee population dynamics model

To relate m_{hf} with colony dynamics, we introduced its estimated lower and upper bounds into Khoury's et al. population model (21). We simulated the fate of a typical colony starting at the oilseed rape flowering period, and under different scenarios of egg-laying rate and of forager exposure to treated oilseed rape (proportion of foragers exposed to treated nectar each day). We chose to start simulations with population sizes of 15,000 to 18,000, i.e. the average values estimated by professional beekeepers at the beginning of the beekeeping season in our study area. Foragers were set to account for 25% of total population. We set natural forager death rate to $0.154 \text{ individuals.day}^{-1}$, assuming an expected forager lifespan of about 6.5 days (21, 27). Other dynamic parameters remained unchanged from Khoury's et al. basic model [Fig. 3 in (21)].

We ran simulations under the hypotheses of (i) constant forager death rate with no forager exposure, and (ii) forager death rate raised by post-exposure homing failure m_{hf} during a 30-days oilseed rape flowering period (29). In the later configuration, exposed foragers were assigned a probability of disappearance combining daily death rate *and* the additional mortality due solely to post-exposure homing failure. The most optimistic and most pessimistic population trajectories were simulated using the lower and upper m_{hf} bounds, respectively.



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RAPPORT D'ESSAIS ACTA-THIAM-11.01/27093

Référence de l'échantillon: SOLUTION SUCREE

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Matrice analysée : SOLUTION SUCREE

Réceptionné le : 21/10/2011 Etat: conforme Température de conservation : +4°C

Angers, le 04/11/2011

Non référencé :

Code dossier: 000005166 Nom de l'échantillon : 11-6121 CODE ETUDE : ACTA-THIAM-11.01

SUBSTANCE ACTIVE	RESULTAT	TECHNIQUE ANALYTIQUE	METHODE	LIMITE DE QUANTIFICATION par substance active	DATE D'ANALYSE
clothianidine	<LD µg/l	MS/MS	méthode interne	0,2 µg/l	03/11/2011
thiamethoxam	67 µg/l	MS/MS	méthode interne	0,2 µg/l	03/11/2011

Fig. S1.

Official report of the thiamethoxam dosage of the experimental solution carried out by an independent biological analysis laboratory. The real solution content (67 µg/l) was close to the expected content intended for intoxication experiments (50 µg/l).

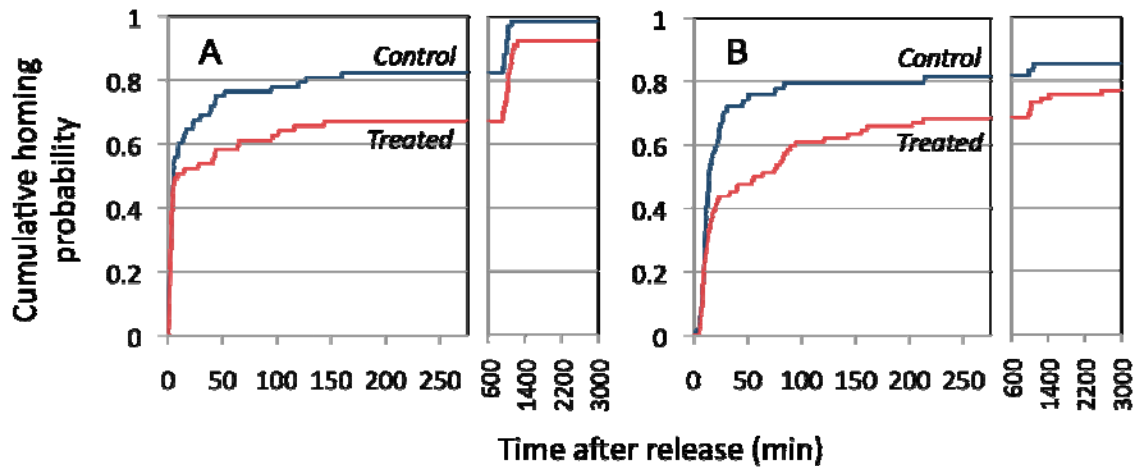


Fig. S2

Cumulative homing probability of released foragers in experiments 3 (A) and 4 (B). Temporal gaps denote the nighttime between the first and second days of release. Homing experiment 3 was carried out in the cereal farming system with foragers released 70 m away from their colony, in a site they were familiar with (A). Homing experiment 4 was carried out in a suburban landscape with foragers released 1 km away from their colony, at random sites regarding their past experience (B). In both cases, treated honey bees that received a non-lethal dose of thiamethoxam returned to the hive in significantly lower proportions than control honey bees (Table S1).

Table S1.

Comparison of homing probabilities between thiamethoxam-*treated* and *control* honey bee foragers, and estimated mortality due to post-exposure homing failure, m_{hf} . Foragers were released 1 km away from their colony, either at a foraging site they are familiar with (Experiment 1), or at a random site regarding their past experience (Experiment 2). Experiment 3 repeats Experiment 1, but at a minimal release distance (70m). Experiment 4 repeats Experiment 2, but in a suburban landscape. In all experiments, homing probabilities in *treated* foragers were significantly smaller than *control* ones, either at 4hrs of release, or after experiments ended.

	Experiment 1: Foragers familiar with release site – 1km, cereal farming system (<i>Treated</i> – <i>Control</i>)	Experiment 2: Foragers released at random sites – 1km, cereal farming system (<i>Treated</i> – <i>Control</i>)	Experiment 3: Foragers familiar with release site – 70m, cereal farming system (<i>Treated</i> – <i>Control</i>)	Experiment 4: Foragers released at random sites – 1km, suburban area (<i>Treated</i> – <i>Control</i>)
Number of released foragers	72 – 74	118 – 118	67 – 68	82 – 54
Homing probability – 4 hours of release (exact binomial test for proportion returned)	68.1% – 81.1% ($P=0.005$)	33.9% – 57.6% ($P<0.001$)	67.2% – 82.4% ($P=0.002$)	68.3% – 81.5% ($P=0.003$)
Homing probability – final values (exact binomial test for proportion returned)	76.4% – 85.1% ($P=0.036$)	56.8% – 83.1% ($P<0.001$)	92.5% – 98.5% ($P=0.003$)	76.8% – 85.2% ($P=0.029$)
Mortality due to post-exposition homing failure (m_{hf})	0.102	0.316	0.061	0.098

Additional Database S1 (separate file)

Data for main results (Fig. 3 and Fig. S2).

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