A common pesticide decreases foraging success and survival in honey bees.

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Version définitive du manuscrit publié dans SCIENCE / Final version of the manuscript published in : SCIENCE, 2012, DOI: *10.1126/science.1215039*

Title: A common Pesticide Decreases Foraging Success and Survival in Honey Bees

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Abstract: Non-lethal exposure of honey bees to thiamethoxam (neonicotinoid systemic pesticide) causes high mortality due to homing failure at levels that could put a colony at risk of collapse. Simulated exposure events on free-ranging foragers labeled with an RFID tag suggest that homing is impaired by thiamethoxam intoxication. These experiments offer new insights into the consequences of common neonicotinoid pesticides used worldwide.

One Sentence Summary: Non-lethal exposure to thiamethoxam leads to high mortality due to homing failure in honey bees.

Main Text:

Colony collapse disorder (CCD) is a recent, pervasive syndrome affecting honey bee (Apis mellifera) colonies in the Northern hemisphere, which is characterized by a sudden disappearance of honey bees from the hive (1). Multiple causes of CCD have been proposed, such as pesticides, pathogens, parasites, and natural habitat degradation (2–4). However, the relative contribution of those stressors in CCD events remains unknown. Some scientists and beekeepers suspect pesticides to hold a central place in colony weakening processes (1) or at least in interaction with other stressors (5, 6). In modern cereal farming systems, honey bees are readily exposed to pesticides because they rely heavily on common blooming crops, like oilseed rape (Brassica napus), maize (Zea mays) or sunflower (Helianthus annuus), that are now
routinely treated against insect pests (3). Systemic pesticides, in particular, diffuse throughout all the tissues as plants grow-up, and eventually contaminate nectar and pollen (7). Foraging honey bees are

therefore directly exposed, but also the rest of the colony as returning foragers store or exchange contaminated material with hive conspecifics (7, 8). Those exposure pathways are of important concern and pesticide manufacturers pay special attention to reduce non-intentional

intoxications in field conditions. Pesticide authorization procedures now require running mortality surveys to ensure doses encountered in the field remain below lethal levels for honey bees.

However, a growing body of evidence shows that sublethal doses, i.e. doses that do not entail direct mortality, still have the potential to induce a variety of behavioral difficulties in foraging honey bees, such as memory and learning dysfunctions and alteration of navigational skills (9). Neonicotinoid pesticides used to protect crops against aphids and other sap-sucking insects are especially liable to provoke such behavioral troubles. They are highly potent and selective agonists of nicotinic acetylcholine receptors, which are important excitatory neurotransmitter receptors in insects (10, 11). Effects of sublethal neonicotinoid exposures in honey bees may include abnormal foraging activity (12–14), reduced olfactory memory and learning performance (15–17) and possibly impaired orientation skills (18). Yet, the consequences of such behavioral difficulties on the fate of free-ranging foragers and on colony dynamics are extremely difficult to assess in the field and remains poorly investigated.

In this study, we tested the hypothesis that a sublethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees. We focused our attention on thiamethoxam, a recently marketed neonicotinoid substance (19), currently being authorized in an increasing number of countries worldwide for the protection of oilseed rape, maize and other blooming crops foraged by honey bees. We proceeded in two steps. First,
we assessed mortality induced by homing failure (mhf) in exposed foragers. This was achieved by monitoring free-ranging honey bees using RFID tagging technology (20). Second, we assessed the extent to which mhf, in combination with natural forager mortality, may upset colony dynamics. For that purpose, mhf was introduced into a model of honey bee population dynamics (21).

We used a custom-made RFID device (20) to monitor the fate of 653 individual free- ranging foragers in the course of four separate treatment-vs.-control homing experiments (22). The study was conducted in an intensive cereal farming system of western France (Zone Atelier Plaine et Val de Sèvre research facility, CEBC) and in a suburban area in Avignon, southern France. To simulate intoxication events, foragers received a field-realistic, sublethal dose of thiamtethoxam (a real dose of 1.34 ng in a 20-µl sucrose solution) and were released away from their colony with a microchip glued on their thorax (Fig. 1A). RFID readers placed at the hive entrance (Fig. 1B) were set to detect on a continual basis tagged honey bees going through the entrance. Mortality due to post-exposure homing failure, mhf, was then derived from the proportion of non-returning foragers. To further discriminate mhf from other causes of homing failure in treated foragers, e.g. natural mortality, predation or handling stress, we simultaneously released equal numbers of control foragers – fed with an untreated sucrose solution. Hence, mhf was calculated as the proportion of non-retuning treated foragers relative to expectations given
by the proportion of returning control foragers. Depending on the experiment, tagged honey bees where released up to 1 km away from their respective colony, i.e. at a distance usually covered by foragers during normal foraging flights (23). Experiments were conducted on individuals from three different colonies (22).

Our strategy was not to get an estimate of mhf per se. Instead we assessed its upper and lower bounds, depending on whether foragers were familiar or not with the foraging site they might get

intoxicated in. Indeed, one might expect that foragers familiar with the pathway back to the colony are less prone to homing failure than unfamiliar foragers. Under field conditions,

many foragers are probably familiar with the pathway back to the colony because they repeatedly forage on the same site (24). However, many others are unfamiliar too. Those include young honey bees at the onset of foraging, scouting honey bees that look for new food sources, and foragers newly recruited by scouting bees on the basis of the dance information (25). Most importantly, systemic pesticides like thiamethoxam are readily present in the nectar and pollen when flowering starts and receive the first visits of honey bees, hitherto unfamiliar with this newly available food source.

To account for individuals' past foraging experience, we conducted two distinct homing experiments. Experiment 1 simulated intoxication at a familiar foraging site and experiment 2 at a random site regarding past foraging experience. These experiments were assumed to return the lower and upper bounds of mhf, respectively. In experiment 1, we referred to as familiar foragers those foragers for which we could make sure they covered at least once the pathway from the release site back to the colony. For that purpose, we selectively captured foragers returning to the colony with pollen loads from a known location, and subsequently released them at that location. To ascertain pollen origin, we sowed beforehand a 1-ha field with scorpion weed Phacelia tanacetifolia, a highly attractive floral resource with bright blue pollen that is easily recognizable (26). Given that no other phacelia fields occurred in the area, we could ensure that phacelia- carrying honey bees came back from our experimental field. The colony was specifically placed 1 km away from the field for subsequent forager release (Fig. 2). In experiment 2, we used the non-phacelia pollen foragers. They were released in equal groups at six sites equally spaced on a 1-km circle around the colony (Fig. 2). Following that design, release sites were considered as random locations regarding the past experience of foragers.

Both experiment 1 and 2 evidenced substantial mortality due to post-exposure homing failure, mhf, with the proportion of treated foragers returning to the colony being significantly lower than that of control foragers (exact binomial tests, $P=0.033$ and $P<0.001$, respectively; Fig. 3, Table S1). Additionally, mhf was greater in treated foragers that tended to be unfamiliar with the foraging site, as indicated by their significantly lower homing proportions compared to familiar foragers (exact binomial tests, $P<0.001$). Experiments 1 and 2 returned mhf estimates of 0.102 and 0.316, respectively, potentially setting the lower and upper bounds for real mhf values. In other words, 10.2% to 31.6% of exposed honey bees would fail to return to their colony after foraging in a treated crop. For the sake of comparison, foragers live about 6.5 days, and therefore die at an average rate of $1/6.5 = 0.154$ individual.day-1 (27). Therefore, the probability that a forager would die due to homing failure after visiting a treated crop (up to 0.316) may attain twice the probability this same forager has to die naturally that day (about 0.154).

Such an additional mortality might represent a heavy burden to bear for colonies exposed to treated crops in their environment. When implementing mhf into a honey bee population dynamics model (21), all the tested scenarios predicted a major deviation from the expected dynamic (Fig. 4). In our simulations, we considered the evolution of a typical colony during the first three months of a beekeeping season, encompassing the oilseed rape blooming period – April-May in our study area (22). At this time of the year, colonies emerge from the wintering period. Population size is rather low (<20,000 individuals) and gradually expands in order to rapidly increase food storage and ensure colony sustainability. The daily egg-laying rate of the queen is a critical parameter in this colony dynamic because it determines the daily egg-hatching rate, and in turn the rate at which honey bees working in the hive will be replaced as they become themselves foragers. We simulated three scenarios with realistic levels of egg-laying rate

(28), namely a rate allowing for a normal colony development (Fig. 4A), a rate ensuring equilibrium population (Fig 4B) and a slightly deficient rate forcing the population to stabilize at a lower size (Fig. 4C). In each case, we also computed the expected trends if most foragers (90%) were exposed to nectar of treated oilseed rape each day, and therefore had a natural mortality increased by a homing failure probability mhf. Regardless of the queens' egg-laying rate, populations from colonies exposed to the treated nectar would follow a marked decline during the blooming period, and would hardly recover afterwards (Figs 4A-4C). When combined with natural forager mortality, mhf raised total forager death rate up to a point that could hardly be compensated for by the rate at which new foragers are recruited. In the worse scenarios, populations would fall down to 5,000 individuals, which is the lowest level one can usually observe in current beekeeping practices. With an exposure rate reduced to 50% of foragers exposed to treated nectar each day (Figs. 4D-F), the model still predicts a major deviation from normal conditions.

In an attempt to verify the applicability of these results to other contexts, we repeated two additional experiments with two different colonies (Fig S2, Tale S1). In experiment 3, we tested whether mhf was still significant when exposure occurred in the least challenging situation, i.e. in the direct vicinity of the colony and with honey bees familiar with the foraging site. Herein, we repeated experiment 1 with phacelia foragers captured from a beehive placed at the phacelia field margin, and released from inside the phacelia field, only 70 m away. Homing failure (mhf = 0.061, Fig. S2A, Table S1) was much reduced compared to experiment 1 (mhf = 0.102), but was still significant (exact binomial test, P=0.003). In experiment 4, we transposed experiment 2 into a different landscape. A beehive was placed in a suburban area in southern France, including a mosaic of mixed farming fields and orchards of moderate size. Foragers were released 1 km away at six equidistant sites. Homing failure (mhf = 0.098, Fig. S2B, Table S1) was significant as well (exact binomial test, P=0.029), but much smaller than in experiment 2 (mhf = 0.316).

Our study clearly demonstrates that exposure of foragers to non-lethal but commonly encountered concentrations of thiamethoxam can impact forager survival, with potential contributions to collapse risk. Furthermore, the extent to which exposures affect forager survival appears dependant on the landscape context and the prior knowledge of foragers about this landscape. Higher risks are observed when the homing task is more challenging. As a consequence, impact studies are likely to severely underestimate sublethal pesticide effects when they are conducted on honey bee colonies placed in the immediate proximity of treated crops. Finally, this study raises important issues concerning exposed solitary bee species, whose population dynamics are probably less resilient to forager disappearance than honey bee colonies.

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Acknowledgments: This study was funded by the European Community program (797/2004) for French beekeeping coordinated by French Ministry of Agriculture (convention FranceAgriMer 11-45R). Special thanks go to L. Belzunces, J.L. Brunet, B. Vaissière, A. Maisonnasse, D. Fortini, Y. Le Conte and C. McDonnell for valuable help and corrections, as well as three anonymous reviewers for useful comments on the manuscript. We are grateful to M. Grijolot for allowing experiments to take place in his fields. RFID devices were designed by Tag Tracing Solutions Inc., Valence, France. Data are in the supporting online material.

Fig. 1. Honey bee RFID monitoring equipment. (A) A pollen-forager honey bee fitted with a 3- mg RFID tag. (B) A hive entrance equipped with RFID readers for detecting returning marked foragers.

Fig. 2. Study area and location of honey bee release sites relative to the colony hive in experiments 1 and 2.

Fig. 3. Cumulative homing probability of foragers released 1km away from the hive. Temporal gaps denote the nighttime between the first and second days of release. Homing experiment 1 was carried out with foragers familiar with the release site (A) and experiment 2 with foragers released 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).

Fig. 4. Comparison of honey bee population dynamics between simulated colonies exposed to thiamethoxam (red lines) or not exposed (blue lines), following six demographic scenarios. L: queens daily laying rate (nb. of eggs per day). Exp: proportion of foragers exposed to treated crops during the day. The non-exposed colony follows either (A and D) a normal development trajectory (at L=2000), (B and E) an equilibrium dynamic (L=1800), or (C and F) a slightly declining trajectory (L=1600). Shaded areas delineate the exposure period (e.g. oilseed rape). Pairs of trajectories in exposed colonies were obtains with the lower and upper bounds of homing failure mortality (0.102 and 0.316), in order to delineate the best and worse estimates for population dynamics, respectively. Dotted lines extend the declining trajectory expected for a sustained exposure. [simulations derive from demographic models in (21)].

Supplementary Materials: Materials and Methods Figures S1-S2
Table S1

External Databases S1

References (1-29)

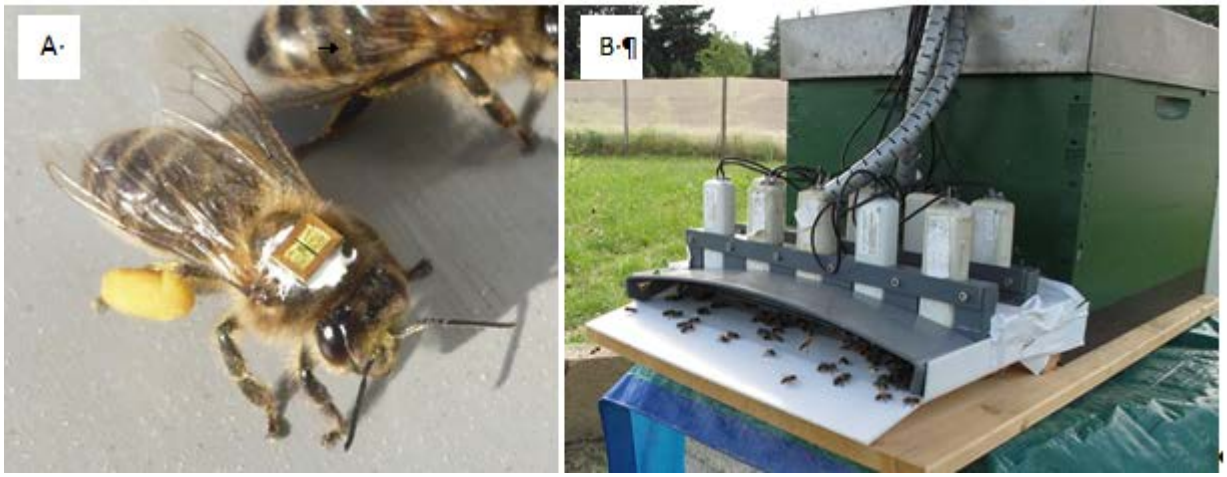

FIG. 1

FIG. 2

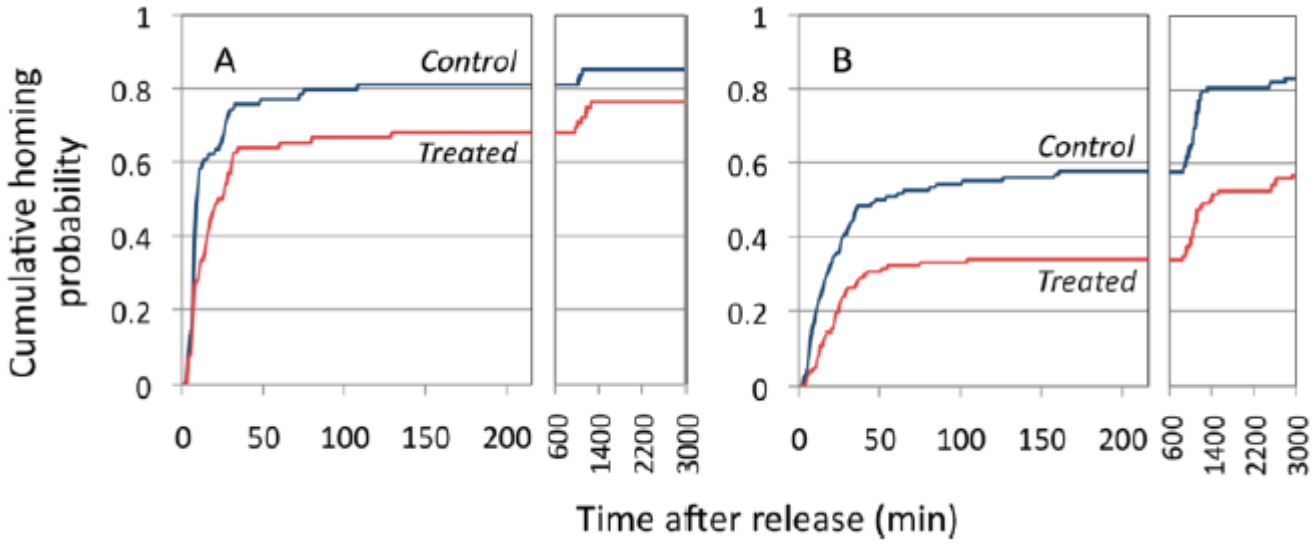

Fig.3

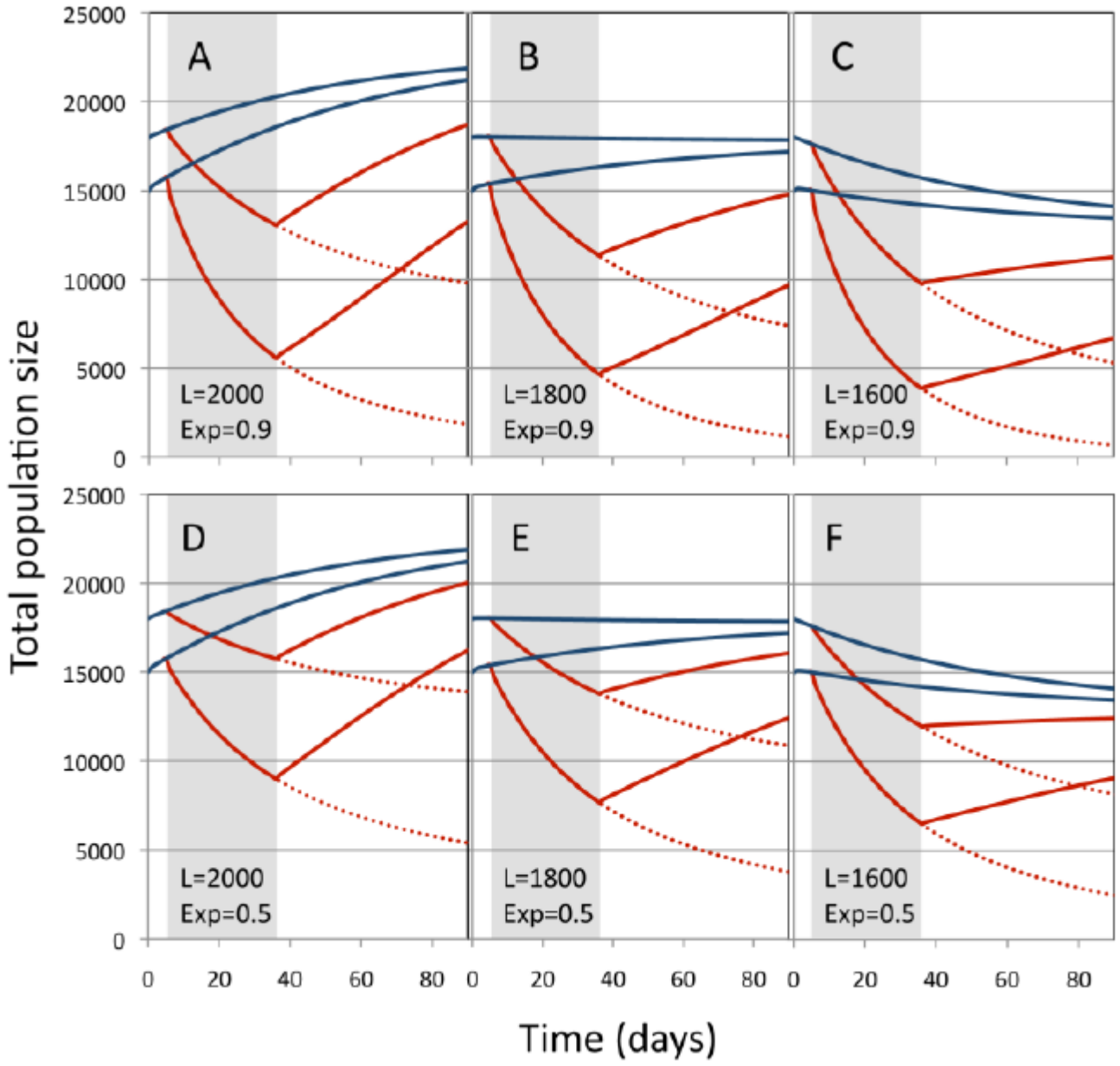

Fig.4