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Science **336**, 348 (2012);

DOI: 10.1126/science.1215039

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A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

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Nonlethal 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 a radio-frequency identification tag suggest that homing is impaired by thiamethoxam intoxication. These experiments offer new insights into the consequences of common neonicotinoid pesticides used worldwide.

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, such as 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, eventually contaminating nectar and pollen (7). Foraging honey bees are therefore directly exposed, but so is 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 nonintentional intoxications in field conditions. Pesticide authorization procedures now require running mortality surveys to ensure that doses encountered in the field remain below lethal levels for honey bees.

However, a growing body of evidence shows that sublethal doses—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 remain 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 (m_{hf}) in exposed foragers. This was achieved by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology (14, 20). Second, we assessed the extent to which m_{hf} in combination with natural

forager mortality, may upset colony dynamics. For that purpose, m_{hf} 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-versus-control homing experiments (22). The study was conducted in an intensive cereal farming system of western France, as a part of the ECOBEE monitoring facility (Zone Atelier Plaine et Val de Sèvre, Centre d'Études Biologiques de Chizé) and in a suburban area in Avignon, southern France. To simulate daily intoxication events, foragers received a field-realistic, sublethal dose of thiamethoxam (a real dose of 1.34 ng in a 20- μ l 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 postexposure homing failure, m_{hf} , was then derived from the proportion of nonreturning foragers. To further discriminate m_{hf} from other causes of homing failure in treated foragers—such as natural mortality, predation, or handling stress—we simultaneously released equal numbers of control foragers fed with an untreated sucrose solution. Hence, m_{hf} was calculated as the proportion of nonreturning treated foragers relative to expectations given by the proportion of returning control foragers. Depending on the experiment, tagged honey bees were released up to 1 km away from their respective colony, 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 m_{hf} per se. Instead, we assessed its upper and lower bounds, depending on whether foragers were familiar or not with the foraging site in which they might get intoxicated. Indeed, one might expect that foragers familiar with the pathway back to the colony are less prone to homing failure than are unfamiliar foragers. Under field conditions, many foragers are probably familiar with the pathway back to the colony because they repeatedly forage on the same

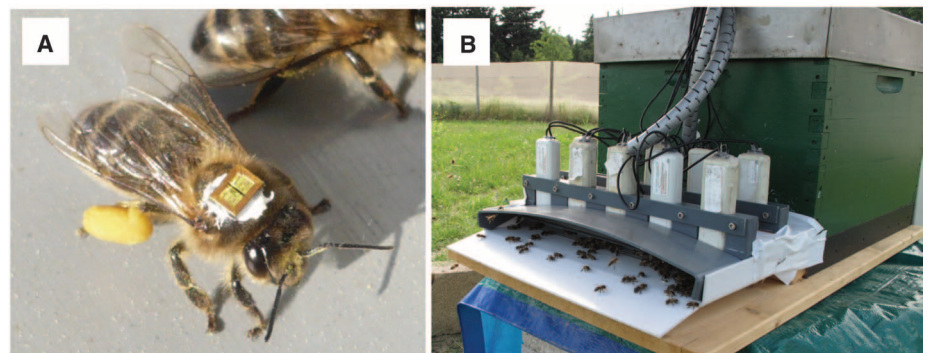


Fig. 1. Honey bee RFID monitoring equipment. (A) A pollen-forager honey bee fitted with a 3-mg RFID tag. (B) A hive entrance equipped with RFID readers for detecting returning marked foragers.

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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 such as 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 m_{hf} re-

spectively. 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 experiments 1 and 2 evidenced substantial mortality due to postexposure homing failure, m_{hf} 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 and table S1). Additionally, m_{hf} was greater in treated foragers that tended to be unfamiliar with the foraging site, as indicated by their significantly lower homing proportions as compared with familiar foragers (exact binomial tests, $P < 0.001$). Experiments 1 and 2 returned m_{hf} estimates of 0.102 and 0.316, respectively, potentially setting the lower and upper bounds for real m_{hf} values. In other words, 10.2 to 31.6% of exposed honey bees would fail to return to their colony when foraging in treated crops on a daily basis. For the sake of comparison, foragers live ~ 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 because of homing failure during a day spent foraging on treated crops (up to 0.316) may attain twice the probability this same forager has to die naturally that day (~ 0.154).

Such an additional mortality might represent a heavy burden to bear for colonies exposed to treated crops in their environment. When implementing m_{hf} 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 3 months of a beekeeping season, encompassing the oilseed rape blooming period, which was April to 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 m_{hf} . Regardless of the queens' egg-laying rate, populations from colonies exposed to the treated nectar would follow a marked decline during the

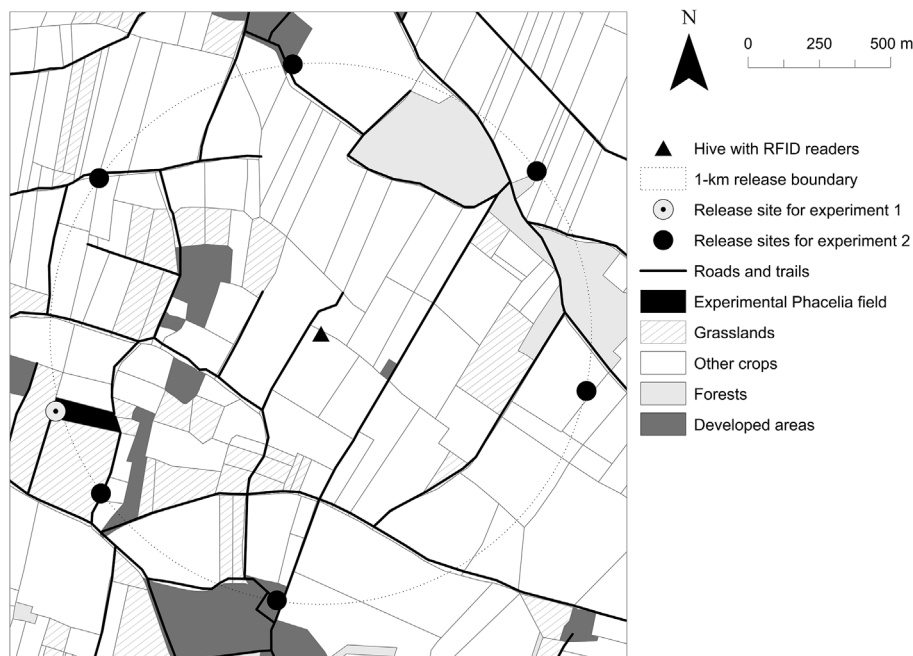


Fig. 2. Study area and location of honey bee release sites relative to the colony hive in experiments 1 and 2.

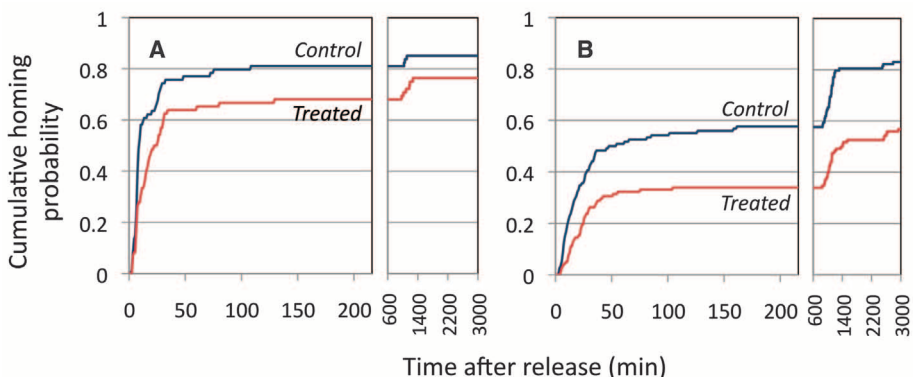


Fig. 3. Cumulative homing probability of foragers released 1 km away from the hive. Temporal gaps denote the nighttime between the first and second days of release. (A) Homing experiment 1 was carried out with foragers familiar with the release site, and (B) experiment 2 with foragers released at random sites regarding their past experience. In both cases, treated honey bees that received a nonlethal dose of thiamethoxam returned to the hive in significantly lower proportions than did control honey bees (table S1).

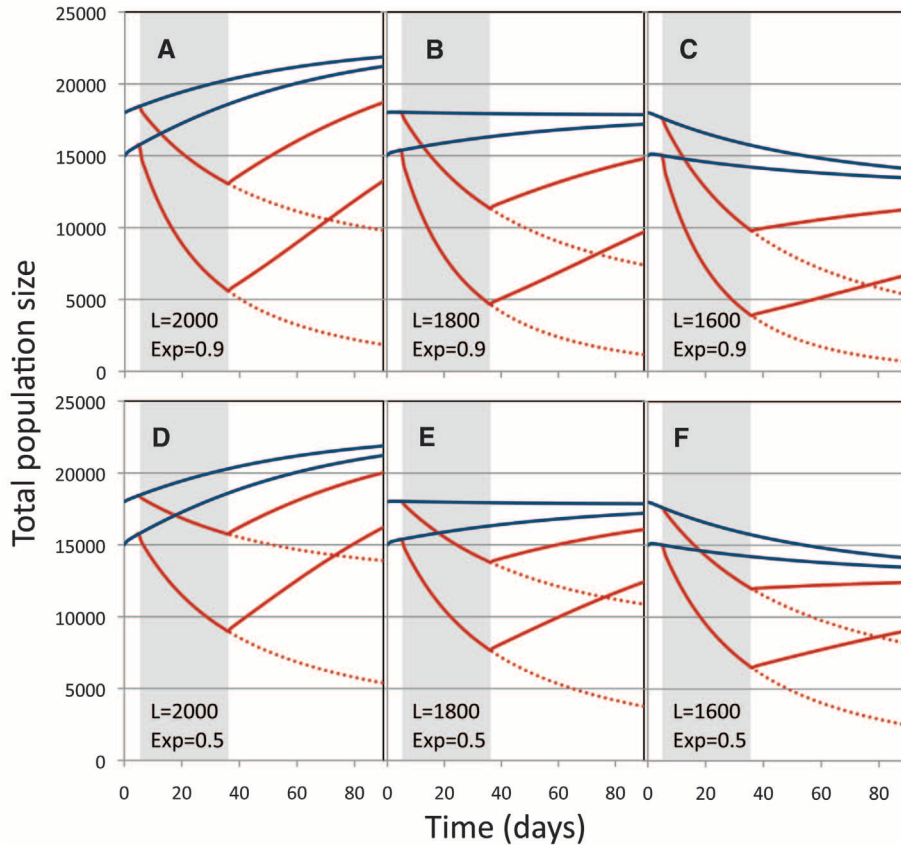


Fig. 4. Comparison of honey bee population dynamics between simulated colonies exposed to thiamethoxam (red lines) or not exposed (blue lines), following six demographic scenarios. L is the queen’s daily laying rate (eggs per day). “Exp” is the proportion of foragers exposed to treated crops during the day. The nonexposed 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 (for example, oilseed rape). Pairs of trajectories in exposed colonies were obtained with the lower and upper bounds of homing failure mortality (0.102 and 0.316) in order to delineate the best and worst estimates for population dynamics, respectively. Dotted lines extend the declining trajectory expected for a sustained exposure. [Simulations derive from demographic models in (21)]

blooming period and would hardly recover afterward (Fig. 4, A to C). When combined with natural forager mortality, m_{hf} 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 5000 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 (Fig. 4, D to 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 and table S1). In experiment 3, we tested whether m_{hf} was still significant when exposure occurred in the least challenging situation: in the direct vicinity of the colony and with honey bees familiar with the foraging site. 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 ($m_{hf} = 0.061$) (fig. S2A and table S1) was much reduced as compared with that of experiment 1 ($m_{hf} = 0.102$) 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 ($m_{hf} = 0.098$) (fig. S2B and table S1) was significant as well (exact binomial test, $P = 0.029$) but much smaller than in experiment 2 ($m_{hf} = 0.316$).

Our study clearly demonstrates that exposure of foragers to nonlethal but commonly encountered doses of thiamethoxam can affect forager survival, with potential contributions to collapse risk. Furthermore, the extent to which exposures affect forager survival appears dependent 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. This study raises important issues concerning exposed solitary bee species, whose population dynamics are probably less resilient to forager disappearance than are honey bee colonies.

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Acknowledgments: This study was funded by the European Community program (797/2004) for French beekeeping coordinated by the 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, C. McDonnell, P. Jourdan, J. P. Vermandère, and V. Bretagnolle for valuable help and corrections, as well as three anonymous reviewers for useful comments on the manuscript. We are grateful to M. Grijot for allowing experiments to take place in his fields. RFID devices were designed by Tag Tracing Solutions, Valence, France, and adapted to honey bees with the help of J. Fourier, F. Brun, J. Devillers, M. Gauthier, and the ECOBEE monitoring facility. Data are in the supporting online material.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1215039/DC1
 Materials and Methods
 Figs. S1 and S2
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10 October 2011; accepted 5 March 2012
 Published online 29 March 2012;
 10.1126/science.1215039