



## Research

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# Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees

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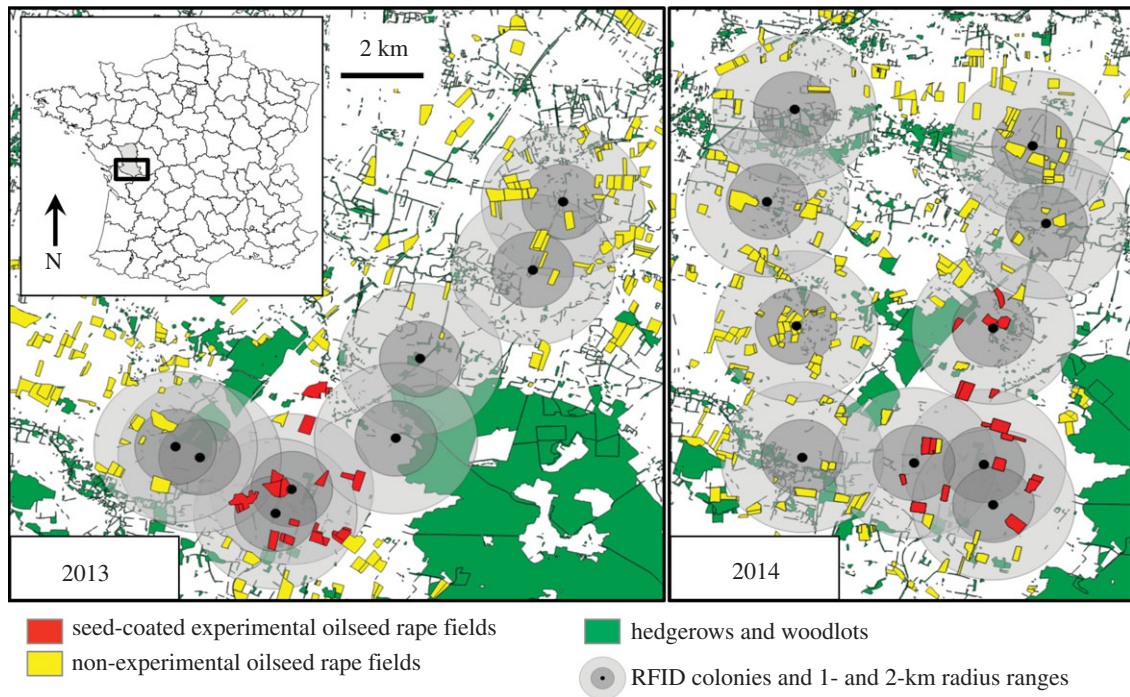
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European governments have banned the use of three common neonicotinoid pesticides due to insufficiently identified risks to bees. This policy decision is controversial given the absence of clear consistency between toxicity assessments of those substances in the laboratory and in the field. Although laboratory trials report deleterious effects in honeybees at trace levels, field surveys reveal no decrease in the performance of honeybee colonies in the vicinity of treated fields. Here we provide the missing link, showing that individual honeybees near thiamethoxam-treated fields do indeed disappear at a faster rate, but the impact of this is buffered by the colonies' demographic regulation response. Although we could ascertain the exposure pathway of thiamethoxam residues from treated flowers to honeybee dietary nectar, we uncovered an unexpected pervasive co-occurrence of similar concentrations of imidacloprid, another neonicotinoid normally restricted to non-entomophilous crops in the study country. Thus, its origin and transfer pathways through the succession of annual crops need be elucidated to conveniently appraise the risks of combined neonicotinoid exposures. This study reconciles the conflicting laboratory and field toxicity assessments of neonicotinoids on honeybees and further highlights the difficulty in actually detecting non-intentional effects on the field through conventional risk assessment methods.

## 1. Introduction

In the current context of global honeybee decline, much attention has been paid to evaluating the possible contribution of neonicotinoid insecticides to colony weakening and collapse [1–3]. These systemic insecticides, which now represent *ca* 30% of insecticide use worldwide [4], pose a particular risk for pollinators, because once the active substance has been taken up in the plant, its residues translocate to the pollen and nectar collected by foragers throughout flowering. However, after 15 years of active research on the side effects of neonicotinoids on bees, a gap has emerged between the results of toxicity assessments in the laboratory and in the field [1–3]. Artificial exposure in laboratory experiments typically consists of providing individuals with contaminated food and comparing relevant physiological and behavioural endpoints with healthy control groups [5]. This approach has led to the identification of a range of sublethal effects, i.e. adverse physiological or behavioural



**Figure 1.** Field exposure experimental design. The maps show the location of the experimental fields with oilseed rape grown from thiamethoxam-treated seeds (23 fields (total 153 ha) in 2013 and 18 fields (135 ha) in 2014). Dots show the positions of beehives fitted with RFID readers, spatially allocated in a way that covers a broad range of field exposure levels. Thiamethoxam field exposure was computed as the sum of all treated surfaces in the territory, with individual field surfaces moderated by an ordinary IDW interpolation. Surfaces of treated fields located farther away than the average honeybee foraging range (1 km) were down-weighted by a  $1/d^2$  multiplicative coefficient, where  $d$  is the distance (km) of the field to the colony. The resulting field exposure values ranged from 1 to 63 (mean =  $15.7 \pm 16.8$  (s.d.)). The most exposed colony (exposure value = 82) showed dramatically high mortality rates compared to the other colonies, but concomitantly developed foulbrood syndromes. It was therefore discarded from the analyses to avoid overstating the excess mortality due to field exposure.

changes measurable at levels well below the 50% lethal dose ( $LD_{50}$ ) currently used as a legal reference threshold for plant protection product approval. Neonicotinoids have been found to have sublethal effects on mobility, orientation, foraging and learning performances [6–11]. However, it is not clear whether these endpoints are reflected in a corresponding effect at field level [12]. The various attempts made so far to appraise the possible consequences of honeybee exposure to treated fields under normal agricultural practices—hereafter called *field exposure*—have failed to detect any noteworthy change in colony performance [13–16]. Without formal identification of deleterious effects in real-field exposure conditions, policy decisions will remain controversial [1–3].

Various explanations have been put forward for the empirical mismatch between laboratory and field experiments. One may argue that laboratory experiments have been conducted at exposure levels much higher than would normally occur in the field [17], or that they have overstated the insecticide effects owing to acute, rather than chronic, exposure. Alternatively, effects in the field may be compensated for by the resilience of honeybee colonies owing to demographic regulation mechanisms and honey storage [1–3]. Exposure effects may also occur after a time lag [18], with colony weakening becoming apparent only later in the season or with lower colony survival the next winter. Space should be taken into account as well as time. For instance, homing failure after artificial exposure has been detected in free ranging bees at the foraging range scale (i.e. 1 km away from the colony [9,10]), whereas field exposure experiments have typically surveyed colonies located in the immediate vicinity of, or even inside, treated crops. Such uncertainty

requires a spatially explicit approach to reconcile laboratory and field exposure experiments.

Here, we show that a landscape-scale increase in exposure to treated oilseed rape fields does indeed entail higher individual mortality. This study follows the recommendations of ANSES, the French food safety agency, to reassess the possible side effects of thiamethoxam [9] under real agricultural usage conditions. We obtained authorization from the French ministry of agriculture to grow winter oilseed rape from seeds treated with thiamethoxam (Cruiser OSR<sup>®</sup> formulation), for experimental purposes. Thiamethoxam is currently prohibited at the national and European levels [19]. With the permission of the land-owning farmers, Cruiser rapeseed was sown in 2 consecutive years on a total of 288 ha (153 ha in 2013 and 135 ha in 2014, figure 1) within the LTER Zone Atelier Plaine & Val de Sèvre area, France. Using RFID technology (radio frequency identification [20,21]), we monitored the life histories of 6847 individual bees in relation to levels of thiamethoxam exposure from the oilseed rape fields, and questioned whether individual survival as well as colony dynamics would vary with field exposure.

## 2. Material and methods

### (a) Study design

The study was initially designed to produce a gradient of real-field exposure to oilseed rape grown from seeds treated with thiamethoxam (figure 1). However, an unexpected concomitant exposure to imidacloprid, another neonicotinoid insecticide, was detected at substantial levels both in the nectar of experimental oilseed rape treated with thiamethoxam, and in the dietary nectar

ingested by foragers (see Results). Therefore, the studied field exposure level referred to in this study actually represents a gradient of combined exposure to both neonicotinoid products.

The field exposure gradient was experimentally achieved by sowing oilseed rape seeds coated with the Cruiser<sup>®</sup> formulation (thiamethoxam content 280 g l<sup>-1</sup>) in a total of 288 ha (153 ha in 2013 and 135 ha in 2014) in a portion of the study area and positioning colonies around at various distances and directions to cover a range of exposure levels to treated fields. The study involved local volunteer farmers and required a special derogation from the French ministry of agriculture due to the current prohibition of any neonicotinoid treatment on oilseed rape.

Eighteen standardized experimental colonies bred from sister queens were set up in 10-frame Dadant hives and monitored in the field for colony dynamics and state of health [22]. Hives were fitted with RFID readers ([21]; electronic supplementary material, figure S1) so as to monitor the life history of a total of 46 cohorts of 100–250 honeybees during the oilseed rape flowering period (one to two cohorts of just-emerged bees per colony and one cohort of adult foragers per colony). We noticed the most exposed colony (field exposure = 82 units, see below) became largely depopulated and showed dramatically high cohort mortality rates unequalled by the other colonies. However, it concomitantly developed foulbrood syndromes. Therefore, we discarded this colony from analyses to avoid any risk of overstating the excess mortality due to field exposure *per se*. Analyses comprise the lifelong monitoring of 6847 individual bees from 17 colonies (1638 bees from 17 cohorts tagged at the foraging stage and 5209 bees from 27 cohorts tagged just after emergence).

## (b) Computation and validation of the field exposure level

Colony field exposure was calculated as the sum of all treated field areas with an ordinary inverse distance weighted (IDW) interpolation (figure 1). Surfaces of treated fields located farther away than the average honeybee foraging range (1 km) were down-weighted by a  $1/d^2$  multiplicative coefficient, where  $d$  is the distance (km) of the field to the focus colony. One field exposure unit is therefore virtually equivalent to 1 ha of treated oilseed rape within a 1-km distance from the colony, or e.g. 4 ha at a 2-km distance.

The resulting field exposure values ranged from 1 to 63 (mean =  $15.7 \pm 16.8$  (s.d.)) for the monitored colonies. Special attention was paid to reducing variability due to landscape composition or configuration. The experiment was carried out in the western part of the study area (*ca* 150 km<sup>2</sup>) characterized by an open field landscape with few semi-natural elements and with homogeneously scattered oilseed rape fields typically accounting for 8–10% of total land cover. Landscape complexity, referring to the amount of non-cropped interstitial habitats around fields (hedgerows and forest edges total length [10]) averaged  $6.6 \pm 2.8$  km km<sup>-2</sup> in the vicinity of the colonies. Total oilseed rape (including treated experimental fields) land cover within a 1-km radius averaged  $29.2 \pm 19.7$  ha. We further ensured that field exposure variations were independent of both total oilseed rape land cover and landscape complexity (Pearson's product-moment correlation,  $r = 0.32$ ,  $p = 0.19$  and  $r = -0.04$ ,  $p = 0.86$ , respectively).

The experimental field exposure gradient was validated by neonicotinoid multi-residual analysis of dietary nectar collected from foragers at each colony entrance (see Results). On three occasions with one-week intervals during oilseed rape blooming, 200 returning honeybees were collected when entering their hive and narcotized with ether in a cage. One by one, their abdomens were gently pressed until their crop nectar content was regurgitated. Nectar samples were then pooled in eppendorf tubes to

get an average value of neonicotinoid residual content per colony and sampling date. The correlative link between field exposure level and neonicotinoid dietary residues was statistically validated by zero-inflated generalized linear mixed models (ZI-GLMM) because an excess of zeroes was detected in the data distribution, thus simple GLMMs did not fulfil model residual normality and homoscedasticity requirements.

## (c) Colony dynamics and radio frequency identification individual monitoring

Colony monitoring comprised systematic inspections for diseases as well as measurements of adult population size, honey reserves and female (worker) and drone (male dispersers) brood production [22]. Measurements were taken at the onset of flowering (18 April 2013 and 25 March 2014), and during the first and the fourth week after the end of flowering. Initial colony state was independent of the field exposure level (Pearson's product-moment correlations, population size:  $r = -0.15$ ,  $p = 0.53$ ; honey reserves:  $r = -0.41$ ,  $p = 0.090$ ; total brood surface:  $r = 0.21$ ,  $p = 0.40$ ; worker brood surface:  $r = 0.22$ ,  $p = 0.37$ ; drone brood:  $r = -0.080$ ,  $p = 0.742$ ). Mean weekly changes of colony parameters were computed for the five-week period referred to as *during* flowering and the three-week period referred to as *after* flowering. Colony changes were analysed against field exposure level using linear models (LM).

The RFID individual lifelong monitoring includes 6847 individual bees (5209 bees tagged just after emergence and 1638 tagged at the foraging stage) assigned to a total of 44 cohorts belonging to 17 colonies. The RFID technology was used to assess three key individual life-history parameters in the context of field toxicology, namely mortality rate, frequency of flight activity and precocious behavioural maturation—with the precocious onset of foraging suspected as a possible mechanism for compensating forager excess mortality [23–25]. The fully detailed information about the RFID system provider, RFID tag characteristics and fixation on honeybees may be found in previously published material [9,10,21]. The cohorts of newly emerged worker bees with homogeneous age were obtained by caging a queen with preselected brood frames containing enough empty cells for eggs [26]. Therefore, each year, newly emerged cohorts were obtained from a single colony independent of the experimental ones, but still originating from sister queens within the same livestock, held at the UE Entomologie of INRA Magneraud. Newly emerged cohorts were tagged and released in colonies during the week preceding the onset of flowering, and with a 5-day interval whenever two distinct cohorts were introduced in the same colony. Returning adult foragers were captured on their way back at the entrance of colonies, RFID-tagged and reintroduced into colonies. The forager tagging sessions occurred *ca* 10–12 days after tagging of the just-emerged bees, i.e. the approximate average duration needed for newly emerged cohorts to perform first flights and become foragers themselves [27–29]. In doing so, newly emerged cohorts and forager cohorts could be simultaneously monitored during their foraging life stage and during the oilseed rape full blooming period. RFID readers record the tag signal of bees passing through the hive entrance and store detection events along with the date and time ( $\pm 0.01$  s). Detections were pooled to the nearest minute, assumed to be the minimal time required for a bee to perform a flight. Therefore, occasional multiple detections of the same individual in less than a minute were considered to be part of the same event.

The hypotheses of (i) increased mortality rate, (ii) precocious behavioural maturation, and (iii) decreased flight activity were investigated using, respectively, the date of the last RFID detection event, the date of the first event (newly emerged cohorts only) and the number of events per day. Tagged honeybees were recorded as alive until the day following last tag detection event. Cox proportional hazard survival models (Cox PH [30]) were then



applied on a daily basis to test whether field exposure was associated with an excess mortality compared to baseline mortality. Cox PH models are semi-parametric analyses specifically designed to test the effect of covariates on the time lapse before occurrence of an event. We applied the Cox PH formula to compare time to last event (increased mortality rate hypothesis) and time to first event (precocious behavioural maturation hypothesis). The non-independency of individuals from the same colony was accounted for by specifying colonies' identity as a grouping cluster. Whenever analysed jointly in the same Cox PH model, cohort types (i.e. foragers versus just-emerged bees) were specified as distinct strata due to their different baseline mortality. Finally, variations of flight activity (number of events recorded per day, normalized with a  $\log[\text{value} + 1]$  transformation) were investigated using GLMMs with colony identity specified as a random grouping variable. Random slopes as a function of date and maximal daily temperature were also allowed to account for the temporal non-independency of repeated measurements as well as the possibly contrasted weather conditions throughout the study. Rainy days (more than 1 mm) were discarded from the flight activity analyses. For all studied life-history traits, inter-annual variations were also explicitly tested, and whenever significant, possible latent interactions with the field exposure level were also inspected. Analyses were performed with the R software for statistical computing [31].

#### (d) Imidacloprid nectar contamination

To investigate the source of imidacloprid contamination in the environment, we also performed neonicotinoid multi-residual dosages on the treated oilseed rape nectar collected with microcapillaries directly from flowers in a subset of nine thiamethoxam experimental fields. Imidacloprid residuals were detected at similar concentrations and frequency in flower nectar samples (0.1–0.9 ppb in six out of nine samples) and honeybee dietary nectar (0.1–1.0 ppb in 13 out of 17 colonies).

Given the substantial levels of imidacloprid residuals unexpectedly found in both oilseed rape flower nectar and in honeybee dietary nectar, we extended the flower nectar survey to as many non-experimental oilseed rape fields as possible within the 450 km<sup>2</sup> study area. A total of 73 oilseed rape fields were thus sampled for nectar between 15 and 24 April 2014. Nectar was collected between 9.00 h and 19.00 h by gently inserting a 5  $\mu\text{l}$  glass microcapillary tube (Drummond Scientific, Broomall, PA, USA) into randomly chosen open flowers [32] until achieving a cumulative volume of 25  $\mu\text{l}$  per field. Nectar samples were collected beyond a 10 m buffer distance from the field margin to avoid any edge effect.

Both flower nectar samples collected from oilseed rape fields and dietary nectar samples collected from forager stomachs were sent to the European Union reference laboratory for neonicotinoid multi-residual analyses (ANSES, Sophia-Antipolis, France). Residues were quantified (limit of detection = 0.1 ppb, limit of quantification = 0.3 ppb) by liquid chromatography with electrospray tandem mass spectrometry [33].

### 3. Results and discussion

In this study we found that individual honeybees near thiamethoxam-treated fields do indeed disappear at a faster rate, but the impact of this is buffered by the colonies' demographic regulation response.

#### (a) Validation of the field exposure design

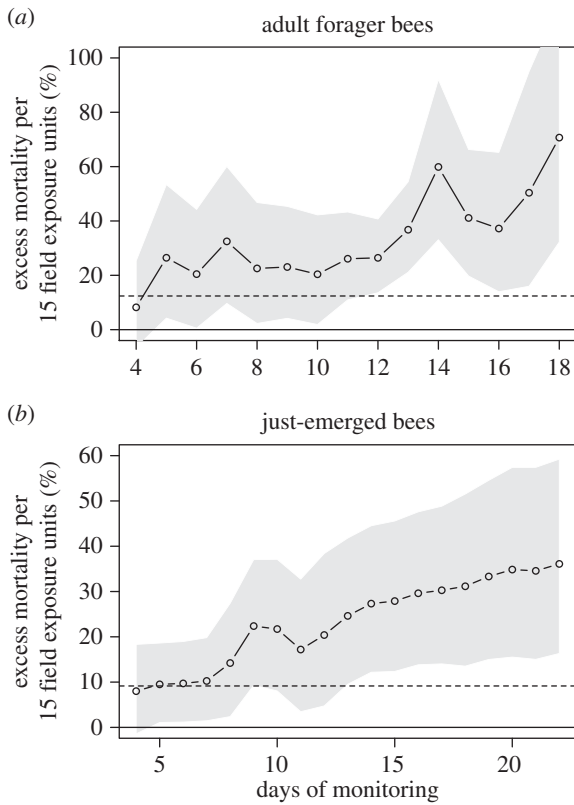
Thiamethoxam residues found in dietary nectar brought back to colonies by foragers increased significantly with experimental thiamethoxam field exposure, validating the

field exposure design (electronic supplementary material, figure S2A). Thiamethoxam residues remained undetected in dietary nectar in colonies with limited field exposure (less than eight field exposure units) and ranged from 0.1 to 0.8 ppb in colonies with the highest field exposure (8–63 units). Furthermore, when dietary nectar thiamethoxam is regressed against field exposure, the intercept is not significantly different from zero (electronic supplementary material, figure S2A), confirming that the least exposed locations may be viewed as thiamethoxam-free environments for a sound basis of comparison. However, we discovered an unexpected and substantial concomitant exposure to imidacloprid (electronic supplementary material, figure S2) in the dietary nectar samples (0.1–1.0 ppb in 13 out of the 17 surveyed colonies). Those residual levels are high enough to potentially exert side effects on bees [34,35]. In France, this neonicotinoid insecticide is currently used as a seed dressing treatment (Gaucho® formulation) for a range of non-entomophilous crops such as wheat, barley and sugar beet, but has been prohibited for sunflower since 1999 and has never been used on oilseed rape [36]. Imidacloprid residues correlated with our experimental field exposure level and *a fortiori* with thiamethoxam residues (electronic supplementary material, figure S2B and C). This unexpected contamination forced us to reconsider the experimental thiamethoxam field exposure as a combined (thiamethoxam and imidacloprid) neonicotinoid field exposure.

#### (b) Honeybee survival and life histories in relation to field exposure

Honeybees disappeared at a faster rate with increasing field exposure and this excess mortality increased over time (electronic supplementary material, table S1). The baseline mortality increased by 10.1% ( $\text{CI}_{95\%} = 3.0\text{--}17.7\%$ ) per 15 field exposure units (Cox PH survival analysis,  $n = 78\,716$  daily records for 6847 bees,  $z = 2.85$ ,  $p = 0.004$ ). However, we detected a highly significant deviation from hazard proportionality ( $\chi^2 = 258$ ,  $p < 0.001$ ), indicating that the excess mortality was not stationary but actually increased with time. We therefore reassessed the field exposure effect, expressing it in interaction with time [30]; this indeed returned a better model fit to the data (Cox PH, field exposure effect:  $z = 3.52$ ,  $p < 0.001$ ). The level of excess mortality was now raised by 5.6% per 15 field exposure units per additional week, i.e. from an average 5.6% excess mortality at the onset of flowering to 22.4% after three more weeks had passed. This time-mediated survival pattern was consistent between the two study years (Cox PH, inter-annual variations:  $z = 0.41$ ,  $p = 0.68$ ), and might be interpreted either as a consequence of the accumulation of neonicotinoid residues in food and hive materials over time, with a delayed effect [34], or as the emergence of a chronic exposure effect [18]. The field exposure effect appeared to be independent of the landscape spatial context (electronic supplementary material, table S2), contrary to what would have been expected from previous studies [10,37].

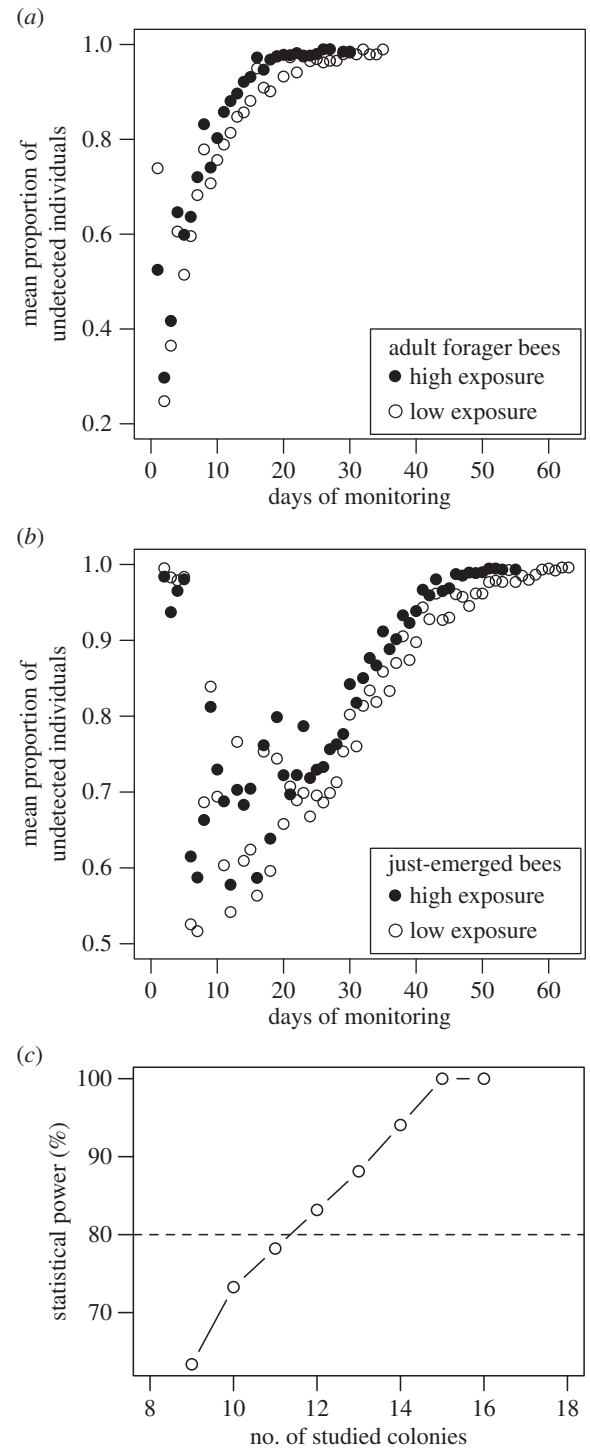
To rule out possible confounding effects due to cohort types, we recomputed separate non-stationary survival models for foragers ( $n = 1638$  bees from 20 cohorts) and just-emerged bees ( $n = 5209$  bees from 24 cohorts). We confirmed that field exposure accelerated the disappearance of both forager and just-emerged bee cohorts (figure 2), but



**Figure 2.** Non-stationary excess mortality due to field exposure during oilseed rape flowering. The honeybee excess mortality level due to field exposure is not stationary, but steadily increases in time. (a) Non-stationary excess mortality due to field exposure in 1638 bees tagged at the foraging stage with RFID microchips. (b) Non-stationary excess mortality due to field exposure in 5209 bees tagged after emergence with RFID microchips. Shaded areas show the 95% confidence envelope of the Cox PH estimate of excess mortality, expressed in per cent of the baseline mortality, and indicating a significant field exposure effect whenever it is above zero. Excess mortality was sequentially reassessed along the temporal axis using a left-censoring procedure, i.e. discarding newly disappeared individuals at each time step. For the sake of comparison, dashed lines show the field exposure effect that would be found on the assumption of stationary excess mortality.

the excess mortality was 2.4 times higher for the former group (11.9% versus 4.9% excess mortality per 15 field exposure units and per week; electronic supplementary material, table S1). As an illustration of the non-stationary hazard during oilseed rape flowering, the excess mortality linked with field exposure increased steadily throughout the survey for both cohort types (figure 2). The rise in mortality is quite clear (figure 3a,b) when average survival below the field exposure threshold that entails no dietary residues of thiamethoxam (field exposure < 8 units,  $n = 24$  cohorts from nine colonies) is compared with average survival above that threshold (more than 8 units,  $n = 20$  cohorts from eight colonies). An *a posteriori* power analysis (figure 3c) established that the field exposure effect as revealed by Cox PH survival analyses was strong enough to be satisfactorily detected (statistical power > 80% [38]) with a subset of 12 out of our 17 surveyed colonies.

Orientation disorders reported by artificial exposure experiments [9,39,40] may contribute to explaining our field exposure results. Conversely, a recent treated versus control field exposure RFID survey reported no effect of foraging on thiamethoxam-treated oilseed rape [41]. However, this study was carried out at a very different spatial scale and



**Figure 3.** Mean cumulative disappearance of monitored individuals under high versus low field exposure. Mean proportion of (a) undetected foragers and (b) undetected just-emerged bees over time with high (8–63 units, black dots) and low (less than eight units, white dots) field exposure values. The high non-detection levels during the first week correspond to the in-hive life stage before first exits in young bees. (c) A posteriori power analysis showing the ability (%) of the Cox PH survival analysis to detect the significant excess mortality due to field exposure out of random subsets of the 17 experimental colonies ( $n = 100$  subsets for dots up to 14 colonies, and  $n = 50$  and 16 subsets for 15 and 16 colonies, respectively). Dashed line shows the recommended 80% statistical power threshold.

with no molecular validation of dietary nectar contamination, therefore precluding any meaningful comparison. In particular, treated and control treatments from that study would both fall in the very initial part of the field exposure range we actually covered here, with fairly low scores (*ca* 1–2

field exposure units, with a single 2-ha treated field and 1-km isolation distances [41]). This study encompasses up to 63 field exposure units, and reports evidence of thiamethoxam residues only beyond eight field exposure units.

An increased forager mortality rate is expected to trigger the precocious recruitment of younger, less efficient, foragers as an adaptive compensatory mechanism [23–25]. We, however, found no evidence that the excess mortality due to field exposure was further echoed by side effects on age at first exit (electronic supplementary material, table S3) or daily rate of flight activity (electronic supplementary material, table S4). Although no significant modification of age at first exit was detected, we noticed a significant non-stationarity, i.e. a significant variation over time, in the rate at which just-emerged monitored bees performed their first flights in relation to field exposure (model 1 in electronic supplementary material, table S3). Up to the 10th day of monitoring, first flights occurred 19.6% *earlier* for an average field exposure of 15 units. But up to the 20th day of monitoring, first flights occurred on average 8.8% *later* in the honeybee life for an average field exposure of 15 units, therefore offsetting the apparent first flight precocity. This non-stationarity of age at first flight along the field exposure gradient suggests that a portion of the monitored young bees indeed became precocious foragers while the others were compelled to spend a longer lifetime as in-hive worker to cope with the increased nursing tasks. Though rather speculative, this tentative scenario is consistent with the observations made at colony level on brood production dynamics (electronic supplementary material, table S5 and figure S3), as detailed below.

### (c) Colony dynamics in relation to field exposure

In spite of the excess mortality measured at the individual scale, highly exposed colonies did not show altered performance *per se* in terms of population growth and honey and brood production. However, there was a change in the way reproductive effort was allocated between female (worker) brood and drone (male disperser) brood. The field exposure did not significantly affect week-by-week changes in the adult population during or after oilseed rape flowering (models 1 and 7 in the electronic supplementary material, table S5), or weekly honey storage or weekly brood production (models 2, 3, 8 and 9 in the electronic supplementary material, table S5), at least within the effect size resolution limits permitted by the study sample size (statistical power analysis, smallest detectable effects estimated to  $\pm 21$ –58% of the mean; electronic supplementary material, table S5). But the field exposure did trigger a significant change in the relative proportions of worker brood versus drone brood production (electronic supplementary material, figure S3). During flowering, the most exposed colonies tended to invest more in worker brood production at the expense of drone brood production (electronic supplementary material, figure S3A). Drone brood development was delayed in exposed colonies; after flowering, drone brood production followed the field exposure gradient, being significantly higher in the more exposed hives (electronic supplementary material, figure S3B).

Rather than a decline in colony performance strictly speaking, these patterns should be viewed as a by-product of colonies' demographic compensation and regulation of reproductive investment [42]. Drones are more costly to produce

and maintain than workers, among others because they do not participate to the foraging task force. Thus, colonies decrease drone production when foraging conditions are poor, either due to resource scarcity [42] or seemingly due to forager excess mortality. Drone production typically peaks in spring [22,42] when virgin queens are most abundant and then when drones are most likely to successfully mate and pass along genes to other colonies. Delayed drone production might somehow disrupt this biological synchrony, and should therefore be addressed in terms of mating success or fitness value of reared drones. This should be further explored along with colony performance metrics using large colony monitoring datasets for enhanced statistical power.

### (d) Imidacloprid nectar contamination

Likewise, more detailed studies on the environmental fate of neonicotinoid residues are urgently needed to properly control for potential confounding effects or synergistic effects between different active substances. Indeed, the concomitant occurrence of imidacloprid residues makes it difficult to assign the excess mortality to thiamethoxam alone or to a combined effect, as has been revealed in various combined exposure trials [43,44]. An increasing number of studies [45–47] report substantial contamination of soil and puddles by neonicotinoid residues that may be subsequently taken up by the next crop in the succession. To investigate the source of imidacloprid contamination of the dietary nectar, we collected and analysed oilseed rape floral nectar samples from 82 oilseed rape fields in the study area in 2014 (nine of the treated experimental fields and 73 additional fields). Imidacloprid was undetected in the nectar of 30 sampled fields (36.6%), and varied from 0.1 to 1.6 ppb (median = 0.4 ppb) in the remaining 52 fields (63.4%). Those results concur with the substantial re-uptake of neonicotinoid residues recently reported in pollen and nectar samples from wild flowers in field margins [48], sometimes at even higher concentrations than in the flowering crop nectar itself. Various hypotheses may be proposed for those observations, including the persistence and accumulation of neonicotinoid residues in the soil throughout one or more annual crop succession cycles, their lateral movement and leaching in adjacent slopes, or even possible contaminations via the seed-coating machinery [48]. The precise pathway by which imidacloprid used on wheat or barley can transfer to oilseed rape nectar later on therefore requires urgent clarification because it is liable to compromise any effort scientists and risk assessors make to reconcile the findings of laboratory and field exposure surveys.

## 4. Conclusion

Overall, our results lead to two main conclusions. First, we found that field exposure to thiamethoxam combined with concomitant imidacloprid contamination is associated with a significant excess mortality in free-ranging bees. This provides a strong and unprecedented link between predictions drawn from artificial exposure experiments [9–11,40] and evidence from real-field surveys. Second, colonies appeared to be able to compensate for the excess mortality so as to preserve unaltered performance in terms of population size and honey production. Instead, the most exposed colonies modified the timing of their reproductive investment, delaying



drone brood production in favour of increased worker brood production. We have now reconciled the conflicting laboratory and field assessments of neonicotinoid toxicity. It is thus urgent that risk assessors take into account the scientific evidence for behavioural disorders triggered by trace levels of neonicotinoids.

**Data accessibility.** Detailed data and statistics supporting the results are available in the electronic supplementary material.

**Authors' contribution.** M.H. and N.C. contributed equally to this work. All authors contributed to reviewing and editing the manuscript. N.C., V.B. and A.D. conceived and designed the study. N.C., J.F.O., P.A., C.R., M.G. and A.P. coordinated the fieldwork and collected the data. M.H., N.C., C.R., M.G. and V.B. analysed the data. M.H., N.C., A.D., J.F.O., P.A. and V.B. prepared the manuscript.

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