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Honeybee tracking with microchips: a new methodology to measure the effects of pesticides

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Abstract Losses of foraging bees are sometimes attributed to altered flight pattern between a meliferous plant treated with an insecticide and the hive. Only a limited number of studies has investigated the impact of pesticides on homing flight due to the difficulty of measuring the flight time between the food source and the hive. Monitoring the flights of the foraging bees needs their individual identification. The number of bees monitored simultaneously and the time span during which observations can be made limit most of the monitoring techniques. However, techniques of automatic tracking and identification of individuals have the potential to revolutionize the study of the ecotoxicological effects of xenobiotics on the bee behaviors. Radio Frequency Identification (RFID) offer numerous advantages such as an unlimited number of codes, a large number of simultaneous recording, and a quick reading, especially through materials (e.g., wood). The aim of this study was to show how the RFID device can be used to study the effects of pesticides on both the behavioral traits and the lifespan of bees. In this context, we have developed a method under tunnel to automatically record the displacements of foragers individualized with RFID tags and to detect the alteration of the flight pattern between an artificial feeder and the hive. Fipronil was selected as test substance due to the lack of information on the effects of this insecticide on the foraging behavior of free-flying bees. We showed that oral treatment of 0.3 ng of fipronil per bee (LD50/20) reduced the number of foraging trips. The strengths of our approach were briefly discussed.

Keywords Honeybee · Pesticides · RFID tags · Life-history traits · Foraging · Automatic monitoring

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Introduction

The behavioral effects of pesticides on the honeybee are largely investigated since the last 10 years (Desneux et al. 2007). This fact relies on depopulations of hives which have been observed by beekeepers near field sowed with seed-dressing treated plants. Beekeepers assumed that foragers collecting nectar and pollen were exposed to low doses of insecticides, during their foraging trips, which induced behavioral effects that subsequently impair homing flight (Maxim and van der Sluijs 2007; Chauzat et al. 2009). Many studies were carried out in order to assess the effects of pesticides on behavioral traits (Desneux et al. 2007), and more particularly on the foraging behavior of

bees treated with the two offending insecticide families, neonicotinoids (e.g., imidacloprid; Decourtye and Devillers 2010) and phenylpyrazols (e.g., fipronil; Kacimi El Hassani et al. 2005; Aliouane et al. 2009). Repeated pesticide exposure during foraging trips can induce disabilities of mobility or spatial orientation, and finally honeybee losses. Many symptoms reveal the effects of a toxic chemical on motor function: knockdown effects, uncoordinated movements (or staggering), trembling, tumbling, abdomen tucking, rotating and cleaning of abdomen while rubbing hind legs together, decreased walking (Desneux et al. 2007). For example, early symptoms of poisoning appeared after oral ingestion of imidacloprid, such as stationary behavior (Medrzycki et al. 2003), staggering, tumbling, hyperactivity and tremors (Suchail et al. 2001). Acute fipronil exposure had no effect on motor activity whatever the route of its administration (oral or topical) (Kacimi El Hassani et al. 2005) but repeated exposure to fipronil induced significant increase in immobility (Aliouane et al. 2009).

More realistic experimental situations were designed in relation to feeding behavior and social communication. An impairment of the homing flight (Cox and Wilson 1984), or of the dance behavior was found after exposure of foragers to insecticides (Schricker and Stephen 1970). In an insect-proof tunnel, where the feeder was located 8 m far from the hive, the homing flight of foragers topically treated with sublethal doses of deltamethrin was altered (Vandame et al. 1995). With similar test design, the impact of imidacloprid on homing flight was evaluated in field with a 500-m-distance between feeder and hive (Bortolotti et al. 2003). At the concentration of 100 μg kg^{-1} foragers fed with imidacloprid-added syrup returned to the hive, but this treatment caused a temporary inhibition of the foraging activity, lasting more than 5 h (Bortolotti et al. 2003). Foragers fed with 500 and 1000 μg kg^{-1} of imidacloprid were seen neither at the hive nor at the feeding site, for the 24 h after the treatment (Bortolotti et al. 2003). Yang et al. (2008) also showed that when bees were orally treated with imidacloprid-added syrup higher than 1.2 μg/l (about 1 μg kg^{-1}), they delayed their return visit to the feeding site and the lowest effective concentration for inducing this effect was found to be 50 μg kg^{-1}.

These studies recorded the trips between a feeder and a hive, and the bees were captured on the feeder and marked with paint or colored number tags. These techniques are limited in the number of individuals simultaneously monitored, and in the time devoted to recording individuals. To correct these inconvenient, we tried to develop an automatic tracking and identification system of the individuals. The use of transponders has the potential to revolutionize the study of insect life-history traits, especially in behavioral ecotoxicology. Different transponder devices have been used on the honeybees: harmonic radar (e.g., Riley and Smith 2002) and Radio Frequency Identification (RFID; Streit et al. 2003). Currently, the RFID tags seem to be the technology offering the most advantages. Among them, we can cite an unlimited number of individual insects that can be tracked, a large number of events recorded in parallel, a quick reading (milliseconds), a detection of tags that can be efficiently made though a variety of substances (propolis, glue, plastic, wood…), and less disturbing effect than harmonic radar (antenna of 16 mm carried by the insect).

With RFID readers localized at the hive entrance and at the feeder site, we tried to measure the behavioral traits of tagged foragers under semi-field conditions, and more precisely the duration of the flight time between the feeder and the hive. Our aim was to prove that such an experimental set-up can be used to study the effects of insecticides on foraging behavior. The insecticide fipronil was chosen as an example because, contrary to imidaclopid (Bortolotti et al. 2003; Yang et al. 2008), no work was reported on the effect of fipronil on the foraging behavior in free-flying bees.

Materials and methods

Insects

Experiments were conducted on a colony of Italian honeybees (*Apis mellifera ligustica* L.) comprising about 20,000 workers and a fertile 1-year-old queen. Honeybees were confined in a 10-comb Dadant hive with 8 combs (four brood combs, two honeycombs and two empty combs). The combs were positioned near the middle of the hive body with a division board on each side. A sanitary control was carried out on the colony that received no chemical treatments (e.g., varroacide) for at least 4 weeks prior to experiments. The experimental colony was maintained in an outdoor tunnel (8 m × 20 m, 3.5 m high) covered with an insect-proof cloth (2 mm × 2 mm mesh) and a ground covered with a double layer of clear polyethylene plastic. The hive entrance was south-facing. Bees were fed with polyfloral pollen which was renewed daily. A quantity of 60 g of pollen per day was offered in a sheltered plastic dish. Pollen came from commercial sources. A sucrose solution (50% w/w) was delivered by a feeder positioned 18 m far from the hive entrance, in a wooden box (26 cm × 26 cm, 30 cm high). The bottom of the box consisted in a reservoir of syrup allowing the feeder to be always filled, to keep bees coming regularly (P. Aupinel, unpublished). Foragers were gradually attracted to go into the box, so as to establish a fixed flying pathway between the hive and the feeding box.

RFID

The RFID technology (Streit et al. 2003) allows detecting each time a tag-equipped bee was passing in nearness of a reader (working distance of 3 mm). A visual check demonstrated that one out of 300 passing was not recorded by the RFID readers (Streit et al. 2003). The principle depends on the emission of a radio signal emitted by the reader which is received by the tag positioned on the bee. The tag is not equipped with a power source (passive function) and it obtains its operating power from the reading process to emit a unique identification code. Reader automatically recognizes a virtually unlimited number (18 × 1018 possible identification codes) of individual insects.

We used RFID tags (mic3®-TAG 64-bit RO, iID2000, 13.56 MHz system, 1.0 mm × 1.6 mm × 0.5 mm; Microsensys GmbH, Erfurt, Germany), weighing about 3 mg (3% of bees' weight) which is low knowing that a honeybee is able to carry up 70 mg of nectar (Ribbands 1953) and 10 mg of pollen (Hodges 1952). An tag-equiped bee passing underneath the reader is identified by the reader that sends the data along with real-time recording to a database. Five readers (iID2000, 2k6 HEAD; Microsensys GmbH, Erfurt, Germany) were placed at the entrance of the hive and the artificial feeder (five readers per recording point, with a total of ten readers). Each reader spanned a tunnel of 80 × 8 mm (7 mm high). As passing underneath the reader both at the hive and at the feeder, the foraging bee is monitored twice, thus determining the direction of target and the walking time between the two recording points. The reader software ("BeeReader", Tag Tracing Solution, Valence, France) records the identification code and the exact time of the event in a notebook (.txt). The data are collected automatically for 6 days, pre-processed and saved in a database for analysis of spatial and temporal information according to treatment modality. We created an easy-to-use interface (TimeBee®, CTIS, Rillieux-La-Pape, France) designing the groups of interest (here: treatment modality) monitoring the experimental progress online and analyzing the data. From the two notebooks—one from the hive and the other one from the feeding station—TimeBee® can provide one raw file per identified honeybee where time is expressed in seconds, or in hours, minutes and seconds. A table providing the life-history traits of each bee can be produced: the time spent within the hive, the time spent within the feeder, the time spent between the feeder and the hive, the number of entries into and exits from the hive, the number of entries into and exits from the feeder.

Experimental procedure

Bees were captured on the sugar syrup feeder, divided into groups of 45–50 individuals and then placed in cages (12 × 11 cm, 8 cm high) equipped with a water supply and a sugar syrup feeder (50% w/w). Three cages of 45–50 bees were used for each dose of fipronil. One cage contained bees used to replace those bees that died or escaped. The bees were kept in the controlled conditions of the laboratory (temperature of 25 ± 2°C and artificial light) while they were labeled with the RFID tags. In order to fit the RFID tags on the bees, they were immobilized without using anesthetic in a holding cage. The transponder was glued to the back side of the thorax in such a way that the movements of the wings were not hampered. Gum Arabic, drying within 1 min, was used. After feeding with contaminated or control sugar syrup (see below) the bees were released back onto the top of the frames into their original colony at the end of the day. These operations out of the tunnel lasted for 6 or 7 h.

Insecticide treatment

Technical grade fipronil (98% pure) was purchased from Cluzeau Info Labo (Sainte-Foy-La-Grande, France). Fipronil was tested at two different concentrations, with a geometrical progression of five, i.e., 0.06 and 0.3 ng per honeybee. The 48 h median lethal dose (LD50) value for oral treatments was 6 ng per bee (Decourtye et al. 2005). So the highest tested concentration corresponded to LD50 divided by twenty. From previous results (Decourtye et al. 2003), it was assumed that this ratio belonged to a sublethal domain. The oral administration was chosen because ingestion of contaminated nectar following plant treatment by seed dressing is the main potential exposure route (CST 2004). Stock solutions were prepared in ethanol. Following the guidelines, this solvent was chosen as a rather generalist solvent (EPPO 1992). Fipronil was added to a sucrose solution (50% w/w). The final concentration of ethanol in the sucrose solution was 1% (v/v). The effects of insecticide-added solutions were compared with those of an untreated sucrose solution (with 1% ethanol v/v). After a 3-h starvation period, each group of tagged foragers received a volume of the contaminated or the control sugar solution, at light and at 25 ± 2°C. The volumes were adjusted for a consumption of syrup estimated to 10 µl per bee. So, the concentrations of fipronil in syrup corresponding to the doses of 0.06 and 0.3 ng per bee were 6 and 30 µg/kg. After complete consumption of the sugar solution, the bees were put back into an incubator (darkness, 25 ± 2°C, 40 ± 10% RH). After a new 2-h starvation period, the bees were provided with an untreated sugar solution ad libitum.

Data analyses

Data were analyzed with R 2.9.1 (R Development Core Team 2009). The analysis of participation time of each tagged individual was performed like a survival analysis

with a Cox proportional hazard model (Cox 1972). In our study, we assume that the last event recorded by RFID revealed the death time for each individual. Thus, the survival distribution was estimated directly from the last time of the records. The interest of the Cox model to define a survival model in the honeybee was previously shown by Dechaume-Moncharmont et al. (2003). The hazard function or missing rate is the instantaneous probability of missing for individuals still alive. The Cox model assumes that the individual hazard function depends on a common baseline hazard and the values of the covariates. Given two individuals with particular values for time-independent covariates, the ratio of the estimated hazards over time is supposed to be constant in time. The individual hazard functions are proportional to hazard function of an individual under treatment i at time s and have the following form:

$$\lambda_i(s) = \lambda_0(s)\exp\{t_i + \alpha_i X_i(s)\}$$

where λ_0 is the unknown baseline hazard function at time s; t_i is the effect of the treatment i; $X_i(s)$ is the proportion of dead bees before time s in treatment i; α is the interaction between the proportion of missing bees and treatment i.

Kruskal–Wallis tests estimated the homogeneity of the behavioral results (homing time, number of bees without return to the hive, number of foraging flights per bee) which allowed us to determine whether the distributions differed between the three treatments (d.f. = 2, $P < 0.05$). This statistic can mean that behavioral endpoint from the three bees' groups differs, but does not reveal which group differ from one another. We used the covariance matrix for the Wilcoxon statistic generated by the R procedure to calculate statistics for each pairwise comparison and a Bonferroni adjustment to stabilize the experiment-wise error rate.

Results

Survival model

The complete model described by the equation described above, where the treatment covariate and its interaction with the proportion of missing bees are taken into account was compared with the null model of interest ($\lambda_i(s) = \lambda_0(s)$). The log-likelihood ratio test between the complete model and the null model nested in the complete model was realized (d.f. = 2). The SL which represents the log-likelihood ratio is 3.67. Consider models, and let the value of the maximized log-likelihood for each model be (Li) and log(Lj), respectively. We compared the two models by their statistical SL = −2[log(Lj) – log(Li)] that has an asymptotic χ^2 distribution under the null hypothesis that the coefficients of the additional variables from Mj to the complete model Mi are zero. The P value associated with the χ^2 is 0.16, showing a no significant difference between the complete model and the null model. The cumulative mortality proportion was plotted in Fig. 1. The treatment effect was not significant. Table 1 gives the coefficient estimates for this treatment effect for each dose. Fipronil at the two doses did not increase the hazard of death of tagged bees. On another hand, no symptoms of poisoning or abnormal behaviours were recorded during the whole trial period in any of the treatment groups.

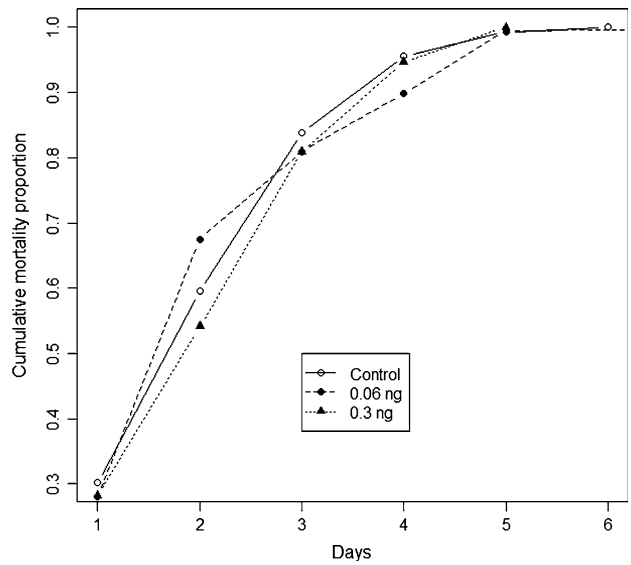

Fig. 1 Cumulative mortality proportion according to day after treatment

Table 1 Coefficient estimates for the treatment effect (ti) and its standard error (SE) on fitting the complete model for each dose of fipronil

Dose of fipronil	ti	SE	Exp{ti}[a]	P[b]
0.06 ng	−0.49070	0.28393	0.61220	0.084
0.3 ng	−0.05754	0.26288	0.94408	0.827

[a] The hazard function of a bee exposed to a chemical was globally multiplied by exp{ti}

[b] This table gives the P value of the Wald's test for the significance of the estimated coefficient in complete model. For all the doses, the value exp{ti} did not differ significantly from 1

Number of foraging flights per bee

The Kruskal–Wallis test supports the implication that the number of foraging flights for the three groups differ ($P < 0.0001$) but does not reveal which group differs from one another. We used pairwise comparisons using Wilcoxon rank sum test and a Bonferroni adjustment to stabilize the experiment-wise error rate. This analysis indicates statistical differences in the number of foraging flights only the first day after releasing the treated bees. There was a

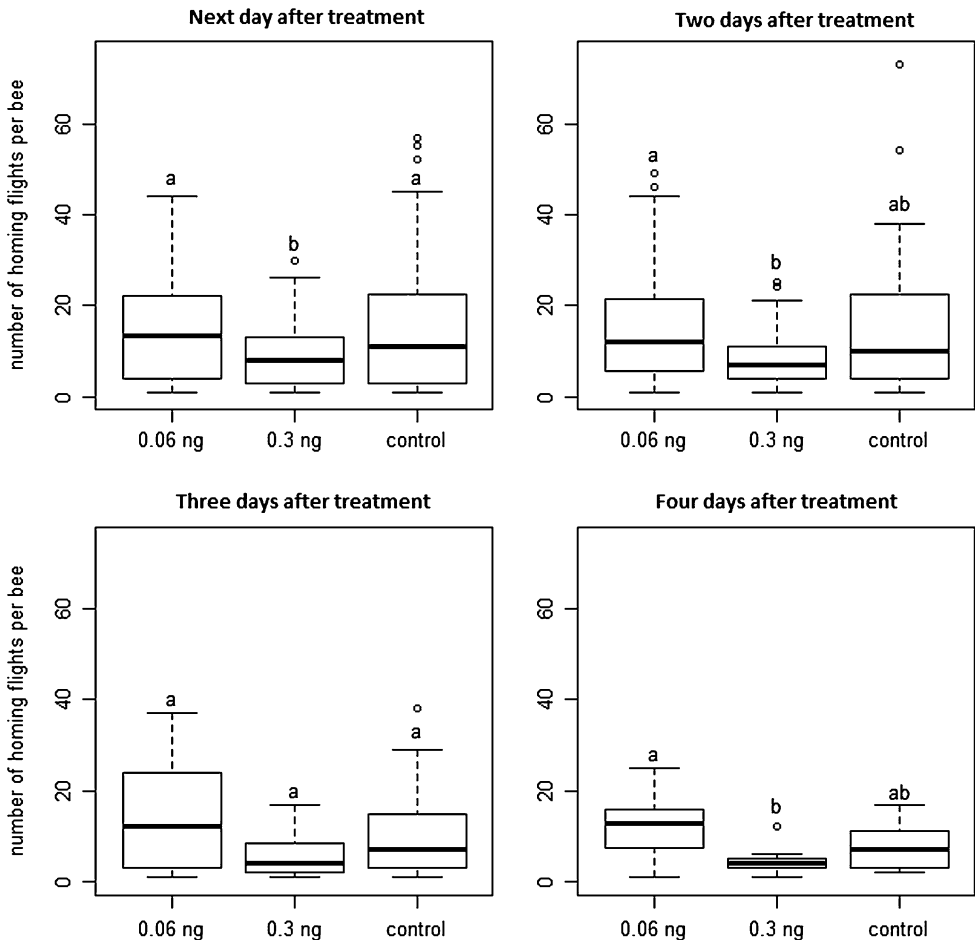

Fig. 2 Number of foraging flights per bee according to treatment. n* for next day after treatment = 103, 122, 105. n* for 2 days after treatment = 64, 59, 65. n* for 3 days after treatment = 26, 31, 27. n* for 4 days after treatment = 12, 19, 9. *Different letters* indicate significant difference with pairwise comparisons using Wilcoxon rank sum test (*P* value adjusted by Bonferroni method: $P < 0.025$).
* n: number of bees for control, 0.06 and 0.3 ng, respectively

significant effect with 0.3 ng of fipronil per bee (Fig. 2). Bees treated at this dose had the lowest number of foraging trips within the first 24 h; after this day, the number of trips for this group resembled those of others groups.

Rate of bees that do not return to the hive

The percentage of disappearing bees between the feeder and the hive (with last hit recorded in feeder) was similar for all treatments (Fig. 3). The homing abilities of the bees for all treatment modalities were not significantly different regarding the number of bees not returning to the hive, as given by the Kruskal–Wallis test across the treatment groups ($P > 0.05$). Despite the small decrease in the second day, the rate of disappearance between the feeder and the hive was roughly regular in time. For the overall period, the percentage of bees not returning to the hive was between 24 and 44%.

Time of homing flight

Fipronil at the dose of 0.3 ng per bee induced an increase in the flight time between the artificial feeder and the hive in comparison to the control group and the group treated with 0.06 ng per bee (Fig. 4). Pairwise multiple comparisons indicated statistical differences on the three first days between the homing times of bees treated with 0.3 ng of fipronil and those of others bees ($P < 0.0001$). The flight time between the feeder and the hive was increased until 3 days after the fipronil treatment at 0.3 ng. The fourth day, the flight times of treated bees were similar to those of control bees. Foragers treated at the highest dose took roughly between 16 and 30 s more within time to return to the hive than control foragers. On the first recording day, the rate of bees returning to the hive within 60 s after the departure from the feeder was 77 and 80% for the control bees and the bees treated with 0.06 ng of fipronil, respectively, whereas those for the bees treated with 0.3 ng was only 51%.

Daily pattern of foraging flights

Foraging activity was temporally restricted from 06:00 to 21:00, with almost no activity between 01:00 and 05:00 (Fig. 5). Activity levels started to increase between 06:00 and 07:00, rising steadily during the morning and reaching

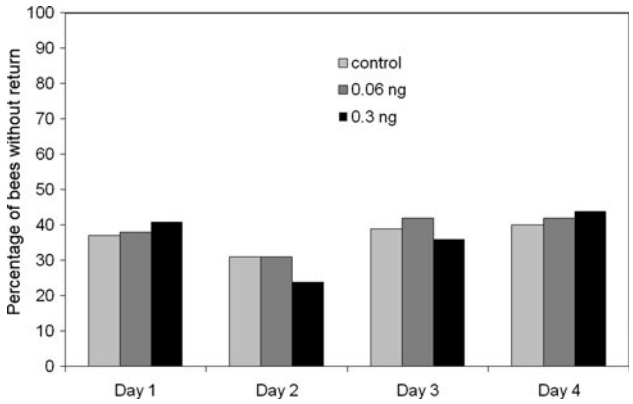

Fig. 3 Percentage of bees without return to the hive according to day after treatment. For each day, no significant difference (*NS*: $P > 0.05$) in the number of bees not returning to the hive was found across the treatment groups (Kruskal–Wallis test, d.f. = 2)

the maximum during midday before decreasing again. Thus, the colony exhibited a diurnal rhythm in its foraging pattern. In the figure illustrating the homing intensity according to hours (Fig. 5), there was no difference between the shapes of the graphs for bees treated with fipronil and those untreated. Although the level was lower for 0.3 ng of fipronil per bee, treated bees presented a daily activity with a similar pattern to control bees.

Discussion

New insights into the behavioral effects of insecticides, including effects on mobility, orientation, foraging and learning, were largely investigated over the last 10 years (Desneux et al. 2007). For example, the proboscis extension reflex assay with restrained workers has been used to investigate the behavioral effects of about 20 pesticides. But it is not clear whether the endpoints tested in these studies can be clearly related to the respective field effect of concern (Thompson and Maus 2007; Decourtye and Devillers 2010). Hence, the ecological relevance of the methods proposed to measure the orientation and the homing ability of bees is better (Schricker and Stephen 1970; Cox and Wilson 1984; Vandame et al. 1995; Bortolotti et al. 2003; Yang et al. 2008). However, these monitoring techniques relying on individual identification of bees with colored and numbered tags are limited by the number of bees monitored simultaneously and the time span during which observations can be made. Conversely,

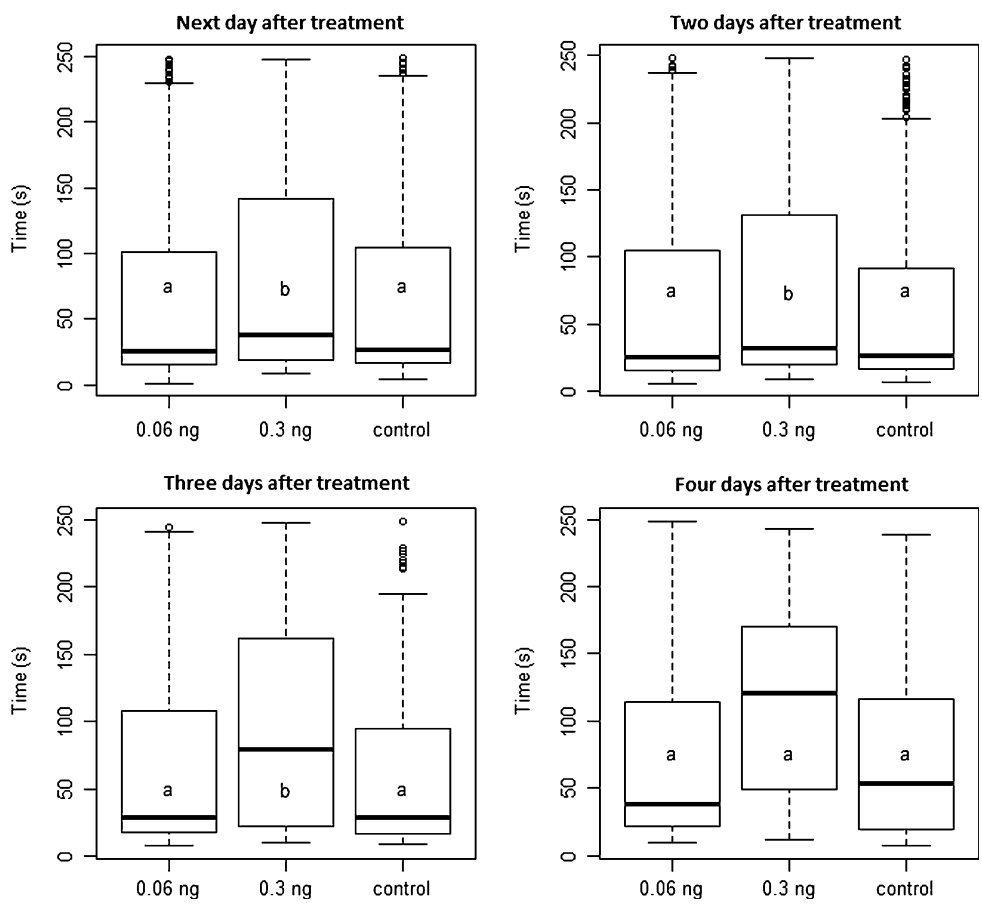

Fig. 4 Time of homing flights according to treatment. n* for next day after treatment = 1537, 1791, 891. n* for 2 days after treatment = 938, 940, 520. n* for 3 days after treatment = 269, 437, 157. n* for 4 days after treatment = 92, 228, 40. *Different letters* indicate significant difference with pairwise comparisons using Wilcoxon rank sum test (P value adjusted by Bonferroni method: $P < 0.025$).
* n: number of trips for control, 0.06 and 0.3 ng, respectively

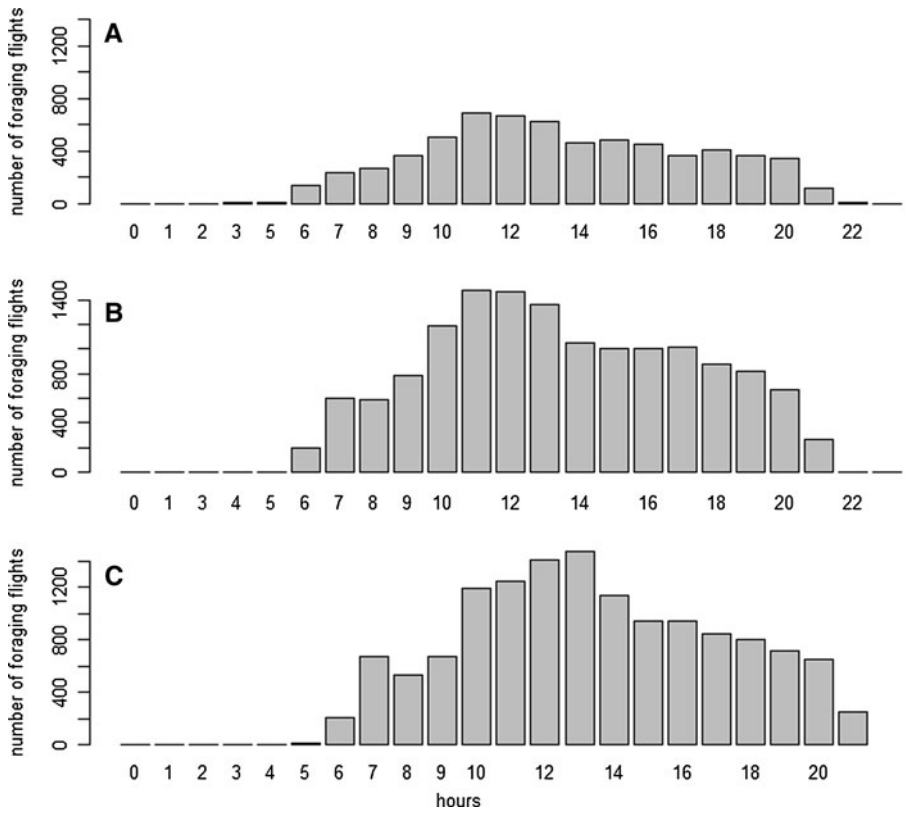

Fig. 5 Daily number of foraging flights. **a** Bees treated with fipronil at 0.3 ng per bee. **b** Bees treated with fipronil at 0.06 ng per bee. **c** Control bees. Each *bar* represents an hour of the day and the *height of the bars* indicates the total number of foraging flights recorded within 4 days

in the present experiment we use automatic tracking and identification of individuals (Streit et al. 2003). With the RFID transponders, we track the tagged bees during their daily foraging activity until their death. We monitor the foraging activity of hundreds of individual workers around the clock for several days and we analyze their foraging rhythms. These advantages of the RFID device allow us to study both the behavioral traits and the lifespan of bees, especially under biotic and/or abiotic stress. The high quantity of data obtained with this technique requires that we create an easy-to-use interface for analyzing the data and providing the life-history traits of each bee. Under semi-field conditions, our approach using RFID microchips provides detectable adverse effects due to an insecticide. Impairment in foraging performances has been shown for a fipronil dose at which no additional mortality occurred. Thus, we assume that the use of this method to evaluate the potential effect of pesticides on the honey bees' foraging can help to assess the toxicity of agrochemicals in a more comprehensive way.

Here, we describe the application of a new technique of automatic tracking and identification of bees that could be useful in the search for the causes of the syndrome found in North America and Europe, where beekeepers have recently claimed a complete absence of adult bees in colonies, with little or no build-up of dead bees in or around the hives (Rortais et al. 2005; Higes et al. 2006; Oldroyd 2007; van Engelsdorp et al. 2008). Among several potential causations, it was assumed that foragers collecting nectar and pollen were exposed to low doses of insecticides during their foraging trips, which induced behavioral effects and subsequently no homing return to the hive. For example, French beekeepers reported that hives located near seed-dressing treated sunflowers with Gaucho® (imidacloprid-based product) or Regent TS® (fipronil-based product), showed a progressive decline in their populations, until a complete loss of the colonies (Colin et al. 2004). Testing the hypothesis of no homing return to the hive implies the monitoring of foraging activity at the individual level. To this aim, RFID provides automatic recording of the date and time of each honeybee passage, as well as the identity of the passing bee. Moreover, the individualization of bees allows us to compare groups receiving several treatment modalities and placed in the same colony, then ensuring homogeneous experimental conditions among bees.

To prove the interest of RFID set-up in studying the effects of insecticide on honeybees, we chose fipronil as an example. For that, we recorded under a tunnel the displacements of foragers trained to forage on an artificial feeder filled with a sucrose solution, with a distance between the feeder and the hive of 18 m. Bees foraging on the syrup were captured, orally treated with fipronil and individually marked with RFID tags. After release, the

life-history traits of the bees were recorded for 4 days. The nonstop recordings of entrances and exits of bees allowed us to analyze the foraging rhythm according to hours. No time difference was observed in diurnal activity of fipronil-treated foragers. The statistical approach based on the comparison of survival data by a Cox proportional hazard model demonstrated no lethal effect of acute treatment with fipronil at 0.06 and 0.3 ng per bee. This result is not surprising as the highest tested dose corresponded to median lethal dose value determined 48 h after the oral treatments (Decourtye et al. 2005) divided by 20, and from previous results it was assumed that this ratio belonged to a sublethal domain (Decourtye et al. 2003). The analysis of survival data can be linked to homing capacities since no return to the hive increases drastically the risk of death of the individual during the next night. Our hypothesis was that a sublethal dose of fipronil can prevent the return to the hive and thus indirectly induces the death of the individual. But, in a coherent way with the survival data, our results show also that the poisoned honeybees were able to return to the hive. So, the tested fipronil doses did not induce disappearance of foragers.

Foragers fed with fipronil-added syrup at the dose of 0.3 ng per bee (LD50/20), but not at 0.06 ng per bee, were delayed in returning to their hive for up to 3 days. The increase in the flight time disappeared the fourth day after ingestion of the supplemented syrup. The highest dose of fipronil induced a lower number of foraging flights per bee the next day after the treatment. When fipronil at a nominal concentration of 2 $\mu g\ kg^{-1}$ was orally administered in free-flying bees confined under insect-proof tunnel, clinical signs of disruptive motor activity, such as convulsions or paralysis, were observed (Colin et al. 2004). In our study, the oral treatment with fipronil did not induce these neurotoxic symptoms, so it cannot explain our results. Previous work found that 1 $\mu g\ kg^{-1}$ of fipronil induced a poor rate of foraging activity and increased bees' flight time through a maze (Decourtye et al. 2009). The impact of fipronil on the flight-time could be related to the action of fipronil on its main targets, the receptors to the neurotransmitter λ-aminobutyric acid (GABA). These receptors are located on the membrane of the muscle cells and play an important role in modulating locomotor and flight activity in insects (Usherwood and Grundfest 1965; Cole et al. 1993). GABA transport inhibitors administered orally yielded a reduction in locomotion and geotaxis in flies (Leal and Neckameyer 2002). We can assume that fipronil may act at the peripheral neuromuscular junction in bees leading to an impairment of flying activity. Besides the action of fipronil at the peripheral system, a biochemical action at the central level can also explain our results. The delayed homing flight might be due to disoriented honeybees temporarily losing their way to the hive. Vandame et al. (1995) showed that insecticide-treated bees were disoriented and flied in the sun direction. More precisely, we have previously shown that orientation capacities of foragers in a complex maze were affected by a treatment with 1 $\mu g\ kg^{-1}$ of fipronil (Decourtye et al. 2009). The neurobiological actions of fipronil on GABAergic and glutamaergic neurotransmissions (Cole et al. 1993; Ikeda et al. 2003) might explain the effects of fipronil on orientation. GABA and glutamate seem to be involved in cognitive processes, particularly in memory function (Maleszka et al. 2000; Kacimi El Hassani et al. 2008, 2009). Several brain areas are involved in orientation including the visual neuropiles and the mushroom bodies, which are the integrative structures of the insect brain (Waddell and Quinn 2001). The neurotransmitter glutamate has been found in some intrinsic neurons of the mushroom bodies' calyces and GABA is mainly present in extrinsic neurons, i.e., interneurons that establish connections between the mushroom bodies and other brain neuropiles (Rybak and Menzel 1993; Gronenberg 1987). These neurons build up gabaergic inhibitory networks inside the brain. Fipronil blocking of gabaergic receptors can lead to a disruption in their contribution to the orientation process.

Losses of foraging bees are sometimes attributed to a failure of foraging flight between a meliferous plant treated with an insecticide and the hive. Thus, we have developed a method under tunnel to automatically record the displacements of foragers individualized with RFID tags and to detect the alteration of the flight pattern between an artificial feeder and the hive. Measurable differences in homing flight are found in such experimental design, so we can suppose that in field conditions an individual would probably suffer from this effect. However this hypothesis had to be proven since the distance between the feeder and the hive is only some meters in our experimental setup. Additional experiments are needed to establish whether foragers exposed to fipronil can negotiate a longer route in a complex environment, such as those in field conditions, or if they are lost, this being a possible cause to the drastic bee population losses as observed by beekeepers.

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