

Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group

	Average Bee Mortality in %							
	First test				Second test			
	4 h	24 h	48 h	72 h	4 h	24 h	48 h	72 h
Control	0	0	5	17	0	0	2	8
Toxic standard	31	100	100	100	12	94	94	98
Sunflower Melody	0	3	25	40	0	0	7	22
Sunflower LG 5660	0	2	5	8	0	0	0	5

Discussion and conclusion.

With no cross contamination possible, some lethal effects on bees were observed following the use of one treated seed and absolutely no effect for the other one. Experimental conditions were satisfactory as there was no wind at all and dust lay down around in the field. A little wind could have blown away the dust into hazardous directions. To ensure a better exposure it will be necessary to sow maize or sunflower insecticide coated seeds around plants placed in the middle of the field.

Following this first study, French authorities set up a 'dust schedule' to seed coating factories limiting the dust discharge to 4 grams per quintal (100 kg) of coated seed which corresponds to the safe variety (LG 5660) in above described test.

Since 2004 no more high mortalities have been attributed to sowing operations in France. This results should be of high interest for other European countries. This methodology should therefore have a place as a guideline in the regulation scheme in European countries.

Sublethal effects of fipronil on the ability of honeybees (*Apis mellifera* L.) to orientate in a complex maze

Axel Decourtye^{1*}, Samuel Lefort¹, James Devillers², Monique Gauthier³, Pierrick Aupinel⁴, Michel Tisseur¹

¹ACTA, ICB-ENVL, 1 avenue C. Bourgelat, 68280 Marcy l'Etoile, France*

²CTIS, 2 chemin de la gravière, 69140 Rillieux La Pape, France

³CNRS, Centre de Recherches sur la Cognition Animale, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse cedex, France

⁴INRA, Unité expérimentale d'entomologie Le Magneraud, 17700 Surgères, France

*Tel.: 334 788 756 22, Fax: 334 788 756 20, E-mail: axel.decourtye@acta.asso.fr

Abstract

Background: The recent fipronil-based pesticide is accused by bee-keepers of causing depopulations in hives of honeybees (*Apis mellifera* L.). Behavioural effects during the flight of foraging honeybees would have been evoked. To test whether the insecticide fipronil may disorientate foragers, its impact on orientation in a maze was examined. Bees had to fly through a sequence of boxes to reach the target, which was a feeder containing a reward of sugar solution. After being trained to associate a green mark with the reward, foragers received 1 µg kg⁻¹ fipronil orally and their capacity to orientate through the maze following the colour mark was tested and compared to control.

Results: The rate of foragers entering the maze, and so responding to the mark placed at the entrance, was reduced with fipronil-fed animals. Before and after treatment, 86-89% of bees equally flew through the whole path and arrived to the goal without mistakes. The rate of fipronil-treated bees finding path without mistakes decreased to 60%. Conversely, the rate of bees with unsuccessful searches for the goal notably increased with treatment (34% in treated bees *versus* 4% in control bees).

Conclusion: Our results show that orientation capacities of foragers in a complex maze were affected by fipronil.

Keywords: *Apis mellifera* L., pesticide, maze, conditioning, visual learning, flight

Introduction

Honeybees can accurately and repeatedly navigate to a food source, and then communicate to their nest mates the distance and direction to reach it.¹ The process of foraging involves learning and memory, communication, navigation, taking into account information from the internal clock and many other flexible responses, e.g. the ability to integrate local landmarks.² These biological functions are potentially affected by pesticides. This is particularly true for the visual learning of landmarks which is important in spatial orientation.^{3,4} One of the major tasks for the honeybee during a foraging flight is to learn and recall many complex visual patterns.⁵ It is well known that honeybees use landmark-based cues to navigate to a goal and to return to the nest. These cues are needed to set the flight direction, to monitor progress to the goal, to provide intermediate guiding landmarks and they finally aid in spatial tracking the target when the bee is in its vicinity.³ Considering the neurobiological functions in orientation processes, it is of great interest to know whether neurotoxic insecticides induce behavioural disturbances and if these alterations exist at low concentration level. It is now well-admitted that sublethal concentrations of pesticides can affect the spatial orientation of the honeybee.⁶ In an insect-proof tunnel (feeder located at 8 m from the hive), Vandame et al. (1995) showed that deltamethrin altered the homing flight of foragers treated topically at sublethal doses.⁷

Accordingly, when insecticide intoxication is suspected, bee losses observed in field conditions could be attributed to alteration of the flight pattern between a contaminated food source and the hive. More significantly, the impairment of homing flight of exposed foragers is a possible cause in the Colony Collapse Disorder. This syndrome was principally found in North America and Europe, where beekeepers have recently claimed to observe a complete absence of adult bees in colonies, with little or no build-up of dead bees in or around the colonies.⁸⁻¹¹ In recent years, French beekeepers reported that hives located near sunflowers, originated from seeds dressed with Gaucho[®] or Régent TS[®], show high levels of damage due to a progressive decline in the hive populations, until a complete loss of the colonies.¹² The imidacloprid- and fipronil-based products are accused by French bee-keepers of causing behavioural effects in foragers and subsequently no homing return to hive. So, many studies were carried out in order to assess the effects of these insecticides on behavioural traits, and more particularly those involving in the foraging. Using conditioning of the proboscis extension reflex in restrained individuals, previous studies showed that fipronil in acute topical application or chronic ingestion impaired olfactory learning of bees.^{13,14} But, it is not clear whether the endpoints tested in these sublethal studies can be clearly related to the respective field effect of concern.^{15,16} In contrast, the ecological relevance is better in the methods on orientation and homing ability.^{6,7,17,18}

To test whether fipronil may disorientate foragers, its impact was examined on orientation of honeybees in a maze under outdoor conditions. Orientation performance of bees in a complex maze relies on associative learning between a visual mark and a reward of sugar solution.¹⁹ We studied whether foragers receiving orally 1 µg kg⁻¹ of fipronil can orientate themselves through the maze.

Materials and Methods

Insects

Experiments were repeated twice, each time with a colony of honeybees (*Apis mellifera* L.) of about 20,000 workers and a fertile 1-year-old queen. Honeybees were confined in a 5-comb hive (2 brood combs, 2 honeycombs and one empty comb). The colony was maintained in an outdoor flight cage (2.5 m × 2.5 m, 2 m high) covered with an insect-proof cloth (2 mm × 2 mm mesh) and a ground covered with a plastic. Any dead bees found on the ground were counted and discarded daily.

Feeding

A feeder was positioned about 1.5 m from the hive entrance, filled with sucrose solution (500 g kg⁻¹) and multi-floral pollen. The sucrose solution was delivered in a dish, 7 cm in diameter, made of a material impervious to ultraviolet rays.

Device of the maze

The maze consisted of a matrix of 4 × 5 identical cubic boxes, each side of 30 cm, with each wall carrying a 4-cm diameter hole in its centre where bees crossed.¹⁹ The maze was placed inside the flight cage on a table, 60 cm height. The boxes were made of white opaque Plexiglas and a metallic grate covered the maze (3 mm × 3 mm mesh).

Principle of the maze

Bees had to fly through a sequence of boxes to reach the goal – a feeder containing a reward of sugar solution. A path through the maze spanned 9 boxes, including 3 decision boxes and 6 non-decision boxes. A non-decision box had two holes, each in a different wall, where the bee entered through one hole and was expected to leave through the other tagged with a green mark. A decision box had three holes, each in a different wall, where the bee entered through one hole and then was expected to choose between two other holes: one with a green mark representing the correct path and another without mark representing the incorrect path which ultimately led to a dead end. Finally, the bee was released from the box in which she was trapped.

Conditioning procedure

During conditioning, bees were collectively taught to associate the mark with a feeder. For that, a green mark was fixed in front of a sucrose solution feeder outside the maze near the entrance during one hour. One additional hour, the feeder was placed in the first box of the path for about one hour, in the second box of the path the next hour, in the third box during one other hour and so on. Then, the feeder was moved on the fifth box during the same time. Finally, the feeder was placed at the end of the path (Fig. 1), in the reward box (9 cm × 9 cm), where all bees that underwent the conditioning procedure were individually marked with colour number tags.

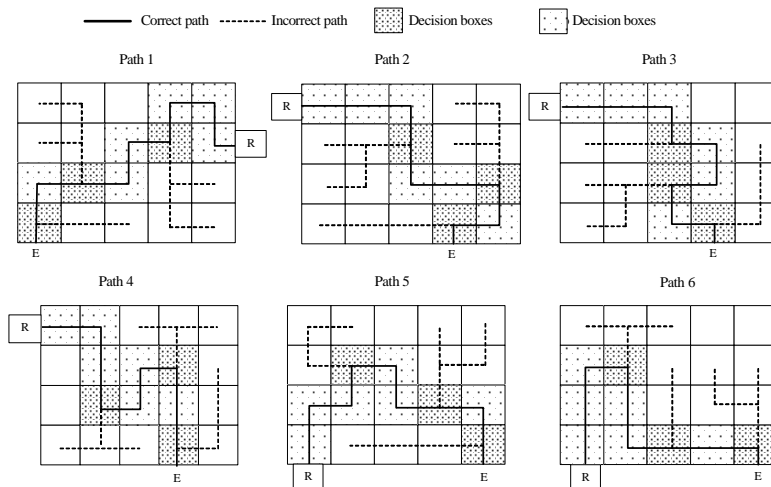


Figure 1 Maze paths used before, during and after treatment. Path 1 was used for the conditioning procedure and other paths were used for the retrieval tests. Each path started with the entrance (E), contained 3 decision boxes, 6 non-decision boxes, and finished with the reward box (R).

After the bee had found the goal, received her reward and was marked, she was released from the reward box and allowed to return directly to the hive (without flying back through the maze). A total of 185 bees were labelled during the untreated periods (100 before treatment: 58 for colony 1 + 42 for colony 2; 85 after treatment: 40 for colony 1 + 45 for colony 2) and 131 bees during treated periods (71 for colony 1 + 60 for colony 2). One-day conditioning period was necessary to train a sufficient number of bees. Each bee was trained only once.

Retrieval tests

After conditioning, the capacity of an individual bee to negotiate a path into the maze was tested. Green marks were affixed below the appropriate hole in each box to indicate the correct path. When a bee entered the maze, an observer noted the number and the colour of the tag, correct decisions, incorrect decisions and turns back. During retrieval tests, five different paths lasting 15-20 min were used (Fig. 1). Successive paths were interspersed with a cleaning containing ethanol to remove possible olfactory cues. During a test, only one bee was allowed into the maze at the same time and she was tested for one of the five path configurations. Bees were tested between 24 h and 32 h after training.

At the end of each day, a path was carried out without green mark inside the maze (only one mark stayed at the entrance). Therefore, any bee arrived at the goal within 5 minutes. This test confirms that green marks were the only internal landmarks used by bees as navigation cues.

Three-stage periods

We compared responses of honeybees before and after exposure to the insecticide on the same colony. Thus, performances of the honeybees were compared under various feeding conditions: sucrose solution without pesticide, with 10 ml l⁻¹ ethanol (before and after treatment) and sucrose solution added with fipronil, with 10 ml l⁻¹ ethanol (treatment). For each condition (controlled and treated), bees were submitted to conditioning and retrieval tests. Data of each period were obtained from different bees.

Oral treatment

Technical grade fipronil (98% purity, CAS RN 120068-37-3), purchased from Cluzeau Info Labo (France), was dissolved in ethanol (95-96% purity) and stock solution was diluted to final concentration in sucrose solution. The final concentration of ethanol was 10 ml l⁻¹. As a control, the sucrose solution was analysed (GIRPA, France) for contamination with HPLC/MS technique (limit of quantification = 0.5 µg kg⁻¹) to detect fipronil and its two main metabolites (MB46136, MB46513). According to these analyses, the sucrose solution contained 1 µg kg⁻¹ fipronil and was free of metabolites.

During the treatment period, fipronil was administered at the end of the conditioning period and before the test, then the honeybees consumed the contaminated syrup between 24 h and 48 h after training. The contaminated sucrose solution (1.2 litre) was delivered in a feeder placed outside the maze, and all the syrup was collected by foragers. During control periods (before and after treatment) honeybees were fed after training with a sucrose solution containing 10 ml l⁻¹ ethanol.

Performance analysis

For each period, the performance of labelled bees, which entered the maze for the first time, was analysed. Four categories of performances were defined and a note was assigned to each of them:

- Bee flows through the path and arrives directly to the goal (reward box);
- Bee flows through the path and arrives to the goal with one or more turns back (bee leaves the box through the hole from which it entered);
- Bee flows through the path with mistake(s) (bee making one or more wrong turns at the decision boxes) but arrives to the goal;
- Bee does not arrive to the goal within 5 min after entering the maze.

- Each bee received a note corresponding to her performance. Performances of control and fipronil-treated bees were evaluated as the mean of notes assigned to bees in each group.

Flight time

The time required to reach the goal from the instant of entering the maze was measured for each bee. Flight time was considered only for bees flying through the whole path within 5 min. Honeybees that did not reach the goal within 5 min were excluded from this analysis.

Statistical analysis

A multifactor ANOVA (Type III sums of squares) was used: the dependent factors were number of dead bees, performance notes or flight times, and the independent factors were colonies, feeding periods (i.e., before, during or after treatment) or paths. We also checked for first-order interactions between the independent factors. For these statistical analyses, the data were log-transformed to achieve normal distribution.²⁰ Tukey's Honest Significant Difference test (THSD test) was performed on all analyses to assess pairwise differences between the feeding periods. Each comparison was carried out according to the Dunn-Sidak method,²⁰ at a critical probability of $\alpha' = 1 - (1 - \alpha)^{1/k}$, where k is the number of intended tests ($\alpha' = 0.0125$). To improve the illustration of performances and the comparability with other studies, we give in the text the percentage of bees ranked in the four performance categories according to the feeding period.

Results

Mortality

No significant differences were found between the two colonies and the three feeding periods (Table 1). The treatment with fipronil did not lead to additional mortality. The pooled number of dead worker bees for the two colonies was 2611 and 1934 for control periods (before and after treatment respectively) and 1982 for treatment period. Therefore, feeding honeybees with sucrose solution added with fipronil 1 $\mu\text{g kg}^{-1}$ could be considered as a sublethal concentration.

Table 1 Effects of independent factors on mortality of honeybees (*Apis mellifera* L.).

	n = 30^a	d.f.	Mean square	F value	p value
Main effects					
Colony		1	0.00	0.21	0.648
Feeding period		2	0.04	1.86	0.177
Error		24	0.02		
Interactions					
Colony \times feeding period		2	0.03	1.65	0.213

Results of multi-way ANOVA with first-order interactions are given. ^aNumber of days where mortality was recorded.

Performance

Data collected from the two colonies and the five paths were pooled in Fig. 2 to show the percentages of bees assigned to each performance category during retrieval paths tests. Control and fipronil-treated bees made no mistakes and consequently category 3 is empty. Before and after treatment, a high percentage of bees flew through the path and arrived directly to the goal (category 1: from 87 to 89%). In the same time, a low percentage of bees made turns back (category 2: from 6 to 9%) or failed in reaching the goal (category 4: from 4 to 5%). Thus, bees without treatment trained to follow colour marks were able to use the same cue to find a new way in a path they had never encountered previously. The rate of fipronil-treated bees reaching the goal directly decreased to 60%. In parallel the rate of bees that did not reach the goal within 5 min notably increased to 35%. In this group, foragers stopped during the trip, remaining in a box and flying inside. The number of turns back (category 2) was not different between control and treated-bees. The number of fipronil-fed foragers entering the maze, and so responding to the mark placed at its entrance, was

reduced. Only 15% of labelled bees were observed into the maze during the treatment period, compared to 34% and 41% before and after treatment, respectively.

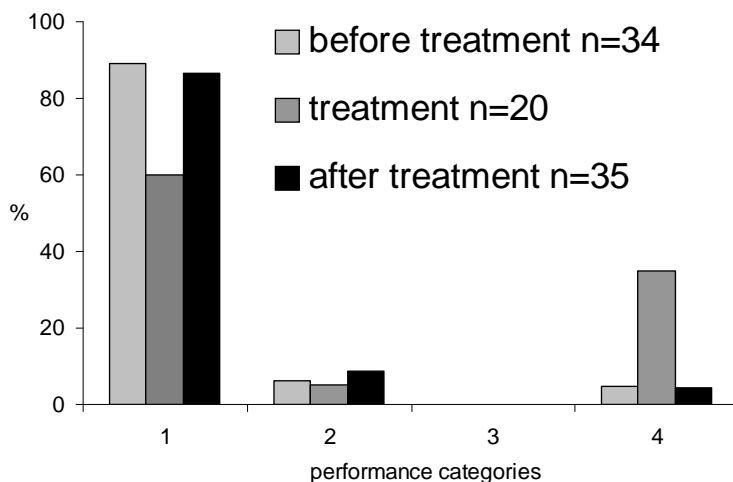


Figure 2 Performance of bees ranked into 4 categories.

Performance analysis with three-way ANOVA showed no significant differences between paths, whereas feeding periods significantly differed (Table 2). This difference was nearly statistically significant between colonies. Honeybees' performance before and after treatment was not significantly different (THSD tests; $p = 0.35$; Table 3). Bees orally exposed to fipronil had significantly lower performances than untreated bees (THSD test; before treatment: $p < 0.001$; after treatment $p < 0.01$). There was significant interaction effect between colony and path (Table 2). But in separate analyses, the performance of both colony 1 ($F = 3.46$, $p = 0.072$) and colony 2 ($F = 2.31$, $p = 0.069$) did not differ significantly between paths.

Table 2 Effects of independent factors on performances of foraging honeybees (*Apis mellifera* L.).

n = 89 ^a	d.f.	Mean square	F value	p value
Main effects				
Colony	1	0.11	3.69	0.057
Feeding period	2	0.22	7.07	0.001
Path	4	0.02	0.61	0.654
Error	120	0.03		
Interactions				
Colony × feeding period	2	0.02	0.69	0.505
Colony × path	4	0.08	2.89	0.025
Feeding period × path	8	0.01	0.53	0.843

Results of multi-way ANOVA with first-order interactions are given. ^aNumber of bees taken into account for performance evaluation.

Flight time

In bees ranked in categories 1 and 2 the time required to reach the goal from the instant of entering the maze was measured. Flight time of forager bees did not differ significantly between colonies and path but differed between feeding periods (Table 4). On average, before and after treatment, bees fled through the maze in 59 s and 40 s, respectively (Table 3). Fipronil induced a significant increase of bees' flight time through the maze ($p < 0.01$). The mean duration of the flight was of 93 s. Thus, the bees' ability to negotiate the maze following a colour mark was reduced by treatment.

Table 3 Performance notes and flight times from honeybees (*Apis mellifera* L.) to three feeding periods.

	Performance notes	Number of bees	Flight times (s)	Number of bees
Before treatment	1.20 ± 0.08 (a)	34	59.48 ± 6.42 (ab)	32
Treatment	2.10 ± 0.32 (b)	20	93.15 ± 20.03 (b)	13
After treatment	1.22 ± 0.10 (a)	35	40.40 ± 4.44 (a)	33

Mean ± s.e.m. and number of bees are given. Letters indicate significant differences (THSD test; $p < 0.01$).

Table 4 Effects of independent factors on flight time of foraging honeybees (*Apis mellifera* L.).

	n = 78 ^a	d.f.	Mean square	F value	p value
Main effects					
Colony		1	0.01	0.10	0.756
Feeding period		2	0.47	5.78	0.004
Path		4	0.08	1.03	0.397
Error		108	0.08		
Interactions					
Colony × feeding period		2	0.14	1.74	0.180
Colony × path		4	0.13	1.60	0.179
Feeding period × path		8	0.02	0.60	0.873

Results of multi-way ANOVA with first-order interactions are given. ^aNumber of bees taken into account for flight time evaluation.

Discussion

Our experiments show that honeybees, in flying situation, can associate a visual mark to a reward, a result already observed by Menzel et al. (1974)²¹, and they can use this associative learning to negotiate a path in a complex maze.¹⁹ The retrieval tests point out the capacities of bees to restore the rule previously learned that the colour predicts the location of food. After treatment with 1 µg kg⁻¹ of fipronil, the ability of bees to perform the task was impaired compared to control bees. The significant features for intoxication are the small number of honeybees entering the maze for the test, the relatively poor rate of honeybees reaching the goal directly with an increasing flight time and the increased rate of honeybees that did not find the goal during the 5-min observation period. Control bees can successfully locate the goal (sugar solution) by flying through paths they have never previously encountered, but this task was more difficult for treated bees. Treated bees that displayed unsuccessful searches for goal remained and flew into a box, without using the local landmarks to reach the goal. They landed on the grid, towards the light and this behaviour probably indicates a modification of phototropism. The fact that insecticide-treated bees fly in the sun direction was previously shown by Vandame et al. (1995).⁷ But, fipronil-treated bees made no more errors and turns back than control bees.

It is possible to divide the fipronil-treated honeybees into three categories: those previously conditioned but which do not come back to the maze for testing, those recorded during testing but which are lost in the maze and those which succeed taking more time to reach the goal. How explain these different reactions to treatment? This complex panel of behavioural modifications we have observed can be linked to different levels of intoxication related to the dose ingested by each bee. According to the model of exposure previously developed by Rortais et al. (2005),¹⁰ a nectar forager would have ingested between 0.03 and 0.11 ng of fipronil in our experimental conditions. These doses are inferior to the median lethal dose value of

fipronil (LD50 determined 48 h after the oral treatments was 6 ng per bee in our laboratory¹⁴), confirming the sublethal character of treatment.

As no extra mortality was associated to fipronil treatment, we can suppose that fipronil decreased the motivation of honeybees to come back to the maze. Fipronil ingested after the training period should be perceived as a repulsive agent, foragers could associate the green mark to a negative reward and avoided it during the retrieval test. These effects are classically attributed to an anti-feeding character of the compound.^{22,23} But a decrease of foraging activity can also be due to processes occurring inside the hive. For example, Kirchner (1999) reported a reduction in the foraging activity on a food source contaminated with imidacloprid (20-100 µg kg⁻¹) due to the induction of trembling dances that prevent other bees from foraging.²⁴ In addition, a lower motivation to perform waggle dances revealed a reduction in the recruitment activity. Thus, the changes in the communication process can also result in a decreased foraging activity.

The mean flight time in the maze ranged from 40 s to 59 s in untreated bees, and reached 96 s in fipronil-treated bees. The impact of fipronil on the flight-time would not be surprising because fipronil's main targets, the receptors to the neurotransmitter γ -aminobutyric acid (GABA) located on the membrane of the muscle cells, play an important role in modulating locomotor and flight activity in insects.^{25,26,27} Fipronil may act at the peripheral neuromuscular junction of muscle fibres in bees, leading to an impairment of flying activity.

Previous studies based on olfactory learning in the honeybee have shown the negative effects of fipronil on memory. Using conditioning of the proboscis extension reflex in restrained individuals, Decourtye et al. (2005) reported a decrease of the response level during the tests compared to the control group after chronic ingestion of fipronil (4.5 µg l⁻¹ corresponding to a dose of 0.15 ng per bee per day).¹⁴ El Hassani et al. (2005) showed that fipronil in acute topical application impaired olfactory learning of bees (0.5 ng per bee) and reduced their sucrose sensitivity (1 ng per bee).¹³ The originality of our results consists in the demonstration of impact of fipronil on the orientation process which is a complex integrated function depending on phototaxis, learning of visual landmarks, memorization of the rule consisting in the association of the green mark to the right way. If our experiments would not allow conclusions about learning and memory impairment, they confirm the negative effects of the insecticide on the ability of bees to find a route.

While we cannot establish a direct link between previous results obtained in laboratory and the disorientation of foragers as suspected by beekeepers, our experimental data can tentatively be related to the field situation of bees exposed to fipronil.^{13,14} In the field, foragers use landmark-based cues to navigate to a target as well as to return to the nest.³ The learning flights that bees perform in order to memorise the location of a target typically cover a limited sector of space around the goal.⁴ So, the memorized landmarks play a prominent role in path recognition during the next foraging trips. This work shows that the administration of 1 µg kg⁻¹ of fipronil leads to disorientation of foragers. Unlike in the maze where the performances are based on the use of limited pertinent cues, the navigation in the field relies on several guidance mechanisms. Bees are capable of recognizing patterns in situations where local landmarks are not reliable.²⁸ Additional experiments are needed to establish whether foragers exposed to fipronil can negotiate a route in a complex environment or if they are lost, this being a possible cause in the drastic bee population losses as observed by beekeepers.

Acknowledgments

This work was supported by grants from the European Community program (1221/97) for French beekeeping co-ordinated by French Ministry of Agriculture.

References

1. Frisch von K, The dance language and orientation of bees, Belknap/Harvard University Press, Cambridge, Massachusetts, 566 pp. (1967).
2. Menzel R, Geiger K, Joerges J, Muller U, Chittka L, Bees travel novel homeward routes by integrating separately acquired vector memories. *Anim Behav* 55: 139-152 (1998).
3. Collett TS, Fry SN and Wehner R, Sequence learning by honeybees. *J Comp Physiol A* 172: 693–706 (1993).

4. Lehrer M, Why do bees turn back and look? *J Comp Physiol A* 172: 549–563 (1993).
5. Capaldi EA and Dyer FC, The role of orientation flights on homing performance in honey bees. *J Exp Biol* 202: 1655-1666 (1999).
6. Cox RL and Wilson WT, Effects of permethrin on the behavior of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environ Entomol* 13: 375-378 (1984).
7. Vandame R, Meled M, Colin ME and Belzunces LP, Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. *Environ Toxicol Chem* 14: 855-860 (1995).
8. Oldroyd BP, What's Killing American Honey Bees? *PLOS Biol* 5: 1195-1199 (2007).
9. VanEngelsdorp D, Hayes J, Underwood RM and Pettis J, A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008. *PLoS ONE*, 3(12): 1-6 (2008).
10. Rortais A, Arnold G, Halm MP and Touffet-Briens F, Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36: 71-83 (2005).
11. Higes M, Martin R, Meana A, *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *J Invertebr Pathol*, 92(2): 93-95 (2006).
12. Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S and Vermandere JP, A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Arch Environ Con Tox* 47: 387-395 (2004).
13. El Hassani AK, Dacher M, Gauthier M and Armengaud C, Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). *Pharmacol Biochem Behav* 82: 30-39 (2005).
14. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S and Pham-Delègue MH, Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Arch Environ Con Tox* 48: 242-250 (2005).
15. Thompson HM and Maus C, The relevance of sublethal effects in honey bee testing for pesticide risk assessment. *Pest Manag Sci* 63:1058–1061 (2007).
16. Desneux N, Decourtye A and Delpuech JM, The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol*, 52: 81-106 (2007).
17. Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S and Porrini C, Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bull Insectology* 56: 63-67 (2003).
18. Yang EC, Chuang YC, Chen YL and Chang LH, Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101(6): 1743-1748 (2008).
19. Zhang SW, Bartsch K and Srinivasan MV, Maze learning by honeybees. *Neurobiol Learn Mem* 66: 267-282 (1996).
20. Sokal RR and Rohlf FJ, *Biometry: The principles of practice of statistics in biological research*, Freeman WH and Co, New York, 859 pp. (1995).
21. Menzel R, Erber J, Andmasuhr T, Learning and memory in the honey bee, in *Experimental Analysis of Insect Behavior*, ed. Barton Browne L., Springer-Verlag, New York, pp. 195–217 (1974).
22. Schmuck R, No causal relationship between Gaucho seed dressing in sunflowers and the French bee symptom. *Pflanzenschutz Nachrichten Bayer* 52: 257-299 (1999).
23. Decourtye A, Devillers J, Cluzeau S, Charreton M and Pham-Delègue MH, Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotox Environ Safe* 57: 410-419 (2004).
24. Kirchner WH, Mad-bee-disease? Sublethal effects of imidacloprid ("Gaucho") on the behavior of honey-bees. *Apidologie* 30: 422 (1999).
25. Cole LM, Nicholson RA and Casida JE, Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic Biochem Phys* 46: 47-54 (1993).
26. Usherwood PN and Grundfest H, Peripheral inhibition in skeletal muscle of insects. *J Neurophysiol* 28: 497-518 (1965).
27. Leal SM and Neckameyer WS, Pharmacological evidence for GABAergic regulation of specific behaviors in *Drosophila melanogaster*. *J Neurobiol* 50: 245-261 (2002).
28. Lindauer M, Time-compensated sun orientation in bees. *Cold Spring Harb Symp* 25: 371-377 (1960).