Sublethal effects of thiamethoxam on the ability of honeybees to orientate in a complex maze

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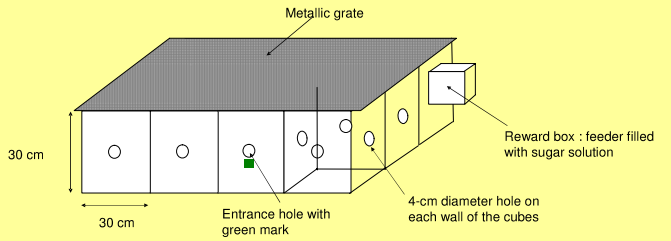
It is well known that honeybees use **landmark-based cues during a foraging** flight to navigate to a food source and to return to the nest. Spatial orientation processes involving neurobiological functions, it can be potentially affected by pesticides. Currently, there is a great interest to know whether neonicotinoid insecticides induce behavioural disturbances in foraging bees and impairment of return flight to the hive **at low concentration level** (1,2). Recently, a neonicotinoid compound **thiamethoxam** was homologated to be used on plant seeds dressing. This molecule acts mostly as an agonist of insect nicotinic acetylcholine receptors. To study whether **thiamethoxam** may disorientate foraging bees, **its impact on orientation performance** was examined in a **complex maze under outdoor conditions**. Orientation in a maze relies on associative learning between a visual mark and a reward of sugar solution (3).

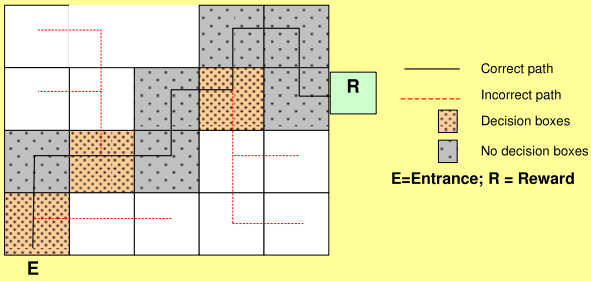
➢ Materials and Methods

Experiments were repeated twice. The maze and the honeybees confined in a 5-comb hive were placed in an outdoor flight cage (2,5 m x 2,5 m x 2 m high) covered with an insect-proof cloth. The maze consisted of a matrix of 4 x 5 identical cubic boxes (3). Device of the maze is described below.



Principle of the maze and conditioning procedure

Bees had to fly through a sequence of **9 boxes** to reach the target: the reward box with a feeder filled with sugar solution. To do so, **bees were first conditioned to associate a green mark to the reward**. In the maze, green marks were fixed under the appropriate hole in each box to indicate the correct path. Along the path, 3 boxes constituted a decision point: the bee had to choose between a marked hole (correct path) and an unmarked hole leading to dead end. Conditioning procedure lasted one day and was repeated three times. At the end of the third one, conditioned bees were captured and transported to laboratory for label and treatment.



Bees label and treatment

Bees were individually marked with colour number tags. A total of **138** bees were labelled (69 for control vs 69 for treated groups) during experimental period. Treated groups orally received a dose of 3 ng of thiamethoxam per bee (LD50/10) in a sugar solution (saccharose 50 % m/m). Control bees received non-contamined sugar solution.

Tests

The day after treatment, **individual orientation performances** in the maze were tested on **three different paths** during two repetitions: experiment 1 and 2. For each labelled bees, **correct decisions, wrong decisions and turn backs** were recorded. **Time to reach the goal** was measured. Above 5 min, bees were considered unsuccessful. Performances were so defined in **4 categories** : direct arrival to the goal (**category 1**), arrival to the goal after turn(s) back (**category 2**), arrival to the goal with mistake(s) (**category 3**), no arrival to the goal within 5 min (**category 4**).
For each test, only the first flight of each labelled bees entering in the maze were considered.

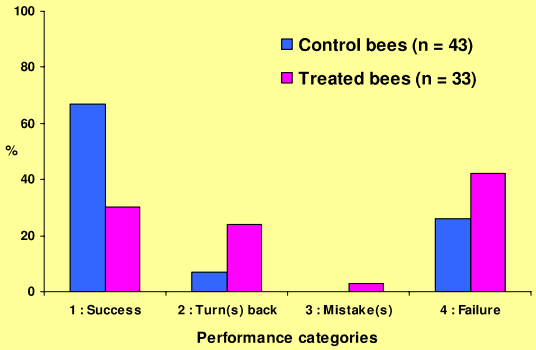
➢ Results

Mortality

A significant difference was found between control and treated bees (Khi^2 test; $P<0.01$). Thiamethoxam treatment has increased mortality **(42 % vs 20 % in control**).

Performance

Performance analysis showed no significant differences between experiments, between days of tests and between paths (Mann-Whitney tests; $P>0.03$). Data from the two colonies were so pooled. **Bees orally exposed to thiamethoxam had significantly lower performances than untreated bees** (Mann-Whitney test; $P<0.01$).
The rate of foragers that successfully reached the reward directly was reduced in thiamethoxam-fed group (**30% vs 67%**). In parallel, a higher rate of treated bees arrived to the goal with one or more turns back (**24% vs 7%**). Only some treated bees arrived to the goal after one or more mistakes (**3 %**). Finally, the rate of bees that did not reach the goal within 5 min was higher in treated bees than in control (**42% vs 26%**).



Flight time

In bees ranked **in categories 1 to 3**, 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 experiments, day of test and between paths (ANOVA; $P>0.05$). **thiamethoxam induced an increase of bees' flight time through the maze**. But this difference was not significant (ANOVA; $P>0.05$).

Modalities	Flight time (s) (Mean ± s.e.m.)	Number of bees
Control bees	75.13 ± 11.42	32
Treated bees	90.11 ± 17.37	19

➢Conclusion

Orientation perfomance of foragers in a complex maze were affected by thiamethoxam. Control bees can more successfully locate the goal (sugar solution) by flying through paths they have never previously encountered. This task was more difficult for treated bees.

The administration of 3 ng of thiamethoxam per bee induced sublethal effects but also lethal effects with more than 40% of mortality in treated modality.

The originality of our results consists in the demonstration of impact of thiamethoxam on orientation which is a complex process depending on learning of visual landmarks, memorization of the rule consisting in the association of the green mark to the right way. If our experiments do not allow concluding to learning and memory impairment, they show a negative effect of the insecticide on the ability of bees to find a route.

Navigation in the field relying on several guidance mechanisms, additional experiments would be needed to establish whether foragers exposed to thiamethoxam can negotiate a route or not in a complex environment.

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