Bulletin of Insectology ***56*** *(1): 59-62, 2003*
ISSN 1721-8861

Effects of imidacloprid administered in sub-lethal doses on honey bee behaviour. Laboratory tests

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Abstract

Under certain circumstances, the effects of imidacloprid on honey bees may not be immediately perceived. The aim of this study was to investigate if imidacloprid, provided in sub-lethal doses, could influence honey bee behaviour in the laboratory.

Imidacloprid (supplied as Confidor®) was offered to bees in 50 % sucrose solution at two different concentrations (100 ppb and 500 ppb of active ingredient). Each dose was administered both as single dose and *ad libitum*, to three sets of 10 honey bee foragers. Bees fed with 50% sucrose solution were used as a control. Feeding occurred in holding cages in an incubator in complete darkness. After administration, in each treatment, the behaviour of the bees was recorded with an IR camera, and then analysed with "The Observer" computer program.

In each treatment bees were significantly less active (in terms of mobility) than bees in the untreated control. Furthermore, in the treated bees, the communicative capacity seemed to be impaired, and this could cause a decline in the social behaviour. Nevertheless, the negative effects appeared only after a certain period of time following administration (30-60 minutes) and vanished after several hours.

Imidacloprid therefore has an inhibiting, even though transitory, effect on honey bees. We assume that the period of time in which honey bee behaviour is altered could negatively affect both the individual and the entire colony.

Key words: *Apis mellifera*, imidacloprid, sub-lethal effects, behaviour, pesticides

Introduction

Imidacloprid (fig. 1) is a systemic insecticide, that acts on the transmission of nervous impulses in the most hazardous phytophagous insects. Imidacloprid-based insecticides are used on *Pomaceae*, on *Drupaceae*, on *Citrus*, on horticultural plants and for seed dressing in corn, sugar beet, sunflower and potato. Because of its high efficacy, its use is increasing worldwide.

It is known that at the field dose, imidacloprid is highly toxic to almost all insects, including honey bees (oral LD_{50} = 0.0037 µg/bee, topic LD_{50} = 0.081 µg/bee; Schmidt, 1996). The producer asserts that in normal conditions honey bees do not get in contact with the substance at toxic levels (Schmuck *et al.*, 2001). Nevertheless, in certain cases, the effects of imidacloprid on honey bees may not be of immediate perception. In the last years, in northern Italy and in France, during the application period of imidacloprid-based pesticides, many bee losses occurred. In fact, at very low doses, the molecule does not cause the death of honey bees, but it can induce behavioural changes, such as foraging activity decrease (Curé *et al.*, 2001; Decourtye *et al.*, 2001) or disorientation (Kirchner, 1998), that can temporarily damage the entire family.

The aim of the present research was to investigate in the laboratory the impact of imidacloprid, administered at sub-lethal doses, on the behaviour of *A. mellifera* foragers, and to analyse how their behaviour changes over time. The attention was focused on the mobility of the insects and on their communicative capacity.

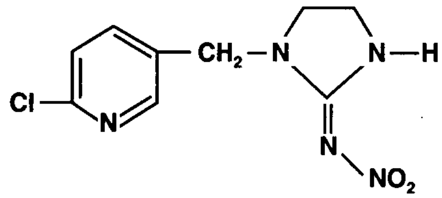

Figure 1. Structure of imidacloprid molecule

Material and methods

To investigate the effects of imidacloprid on honey bee behaviour, the formulated product Confidor® was administered to three replicates of 10 foragers by ingestion at two different concentrations, 100 ppb and 500 ppb of active ingredient, respectively, and each concentration was administered both as "single dose" (20 µl / bee) and "*ad libitum*". Known amounts of the formulated product were dissolved in sucrose solution (50% weight) to obtain the desired pesticide concentrations. The feeding solution for untreated bees (control) was sucrose solution (50% weight). In the "single dose" administration, a common feeder containing the test solution (200 µl sucrose solution added with imidacloprid at known concentration) was provided. Once the bees had consumed all the test solution, an additional feeder with pure sucrose solution was introduced into the holding cages. In the *ad libitum* administration, the test solution was offered for the entire trial period.

Foraging honey bees were captured from the flight board at the entrance of an experimental hive. Prior to being transferred into the holding cages, bees were chilled for 30 minutes at +4°C, to facilitate handling. Holding cages consisted of small plexiglas cages (13 cm x 6 cm x 11 cm – height) with two transparent side walls. Each cage was provided with a feeder and a piece of empty honey comb taken from a super of the experimental hive. The holding cages were then placed in an incubator at 25 ± 1 °C, 60 - 70 % RH, and in complete darkness (L:D=0:24).

In each treatment, the activity of the honey bees was monitored with an infrared camera and video-recorded over a period of 24 hours in order to follow bee behaviour evolution over time (fig. 2). Due to a narrow recording angle of the IR camera, only two cages were monitored simultaneously: one with treated bees, and one with untreated bees (control). Bee behaviour, recorded with the IR camera, was subsequently analysed with the help of "The Observer®" computer program, from Noldus Information Technology B.V.

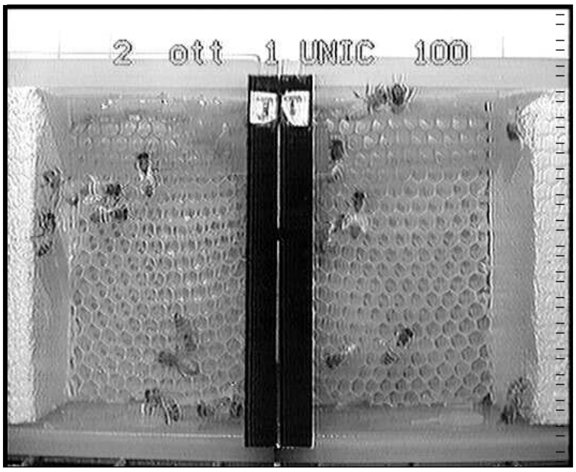

Figure 2. Video recording of honey bee behaviour with IR camera. Two holding cages, each containing 10 bees, a fragment of honey comb and, in the bottom, the common feeder for the "single dose" administration can be seen.

The following three different behaviours related to mobility were identified:

- "stationary": the bee is immobile either on the comb, or on the walls of the holding cage, or in a cell;
- "walking": the bee moves slowly;
- "running": the bee moves at high velocity.

After a preliminary screening of all three behaviours over 24 hours, we decided to analyse the time spent by the bees in each cage being stationary in the 5 recording intervals: from 0 to 30 minutes, from 30 minutes to 1 hour, from 1 to 2 hours, from 6 hours 30 minutes to 7 hours, and from 23 hours to 23 hours 30 minutes after administration. Furthermore, in the third recording interval (from 1 to 2 hours after administration), in each cage, the time spent by the bees performing the three different behaviours, was recorded. Also bee mortality (after 24 hours) was measured in each cage. Mean values (time and mortality) were calculated for each treatment.

The Mann-Whitney U-test was used to compare the data recorded for each treatment with those obtained in the respective control.

To analyse whether in each cage the bees manifested activity in a co-ordinated or random manner the number of bees running at the same moment was registered in the third recording interval (1h-2h after administration). The Brown-Forsythe HOV test, applied to the variance of the number of running bees, was used to compare each treatment with the respective control.

Results and discussion

As far as the stationary behaviour is concerned, in all four treatments (single dose and *ad libitum* administration of 500 and 100 ppb imidacloprid, respectively) significant differences between treated and untreated bees emerged during the first two hours after administration (fig. 3): in all the treatments, in the third recording interval (1h-2h after administration), the stationary behaviour was significantly more frequent in treated bees than in untreated bees; in the second recording interval (30min-1h) significant differences emerged in all treatments except in the *ad libitum* administration of 100 ppb imidacloprid, whereas in the first recording interval, the stationary behaviour was more frequent only in bees fed *ad libitum* with 500ppb imidacloprid. In the fourth and fifth interval (6h 30min-7h, and 23h-23h 30 min), differences between treated and control bees were not significant any more, indicating that the behavioural changes may be transitory. Transitory character of imidacloprid influence on honey bee behaviour was asserted also by Curé *et al.*, 2001.

In the third recording interval (from 1 to 2 h after administration), significant differences between treated and control honey bees emerged for all the behaviours, at both concentrations and at both administrations: treated bees ran and walked less and spent more time being stationary than control bees (U-test: $p<0.05$, in all cases; fig. 4). Imidacloprid had a negative effect on honey bee mobility, which could be revealed only sometime after ingestion, and persisted for at least one hour. Further analyses are in progress to establish when exactly the effects of sub-lethal doses of imidacloprid on bee mobility vanish.

In each treatment, mortality rates did not differ significantly from those registered in the respective control (U-test: $p>0.05$, in all cases), confirming that we actually used sub-lethal doses.

In the third recording interval (from 1 to 2 h after administration), in each treatment, the standard deviation of the number of running honey bees was significantly higher in treated than in untreated bees (fig. 5), indicating that treated bees manifested activity in a more random manner. We observed that each single bee switched back and forth from periods of high mobility (running, walking) to periods of low activity (being stationary) Nevertheless, control bees had a regular tendency to perform those behaviours in a co-ordinated way, that is: almost all of them running vs. almost all of them being stationary. This phenomenon was not observed in the treated bees, where each single bee did not seem to be influenced by the behaviour of the other bees. This could result in a decreasing communicative capacity in treated bees, and in a subsequent decline in the social behaviour.

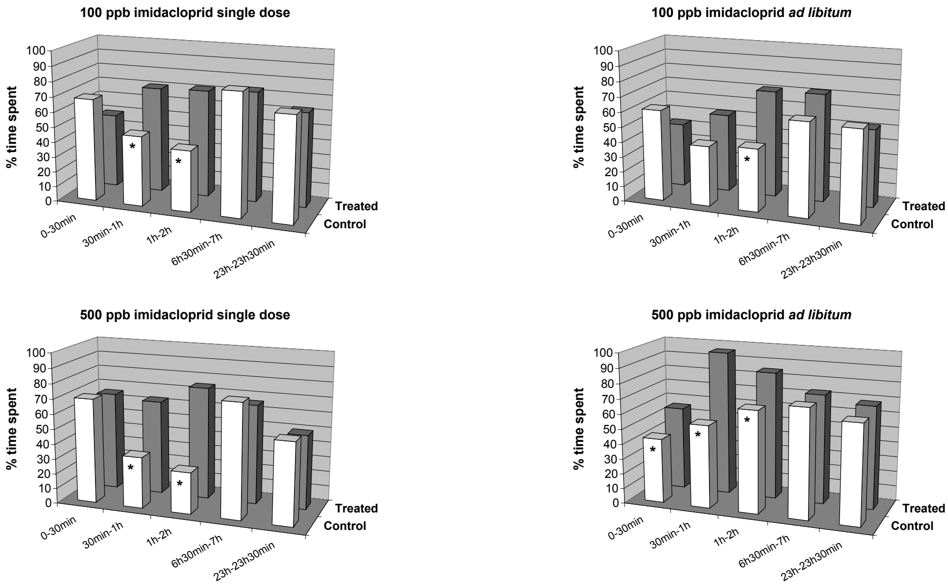

Figure 3. Mean percentage of time spent by treated (with imidacloprid at 100 and 500 ppb, administered as single dose and *ad libitum*) vs. control honey bees being stationary, in the 5 different recording intervals. * - Mann-Whitney U-test; p<0.05

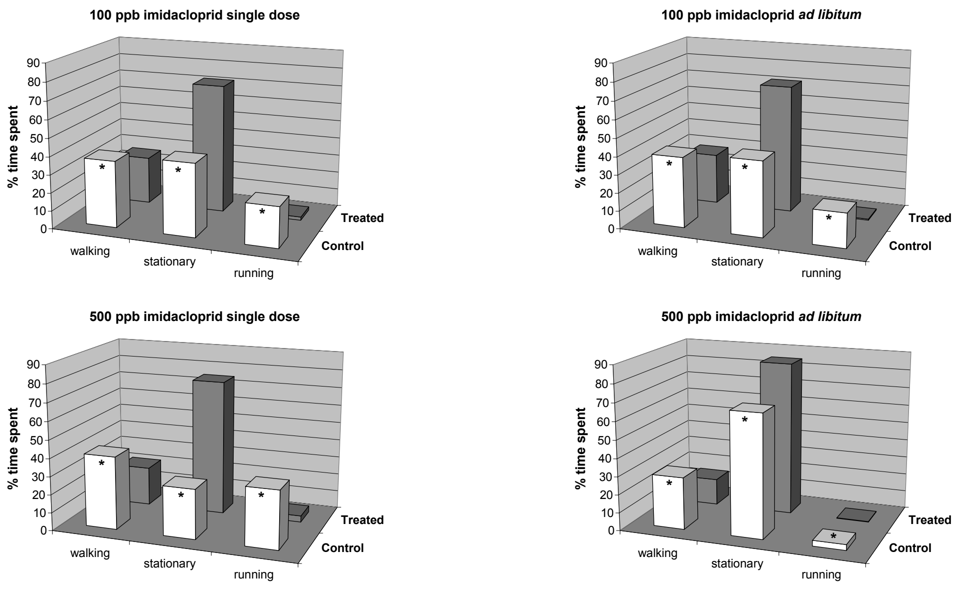

Figure 4. Mean percentage of time spent by treated (with imidacloprid at 100 and 500 ppb, administered as single dose and *ad libitum*) vs. control honey bees performing the three different behaviours, in the third recording interval (from 1 to 2 hours after administration). * - Mann-Whitney U-test: p<0.05.

Figure 5. Standard deviation of number of running honey bees in treated (with imidacloprid at 100 and 500 ppb, administered as single dose and *ad libitum*) vs. control bees, in the third recording interval (from 1 to 2 hours after administration). (Brown-Forsythe HOV test).

Conclusions

Some aspects of the impact of imidacloprid on honey bee behaviour were evidenced, in particular:

- Imidacloprid affects honey bee mobility, reducing both the moving time ratio and the speed of movements.
- The effect starts 30-60 minutes after ingestion and vanishes after a few hours.
- Treated bees seem to loose their communicative capacity, which could impair the social behaviour within the colony.

We therefore believe that bees, accidentally intoxicated in a field with imidacloprid, could find difficulties in returning to the hive, thus depriving the colony of foragers and harming the entire colony.

Further studies are needed to investigate when exactly the effects of imidacloprid vanish, and to establish the impact of sub-lethal doses of imidacloprid on social behaviour.

Acknowledgements

The authors would like to thank Dr Edith Ladurner for her help in revising this paper.

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