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# Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees

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## Abstract

For several years, reports by French and Italian beekeepers have been suggesting a lethal effect of imidacloprid on honey bees; in particular, the molecule has been related to honey bee mortality and decrease of hive populations, affecting the orientation and ability of honey bees to return to the hive.

In this paper we investigate the effects of sub-lethal doses of imidacloprid on foraging activity and homing ability of honey bees. Honey bees from one hive were trained to forage on an artificial feeder filled with a 50% sucrose solution. The feeder was gradually moved up to a distance of 500 meters from the hive. Thirty bees, foraging on the sucrose solution, were captured, individually marked with coloured number tags and transferred into a flying cage, acting as control. The feeder was then replaced with a new one, filled with an imidacloprid supplemented sucrose solution. Again, thirty bees foraging on this feeder were captured, individually marked with different coloured number tags and transferred into an other flying cage. Three concentrations of imidacloprid were tested: 100 ppb, 500 ppb and 1000 ppb. The solutions at 500 ppb and 1000 ppb of imidacloprid had a repellent effect and the bees stopped visiting the feeder, hence only 10 and 20 honey bees, respectively, were captured for the two doses. Since the effects of imidacloprid start half an hour to one hour after ingestion, bees were released from the flying cage 1 hour after confinement. After the release, the behaviour of the bees was followed for 2 hours: two observers at the hive and one observer at the feeding site recorded the arrival and the departure of the marked bees. The presence of the bees at the hive and at the feeder was also recorded for one hour, 5 and 24 hours after the release.

The results show that almost all the control honey bees returned to the hive, and started again visiting the feeder between 2 to 5 hours after the release. Honey bees fed with the concentration of 100 ppb also returned to the hive, but they returned to visit the feeder only 24 hours after the release. Honey bees fed with 500 ppb and 1000 ppb completely disappeared after the release, and they were not seen during the following 24 hours, neither at the hive nor at the feeding site.

**Key words:** imidacloprid, *Apis mellifera*, sub-lethal doses, behavioural effects, foraging, orientation.

## Introduction

In recent years, many beekeepers from European and American countries have been complaining about unusual bee mortality rates and high honey bee losses. In France, one molecule was indicated as the main responsible for these damages, imidacloprid, particularly in its formulation Gaucho®. French and Italian beekeepers report that hives placed near sunflower (in France) and maize (in Italy), originated from seeds dressed with Gaucho®, show high levels of damage due to a progressive decline in the hive populations (the so called "disappearing disease"), until the complete loss of the colonies.

Trying to connect the beekeepers' complaints with the observed effects of imidacloprid, one question arises: can sub-lethal doses of imidacloprid be responsible for the "disappearing disease"? Is there a link between the use of imidacloprid and the effects reported by beekeepers?

In a first experiment, during summer 2001, our group investigated the effects of sub-lethal doses of imidacloprid on the foraging behaviour of honey bees. Bees were trained to forage on an artificial feeder, then orally treated with two concentrations of imidacloprid (100 ppb and 1000 ppb) and immediately released. We recorded the number of honey bees returning to the hive and their foraging activity on the feeder for the following two days. We found a decrease in the foraging activity of bees treated at the two doses, lasting until the second hour after the treatment, but we did not find any effect on the honey bees' homing ability. All the treated bees were able to return to the hive within two hours from the treatment (Bortolotti *et al*., unpublished data).

Our results were coherent with those of a comprehensive field research on the effects of imidacloprid on honey bees, presented by Curé *et al*. (2001). In this study the authors found that imidacloprid at a concentration of 100 ppb, disrupts the communication of the location of the food source between foraging bees and house bees, causing a temporary reduction or the end of the foraging activity. However, foraging activity is restored the day after the treatment, and no other adverse effect was noticed.

One hypothesis to explain these results is that treated honey bees, returning immediately to the hive, regurgitate the content of their stomach and therefore the active ingredient does not have enough time to enter the bees' body fluids. In fact, from the results of a laboratory study, we know that the active ingredient starts to affect the behaviour of treated bees only 30-60 minutes after ingestion (Medrzycki *et al*., 2003).

According to these considerations, in summer 2002 we outlined a new experiment, where honey bees, exposed to similar concentrations of imidacloprid, (100 ppb, 500 ppb, and 1000 ppb) were not allowed to return to the hive immediately after ingestion, but they were forced to remain in the field for enough time to allow the active ingredient to enter the bees' body fluids and manifest its effect.

## Material and methods

### Bees

One healthy honey bee (*Apis mellifera* L.) colony with a single queen was used. The hive was placed in an area without any other honey bee colony within a range of 3 km, to avoid the presence of foreign bees disturbing the experiment.

### Training

Honey bees were trained to forage on an artificial feeder, filled with a 50% sucrose solution, that was gradually moved from the hive up to 500 meters distance. The flying path from the hive to the feeder was very linear and without obstacles or barriers. After the training and prior to the beginning of the trial, honey bees were allowed to forage on the feeder for one week.

### Test design

On the trial day, 30 honey bees that had been feeding on the feeder filled with sucrose solution, were captured and labelled with coloured and numbered tags. These bees, acting as control, were then transferred into a flying cage (60x60x60 cm) and kept in the shadow near the feeding site. Once the 30 control bees were inside the cage, the feeder was replaced with a new one, filled with sucrose solution at the lowest concentration of imidacloprid (100 ppb); 30 bees that had been feeding on this solution were again captured, labelled with coloured numbered tags and transferred into an other flying cage. The same procedure was repeated with the higher concentrations of imidacloprid. Bees remained inside the flying cage for one hour.

### Imidacloprid concentrations

Three concentrations of imidacloprid containing 100, 500, and 1000 ppb of the active ingredient (obtained from the commercial product Confidor®) were tested. The solutions at 500 and 1000 ppb of imidacloprid had a repellent effect on the honey bees: they gradually stopped visiting the feeder, and consequently we could not collect 30 honey bees on the feeder. Furthermore, the two concentrations 1000 and 500 ppb were tested at a week distance from each other: in the first test, 30 control bees and 20 bees treated with 1000 ppb were collected; in the second test, 29 control bees, 30 bees treated with 100 ppb, and 10 bees treated with 500 ppb were collected.

### Observations

Observations started as soon as the control bees were released from the cage. At that moment, all the treated bees were already inside the cage and the feeder was filled with sucrose solution. One observer at the feeding site and two observers at the hive recorded on audio tape all the labelled bees that were seen foraging on the feeder, and entering and leaving the hive, respectively. During the test, the hive was equipped with a pollen trap to slow down entering bees and facilitate observations. The observer at the feeding site was also in charge of opening the flying cages of the treated bees, after the period of one hour had expired. The behaviour of the bees leaving the cages was also recorded.

Three observation turns were run: the first from the release up to two hours; the second from the fourth to the fifth hour after the release; the third on the first day after the test (24 hours after the treatment) for one hour.

## Results

The percentages of honey bees that returned to the hive and to the feeding site in each treatment during the three periods of observation are reported in figures 1 and 2, respectively.

During the first observation turn (after two hours from the release), 80% and 72% of the control bees returned to the hive, and 33% and 31% returned to forage on the feeder (Control 1 and Control 2, respectively), whereas in the honey bees treated with 100 ppb, 57% returned to the hive and only 3% returned to the feeding point.

In the second observation turn (from the $4^{th}$ to the $5^{th}$ hour after the release), 87% and 79% of the control bees returned to the hive, and 77% and 76% restarted to forage on the feeder (Control 1 and Control 2, respectively); the percentage of bees treated with 100 ppb that returned to the hive did not increase in this second observation turn, whereas 7% returned to forage on the feeder.

After the third observation turn (24 hours after the treatment), the percentage of bees returning to the hive increased to 90% and 87% in Control 1 and 2, respectively, and in the bees treated with 100 ppb, 84% returned to the hive and 73% restarted foraging on the feeder.

None of the bees treated with 500 and 1000 ppb were seen either at the hive or at the feeding site in none of the three observation turns. These bees completely disappeared.

Despite the time spent to return to the hive and/or to the feeder, most of the control bees and of the bees treated with 100 ppb (more than 70%) returned to the hive, and restarted to forage on the artificial feeder within 24 hours after the treatment (figure 3). A small percentage, 7% and 13%, respectively, returned to the hive within 24 hours, but probably changed the food source, since they were seen entering the hive, but never visiting the artificial feeder. Only a few control bees and bees treated with 100 ppb completely disappeared.

On the contrary, bees treated with 500 and 1000 ppb completely disappeared both from the hive and from the feeder within 24 hours after the treatment. In fact, during the following days, none of these bees was seen, dead or alive, either in front of the hive or at the feeding

site. Probably they were not able to return to the hive and died somewhere in the field.

Increasing concentrations of imidacloprid also influenced the capability of the honey bees to leave the cages. Once that the cage had been opened, the control bees left it immediately; the last bee treated with 100 ppb left the cage after 10 minutes; bees treated with 500 ppb needed 45 minutes to leave the cage; it lasted 75 minutes before all the bees treated with 1000 ppb left the cage. Treated honey bees also showed anomalous flying behaviour: they often fell in the grass and their flight direction was not towards the hive. Treated bees seemed to be disoriented, and that could be the cause of their disappearance.

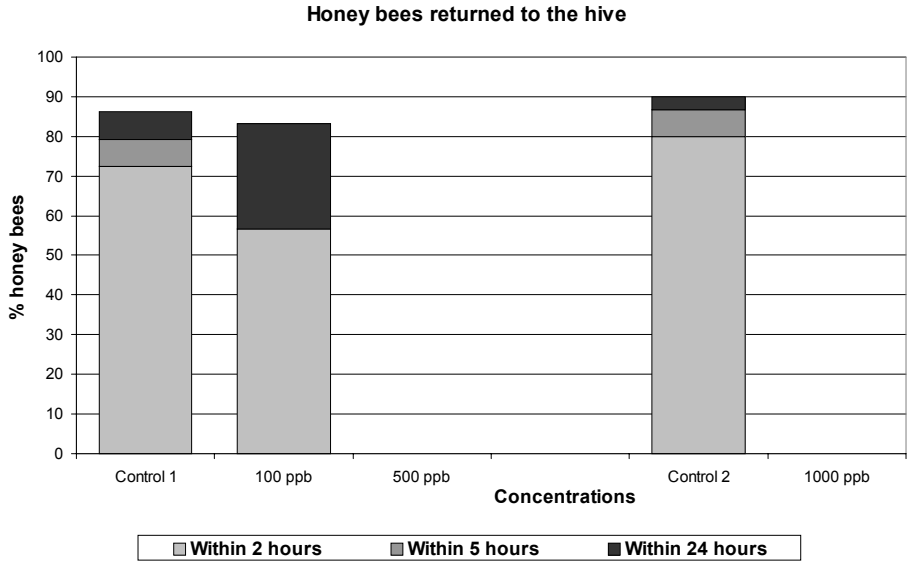


**Figure 1.** Percentage of honey bees in each treatment that returned to the hive during the three observation turns (within 2 hours, after 4 hours and 24 hours).

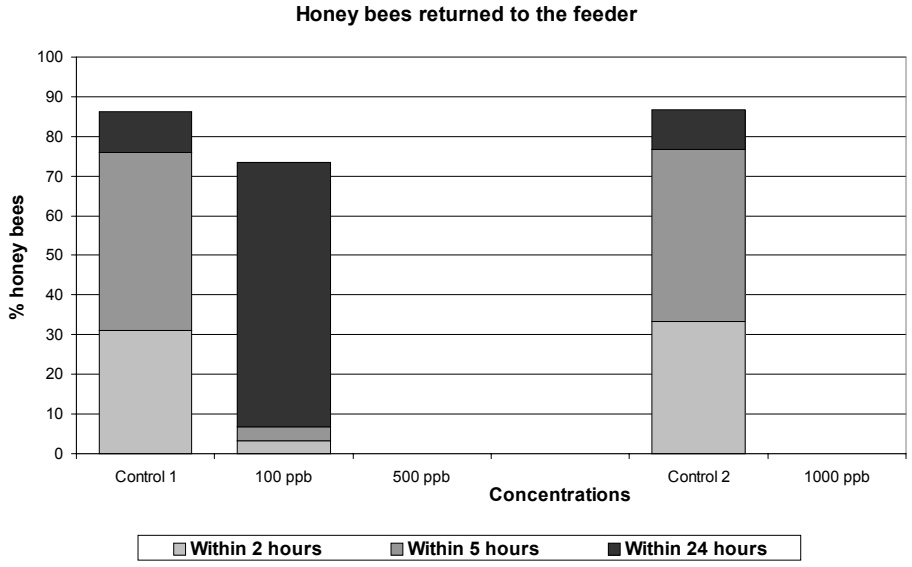


**Figure 2.** Percentage of honey bees in each treatment that returned to the feeding site during the three observation turns (within 2 hours, after 4 hours and 24 hours).

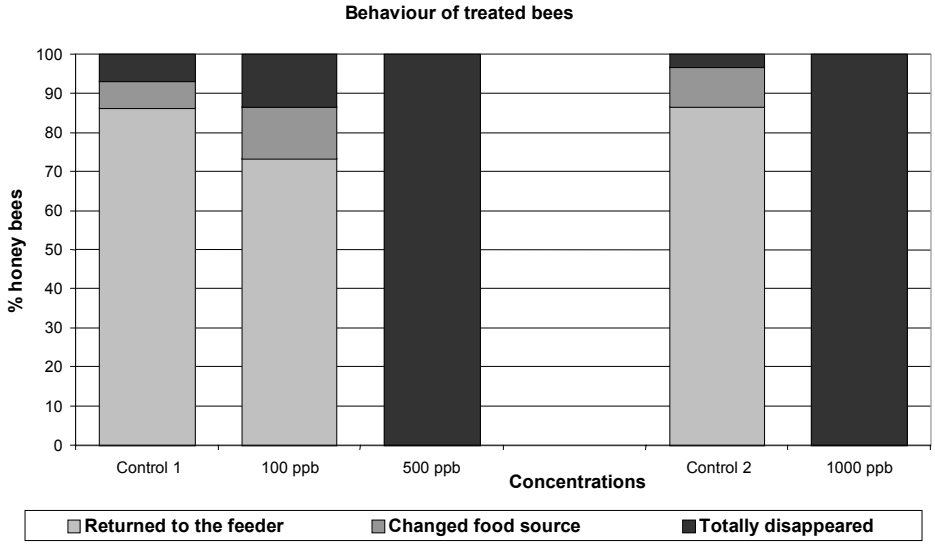


**Figure 3.** Percentage of bees in each treatment that returned to forage on the feeder, changed food source or disappeared completely.

## Discussion and conclusions

Due to the beekeepers' complaints regarding honey bee losses, many researches were prompted in different countries, to understand whether imidacloprid could be responsible for these damages.

The results of seven European laboratories show that the molecule is very toxic to bees in the laboratory: oral 48h $LD_{50}$= 41-81 ng/bee; contact 48h $LD_{50}$= 49-102 ng/bee (Nauen *et al*., 2001). Other laboratories found even lower values: oral 48 h $LD_{50}$= 3.7-40.9 ng/bee; contact 48h $LD_{50}$= 59.7-242.6 ng/bee (Schmuck *et al*., 2001).

Nevertheless, tunnel and field researches demonstrated that honey bees hardly get in contact with toxic concentrations of imidacloprid on seed dressed crops. Pollen and nectar in sunflower plants originating from imidacloprid dressed seeds, show very low residual levels of the active ingredient (1.9 ppb in nectar and 3.9 ppb in pollen, Schmuck *et al*., 2001). Honey bees foraging on *Phacelia tanacetifolia* plants, whose seeds were treated with the same amount of imidacloprid used in sunflowers, show a residual amount of the active ingredient less than 10 ppb in the honey sac and less than 3 ppb in the collected pollen bread (Wallner, 2001).

Since there is a great variability in the $LD_{50}$ values of imidacloprid reported by different authors (Suchail *et al*., 2001), we performed preliminary laboratory tests to choose the sub-lethal doses. Even at a concentration of 1000 ppb, imidacloprid did not cause mortality, but only a temporary knock-down effect on some of the treated bees. Concentrations similar to those we used in our experiment (100 ppb and 1000 ppb), were also tested by other authors in a field study on the foraging behaviour (Guez *et al*., 2001).

In our experimental conditions, the concentration of 100 ppb causes a delay in the homing behaviour of honey bees compared to the control. After the release from the cage, almost half of the honey bees needed more than five hours to return to the hive, whereas most of the control bees returned to the hive within two hours. The same concentration causes a temporary inhibition of the foraging activity, lasting more than five hours. The normal foraging activity was restored only on the following day. The transitory effect observed at 100 ppb does not seem to affect the hive population.

The concentrations of 500 and 1000 ppb cause the complete disappearance of treated honey bees. The bees were seen neither at the hive nor at the feeding site, for 24 hours after the test. It is likely that these bees got lost and died somewhere in the field. An exposure to such concentrations could therefore strongly affect the hive population.

Our results are consistent with those reported by other authors. Guez *et al*. (2001) report that at a concentration of 1000 ppb foraging activity ceases, whereas at 100 ppb it is not affected. Curé *et al*. (2001) observed an effect of imidacloprid at the concentration of 100 ppb on the communication of the food source between bees, causing a temporary reduction or interruption of the foraging activity, which was restored the day after the treatment. Decourtye *et al*. (2001) noticed an effect of the active ingredient on the flight activity and on the learning performances of honey bees at an even lower concentration (50 ppb). At an individual level, the molecule affected the olfactory learning performances, measured with the proboscis extension reflex bioassay; at a colony level, it lead to a decrease in the flight activity and in the olfactory discrimination performances, evaluated by using artificial flower feeders in a flight cage. None of these authors observed the disappearance of honey bees, because they allowed the bees to return to the hive immediately after the release or because they worked in a protected environment (cage or green-

house). In such conditions, disorientation effects are less evident and the decrease in foraging activity is temporary.

Also our observations on the behaviour of bees after the release from the cage are similar to those reported by other authors. Suchail *et al*. (2001) noticed behavioural abnormalities, such as trembling, tumbling and lack of co-ordination, 24 hours after exposure of honey bees to imidacloprid and its metabolites. In a field test, Guez (2001) observed in imidacloprid treated honey bees legs trembling, movement difficulties, transitory immobility and inhibition of flight ability. Medrzycki *et al*. (2003) state that imidacloprid has a negative effect on the insect's mobility and that treated bees behave in an uncoordinated and unsynchronised way. According to the authors, this could indicate a decreasing communicative capacity of treated bees with a subsequent decline in the social behaviour.

In conclusion, our research shows that, in certain conditions, the administration of imidacloprid can lead to the disappearance of honey bees from the hive, probably due to the disorientation caused by the substance. This effect can arise if the concentration of imidacloprid to which honey bees are exposed exceeds 100 ppb, and if after ingestion, honey bees are not allowed to return immediately to the hive (because of the distance between the hive and the food, obstacles in the landscape, climatic conditions or any other reason). Further studies are needed to investigate if such conditions can actually occur in the field.

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