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RESEARCH ARTICLE

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Effects of imidacloprid on stretching and production of wax in domestic bees *Apis mellifera intermissa* in North Africa (Algeria)

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Imidacloprid is a well-known systemic insecticide, which has a deleterious impact on honeybees. Beekeepers in the Wilaya of Tizi-Ouzou (Algeria) reported unusual losses and deaths of bee colonies in the places where imidacloprid insecticide was used. Even at sublethal doses, insecticide can affect tasks performed by workers in a colony, such as comb building. The study investigated the effect of an imidacloprid based insecticide (Confidor®Supra) on the production of wax by the honeybee *Apis mellifera intermissa.* After the imidacloprid LD_{50} was determined in controlled conditions, three sublethal doses were tested. The mortality of the bees, syrup consumption and the weight of the wax generated were recorded. The imidacloprid insecticide LD_{50} at 48 hours was 3.5 ng bee^{-1} for 4-day old spring worker bees. The three sublethal doses (0.175 mg L^{-1}, 0.087 mg L^{-1} and 0.035 mg L^{-1}) had an impact on the syrup consumption and the wax production of adult bees. Bees exposed to sublethal doses of insecticide consumed less syrup and produced less wax (between 0.040–0.415 g) than the bees in the control (0.745 g). A dose response was observed regarding the production of wax. This impact could have harmful consequences for bee colonies, as wax production is the basis of comb building. The physiological causes of the reduction in wax production remain to be investigated.

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INTRODUCTION

In intensive agriculture, pesticides are commonly used for plant protection and the increase of crop production. Many modern pesticides used around the world belong to the neonicotinoids, a systemic insecticide family that move between the seed, soil and whole plant (Nauen et al. 1999). Imidacloprid, an active ingredient of widely used commercial insecticides, is present in 14 different formulations (e.g. Confidor, Admir, Commando, etc.). It is a well-known neonicotinoid used against plant and root pests (Elbert et al. 1998). Since its commercialization in the 1990s, the presence of this insecticide has been identified in tomato (Banerjee et al. 2012), corn and sunflower, in concentrations ranging from 1–20 µg kg^{-1} (Bonmatin et al. 2003), or 1.9 ppb. (Schmuck et al. 2001). More specifically, studies have shown that after treatment, the pollen from melliferous plants has imidacloprid concentrations of 0.1–18 ppb, with comparable concentrations in nectar (approximately 0.8 ppb) (Bonmatin et al. 2005; Schmuck et al. 2001; Scott-Durpee & Spivak 2001). Consequently, the pesticide found in pollen, harvested by honeybees, has an aggregate value of 1.1–5.7 µg kg^{-1} (Chauzat et al. 2006), even when harvested from wild plants. The foraging bees brought contaminated pollen and nectar back to the colony, which can lead to lethal, or sublethal exposures of imidacloprid (Halm et al. 2006).

Imidacloprid is very toxic to bees (Suchail et al. 2000) in which it stimulates the synapses, which can lead to nervousness, convulsions, paralysis and death (Nagata et al. 1998). Suchail et al. (2001) reported that an imidacloprid concentration of 3.7–40 µg kg^{-1} would induce acute toxicity, while a dose of 3.7–102 ng bee^{-1} would kill 50% of the bee population (LD_{50}) (Suchail et al. 2001). These lethal values correspond to a concentration of 0.1 and 1.6 mg kg^{-1} in the food. In chronic exposure tests, doses between 48–96 ppb were shown to be fatal to worker bees in cages (Decourtye et al. 2003).

Sublethal effects were also described in several studies, such as the orientation of the bee (Lambin et al. 2001), the waggle dance for the recruitment of foragers (Kirchner 1999), or the number of visits to a source of sugar. These impacts were triggered by concentrations of imidacloprid ranging from 6–50 ppb (Colin et al. 2004). This can be explained by the fact that this insecticide is known to reduce olfactory flight and olfactory performance (Decourtye et al. 2001), affect learning (Guez 2001) and cause orientation disorders (Vandame et al. 1995). Repeated ingestion of low levels of imidacloprid may also lead to immune deficiency, bee diseases (Glinski & Kauko 2000) and other physiological disturbances (Atkins & Kellum 1986). Some insecticides have affected the hypopharyngeal glands, which could influence nurse bees and consequently brood care. The effect on *Apis mellifera intermissa* wax bees, which synthetize wax from hydrocarbons, esters, fatty acids and proteins is unknown. These workers with developed wax glands have an important role in the colony, as they are responsible for comb building (Winston 1991). The effects of insecticides observed on the different categories of honeybees (nurse, wax or forager workers) weaken the colonies and could be involved in the observed worldwide colony losses.

Like other countries, Algeria recorded colony mortalities, with colony losses greater than 30% per year. More specifically, beekeepers in the Wilaya of Tizi-Ouzou reported unusually high losses and deaths of bee colonies of approximately 37% in 2015–2016 (DSA 2016). The Wilaya of Tizi-Ouzou

is a mountainous area in Algeria, and one of the most important areas for intensive agriculture and beekeeping in the country, with 115 779 colonies present (DSA 2018). Interestingly, the colony losses are more common near agricultural areas, where 'Confidor®Supra' (based on imidacloprid) is widely used (Phytosanitary Inspection 2016). In this region however, little is known about the impact of imidacloprid on *intermissa* worker bees, especially bees performing in-hive activities. The objectives of the present study were to determine the chronic effects of ingesting sucrose syrup contaminated with an imidacloprid-based pesticide at sublethal doses, and to determine the behaviour of stretching and production of wax by the honeybee *Apis mellifera intermissa*.

MATERIALS AND METHODS

The insecticide 'Confidor®Supra', which is 70% imidacloprid, *(NE)-N*-[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]nitramide was bought at a local phytosanitary shop. This insecticide was chosen, as it is the most commonly used insecticide in the Wilaya of Tizi-Ouzou. To obtain the required concentrations of imidacloprid, 5 mg of product (i.e. equivalent to 3.5 mg of imidacloprid) were dissolved in one litre of 50% sucrose syrup (v:v) and homogenize using a magnetic stirrer. The different concentrations used in the study were obtained by diluting the stock solution. Six imidacloprid concentrations were used for the estimation of the LD_{50} (viz. 0.80, 0.70, 0.60, 0.50, 0.40, 0.30 and 0.20 ng μL^{-1}). Three sublethal doses were chosen arbitrarily after determination of the LD_{50} and tested in the study on wax production. In this bioassay, the highest dose of 0.175 mg L^{-1} was 20 times less concentrated than the LD_{50}, while the other two doses were 40 (0.087 mg L^{-1}) and 100 (0.035 mg L^{-1}) times less concentrated than the LD_{50}. These solutions were stored in a refrigerator at 4 °C and protected from light until used.

The study was carried out with 48-hour old worker bees from the *A. mellifera intermissa* apiary of the faculty of biological and agronomic sciences at the University of Tizi-Ouzou (Algeria)[36°43′00″ N and 4°03′00″ E]. The apiary is located far from agricultural areas treated with phytosanitary products. Closed brood frames ready for hatching, were taken from hives apparently free from diseases. Hives were treated against *Varroa destructor* Anderson & Trueman, 2000 with Bayvarol (flumethrin) [Bayer] at 3.6 mg/strip, as per the supplier's recommendations. No other treatment was administered to the honeybees. Frames were taken from three hives between May and June 2016, and then kept in an oven at 33 ± 2 °C. Nascent bees were divided into pools of 100 individuals and kept in experimental cages (Pain 1966) for two days before the start of the experiment. Two-day old bees were chosen for the study, as this age corresponded to early wax gland development and comb building (Winston 1991). Two 5 mL plastic haemolysis tubes, perforated at the bottom to allow the bees to consume the syrup, were used as feeders. The cages were kept in a dark oven at 33 °C ± 2 °C and a relative humidity of 60%, until the bees were 18 days old. This temperature was higher than recommended for the standard method of toxicity testing (CEB n°95 1995) (i.e. 25 ± 2 °C), but was in the range of optimum temperatures for wax stretching described by authors and used in their laboratory bioassays (Darchen 1962; Hepburn & Muller 1988).

Determination of LD_{50}

As the imidacloprid LD_{50} has never been measured at 33 °C on *A. m. intermissa*, and since variability has been observed between studies (Suchail et al. 2001; Nauen et al. 2001; Schmuck et al. 2001), the first step was to determine the LD_{50} at this temperature.

Following the recommendations of the Biological Testing Commission (CEB n° 95), we defined the oral acute 48 h LD_{50} (i.e. the LD_{50} determined 48 h after ingestion of the insecticide) on adult female workers 48 h old (i.e. on workers 96 hours after hatching).

For this step, each of the six doses (treatments) were tested on three cages of 100 bees (replicates) from the three different colonies (pseudoreplicates). After 48 h of ad libitum feeding, the bees were fasted for two hours before 1 mL of contaminated syrup was introduced in the cage (i.e. an average 10 µL per bee). The fasting ensured that the syrup consumption started at the same time for each group of bees (Suchail et al. 2000).

For the LD_{50} determination, the mortality observed in different experiment treatments were corrected by the natural mortality observed in the control group based on the Schneider-Orelli formula, which is a variant of the Abbott formula (Abbott 1925; Püntener 1981).

$$CM(\%) = (M_t - M_c/(100 - M_c)) \times 100 \quad (1)$$

where
CM: corrected percent mortality
M_c: percent mortality in the control population
M_t: percent mortality observed in treated populations

The corrected mortality percentages (CM) were then transformed in probits and the doses into decimal logarithms. This allowed for the analysis of a linear regression curve of the type:

$$\text{Probit of corrected mortality} = a \times \text{logarithm of doses} + b \quad (2)$$

This linear regression was used to determine the LD_{50}.

Sublethal treatments and wax production

After the determination of the acute oral LD_{50}, three sublethal doses were tested on cages of 100 bees from three different honeybee colonies. Using paraffin, a 2 g, 7 × 4 cm rectangle of wax was glued to the upper parts of the experimental cage before the start of the experiment. As in the LD_{50} bioassay, the bees were fed ad libitum for two days with a 50% sucrose syrup before being fasted for two hours. They were then fed for 16 days with the contaminated, or control syrup, according to their treatments. Each treatment modality was repeated three times, on the three cages, from three different colonies (i.e. total of nine cages by treatment). The bees, the cages and the imidacloprid solutions were renewed between repetitions. Mortality and syrup consumption were recorded daily. Immobile bees were considered dead and were removed from the experimental cages. On the 16th day, which is the peak of wax production (Hepburn et al. 1991), the cages were put in a freezer for 10 minutes in order to kill the bees quickly. The rectangles of wax were then recovered, soaked in water to remove any reserves of syrup, dried in the open air and reweighed.

Statistical Analysis

Statistical analyses were performed in R version 3.4.3 (R Core Team 2017). Graphs were generated using the same software. Syrup consumption was analysed using linear models with time, survival of bees and treatment as explanatory factors. The number of surviving bees was analysed following the same method using the colony, the time, the condition and the interactions between both variables as explanatory variables. The number of surviving bees and total consumption of syrup on the 16th day were also analysed using a linear model to check if survival depended on the colony or the treatment.

Finally, the total production of wax on the 16th day was based on the total consumption of syrup, as it affects the amount of imidacloprid ingested. The ratio obtained was analysed using a linear model with the number of surviving bees, the treatment and the colony of origin as explanatory variables. For each analysis, the final model was selected based on the minimization of the Akaike information criterion. The residuals were plotted

to check graphically for the normality, homoscedasticity and the absence of outliers.

RESULTS

Determination of LD_{50}

Approximately 20 min after ingestion, the bees that ingested the syrup containing the high doses of imidacloprid (0.8 and 0.6 ng 10 μL^{-1}) showed symptoms of intoxication. At first the bees showed aggressiveness between themselves, followed by hyperactivity and tremors, before finally becoming listless. Many bees died approximately 2 h after intoxication (Table 1).

We were able to extract interesting toxicological values from the mortality rates measured, namely the LD_{80}, LD_{50} and the LD_{20}. The LD_{50} at 48 h for two-day old caged spring bees *A. m. intermissa* was 3.5 ng μL^{-1} (consumed by one bee).

Chronic mortality

According to the statistical analysis, the survival of bees was significantly dependent on time. Even though the evaluation of mortality throughout the study differed slightly, but significantly, between colony and treatment, the final mortality on the 16th day was neither impacted by the colony, nor the treatment, so the doses tested do not seem to cause any lethal effects (Figure 1). The cumulative mortality recorded for the control group is 9.67 ± 2.19%, which is less than the 10% recommended by the European and Mediterranean Plant Protection Organization (EPPO 1992) for that type of study. For the lowest dose of imidacloprid, which was 0.035 mg L^{-1} of syrup, the mortality was 10.78 ± 0.58%. It should be noted that for the second dose (0.087 mg L^{-1}), the mortality rate averaged 11 ± 0.43%, but 10.78 ± 0.52% for 0.175 mg L^{-1}.

Syrup consumption

During the treatment period, the daily syrup consumption averaged 22.6 ± 0.8 µL bee^{-1} day^{-1} for the control group, whereas the treated individuals consumed between 16.1–17.5 µL bee^{-1} day^{-1}. The cumulated amount of syrup consumed significantly increased with time and reached 361.1 ± 22.1 µL bee^{-1} on the 16th day for the control condition, and 264.9 ± 19.1, 280.3 ± 10.6 and 257.3 ± 15.1 µL bee^{-1} for the groups treated with low, medium and high concentrations of imidacloprid, respectively. The cumulated amount of syrup consumed was significantly dependent on the treatment (Figure 2) and on the number of surviving bees present in the cage.

If the amounts of insecticide ingested during the 16 days of experimentation was considered, the highest exposure was observed for the 0.175 mg L^{-1} group, with a total of 45.03 ± 2.64 ng bee^{-1}. For bees treated with a chronic oral exposure to syrup containing 0.087 mg L^{-1} of imidacloprid, the average exposure per bee was 24.39 ± 0.9 ng bee^{-1}. The lowest amount, 9.27 ± 6.7 ng bee^{-1}, was ingested by the group of bees fed with syrup containing 0.035 mg L^{-1} of imidacloprid.

Table 1: Mortality rates of honeybees *Apis mellifera intermissa*, based on doses of imidacloprid

Doses (ng 10 µL^{-1})	Log dose	Time (hours)	Gross Mortality (%)	Corrected Mortality (%)	Probits
Control	NA	6	1	NA	NA
		24	2	NA	NA
		48	2.5	NA	NA
		72	2.5	NA	NA
D1 = 0.8	0.90	6	80	79.80	5.81
		24	100	100	8.09
		48	100	100	8.09
		72	100	100	8.09
D2 = 0.6	0.78	6	55	54.55	5.12
		24	80	79.59	5.81
		48	100	100	8.09
		72	100	100	8.09
D3 = 0.5	0.70	6	46	45.45	4.88
		24	70	69.39	5.50
		48	85	84.62	6.02
		72	90	89.74	6.23
D4 = 0.4	0.60	6	23.8	23.03	4.26
		24	46.7	45.61	4.89
		48	48.7	47.38	4.93
		72	48.7	47.38	4.93
D5 = 0.3	0.48	6	10	9.09	3.69
		24	23	21.43	4.21
		48	24	22.05	4.23
		72	24	22.05	4.23
D6 = 0.2	0.30	6	1	0	NA
		24	3	1.02	2.67
		48	5	2.56	3.04
		72	5	2.56	3.04

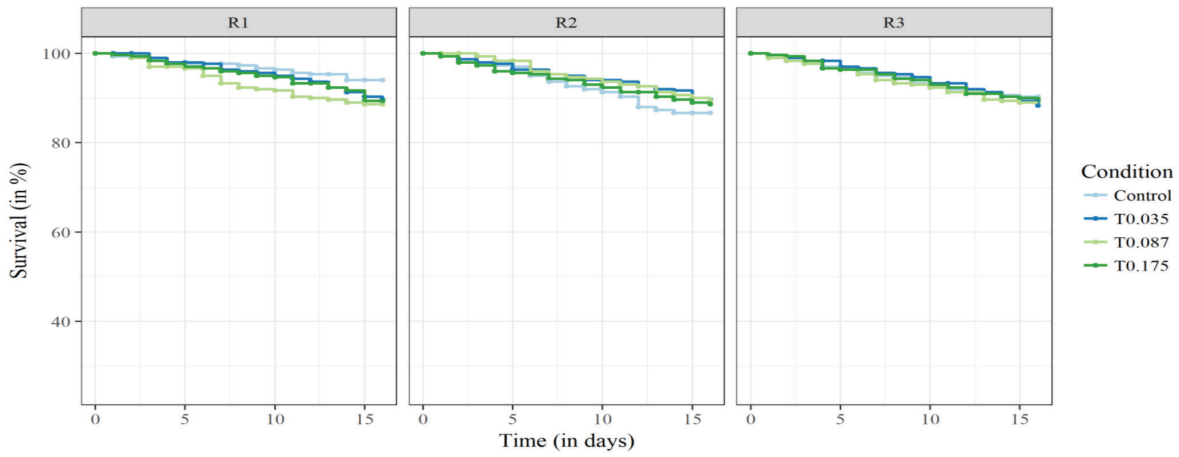

Figure 1. Evolution of the mortality rates during the 16 days of experiments, in relation to the colony of origin of the bees and the treatments applied.

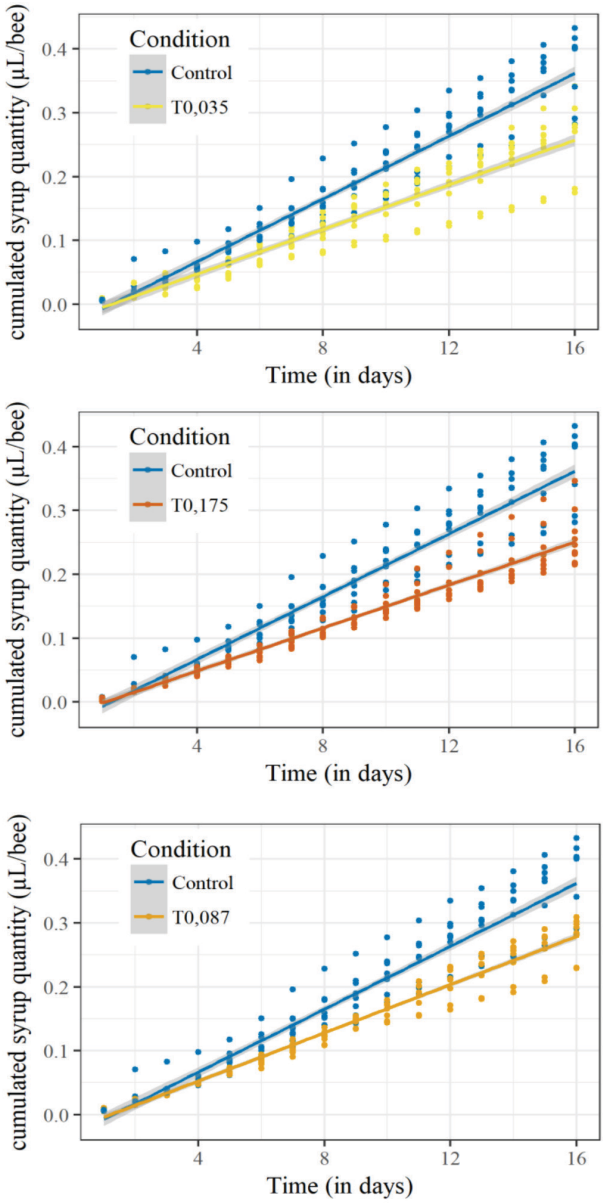

Figure 2. Evolution of the cumulated amount of syrup ingested by a bee during the test period, in relation to the treatment administered. The slopes were fitted using a linear model, while the thin grey areas represent the 95% confidence intervals of the slopes.

Quantities of wax produced

The exposure to the pesticide on the 16th day varied within a treatment group, because of the fluctuations in syrup consumption. As the amount of food ingested was known to influence directly the comb building, the raw wax production values were divided by the total amount of syrup ingested and this ratio was analysed. The statistical model retained included the treatment ($p > 0.001$) and the survival of bees ($p > 0.05$) as significant explanatory factors. More precisely, the amount of wax produced was reduced significantly by the oral exposure to imidacloprid. Comparisons between the amounts of wax produced by the control bee group and the treated bee groups revealed differences (Figure 3). The control bees produced an average of 0.745 ± 0.19 g of wax, whereas the bees treated with a 0.035 mg L^{-1} syrup solution produced an average of 0.415 ± 0.04 g of wax. The bees in the 0.087 mg L^{-1} treatment produced 0.26 ± 0.11 g of wax. Finally, of the wax production was measured at 0.04 ± 0.016 g for bees in the 0.175 mg L^{-1} group. As in the previous models, the survival rate was significant, as the ratio includes the syrup consumption, which depended on the number of surviving bees.

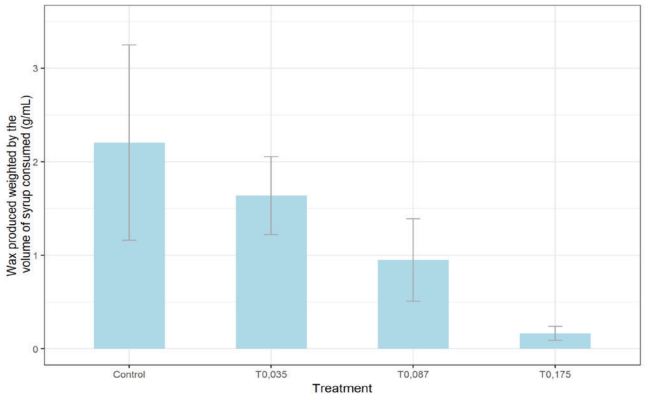

Figure 3. Ratio of the quantity of wax produced divided by the amount of syrup consumed, in relation to the treatments administered to the bees.

DISCUSSION

Honeybees are exposed to neonicotinoids through the pollen and nectar brought back to the colony (Botias et al. 2015). Imidacloprid is the insecticide that has the highest acute toxicity to the honeybee *A. mellifera* (Suchail et al. 2000). It is known to be toxic to bees and cause both lethal and sublethal effects (Sanchez-Bayo et al. 2017). Imidacloprid is still used in modern agriculture, especially in North Africa, yet toxicity data on the North African species of bee *A. m. intermissa* are unknown.

In beginning of this study, the acute oral LD_{50}, of a commercial form of imidacloprid, on this subspecies of honeybee was determined. The result obtained, with two-day old honeybees, of 35 ng of imidacloprid per bee remains within the range of values reported in other studies, namely between 3.7–40.9 ng bee^{-1} (Schmuck et al. 2001). The large variability of LD_{50} values reported in the literature can be attributed to the subspecies. For instance, the imidacloprid oral LD_{50} for *A. mellifera mellifera* and *A. mellifera caucasia* is the same, whereas the contact LD_{50} was 14 ng bee^{-1} for *A. m. caucasia* and 24 ng bee^{-1} for *A. m. mellifera*. Age can also be a source of variability, with young bees (Suchail et al. 2000) less susceptible to malathion, but more sensitive to DDT and carbaryl than older workers (Johansen et al. 1983). Furthermore, even if the concentrations tested were calculated specifically for imidacloprid, the pesticide used was in its commercial form (i.e. Confidor®Supra, 70% imidacloprid as the active ingredient), so we cannot rule out the effect of the adjuvants present in this formulation. It should be noted that imidacloprid does not act alone when ingested. Many of its metabolites, such as olefin, 5-hydroxy-imidacloprid and 4-5-hydroxy-imidacloprid may be more toxic to bees than the parent molecule (Suchail et al. 2001).

Most of the time in natural conditions, the honeybees are confronted with sublethal concentrations of insecticides. Therefore, it is extremely important to characterize the effects of such doses on them. In this study, three doses (0.035 mg L^{-1}, 0.087 mg L^{-1} and 0.175 mg L^{-1} of imidacloprid per litre of syrup) were chosen based on the LD_{50}. The highest dose tested was equivalent to $LD_{50/20}$ (0.175 mg L^{-1}). Indeed, on the 16th and last day, the mortality rates were low in all the groups of honeybees. The chosen doses were therefore confirmed to be sublethal. All conditions registered a mortality rate of approximately 10%, which was comparable or lower than the percentages obtained in similar studies. For example, Decourtye et al. (2003) measured mortality rates of 13% for an imidacloprid concentration of 12 ng bee^{-1}, 11% for 0.12 ng bee^{-1} and 12% for the control. In another study on three-day old honeybees that ingested a syrup contaminated with imidacloprid for 11 days, the mortalities were 16.1% for 24 ppb and 20.5% for 48 ppb. The variability could have been as a result of the different subspecies of bees

used, or the different designs between studies (e.g. temperature, purity of insecticides).

The amounts of syrup ingested were also recorded daily, with differences observed between treatments, both for the evolution of syrup consumption through time and for the total consumption after 16 days. The control bees consumed more syrup than the bees from the treated groups. The values obtained in this study for *Apis mellifera intermissa* were generally lower than the values reported for *Apis mellifera ligustica*. The daily consumption of 4-day old bees exposed to imidacloprid for 11 days was estimated to be between 23.5 and 40 µL bee^{-1} (Decourtye et al. 2003), which is higher than the control condition in our study. The feeding rates are known to vary according to the level of activity of the bee, but also to its age and role, or to the composition of the food solution (Barker & Lehner 1974; Rortais et al. 2005; Winston 1991). Even if there is to our knowledge no study on the subject, this difference may also be explained by the variety of the bees used in the different works. Regarding the reduction of the syrup consumption between the control and the treated conditions, this could be due to the anti-feeding effect of the pesticide. This effect has already been observed in other studies on honeybees and on Aphids treated with imidacloprid at even lower concentrations (Nauen et al. 1998; Ramirez-Romero et al. 2005). Repellent effects have been described for phytosanitary formulations. Formulation adjuvants would be perceived by the olfactory system and trigger an avoidance response in foragers (Delabie et al. 1985). Thus, the absence of anti-feeding effects in the Decourtye et al. (2003) study could be attributed to their use of pure imidacloprid, in contrast to our results obtained for a commercial formulation. It is also important to note that even if it has not been investigated yet, the feeding status of the bees can probably impact their sensitivity to chemical compounds, as suggested by Goulson et al. (2015).

Wax production is an important activity of worker bees, as it is the basis of comb building. Any impact on this activity could have severe consequences for the colony, such as decreased egg laying and storage of pollen and honey in the combs. In this study, wax production was greatest in the control group (i.e. 745 mg), corresponding to 0.41 mg bee^{-1} day^{-1}. This value is consistent with the value of 0.73 mg bee^{-1} day^{-1} reported by Barker and Lehner (1978), or to the 8.62 mg in 16 days (0.54 mg bee^{-1} day^{-1}) reported by Chauvin (1976). It should be noted that the amount of wax produced individually by the bees can be influenced by the age, by the season, a colony's need for wax, the intensity of egg laying by the queen (Pratt 2004; Whiffler & Hepburn 1991; Ledoux et al. 2001), the temperature (Whiffler & Hepburn 1991), or the food source as sugar syrup (Hepburn 1991). All these parameters were controlled in our study, as spring bees of the same age were maintained in experimental cages at a temperature of 33 ± 2 °C. However, the rate of wax production in a colony was also considered as dependent on the amount of food brought back to the nest (Hepburn et al. 1984; Taranov 1959). In this study, the raw wax production indeed depended on the sugar consumption, even when a subset of the data containing only the control group was analysed. As the sugar consumption in the treated groups also directly influenced the exposure to imidacloprid, we analysed a ratio of the wax production to the sugar consumption. This ratio was impacted by the treatment, but also by the number of surviving bees. This last result was consistent with other studies that showed that the number of workers in a colony has an impact on wax production (Taranov 1959). It is the first time that imidacloprid ingestion is shown to have an impact on the production of wax. Indeed, even when the sucrose consumption is taken into the account, the treated groups produced less wax and the response seemed proportional to the dose. It should be noted that to study the effect on stretching and wax production, the insecticide used was 'Confidor Supra', with the active ingredient, imidacloprid, comprising 70% of the formulation. The causes of the reduced wax production remain unknown, but may be related to alteration of the wax glands, or from a behavioural effect on the bees. Other bee glands are known to be damaged by exposure to pesticides. For example, diflubenzuron is known to influence the development of the hypopharyngeal glands in *Apis cerana indica* and *Apis mellifera* Linnaeus, 1758 (Gupta & Chandel 1995). Similarly, sublethal doses of cypermethrin affected the degeneration of the mandibular and hypopharyngeal glands of the foragers (Bendahou et al. 1999). The hypothetical effect of imidacloprid on wax glands should, however, be qualified by the fact that other substances, such as queen pheromones, influence comb building without influencing wax secretion (Whiffler & Hepburn 1991; Ledoux et al. 2001).

In conclusion, the results reported in this study revealed for the first time that three doses of imidacloprid (viz. 0.035 mg L^{-1}, 0.087 mg L^{-1} and 0.175 mg L^{-1} of imidacloprid per litre of syrup) had an effect on bees. The fact that wax production was reduced by imidacloprid in a dose response manner implied that colonies exposed to imidacloprid would possibly suffer from a limited production of wax and comb building. If this is verified in natura, this could mean that the nest size and activity (and productivity) would also be reduced. This would lead to a reduction in the stocks of pollen and honey stored by the colony, and the amount of brood (Darchen 1980).

The physiological causes of the decrease in wax production need to be investigated, while field experiments are needed to evaluate this phenomenon and to define its negative impact on the functioning of a honeybee's colony.

It is evident, that the use of neonicotinoids in Africa needs to be reduced and placed within the framework of an integrated pest management strategy (ASSAF 2021). Imidacloprid, the most widely used insecticide in agriculture (Zhu et al. 2017), has been detected in the pollen and nectar of various plants by many studies (Wood & Goulson 2017). As this insecticide has the highest acute toxicity to the honeybee *A. mellifera* (Suchail et al. 2000), its use must be regulated.

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