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Original article

Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach[1]

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Abstract – In a comparative approach, we evaluated the effects of Cry1Ab protoxin, deltamethrin and imidacloprid insecticides on mortality, syrup consumption, foraging activity and olfactory learning capacities of free-flying honeybees. In an indoor flight cage we exposed bee colonies to different syrups containing Cry1Ab protoxin, deltamethrin or imidacloprid at 1000 µg/kg, 500 µg/kg and 48 µg/kg, respectively. Cry1Ab did not affect mortality, syrup consumption or learning capacities. However, foraging activity was reduced during and after the treatment. Deltamethrin and imidacloprid both affected syrup consumption and foraging activity. Deltamethrin also induced a reduction in learning capacities. With the tested concentrations, our study suggests that for honeybees, synthetic insecticides such as deltamethrin may induce a greater hazard than Cry1Ab protein, potentially expressed in Bt corn pollen at concentrations lower than 1000 µg/kg.

***Apis mellifera* / Cry1Ab protoxin / insecticide / behaviour / risk assessment**

1. INTRODUCTION

In crop systems the control of insect pests is important to ensure good yields. For this purpose, insecticides (biological or synthetics) are used currently, and alternative methods, such as the use of genetically modified (GM) plants, are used increasingly (Jouanin et al., 1998; James, 2004).

Nowadays the efficiency of insecticides and GM plants for controlling pests is well-known; however their effects on non-target or auxiliary insects such as honeybees remain under discussion (Flexner et al., 1986; Decourtye and Pham-Delègue, 2002; Ervin et al., 2003).

Pollination activity by honeybees (*Apis mellifera* L.) is of benefit to ecosystems especially to agroecosystems. During foraging activity, honeybees could be exposed to synthetic insecticides or GM plants, perhaps with subsequent effects on their survivorship or behaviour. This is why the effects of synthetic insecticides or GM plants have been studied in the honeybee [for reviews see Decourtye and Pham-Delègue (2002) and Malone and Pham-Delègue (2002)].

Cry1Ab protein is naturally produced by the bacterium *Bacillus thuringiensis* (Bt) during the stationary phase of its growth (Schnepf et al., 1998). Maize plants have been genetically modified to express the gene encoding the

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Cry1Ab protein (Koziel et al., 1993), which is insecticidal to the European corn borer (*Ostrinia nubilalis*). Most studies carried out to understand the mode of action of Bt proteins have been conducted with lepidopteran species, and little is known about their effects on Hymenoptera. In susceptible insects, after ingestion these proteins are dissolved and proteolytically cleaved by gut proteases. The resultant active forms bind to specific receptors of the insect midgut, producing cell lysis and eventually insect death (Knowles and Dow, 1993).

Studies with Cry proteins and honeybees, have reported no differences between treatment and control groups. The parameters observed included: larval survival and pupal dry weight (CryIIIB: Arpaia, 1996), mortality (Cry1Ac: Sims, 1995), longevity and food rate consumption (Cry1Ba: Malone et al., 1999), timing of first flights and the duration of the flight activity (Cry1Ba: Malone et al., 2001). Hanley et al. (2003) reported no effect of Cry1Ab protein on honeybee larvae and pupal mortality and weight when larvae were fed Bt-corn pollen. These authors reported no differences in the haemolymph protein contents of newly-emerged adult bee. However, cellular, physiological or behavioural effects of Cry1Ab protein or Bt-corn pollen on honeybees have not yet been investigated.

The objective of our study was to assess the effects of the Cry1Ab protein on honeybees by testing a concentration similar to that found in Bt-corn pollen, and comparing these effects to that of two synthetic insecticides, deltamethrin and imidacloprid, using the same experimental procedures.

Deltamethrin is a type II pyrethroid insecticide which acts mainly by altering the inactivation kinetics of the voltage-dependent sodium channel (Soderlund and Bloomquist, 1989). In honeybees, some studies have shown high toxicity in acute toxicity tests (Atkins et al., 1981; Faucon et al., 1985a). Some sub-lethal effects produced by deltamethrin have also been reported, such as the paralysis of foragers (Faucon et al., 1985b), the decrease of foraging activity (Bocquet et al., 1982; Decourtye et al., 2004) and a disruption of the homing-flight of foragers (Vandame et al., 1995).

Imidacloprid is a nicotine-related insecticide for which toxicity is correlated with its competitive agonist property onto the nicotinic acetylcholine receptor (nAChRs) of insects (Liu and Casida, 1993; Matsuda et al., 2001). In honeybees, imidacloprid was highly toxic in acute laboratory tests (Suchail et al., 2000; Schmuck et al., 2001). As for sub-lethal effects, some studies have reported that imidacloprid could affect foraging activity (Kirchner, 1999; Decourtye et al., 2004) and learning performance (Guez et al., 2001; Decourtye et al., 2003). However, other studies showed no deleterious effects on the development of exposed colonies (Schmuck et al., 2001).

Here we compare the mortality, rate of syrup consumption, foraging activity and learning performances of free-flying honeybees from colonies fed with syrups containing Cry1Ab protoxin, deltamethrin or imidacloprid with bees from the same colonies given syrups without additives. In addition, to estimate the dynamic of Cry1Ab protoxin in the hive, we present data obtained when honey, larvae and bee foragers were analysed using immunological tests (ELISA).

2. MATERIALS AND METHODS

2.1. Tested syrups

Cry1Ab protoxin was provided by the INRA laboratory 'Unité Génétique Microbienne et Environnement', Guyancourt, France. The Cry1Ab protoxin was 131 kDa in size (Gohar, personal communication), and was obtained from a gene cloned from the *aizawai* 7.29 strain in the pHTA1 plasmid (Sanchis et al., 1988).

Deltamethrin (99% purity, sample: 81112) and imidacloprid (98% purity, sample: 90830) were provided by Cluzeau InfoLabo (Sainte-Foy La Grande, France).

The sugar syrups without additives contained sucrose (500 g/L) and 0.15% (v/v) of solvent (Na_2CO_3 for Cry1Ab protoxin, and acetone for the synthetic insecticides).

For the Cry1Ab test, the sugar syrup (500 g/L) contained the Cry1Ab protoxin at a final concentration of 1000 µg/kg. This amount of Cry1Ab protein is higher than that recorded for Bt-corn pollen of variety MON810 (~2 µg/kg) (Wraight et al., 2000) and lower than that of Event 176 corn pollen (~5000 µg/kg) (Fearing et al., 1997). For the deltamethrin test, the sugar syrup contained 500 µg/kg

deltamethrin which is within the range of concentrations of this insecticide measured in pollen of oilseed rape after spraying with Decis Micro (240 to 650 µg/kg: Faucon et al., 1985b). For the imidacloprid test, the sugar syrup contained imidacloprid at a final concentration of 48 µg/kg. This concentration was chosen because Decourtye et al. (2003) showed it had sublethal negative effects. It is worth noting that it is about 16 times the mean concentration reported in sunflower pollens (Bonmatin et al., 2003).

Fresh syrups were prepared before each weekly observation period, frozen at –20 ± 2 °C for two days and defrosted at ambient temperature before use. During the observation period, sugar syrups were stored at 3 ± 1 °C.

2.2. Experimental protocol

Experiments were performed on colonies of *Apis mellifera* L. (about 10 000 bees per colony) with a one-year queen and 3 brood combs. These colonies received sanitary control weekly (i.e. control for the mite Varroa destructor) and no chemical treatment at least one month before experiments started. Every week, hives were opened to check the presence of the queen and larvae.

Experiments were done in a flight cage (2.5 × 2 × 2 m) placed in an acclimatized room (23 ± 1 °C, 50% RH, photoperiod: 12:12 (L:D), 400 lux artificial lighting during observation periods, and 200 lux after observation periods).

Because colony activity and foraging intensity may vary among colonies (Page et al., 1995), we did not compare independent control and treated colonies. The detection of possible abnormal behaviours was conducted on the same colony, subjected to various feeding treatment along time. This protocol was based on the assumption that under normal conditions of feeding, the foraging activity would remain constant, while food sources with toxic compounds would affect the activity of the colony. Previous work by Bailez (1996) demonstrated that under the same experimental conditions as those described herein, one honeybee colony could be kept under control conditions with a foraging activity of respectively 496.8 ± 58.6, 440 ± 14.1, 435 ± 6.8 number of visits per week, along three following weeks. Thus, we may assume that although a slow decrease of activity might occur overtime, it would not surpass 15%. In this work, we compared parameters measured during three observation periods of 4 days each: before treatment (colony fed non-contaminated syrup), during treatment (colony fed contaminated syrup) and after treatment (colony fed non-contaminated syrup). A disruption of 3 days, during which the colony was provided with non-contaminated sugar syrup, was allowed between each period to let the colony recover. Three colonies were tested in total, one for each substance of interest.

2.2.1. Mortality and syrup consumption

For each colony, all dead bees found on the ground (both non marked bees and marked bees; i.e., those having visited the experimental device, the last category representing ca. 80% of the bees found dead) were counted over four consecutive days along each observation period. Syrup was renewed daily. The standard feeding device (glass bottle) was positioned 1.5 m from the hive entrance. Syrup consumption was estimated daily by measuring the difference in volume on each of four consecutive days for each observation period.

2.2.2. Foraging activity and olfactory learning performance

The foraging activity and learning performances were evaluated using an artificial flower device described by Pham and Masson (1985) and modified by Decourtye et al. (2004). This flower device was comprised of 6 artificial flowers set around the periphery of a circular tray (50 cm diameter), each flower placed in a plastic Petri dish (5 cm diameter) containing glass marbles. When used as a conditioning device, a syrup (contaminated or not) was offered in association with an odour stimulus as the conditioning stimulus (CS) (20 µL of pure linalool, 95–97% purity, Sigma). When the flower device was used to test learning performance (testing device), the flowers did not contain the syrup and only three out of six flowers provided the odour stimulus. The flower device was placed 1.5 m from the hive entrance.

Before each experiment, about 100 workers were put on the feeding device to trigger the recruitment of foragers. To evaluate foraging activity, bees were allowed to visit the conditioning device over 4 days, for a 2 hours observation period the first 2 days (from 2:00 to 4:00 pm GMT), and 1 hour observation period the following 2 days (from 2:00 to 3:00 pm GMT). All bees spontaneously visiting the device during the observation periods were marked by a colour dot on the thorax and counted.

After the first two days of recording foraging activity, olfactory learning performance was tested (from 1500 to 1600 GMT) during the 2 subsequent days, just after the foraging activity recording period. This testing procedure consisted of a conditioning phase (15 min) where the conditioning device was offered (and the new bees visiting the device were marked as in the period of foraging activity recording) alternating with a testing phase (5 min) using the testing device (for a detailed description of

the technique, see Picard-Nizou et al., 1997). The learning performance was evaluated by recording the number of marked bees visiting the flowers delivering the CS on the testing device. After each testing phase, the tray was cleaned with ethanol and distilled water, and the Petri dishes were rotated to avoid the recognition of marking scents. A total of 4 conditioning and 4 testing periods per day were conducted.

The volume of syrup administered during experiments was noted and the volume of remaining syrup was measured. The volume consumed was pooled with the values obtained from the experimental period to complete the syrup consumption estimates (see above).

2.3. Detection of Cry1Ab in bees, larvae and honey by ELISA tests

Foraging bees, larvae and honey were sampled from the colony exposed to Cry1Ab protoxin during and after treatment.

After four days of exposure to the contaminated syrup, we opened the beehive and extracted six honey samples of 2–3 mL each. At the same time, we took two samples of 5–6 larvae each from the brood. For foraging bees, we individually analysed about 15 marked bees caught when they visited the food source. Analyses were performed on whole individuals of both larvae and foraging bees. The volume of honey collected was evaluated using a 1 mL micropipet and larvae and foraging bees were weighed to the nearest 0.01 mg. All samples were kept at -20 ± 2 °C until analysis. One week after the Cry1Ab treatment, we repeated the sampling procedure.

We used the EnviroLogix Quantiplate™ Kit for Cry1Ab/Cry1Ac detection. In this test the Cry1Ab present in the sample extracts is related to an optical density (OD). Three calibrators (known Cry1Ab concentrations: 500, 2500 and 5000 µg/kg) are used to establish a linear curve which allows to calculate the Cry1Ab concentration of each sample using its OD. Quantification of sample concentration is only possible if the OD of the sample occurs within the range of the calibrators' ODs. The limit of detection of this test is 140 µg/kg Cry1Ab.

2.4. Statistical analyses

Mortalities recorded at each observation period (before, during and after treatment) within colonies, were compared using the Friedman's analysis of variance test. When significant differences were found ($P < 0.05$), multiple comparisons for non-parametric analysis of variance were made (Zar, 1998).

Syrup consumption and foraging activity at each observation period within colonies were compared using a repeated-measures analysis of variance. Foraging activity was statistically analysed taking into account values recorded from the time that activity reached stability (i.e. after two days of the beginning of the observations). When significant differences were found ($P < 0.05$), multiple comparison procedures for repeated-measures analysis of variance were performed (Zar, 1998).

Olfactory learning performance was evaluated over the three observation periods comparing the number of visits to the scented or unscented sites with the hypothesis of an equal distribution (50% of foragers on either site) using a chi-square analysis.

The optical densities recorded after the ELISA analysis were compared between the Cry1Ab treatment period and the period after treatment using the Mann-Whitney test. Analyses were performed using Systat® software (SPSS, 2000).

3. RESULTS

3.1. Mortality and syrup consumption

When mortality was compared among observation periods, no significant difference was found when colonies were exposed to Cry1Ab protoxin ($\chi^2 = 1.6$; 2 df; $P = 0.4$) or imidacloprid ($\chi^2 = 1.6$; 2 df; $P = 0.4$). In contrast, a significant difference was observed when deltamethrin was tested ($\chi^2 = 6.5$; 2 df; $P = 0.04$); the mortality recorded before treatment was significantly higher than mortality recorded after treatment (Tab. I). Regarding syrup consumption, in the Cry1Ab experiment, the solution uptake was not significantly modified among experimental periods ($F = 0.9$; 2 df; $P = 0.4$). In contrast, when deltamethrin was tested, syrup uptake was significantly lower during and after treatment than before treatment ($F = 46.9$; 2 df; $P < 0.01$). In the case of the colony exposed to imidacloprid, syrup consumption was significantly lower during treatment than before and after treatment ($F = 33.6$; 2 df; $P = 0.001$) (Tab. II).

3.2. Foraging activity and olfactory learning performance

The mean foraging activity for each observation period is reported in Figure 1. When Cry1Ab was tested, foraging activity was

Table I. Mortality in relation to treatment*. Data represent mean number of dead honeybees per day (± SEM) which were found on the ground of the flight chamber. Mortality was recorded over 4 days per week in all treatments.

Treatment	Before treatment	During treatment	After treatment
Cry1Ab	148 ± 37.4a	91.8 ± 20.1a	122.5 ± 29.0a
Deltamethrin[a]	169.3 ± 18.3a	109.5 ± 24.8ab	102.75 ± 16.3b
Imidacloprid	218.5 ± 12.8a	169.8 ± 37.3a	218.5 ± 20.0a

* Different letters following the means within a row indicate significant differences.

[a] Differences in mortality among observation periods for deltamethrin were significant ($\chi^2 = 6.5$; 2 df; $P = 0.04$).

Table II. Syrup consumption for each treatment*. Data represent the mean value of syrup consumption (mL) per day (± SEM). Syrup consumption was recorded along 4 days per week.

Treatment	Before treatment	During treatment	After treatment
Cry1Ab	227.3 ± 23.6a	217.1 ± 23.5a	189.3 ± 8.8a
Deltamethrin[a]	110.1 ± 7.6a	44.8 ±1.7b	64.9 ± 5.6b
Imidacloprid[b]	178.4 ± 7.7a	105.3 ± 7.1b	211.5 ± 9.8a

* Different letters following the means within a row indicate significant differences.

[a] Differences in syrup consumption among observation periods for deltamethrin were significant (F = 46.9; 2 df; $P < 0.01$).

[b] Differences in syrup consumption among observation periods for imidacloprid were significant (F = 33.6; 2 df; $P = 0.001$).

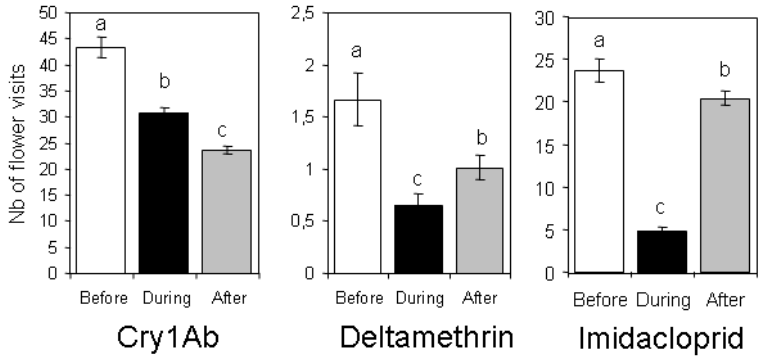

Figure 1. Foraging activity of honeybees on the artificial flower device in relation to treatment. Bars give the mean (± SEM) number of foraging honey bees recorded on the feeding sites every 5-min during 2-h (conditioning period) or 1-h (testing period) per day. Bars correspond to the periods of feeding with control syrup before treatment (white), or after (grey), or with the contaminated syrup (black). Each foraging activity observation period was conducted over 4 days, separated by a period of 3 days of inter-treatment recovery. Mean foraging activity for the period before treatment was calculated using values from the previous two days (i.e. when foraging activity reached stability) whereas mean foraging activity for the periods during and after treatment were calculated using values from the four days. Different letters among periods for each substance indicate significant differences. For Cry1Ab test: F = 32.4; 2 df; $P < 0.01$. For Deltamethrin test: F = 11.7; 2 df; $P < 0.01$. For Imidacloprid test: F = 61.9; 2 df; $P < 0.01$.

significantly higher before treatment in comparison to during and after treatment (F = 32.4; 2 df; $P < 0.01$). We recorded a mean number of visits of 43.4 (± 1.9 SEM), 30.7 (± 1.2 SEM) and 23.7 (± 0.8 SEM) before, during and after treatment respectively. The percentage of decrease in the number of visits was of ca. 29% between the week before treatment and the week of treatment, and of ca. 23% between the week of treatment and the week after treatment. In the deltamethrin test, foraging activity was significantly higher before treatment than during and after treatment (F = 11.7; 2 df; $P < 0.01$). The mean number of visits before treatment was 1.7 (± 0.3 SEM), 0.7 (± 0.1 SEM) during the treatment and 1.0 (± 0.1 SEM) after treatment. The decrease due to the treatment was of ca. 60% visits, and the recovery after treatment led to an increase of ca. 35% visits. For imidacloprid, the foraging activity was significantly lower during treatment than before and after treatment (F = 61.9; 2 df; $P < 0.01$). The mean number of visits during the treatment was of 4.8 (± 0.4 SEM), and of 23.7 (± 1.3 SEM) and of 20.4 (± 0.8 SEM) before and after treatment respectively. The treatment led to a decrease of ca. 20% visits, and the release after treatment led to an increase of ca. 24% visits. In all cases, the variations between the treatment and the non-treatment periods surpassed the maximum of 15% decrease of visits from one week to the following one, as observed in a non-treated colony under the same experimental conditions by Bailez (1996).

Before treatment, when syrup without additives was delivered, the number of bees visiting the scented sites was significantly higher than the hypothesized equal distribution of landings, showing that foragers were well conditioned to the odour under these experimental conditions.

In the Cry1Ab protoxin test, landings on scented sites were not modified at any observation period ($P < 0.01$) suggesting no deleterious effects of Cry1Ab on olfactory learning performances (Fig. 2). In the deltamethrin experiment, olfactory learning performance was strongly reduced during the treatment period and the level of visits was not significantly different from a randomised distribution between scented and unscented sites ($P = 0.1$ and $P = 0.5$). Similarly, after treatment, the visits on scented sites were not significantly different from that on unscented sites ($P = 0.1$ and $P = 0.1$). In the case of imidacloprid, the percentage of foragers visiting the scented sites during the treatment period (76.8 and 78.2%) were lower in comparison to the percentages of visits before treatment (90.9 and 81.8%) and after treatment (83.8 and 90.6%). Nevertheless, this level always remained significantly higher than a randomised distribution between scented and unscented sites ($P < 0.01$) which suggests a non significant deleterious effect of imidacloprid on the olfactory learning performances under these conditions.

3.3. Detection of Cry1Ab in bees, larvae and honey by ELISA tests

Quantification of Cry1Ab was only possible with ODs obtained on foraging bees exposed to Cry1Ab and on larvae sampled after treatment. These estimated values were of 8.9 (± 1.0) ng Cry1Ab/g tissue for foraging bees and of 1.2 (± 0.02) ng Cry1Ab/g tissue for larvae. As for imidacloprid, it has been shown in previous studies, that when imidacloprid at 50 µg/kg was delivered in a food source to a honeybee colony under similar conditions as those described herein, the molecule was not detected in stored honey one week after exposure (Decourtye and Pham-Delègue, unpublished data). For deltamethrin, the work by Faucon et al. (1985b) indicated that this molecule may be found in stored honey at concentrations of 23 µg/kg after treatment of oilseed rape with the insecticide Décis®.

4. DISCUSSION

In this study we conducted toxicological observations at colony level under controlled flight room conditions. Such experimental conditions have been used previously by Picard-Nizou et al. (1997) to study the effects of trypsin inhibitors on olfactory learning performances of free flying bees. To measure the acute toxicity of agrochemicals on honeybees, laboratory experiments are classically conducted on small groups of caged worker bees. These studies only provide data on lethal effects obtained under artificial conditions. On the other hand, field trials are also conducted to assess the effect of various pesticides such as

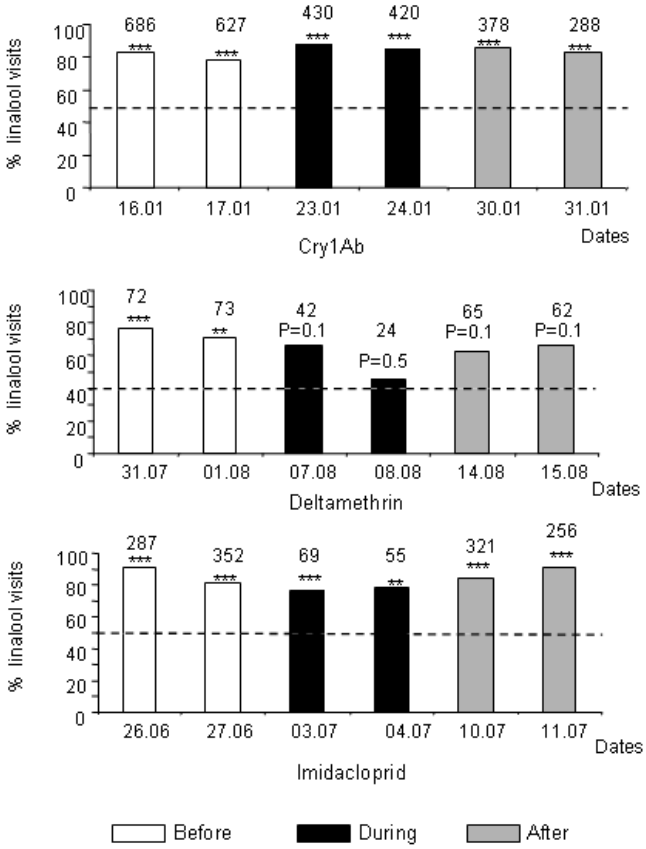

Figure 2. Olfactory learning performance of free-flying foragers in relation to treatment. After conditioning to linalool (scented sites + reward syrup), the visits of foragers on either the scented sites or the unscented ones were noted every 30-s during 3 periods of 5-min per day. Bars give the percentage of foragers visiting the scented sites after conditioning to linalool. White and grey bars correspond to the periods of feeding with the control syrup before and after the treatment, respectively. Black bars are related to the period with the contaminated syrup. Each testing period was conducted over 2 days, separated by 2 days of recording foraging activity and 3 days of inter-treatment recovery. The total number of foragers visiting the testing device is indicated above the bars. The observed numbers of visits were compared to a hypothetical equal distribution of landings on the scented sites and unscented sites, shown as the 50% dotted line (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

imidacloprid (Schmuck et al., 2001) and deltamethrin (Faucon et al., 1985b). Unfortunately due to the variability of field conditions the information from such studies is not easy to interpret.

Therefore, we decided to design experiments under intermediate conditions where a colony is subjected to contaminated food sources on which bees can forage freely, in a flight cage maintained in controlled conditions. Our method relied on the hypothesis that the foraging activity will not decline within the experimental period, which is in accord to Bailez' results (1996). We found that the effects of the two molecules on the colony activity were consistent with those obtained by Decourtye et al. (2004), using the same protocol. Therefore, such experimental conditions on a colony subjected to alternating control and exposure periods appeared repeatable in different studies, and can be considered as satisfactory to test effects of a toxin at a colony level. In addition, deltamethrin and imidacloprid may be used as toxic references at adequate concentrations. In our protocol, the dynamics of the colony responses under the different exposure conditions is the key parameter to compare products. However, further work is necessary to investigate whether the toxic effect of a given molecule would be similar for different colonies with different levels of activity. In addition, it should be noted that our experiments were not performed in the same season, with the chemical insecticides being tested during summer, while the Bt toxin was tested in winter. Although experiments were conducted in an indoor flight room under controlled environmental conditions, we cannot exclude the hypothesis of a seasonal effect on the levels of foraging activity or on the susceptibility of bees to the toxins. Indeed, other works comparing the effects of imidacloprid on learning abilities

of individual worker bees subjected to a conditioned proboscis extension response procedure using summer bees or winter bees, reported a higher susceptibility of summer bees to the insecticide (Decourtye et al., 2003). This suggests that the effect of Cry1Ab tested herein on winter bees might have been underestimated. Complementary work testing summer bees towards the Bt toxin should be carried out to document this question.

In the present study no lethal effect on honeybees was observed after exposure to Cry1Ab protoxin (1000 µg/kg), deltamethrin (500 µg/kg) or imidacloprid (48 µg/kg). Adversely Decourtye et al. (2004) reported that deltamethrin at 500 µg/kg caused significant mortality on colonies under outdoor conditions. This discrepancy concerning deltamethrin may be explained by a lower activity of the colony tested under indoor conditions compared to outdoor colonies; the low activity could lead to lower colonial contamination and consequently to lower mortality. For this reason, the comparison of our 3 tested colonies may be questionable since they showed different levels of foraging activity.

Additionally, various sublethal effects were observed in our study. Regarding syrup consumption, upon exposure to deltamethrin and imidacloprid, honeybees reduced their syrup uptake, whereas Cry1Ab protoxin exposure did not modify this parameter. The reduction in syrup uptake may be related to repellent properties of the products. Indeed this property has been mentioned for deltamethrin after field treatment with Décis at rates of 7.5 g a.i./ha (Bocquet et al., 1982). This reduction in syrup consumption is also in agreement with results obtained under outdoor conditions testing the same concentration of deltamethrin and a lower concentration of imidacloprid (24 µg/kg) (Decourtye et al., 2004).

As for foraging activity, deltamethrin and imidacloprid induced a significant decrease in foraging activity during the exposure period with a significant recovery of foraging activity after the treatment. Obviously, the two synthetic insecticides produced after ingestion a disruption in foraging activity related to their repellent effect. This disruption on foraging activity as a result of exposure to these insecticides was also reported by Decourtye et al. (2004) using the same protocol and similar concentrations. It may be assumed that in terms of short-term effects, both compounds would have the same type of effect; i.e., a strong repellency. However, while deltamethrin has been tested at a concentration potentially found in pollen of treated crops, the experimental concentration of imidacloprid (48 µg/kg) was about 16 times higher than the mean expected concentration in sunflower pollen (Bonmatin et al., 2003). Therefore, further work using imidacloprid at lower concentrations is needed to assess the no effect concentration. A strong repellency, as that found with deltamethrin, may result in a less deleterious effect on bees, which should consume less of this substance. However, if the effect lasts too long after exposure, bees may die from starvation.

Concerning Cry1Ab protoxin, foraging activity decreased during and after exposure to Cry1Ab protein, however this effect was not related to repellence since the consumption of syrup during and after treatment was not significantly reduced. This decrease in the foraging activity appeared to be related to the exposure to the product in the syrup since Cry1Ab protein was still detected in honey after treatment, as shown using ELISA detection technique. Surprisingly, although the number of foragers decreased, those visiting the device were still exhibiting olfactory discrimination abilities. This suggests that different behaviours, such as communication processes leading to the recruitment to the food source, and individual learning ability expressed by the recognition of scented sites, could be differentially affected by the toxin. The physiological mechanisms underlying such differential effect cannot be explained by what is known of Bt's mode of action (Schnepf et al., 1998) but negative effects of Cry1Ab protein on bees can not be excluded over time. Moreover Cry1Ab should be tested in a worse case study using the maximum concentration reported in G.M. varieties i.e. 5000 µg/kg.

In contrast, deltamethrin affected significantly the learning performances during and after treatment. For imidacloprid, no significant effect on olfactory discrimination was found, consistent with other studies using the same paradigm under outdoor conditions (Decourtye et al., 2004). Finally, only deltamethrin induced a clear negative effect on learning. This may be related to a disruption in the

orientation ability of bees after exposure to deltamethrin, as shown by Vandame et al. (1995). Olfactory learning and discrimination are part of the process which leads to the homing flights; therefore a toxic effect of deltamethrin may affect both parameters. Interestingly there may be some link between our results with deltamethrin used at a realistic concentration and the bee losses reported by beekeepers as caused by this substance (Faucon et al., 1985b).

In conclusion, our study suggests that for honeybees, synthetic insecticides such as deltamethrin may induce a greater hazard than Cry1Ab protein, potentially expressed in Bt corn pollen at concentrations lower than 1000 µg/kg.

Our method, using indoor flight rooms may be considered as a semi-field test for studying specific sublethal effects of agrochemicals on honeybees. Its use could be recommended in the course of the regulatory ecotoxicological procedure before a substance can be registered. Finally, studies which compare different control methods simultaneously seem important in the perspective to develop pest control strategies with reduced deleterious environmental effects, especially on non-target insects such as honeybees.

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Résumé – Étude comparative de l'action de la protoxine Cry1Ab, de la deltaméthrine et de l'imidaclopride sur l'activité de butinage et les performances d'apprentissage de l'Abeille domestique, *Apis mellifera.* Parmi les stratégies de protection des cultures, l'usage d'insecticides de synthèse constitue la pratique la plus courante, mais les méthodes alternatives comme les plantes transgéniques sont de plus en plus utilisées. Le pollen de maïs-Bt exprime la protéine Cry1Ab, active contre la pyrale du maïs (*Ostrinia nubilalis*) aux doses de 2 µg/kg ou de 5000 µg/kg selon la variété utilisée. Mais les effets sur les insectes non-cibles comme les abeilles domestiques sont toujours débattus. Dans une approche comparative nous avons évalué l'action de la protéine Cry1Ab, de la deltaméthrine et de l'imidaclopride sur la mortalité, la consommation de sirop, l'activité de butinage et les capacités d'apprentissage olfactif d'abeilles en vol libre. Dans une cage de vol d'intérieur nous avons offert à des colonies d'abeilles différents sirops renfermant la protoxine Cry1Ab, la deltaméthrine ou l'imidaclopride aux doses respectives de 1000 µg/kg, 500 µg/kg et 48 µg/kg. L'exposition au sirop contaminé a duré 4 j ; elle était précédée et suivie d'une période de nourrissement de 4 j avec du sirop non contaminé. Le nourrissement au sirop à la Cry1Ab n'a eu aucun effet sur la mortalité (Tab. I) ou la consommation de sirop (Tab. II) ni sur les capacités d'apprentissage olfactif. Par contre l'activité moyenne de butinage pendant et après le traitement s'est vue réduite (Fig. 1). Le nourrissement au sirop à la deltaméthrine et à l'imidaclopride a réduit l'activité de butinage (Fig. 1) et la consommation de sirop (Tab. II) pendant la période de traitement. Dans le cas de la deltaméthrine nous avons observé une diminution significative des capacités d'apprentissage pendant et après le traitement (Fig. 2). On peut penser que les deux insecticides de synthèse ont les mêmes effets à court terme. Pourtant la deltaméthrine a été testée à une dose potentiellement utilisée en conditions agronomiques, alors que la dose d'imidaclopride (48 µg/kg) était au moins 16 fois supérieure aux quantités que l'on peut trouver dans le pollen de tournesol. Aux concentrations testées, notre étude suggère que les insecticides de synthèse tels la deltaméthrine peuvent représenter pour les abeilles domestiques un danger plus grand que la protéine Cry1Ab, qui s'exprime dans le pollen de maïs-Bt à des concentrations inférieures à 1000 µg/kg. Des effets négatifs de Cry1Ab ne sont pourtant pas à exclure sur le long terme ou à des concentrations plus élevées (dans la pire des situations).

***Apis mellifera* / protoxine Cry1Ab / insecticide / évaluation du risque / comportement**

Zusammenfassung – Die Auswirkung von Cry1Ab Protoxin, Deltamethrin und Imidacloprid auf die Sammelaktivität und das Lernverhalten von Honigbienen *Apis mellifera* in einem vergleichenden Versuchsansatz. Bei Pflanzenschutzstrategien ist die Anwendung synthetischer Insektizide am meisten verbreitet, zunehmend werden auch andere Methoden wie transgene Pflanzen genutzt. Bt-Mais erzeugt das Cry1Ab Protein, das je nach der verwendeten Varietät in Dosierungen von entweder 2 µg/kg oder 5000 µg/kg für den Europäischen Kornbohrer (*Ostrinia nubilalis*) wirksam ist. Mögliche Wirkungen auf Bienen sind allerdings noch nicht bekannt. In einem vergleichenden Versuchsansatz untersuchten wir die Wirkung von Cry1Ab Protein, Deltamethrin und Imidacloprid auf Sterblichkeit, Zuckerwasserverbrauch, Sammelaktivität und Dufterlernungsfähigkeit freifliegender

Honigbienen. In einem Gebäude befindlichen Flugkäfig boten wir den Bienen Zuckerwasserlösungen mit Cry1Ab Protoxin, Deltamethrin oder Imidacloprid in Konzentrationen von 1000 µg/kg, 500 µg/kg bzw. 48 µg/kg. Den Bienen wurden die mit den Wirkstoffen versetzte Zuckerwasserlösung vier Tage lang geboten, davor und danach wurden sie jeweils 4 Tage lang mit reinem Zuckerwasser gefüttert. Bei den mit Cry1Ab Protein-Zuckerwasser gefütterten Bienen fanden wir keinen Effekt auf die Sterblichkeit (Tab. I), den Zuckerwasserverzehr (Tab. II) oder die Fähigkeit, Düfte zu lernen. Allerdings war die Sammelaktivität im Mittel während und nach der Behandlung vermindert (Abb. 1). Bei den mit Deltamethrin oder Imidacloprid haltigem Zuckerwasser gefütterten Bienen war die Sammelaktivität (Abb. 1) und Zuckerwasseraufnahme (Tab. II) während der Behandlung vermindert und im Falle von Deltamethrin beobachteten wir eine signifikante Verminderung der Lernfähigkeit während und nach der Behandlung (Abb. 2). Es kann angenommen werden, dass beide synthetische Insektizide den gleichen Kurzzeiteffekt haben. Allerdings war Deltamethrin in einer Dosis getestet worden, wie sie unter landwirtschaftlichen Bedingungen möglicherweise auftreten kann, während Imidacloprid (48 µg/kg) mindesten 16 mal höher dosiert war als die möglicherweise in Sonnenblumenpollen auftretenden Mengen. Unsere Untersuchung weist darauf hin, dass bei den getesteten Dosierungen synthetische Insektizide wie Deltamethrin eine höhere Gefährdung für Honigbienen darstellen als Cry1Ab Protein, das in Bt Maispollen in Konzentrationen unter 1000 µg/kg auftritt. Allerdings können negative Effekte von Cry1Ab über längere Zeit oder in höheren Dosierungen nicht ausgeschlossen werden (d.h. in einer, worst case' Situation).

***Apis mellifera* / Cry1Ab Protoxin / Insektizid / Verhalten / Risikoabschätzung**

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