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Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test

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Abstract: A laboratory feeding test was conducted on queenless micro-colonies of three bumblebee workers (*Bombus terrestris* L) to study the effects of low doses of imidacloprid on pollen and syrup consumption, worker survival, brood size and larval development. Two doses were used: D1 = 10 μg AI kg^{-1} in syrup and 6 μg AI kg^{-1} in pollen; D2 was 2.5 times higher in syrup and 2.7 higher in pollen. During 85 days 27, 30 and 29 micro-colonies were reared for control, D1 and D2 treatments respectively.

Food consumption was not affected by either dose. During the 5-day pre-oviposition period the mean insecticide intake was 4.8 ng per day per worker in treatment D2. Both doses slightly but significantly affected worker survival rate by 10% during the first month, without any dose-effect relationship. Brood production was significantly reduced in D1 treatment and larval ejection by workers was significantly lower in D1 and D2 than in control. No significant effect of D1 and D2 treatments on the duration of larval development was revealed. No residue could be detected in workers still alive after 85 days.

It was concluded that the survival rate and reproductive capacity of *B terrestris* was not likely to be affected by prolonged ingestion of nectar produced by sunflower after seed-dressing treatment with imidacloprid (Gaucho), since honey or pollen collected by honeybees foraging treated sunflower never revealed concentrations of imidacloprid higher than 10 μg kg^{-1}.

Keywords: imidacloprid; reproduction; survival rate; feeding test; chronic toxicity; brood; *Bombus terrestris*

1 INTRODUCTION

Cultivated plants can be protected against root or foliage pests by systemic insecticides which are absorbed by the roots and conveyed to other parts of the plant through its vascular bundles. One of these systemic insecticides is imidacloprid, which belongs to the new chemical family of chloronicotinyls. It is known under various trade names: Confidor, Admire, Gaucho. Since 1993, Gaucho has been extensively used in France at the registered dose of 700 μg AI per seed, as an aphicide treatment for sunflower.

Imidacloprid proved highly toxic to honeybees in feeding tests, with an oral LD_{50} of 3.7 ng per bee, which is nearly 22 times less than the contact LD_{50} according to Schmidt.[1] Despite the toxicity and systemic properties of imidacloprid this author suggested that its residues in sunflower nectar and pollen may be too low to affect honeybees. His assumption was supported by a field experiment where a seed treatment with Gaucho at the registered dose did not modify significantly mortality, flower visitation or colony development of honeybees foraging sunflower.

However, French bee-keepers have suspected for several years that sunflower seed treatment with Gaucho may result in losses of workers and abnormally low yields in sunflower honey. It was thus hypothesized that imidacloprid could migrate into nectar as other insecticides do: schradan in borage,[2], thiometon in cotton,[3] dimethoate in lemon[4] and aldicarb in lucerne.[5] In addition, some behavioural or physiological traits of honeybees might be affected by low amounts of imidacloprid, as is communication dance by parathion,[6] homing behaviour by deltamethrin,[7] nectar foraging by diazinon,[8] fecundity by dimethoate,[9] larval growth by carbofuran and dimethoate,[10] and longevity by diazinon and malathion.[11]

Several wild bee species are also attracted to sunflower and may be as much at risk as honey bees. We have therefore conducted an experiment on the effects of low doses of imidacloprid on *Bombus terrestris* (L) which is the most abundant wild pollinator of sunflower in France.[12] A laboratory feeding test was performed in order to estimate whether syrup and pollen contaminated with doses close to the detection threshold could affect the food consumption of adults,

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worker survival, the size of the brood and delay of adult emergence.

2 MATERIALS AND METHODS

2.1 Insect origin and management

Two hundred and fifty-eight callow workers, less than one day old, were taken from several colonies of the local ecotype of *B terrestris* reared in the laboratory. Eighty-six queenless micro-colonies of three workers were set up in plywood boxes ($11.3 \times 4.5 \times 4.3$ cm) with a screened bottom and a transparent cover. During the experiment, which lasted 85 days, the micro-colonies were maintained in a climate room at 27 °C, 70% RH, with a 8:16 h L:D photoperiodic regime. A commercial syrup containing 37% sucrose and 38% fructose + dextrose was supplied in plastic feeders, and a pollen dough was prepared by mixing honeybee pollen loads with syrup. Balls (1–2 g) were then dipped into liquid wax to prevent drying. Syrup feeders and pollen balls could be easily removed and weighed.

In each micro-colony, a worker became dominant, developed its ovaries and laid eggs within a week, thus playing the role of a queen. The two other workers helped the false queen for brood care, which mainly consisted in feeding larvae, building and heating cells, and sometimes ejecting larvae when egg laying was too intense. As the false queen was not inseminated, its brood always resulted in a haploid male progeny.

2.2 Chemical and treatments

Pure imidacloprid (Bayer) was used to contaminate syrup and pollen. In treatment D1, syrup and pollen contained 10 μg AI kg^{-1} and 6 μg AI kg^{-1} respectively. In treatment D2, doses were 25 μg AI kg^{-1} and 16 μg AI kg^{-1}. Control food was non-contaminated syrup and pollen. All micro-colonies were supplied *ad libitum* during the test. The food required for the whole experiment was prepared on the same day and stored at −20 °C. 27, 30 and 29 micro-colonies were reared for control, D1 and D2 treatments respectively.

2.3 Observations

During the whole test checks were made every day on: the mortality of workers, the number of larvae ejected from brood cells; and the emergence of males, which were removed from the box.

In order to assess the food consumed by workers before the start of brood care, pollen balls and syrup feeders were weighed before the trial and on the fifth day, ie before the first egg cell was built. Sample sizes (n) indicated in the three tables herein differ from the number of micro-colonies at the beginning of the experiment for specific reasons: when a syrup feeder leaks it is not possible to consider the micro-colony for food consumption assessment (Table 1). In evaluating other parameters, the number of ejected larvae, the number of adult males and the number of larvae, the larval ejection behaviour has to be considered since it is highly inconstant, ranging from 0% to 100% of the larvae. We observed no ejection in 6, 13, and 9 micro-colonies in the control, D1 and the D2 treatments, respectively, so that ejection comparison was performed only on the others. Ejection rate was 100% in 9, 12 and 12 micro-colonies in the control, the D1 and the D2 treatments, which could not produce any males and were not taken into account in the comparison of male production.

On the 85th day, the surviving workers, ie 38, 48 and 49 in the control, the D1 and the D2 treatments, respectively, were frozen, split into six samples of 3.8 to 5.5 g each and sent to a laboratory (GIRPA, Angers, France) for residue analysis. The technique used could detect and quantify residues above the threshold of 20 μg kg^{-1} for imidacloprid and 5 μg kg^{-1} for total residues, expressed as chloronicotinic acid.

2.4 Statistical evaluations

Statistical analysis was realized with SAS. PROC GLM was used for ANOVA and PROC LIFE TEST to test for the homogeneity of the three survival curves of worker micro-colonies which produced broods. A Wilcoxon chi-square was calculated to test for equality.

3 RESULTS

3.1 Food consumption (Table 1)

Only micro-colonies where no worker died and no leak occurred within the pre-oviposition period were considered for estimating food consumption. During this period, ie approximately 5 days, there were no significant differences in pollen and syrup consumption between the control and the two treatments D1 and D2 ($P = 0.19$ and $P = 0.15$ respectively). Syrup

Table 1. Daily consumption of food contaminated with imidacloprid during the pre-oviposition period of *Bombus terrestris* workers

	Control (n = 22)[a]		*D1 (n = 25)*[a]		*D2 (n = 24)*[a]		*F*	
	Pollen	*Syrup*	*Pollen*	*Syrup*	*Pollen*	*Syrup*	*Pollen*	*Syrup*
Active substance dose (μg kg^{-1})	0	0	6	10	16	25		
Amount of food consumed (mg per worker per day) (±SE)	30.3 (±1.1)	193.7 (±8.8)	26.6 (±1.2)	199.0 (±9.9)	30.0 (±2.2)	173.0 (±10.8)	1.68 $P = 0.19$	1.95 $P = 0.15$
Active substance consumed (ng per worker per day) (±SE)	—	—	0.159 (±0.007)	1.990 (±0.098)	0.476 (±0.036)	4.330 (±0.010)		

[a] Number of three worker queenless micro-colonies (syrup leakage occurred in 15 micro-colonies which could not be taken into account).

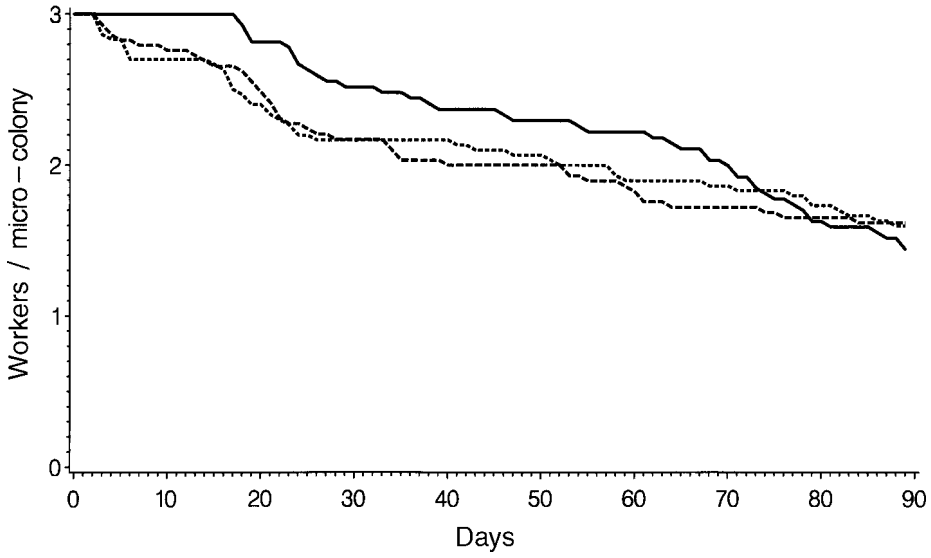

Figure 1. Survival of *Bombus terrestris* workers in relation to the contamination of food with imidacloprid. (—) Control, 27 micro-colonies; (----) D1, 6 μg AI kg^{-1} (pollen), 10 μg AI kg^{-1} (syrup), 30 micro-colonies; (– – –) D2, 16 μg AI kg^{-1} (pollen), 25 μg AI kg^{-1} (syrup), 29 micro-colonies.

intake was 5.7 to 7.5 times higher than that of pollen. Before the first egg-laying, the daily intake of imidacloprid from pollen and syrup was 2.15 ng and 4.81 ng per worker in D1 and D2 treatments respectively.

3.2 Survival of workers

Figure 1 shows that, during the first 2 weeks of the test, a 100% survival rate was observed in the control population, whereas in treatments D1 and D2 mortality increased from day 3 to day 15, reaching 10% at the end of the 2 weeks. After 30 days the mortality rates were 16.0% in the control and 27.7% in the D1 and D2 treatments. These figures show that mortality evolved similarly in the three treatments from day 15 to day 30 and that the difference in mortality was initiated during the first 15 days. After 85 days, at the end of the experiment, the number of micro-colonies with three workers was 6, 8 and 8 in the control, the D1 and the D2 treatments, and the total number of workers in micro-colonies with one, two or three individuals was 38, 48 and 49, respectively, ie 47%, 53% and 56% of the workers present at the beginning.

The life test procedure tested the homogeneity of survival curves during the first period of 30 days. The value of the Wilcoxon chi-squared statistic was 7.04, indicating that the survival rates in both treatments D1 and D2 were significantly lower than in the control ($P = 0.03$).

Table 2. Emergence delays of male progeny according to the level of food contamination

	Control (*n* = 17)[b]	*D1*[a] (*n* = 16)[b]	*D2*[a] (*n* = 17)[b]	*F*
Mean emergence delay of the first male (days) (±SE)	38.2 (±2.1)	41.3 (±1.8)	43.9 (±3.2)	1.39 *P* = 0.26

[a] Dose indicated in Table 1.
[b] Number of micro-colonies producing a male progeny (no observation in three samples).

3.3 Emergence of the first males (Table 2)

There was no significant difference ($P = 0.26$) between the mean emergence delays of the first male in the three treatments.

3.4 Brood production (Table 3)

The number of larvae ejected by workers was significantly higher in the control than in treatments D1 and D2 ($P = 0.002$). The number of adults produced per micro-colony was significantly lower in D1 treatment than in the control. Considering the number of larvae produced (non-ejected plus ejected larvae) there was a significant difference between

Table 3. Brood production by micro-colonies of *Bombus terrestris* within an 85-day period

	Control[b]	*D1*[a,b]	*D2*[a,b]	*F*
Mean number of ejected larvae per colony (±SE)	6.9 (±0.9)*a*	3.1 (±0.7)*b*	3.7 (±0.6)*b*	7.12 (*P* = 0.002)
Sample size	21	17	20	
Mean number of adult males per colony (±SE)	10.6 (±1.5)*a*	6.3 (±0.8)*b*	10.1 (±1.1)*ab*	4.22 (*P* = 0.02)
Sample size	18	18	17	
Mean number of larvae produced per colony (±SE)	14.6 (±1.8)*a*	8.3 (±1.2)*b*	11.2 (±1.3)*ab*	4.41 (*P* = 0.016)
Sample size	23	20	22	

[a] Dose indicated in Table 1.
[b] Figures bearing the same letter are not significantly different according to Scheffe comparison test for $P = 0.05$.

control and D1 treatments ($P = 0.016$). The number of larvae and adults in D2 treatment was not significantly different from the control or D1 treatment.

3.5 Imidacloprid residues in workers

No residues of imidacloprid and its metabolites could be detected in any of the six batches of 20 to 24 workers still alive on day 85.

4 DISCUSSION AND CONCLUSION

The use of micro-colonies of three queenless workers is appropriate for testing the effects of systemic insecticides on bumblebees. Our procedure allows the handling of small homogeneous units in standardized conditions and performing adequate comparisons between contaminated food treatments.

In our experiment, low doses of imidacloprid did not affect food consumption significantly, nor the larval development of males. This was not the case with deltamethrin contamination of larval food of the leaf cutting bee *Megachile rotundata* F at the dose of $100\,\mu g\,kg^{-1}$, which increased the duration of larval instars.[13] Imidacloprid intake affected worker survival rates within the first half-month, without any dose-effect relationship. In both treatments in which food was contaminated, 10% of workers died during the first 2 weeks. Thereafter mortality evolved at the same rate until the end of brood rearing, which suggests that chronic mortality is a slight phenomenon restricted to young workers. Similar results were obtained in a 10-day chronic oral test with honeybees fed with a syrup contaminated with imidacloprid at $10\,\mu g$ litre^{-1}, where the authors observed mortality only after 3 days.[14] Moreover these authors did not find a dose-effect proportionality, which is consistent with our results, showing either a lack of significant effects of the higher dose D2, or similar effects with D1 and D2 treatments. These discrepancies may be due to the low dose ratio of 2.5 and the low susceptibility of *B terrestris* to doses of imidacloprid close to the detection threshold.

Fecundity of dominant workers was affected by imidacloprid, as was also demonstrated in the leaf cutting bee *M rotundata* after a topical application of $2 \times 10^{-3}\,\mu g$ deltamethrin per female bee. The reduced larval ejection rate in both treatments should not be considered a beneficial effect of imidacloprid, but rather a consequence of the reduction of brood size. In other terms, the more larvae per brood, the more workers are stimulated to eject a part of them in order to feed the remaining individuals adequately. Imidacloprid may be metabolized rapidly in live workers since no residues could be found in bumblebees which have consumed 4.8 ng per day. Considering the weight of a worker, absorption of this amount corresponds to a concentration of $37\,\mu g\,kg^{-1}$ in the bumblebee body, which is above the detection limit of the chemical analysis technique. It has been found that 5 ng per honeybee was thoroughly transformed in 24 h into 5-hydroxy-imidacloprid and olefin.[15] A similar breakdown has probably occurred in bumblebees and the two metabolites may also have been at least partly degraded.

Numerous analysis of imidacloprid and its metabolites have been recently performed in France on honey and pollen collected by honey bees foraging on sunflower treated with Gaucho.[16] As this study did not reveal concentrations higher than $10\,\mu g\,kg^{1}$, bumblebee reproductive capacity is not likely to be significantly affected by prolonged ingestion of pollen and nectar produced by treated sunflower.

Apart from reproduction, sublethal effects may concern behavioural traits,[6–8] and it would be worth studying how flower visitation and homing ability of queenright bumblebee colonies might be affected by foraging on sunflower fields.

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