

Long-term toxicity assessment of imidacloprid to evaluate side effects on honey bees exposed to treated sunflower in Argentina

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Abstract

A long-term field trial (226 days) was conducted to assess the effect of imidacloprid on population development and honey production of the beehives exposed to sunflower treated with Gaucho®.

The trial was conducted in two plots of 24 ha each, sown with 60.000 seeds/ha of sunflower, commercial hybrid (DEKASOL 3915 G3). The seeds of the test plot were treated with 0.24 mg imidacloprid (a.i.) per seed and the seeds sown in the control plot were left untreated.

Apis mellifera ligustica (Spin.) populations and sister queens of the same age were placed in 16 hives, consisting of two hive bodies and an excluder each. At the beginning of the study, the shape and population structure of the hives was uniform.

Beehives were placed at the center of each parcel when sunflower came into bloom, and remained there during the flowering period, according to general pollination practices. After bloom, the beehives were transferred to a natural pasture and their development was assessed during the subsequent 216 days. The control of bee parasites and diseases was done according to the usual beekeeping practice in Argentina, and the sanitary status of the hives as well as the whole study, were audited by personnel of the National Service of Health and Quality of Food and Agricultural Products (SENASA).

The evolution of the hives was assessed by analyzing population parameters sensitive to factors affecting the development of the hives: beehive weight, nectar, pollen, brood and honey production, foraging activity, pollen entrance and mortality. Residue analysis (HPLC – Mass Spectrometry) of imidacloprid and its main metabolites in soil, sunflower heads and seeds, wax, honey and pollen completed the study. In addition, honey and pollen samples were analyzed to determine the sunflower pollen content in the hives. No residues of imidacloprid or of its main secondary metabolites olefin-imidacloprid and hydroxi-imidacloprid were detected (<1.5 µg/kg) in any of the components of the beehives 10 days after their exposure to the treated sunflower.

The populations from treated and control hives presented no significant differences in their development regarding pollen entrance and pollen in the hives, nectar and mortality. However, treated hives were more productive in terms of average weight, honey production, foraging activity, worker brood and comb foundation probably due to the better physiological state of the treated crop. A high proportion (>20%) of sunflower pollen in the honey and pollen samples obtained after the exposure to sunflower plots in control and treated hives, revealed that bees foraged actively on the sunflower.

No side effects were observed, in the short (10 and 28 days) or in the long-term (216 days) analysis, on the hives exposed to the sunflower plot treated with imidacloprid. The development of the hives or the individual bees was not affected by their exposure during bloom to sunflower plants originated from seeds treated with Gaucho®, under the conditions of the trial.

Key words: imidacloprid, Gaucho®, honey bees, *Apis mellifera*, field trial, toxicity.

Introduction

The systemic insecticide Gaucho® has been used intensively to protect seeds from soil insects that affect the establishment and development of sunflower. Testimonies on side effects of the product on the structure and honey production of the beehives used to pollinate treated sunflower arouse in the 90's (Curé *et al.*, 1999). Consequently, several research projects were carried out in Germany and France to determine if the active ingredient imidacloprid was responsible for the reduction in honey production and what the French beekeepers called the French bee illness.

In 1999, Argentine beekeepers also gave testimonies of symptoms related to the French bee illness. Consequently, an ad hoc commission was created at the National Service of Health and Quality of Food and Agricultural Products (SENASA), the "Working Group to Evaluate Possible Side Effects of Gaucho® on Honey Bees". This commission supervised a field trial conducted by the LPE - CONICET – LIBIQUIMA (University Comahue-Argentina) research team to assess long-term side effects on honeybees of Gaucho® (imidaclo-

prid 60%) used as sunflower seed treatment in Argentina.

The objective of the study was to assess the effect of imidacloprid on population dynamics, behavior and honey production of the beehives exposed to sunflower treated with Gaucho®, in a long-term field trial, conducted in the framework of a protocol based on BBA (1980) and OEPP / EPPO (1992), in accordance with GLP standards.

Materials and methods

The field trial was conducted in the Estancia "La Catalina" (San Gregorio, Buenos Aires, Argentina) in two plots of 24 ha each, sown with 60.000 seeds/ha of sunflower commercial hybrid (DEKASOL 3915 G3). The seeds of the test parcel were treated with Gaucho® FS (imidacloprid 60%) (600 ml/100 kg of seed) at a dose of 0.24 mg imidacloprid per seed and the seeds sown in the control plot were left untreated.

The management of both plots was done according to the usual agricultural practices. Crops and wild flora in

adjacent fields was monitored during the whole study and spots of flowering plants were removed.

Apis mellifera populations of 20.000 individuals and sister queens of uniform age and characteristics were used for the study and placed in 16 hives, consisting of two hive bodies divided by an excluder (to avoid oviposition on the upper hive body) each. At the beginning of the study, the population structure of the hives was uniform, containing 7 empty combs, 1 feeder and 2 combs filled with honey. The management of bee parasites and diseases was done according to the usual bee-keeping practice in Argentina.

Five assessments (T1 – T5, figure 1) of the parameters to evaluate population dynamics, behavior and honey production of the beehives were performed according the scheme in figure 1.

In 10% sunflower bloom (T2 – figure 1), beehives were placed at the center of each parcel and remained there during the flowering period, according to the local pollination practices. After bloom, 80% flowers without pollen (T3), all the beehives were transferred to a natural pasture (“El Gabi” Farm, La Plata, Buenos Aires province) and their development was monitored during the subsequent 216 days.

The different parameters related to the development of beehives were evaluated four times during the study: when the hives were transferred to the sunflower parcels (T2), 10 days after exposure of the hives to sunflower, before being removed from the test

plots (T3), 28 days after removal (T4) and 216 days after removal (after overwintering) (T5) (figure 1). During these sampling events, beehives were weighed and opened in order to take samples and evaluate comb coverage. The following population parameters, related to the development of beehives were analyzed throughout the study:

- Beehive weight: by weighing hives.
- Honey, nectar and pollen storage and brood: by estimating the percentage of cells occupied by honey, pollen or brood on both sides of each comb sampled.
- In addition, during the period in which the hives remained in the sunflower plots (T2-T3), the following observations were conducted:
 - Mortality: the number of dead bees on a 1m² fine mesh placed on the ground in front of the hive entrance was determined every 24 hours.
 - Foraging Activity: was observed twice daily. Four observation points were established in each plot where bees foraging for pollen and nectar were observed in 100 sunflower heads in the morning (09-12 a.m) and in the afternoon (02-05 p.m).
 - Pollen entrance: the number of bees entering the hive with pollen loads was recorded daily in a 3-minute interval, simultaneously with the foraging activity assessment.

The results of the evaluation of the population parameters were analyzed with ANOVA.

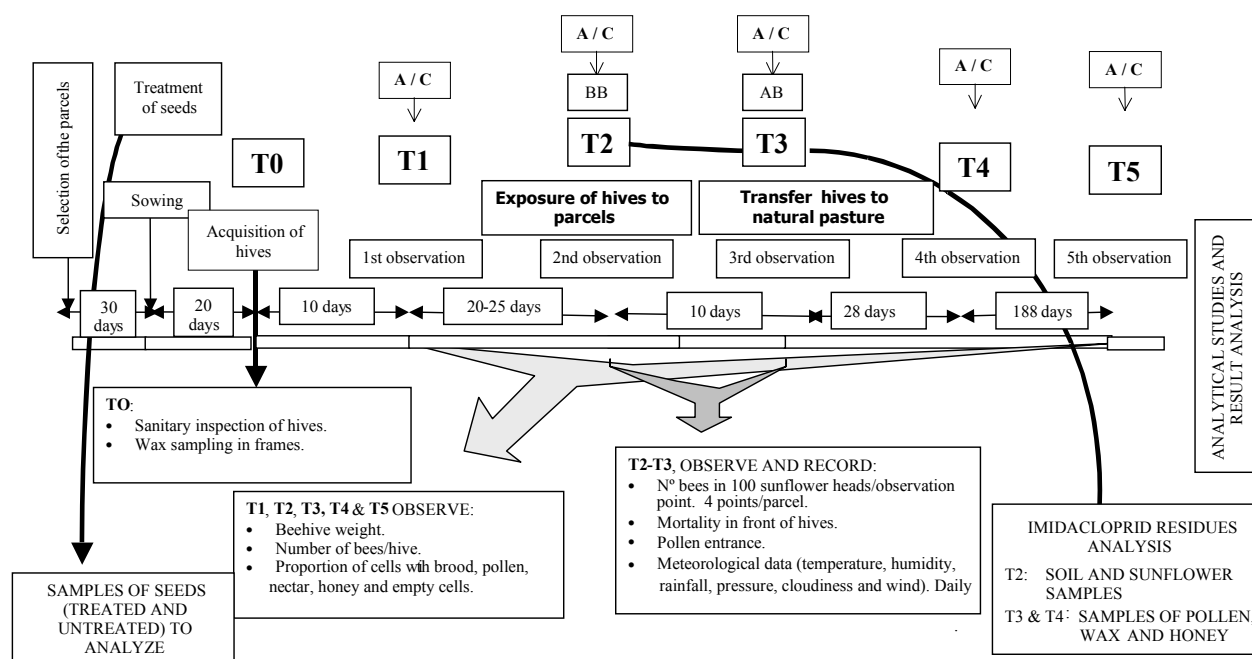


Figure 1. Study design. AC: Audit-Certification, BB: Begins Bloom 10%, AB: After Bloom- End Bloom.

Analytical studies on imidacloprid residues and pollen

According to the Standard Operating Procedures prepared specifically for this study (Stadler, Martínez-Ginés, 2000), samples were taken for analytical studies.

Residue analysis. The residue analysis of imidacloprid and its main metabolites in soil samples and sunflower heads collected at T2, and in pollen, wax, study: Beekeeping Health Program, National Agrifood Service (and honey samples collected at T3 and T4 was done by High Performance Liquid Chromatography (HPLC) with Mass Spectrometry detection (Schöning, 1997).

Pollen analysis. Pollen samples were taken 10 days after exposure of the hives to sunflower (T3), and 28 days after being removed from the sunflower plots (T4), to determine the proportion of pollen in the hives. The palinological analysis was done following the Louveaux et. al. (1978) technique and observing a minimum number of 1000 pollen grains.

Complementary information

This multidisciplinary research project required expertise in different fields. Experts from different Institutes were summoned by the scientific coordinator of the study, the Laboratory for Parasitology and Ecotoxicology (LPE) Univeristy Comahue, Argentina:

- Statistical Analysis: Chair of Biometrics, Department of Biology, Faculty for Exact and Natural Sciences-University Buenos Aires, Argentina.
- Palinological Analysis: National Research Council, Argentina (CICYTTP-CONICET).
- Analytical Chemistry: Institute for Chemistry and En-

vironmental Sciences (INQUIMAE) Faculty for Exact and Natural Sciences-University Buenos Aires, Argentina and Laboratory for Biochemistry and Environmental Sciences (LIBIQUIMA) University of Comahue, Argentina.

- Sanitary Inspection of the hives and audit of the SENASA), Argentina.

Results and discussion

Sunflower density and phenology assessment of in the test plots

When the beehives were transferred to the test plots (T2), the plant density was higher in the plot treated with Gaucho® (50.300 plants/ha) than in the control plot (41.500 plants/ha). This fact is probably related to the pesticide treatment of the seeds with the product tested.

The number of blooming plants was similar in control and treated plots. However, more plants without pollen were observed in the control plot than in the treated one after the first week of the study (figure 2).

Bee activity and mortality assessment during the exposure of the beehives to treated sunflower plots (T2 –T3)

Foraging activity (mean number of bees foraging on sunflower) was significantly higher in the treated plot in comparison with the control plot (table 1).

No significant differences in pollen entrance were observed (table 1).

Mean mortality in front of the treated and control hives was not statistically different (table 1).

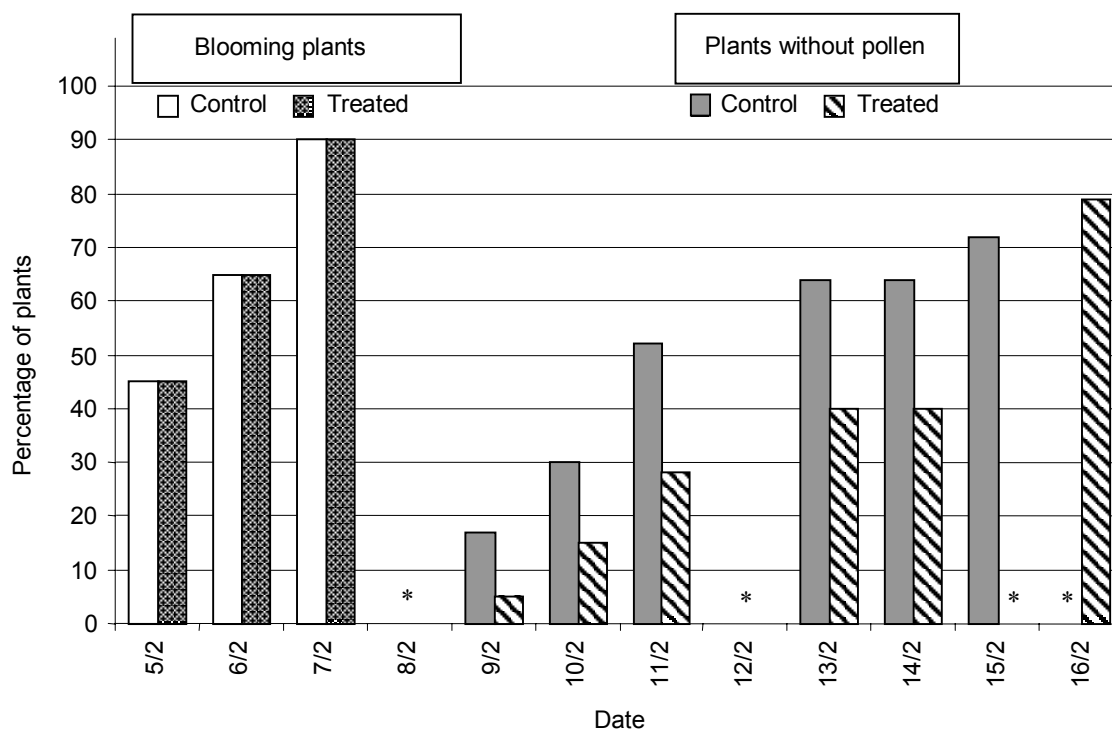


Figure 2. Percentage of blooming sunflower plants and plants without pollen during the exposure of the beehives to sunflower plots. *Data not available.

Table 1. Qualitative comparison of the mean values obtained for the parameters evaluated. * = p > 95%

Hive location Observation	Sunflower plot		La Plata – natural pasture	
	T1	T2	T3	T4
Parameter observed				
Hive weight	Control~Treated*	Control<Treated*	Control<Treated	Control<Treated
lower hive body	Control~Treated*	Control~Treated*	Control~Treated*	Control~Treated*
Cells occupied with honey and nectar (%)	upper hive body	----	Control<Treated*	Control<Treated
upper hive body	Control~Treated*	Control~Treated	Control<Treated*	Control<Treated
Cells occupied with pollen (%)	lower hive body	Control~Treated*	Control<Treated*	Control~Treated
Cells occupied with worker brood (%)	upper hive body	Control~Treated*	Control<Treated*	Control<Treated
Empty cells (%)	hive	Control~Treated*	Control~Treated*	Control<Treated
Foraging activity	Control<Treated*			
Pollen entrance	Control~Treated*			
Mortality	Control~Treated*			

Table 2. Results of the residue analysis.

Sample	Date	Imidacloprid		Olefin-imidacloprid		Hydroxi-imidacloprid	
		Control	Gaicho	Control	Gaicho	Control	Gaicho
soil	04-Feb	<6	<6				
Lower quantification limit (µg/kg)		6					
Lower detection limit (µg/kg)		2					
Sunflower	04-Feb	<1.5	<1.5	<3	<3	<1.5	<1.5
Honey	14-Feb	<1.5	<1.5	<3	<3	<1.5	<1.5
Pollen	14-Feb	<1.5	<1.5	<3	<3	<1.5	<1.5
Wax	14-Feb	<1.5	<1.5	<3	<3	<1.5	<1.5
Honey	15-Mar	<1.5	<1.5	<3	<3	<1.5	<1.5
Pollen	15-Mar	<1.5	<1.5	<3	<3	<1.5	<1.5
Wax	15-Mar	<1.5	<5	<3	<3	<1.5	<1.5
Lower quantification limit (µg/kg)		5		10		5	
Lower detection limit (µg/kg)		1.5		3		1.5	

Development of beehives population structure

T 3 . At the end of the exposure period, an increase in the mean weight, brood, nectar, pollen and honey production was observed. This increase was significantly greater in the hives of the treated plot (table 1).

T 4 . 28 days after the hives were removed from the sunflower plots, the frames surface covered with pollen, nectar and honey was significantly greater in the hives of the treated plot (table 1).

T 5 . 216 days after the hives were removed from the treated plots (after overwintering), both groups of hives (treated and control) presented a similar population development, although differences in mean weight, worker brood, comb foundation and honey production, in the upper hive body, that had been observed in T4 before winter were observed as well.

Sunflower pollen content in honey and pollen samples

According to Maurizio and Louveaux, (1963) and Ricciardelli d'Albore (1997), a high proportion (>20%)

of sunflower pollen in the pollen (figure 3) and in the honey (figure 4) samples obtained after the exposure to sunflower plots in control and treated hives (T3), revealed that bees foraged actively on the sunflower. Furthermore, the honey was identified as “sunflower honey” according to the organoleptic properties of the samples obtained from the hives of the study.

Imidacloprid residues in soil, sunflower heads, honey, pollen and wax samples

No residues of imidacloprid or of its main secondary metabolites olefin-imidacloprid and hydroxi-imidacloprid were detected (<1.5 µg/kg) in any of the components of the beehives analyzed 10 days after their exposure to the treated sunflower.

No quantifiable levels of imidacloprid residues (<5 µg/kg) were found either in soil samples obtained in T2 or in pollen, honey and wax in T3 and T4. Residue findings of imidacloprid and its metabolites relevant for the honeybee exposure in sunflower fields are summarized in table 2.

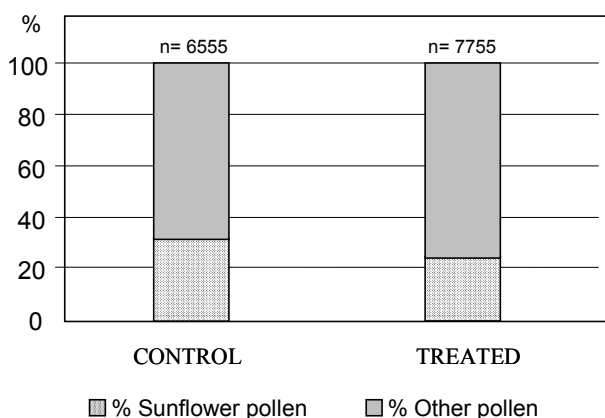


Figure 3. Main percentage of sunflower pollen grains in pollen samples taken from hives at T3 (n: number of grains identified).

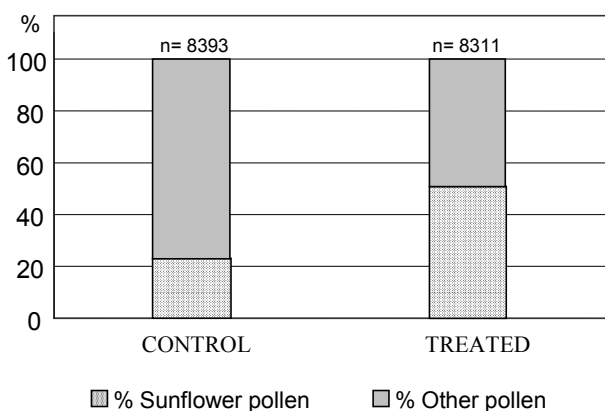


Figure 4. Main percentage of sunflower pollen grains in honey samples taken from hives at T3 (n: number of grains identified).

Conclusions

The hives exposed to sunflower treated with Gaucho® were more productive than the control hives. After removal of the hives from the sunflower plots, both groups (treated and control) presented a similar population development although pollen and honey production was greater in the hives exposed to the treated sunflower.

The differences observed in the population development of the hives in both plots are probably related to the differences observed in foraging activity and this in turn with larger pollen storage during sunflower bloom.

The palinological analysis of honey and pollen samples obtained after the exposure of beehives to sunflower plots revealed that bees foraged actively on the sunflower. Even though bees foraged actively on treated sunflower, no residues of imidacloprid or of its main secondary metabolites olefin-imidacloprid and hydroxi-imidacloprid were detected (<1.5 µg/kg) in any of the

components of the beehives 10 days after their exposure to the treated sunflower. Further, no side effects related to imidacloprid toxicity were observed in the short term (T2-T3) nor in the long-term (T2-T5) analysis of the bees or hives exposed to treated sunflower.

According to the results obtained, the development of the hives or the bees individually was not affected by their exposure to sunflower plants originated from seeds treated Gaucho®, under the trial conditions.

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