Hazards of Imidacloprid Seed Coating to *Bombus terrestris* (Hymenoptera: Apidae) When Applied to Sunflower

Author(s): J. N. Tasei, G. Ripault, and E. Rivault
Source: Journal of Economic Entomology, 94(3):623-627. 2001.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0022-0493-94.3.623
URL: http://www.bioone.org/doi/full/10.1603/0022-0493-94.3.623

Hazards of Imidacloprid Seed Coating to *Bombus terrestris* (Hymenoptera: Apidae) When Applied to Sunflower

J. N. TASEI, G. RIPAULT, AND E. RIVAULT

Unité de Recherche de Zoologie, INRA, 86600 Lusignan, France

J. Econ. Entomol. 94(3): 623–627 (2001)

ABSTRACT Seed coating treatments of sunflower by the systemic insecticide imidacloprid was suspected of affecting honey bees and bumblebees. The hypothesis raised was whether imidacloprid could migrate into nectar and pollen, then modify flower attractiveness, homing behavior, and colony development. Our greenhouse and field experiments with *Bombus terrestris* L. were aimed at the following: the behavior of workers foraging on treated and control plants blooming in a greenhouse, the homing rate of colonies placed for 9 d in a treated field compared with colonies in a control field, and the development of these 20 colonies under laboratory conditions when removed from the fields. In the greenhouse, workers visited blooming heads of treated and control plants at the same rate and the mean duration of their visits was similar. In field colonies, analysis of pollen in hairs and pellets of workers showed that in both fields 98% of nectar foragers visited exclusively sunflowers, whereas only 25% of pollen gatherers collected sunflower pollen. After 9 d, in the control and treated field, 23 and 33% of the marked foragers, respectively, did not return to hives. In both fields, workers significantly drifted from the center to the sides of colony rows. During the 26-d period under field and laboratory conditions, the population increase rate of the 20 colonies was 3.3 and 3.0 workers/d in hives of the control and treated field, respectively. This difference was not significant. New queens were produced in eight colonies in either field. The mean number of new queens per hive was 17 and 24 in the control and treated field, respectively. Their mating rate was the same. It was concluded that applying imidacloprid at the registered dose, as a seed coating of sunflowers cultivated in greenhouse or in field, did not significantly affect the foraging and homing behavior of *B. terrestris* and its colony development.

KEY WORDS *Bombus terrestris,* imidacloprid, seed dressing, sunflower, side effects

MANY CULTIVATED PLANTS such as cereals, maize, canola, and sunflower can be protected from pests by use of seed coating insecticides. Gaucho, which contains the active substance imidacloprid (neonicotinoid insecticide), has been extensively used in France since 1993. It has systemic properties and is a highly effective aphicide during the early stages of plant growth. Although treated seeds are coated with a low amount of imidacloprid (0.7 mg/seed on average) and despite Schmidt's results (1996) showing that seed coated with imidacloprid affects neither sunflower visitation by honey bees nor colony development, since 1996 French beekeepers have suspected sunflower treatments to result in great losses of honey bee foragers and abnormally low yields of sunflower honey.

It was hypothesized that imidacloprid could migrate into nectar, as already demonstrated in the systemic insecticides dimethoate and phosphamidon after soil or foliar application to various crop plants (Jaycox 1964, Waller and Barker 1979; Waller et al. 1984). Because imidacloprid is a potent neurotoxic, sublethal amounts of this active substance or its metabolites could modify foraging and homing behavior and consequently honey production and colony development. Such sublethal effects of insecticides on insect behavior have been reviewed by Haynes (1988). In honey bees, laboratory and semifield studies showed that sublethal doses of carbofuran and dimethoate affected larval development (Davis et al. 1988) and that sublethal doses of deltamethrin reduced homing flight capacities (Vandame et al. 1995).

Apart from the honey bee, the bumblebee *Bombus terrestris* L. is the most abundant pollinator insect visiting sunflowers in Europe. Because the susceptibility of bumblebees to insecticides seems close to that of *Apis mellifera* (Stevenson and Racey 1967, Mayer et al. 1994), *B. terrestris* could be affected similarly by imidacloprid applied to seeds.

We studied the potential effects of treated sunflowers on *B. terrestris.* The four objectives of greenhouse and field experiments conducted in 1998 were as follows: (1) Setting up a semifield and field method adapted to bumblebees. (2) Comparing the foraging behavior of *B. terrestris* on treated and control plants of sunflower. (3) Estimating the homing rate of colonies placed in treated and control sunflower fields. (4) Comparing their development in standardized conditions after the period in field.

Materials and Methods

Greenhouse Lay Out. In April we cultivated the sunflower variety "Rigasol" in 12-liter pots. Three rows of 16 pots each, containing vegetable mold were placed in a greenhouse compartment (3 by 6 m). Three seeds per pot were sown and after germination, only one seedling per pot was grown as a test plant. Treated and control plants alternated within each row. Pots were irrigated daily. When the heads started to bloom a colony of *B. terrestris* containing ≈100 workers was introduced and observations were conducted as follows: (1) Over a 4-d period (16–19 June) the total number of foragers visiting the heads at each blooming stage was recorded through four to eight counts covering flight hours (8.15–16.30) For treated and untreated heads daily cumulative figures are given. (2) During the same period the mean visit duration/head was estimated for 75 foragers on 52 control heads and 52 treated heads. We considered the frequencies of short visits (<50 s) and those of longer ones.

Field Experiment. Two sunflower fields of the variety 'Rigasol' in the Department of Deux-Sèvres (West Center France) were chosen where seed coating of sunflower with imidacloprid was forbidden except in one experimental area, where 90% of sunflower acreage was treated. This area (3-km radius) contained 445 ha of sunflowers, including the experimental treated field of 16 ha in its center. The control field (18 ha, 20 km away) was in the center of a nontreated similar area where 420 ha of sunflowers were grown. The seeds of the preceding crop (cereal) in the control field had not been coated with imidacloprid, whereas they had been coated in the case of the treated field, which was a worse case, because of the very high persistence of imidacloprid in soil. The active substance was applied at 0.7 mg/seed by a standardized process implemented by a private firm. The two experimental fields were sown beginning of May at 70,000 seeds/ha and started to bloom on 4 July and 8 July, respectively. Full bloom was 78 d and 73 d after sowing, respectively.

In June, 20 colonies of *B. terrestris* with ≈50 workers each were purchased from a private firm producing bumblebees for tomato pollination. On 8 July, the day they were moved to the field, all the workers were counted and marked with a colored spot on the thorax. Then 10 colonies were placed at regular intervals in each experimental field in a single row 10 m long, along an aisle between two lines of plants next to the border of the field. Their entrance was oriented to the south. The position of each colony within the row was defined by a rank from 1 to 10. Observations started on 9 July and were completed on 17 July when we moved the hives to the laboratory after the end of the daily foraging activity. During the period in field, we sampled 108 nectar gatherers and 133 pollen gatherers returning to their nest in order to identify the plant species that had been foraged. After pollen sampling, these workers were released. The total number of nectar and pollen gatherers sampled per colony ranged from 6 to 14 in control colonies and from 10 to 15 in treated ones. Capture sessions were performed in the morning and in the afternoon for 6 d. We trapped the workers in plastic tubes inserted in the entrance tunnel. In each tube a roll of filter paper served as a pollen retainer. Only one pellet per pollen gatherer was removed, whereas in the case of nectar gatherers the filter paper inside the tube retained enough pollen grains from hairs to enable the identification of plants visited. We used as many tubes as bumblebee samples. Pollen pellets and pollen grains on the paper roll were treated according to the Maurizio's and Louveaux' method (1965). Some samples were acetolyzed according to Faegri's and Iversen's method (1964) to facilitate their identification. After examination of microscopic slides we identified which was the major pollen species in each sample, i.e., the species representing >70% of the total mass of pollen grains. This was rapidly assessed by scanning the whole slide and estimating the relative area covered by the main pollen type.

When the closed colonies were returned to the laboratory, they were fed with standard syrup and pollen paste, and after 26 d we counted marked and unmarked workers to estimate their homing rate during the field period and the growth rate of each colony under standardized conditions. At the end of the colony life cycle, new queens were caught, counted, and kept in cages containing males for mating.

Statistical Analyses. Data of counts on workers visiting treated and untreated sunflower heads in the greenhouse and those of marked and unmarked foragers in field colonies were analyzed by the chi-square procedure. Workers and queens populations produced within 26 d after D0 were compared between fields by the *t*-test procedure. Chi-square and *t*-test were from Minitab (1998). In the field experiment the population of marked workers seemed to vary with the relative position of the hives in the row. We used analysis of variance (ANOVA) and analysis of covariance (ANCOVA) methods to test the absolute and relative differences in the number of marked individuals in each colony between D0 and D0 + 9 d. The values attributed to the position variable ranged from 1, for the two colonies in the center of the row, to 5, for the two colonies on both sides. Analyses were done on Systat (Wilkinson 1989).

Results

Greenhouse Experiment on Foraging. On 16 June and 17 there were the same number of heads at stage 1 (one to three floret circles opened) on both treated and untreated plants. On 18 June and 19 the first heads at stage 2 appeared (floret circles opened at half radius) and flowering continued to be similar on treated and control plants.

The number of workers visiting blooming heads increased from 16 to 18 June and did not differ between treated and control plants significantly (Table 1).

The duration of individual visits on heads ranged from a few seconds to 4 min. The number of short

Table 1. Foraging population of bumblebees on control and treated sunflower heads in the greenhouse

Date	No workers		Chi-square value
	Control	Treated	
16 June	12	13	
17 June	34	42	0.4
18 June	68	82	(P = 0.9)
19 June	59	80	

visits, <50 s, and long visits, >50 s, were not significantly different on treated and control plants (table 2).

Field Experiment. Nectar gatherers of our experimental colonies visited sunflowers almost exclusively in both fields, because 98% of trapped individuals carried sunflower pollen, as the unique or major species (Table 3). Only 24 and 25% of pollen gatherers in the control and treated field, respectively, carried sunflower pollen (pure or major). Other pellets contained pollen collected from a total of 17 wild or cultivated plants. In both fields the main competitive plants were the same: *Medicago sativa*, *Solanum* sp., *Rubus* sp., and *Papaver rhoeas.* Other species, detected in either of the fields, belonged to the following 13 genera: *Trifolium, Tilia, Sinapis, Mercurialis, Filipendula, Borrago, Verbascum, Centaurea, Dipsacus, Cirsium, Crepis, Bryonia,* and *Hypericum.*

In control and treated fields, nectar gatherers accounted on average for 40 and 50% of the foragers, respectively (Table 3). Considering nectar and pollen gatherers visiting sunflowers in both fields, it can be inferred that 53% of foragers visited sunflowers in the control field, compared with 62% in the treated field.

Because the workers were marked, estimates of the loss of workers during the 9-d foraging period in field could be made. The mean number of marked workers per colony, after 9 d, was lower than at the beginning of the study. (Table 4). Colonies in the treated field lost 33.5% of marked workers compared with 23.1% in the control field. The difference was not significant (P = 0.63) if a chi-square test was applied to the number of marked workers at day of introduction D0 and D0 + 9 d. Fig. 1 shows a similar tendency in both fields where colonies on the sides of the row gained marked workers from colonies placed in the center during the 9-d period in the fields. The treatment with imidacloprid had no significant interaction with the position of the hive (ANOVA, P = 0.62). The treatment had no significant effect (ANCOVA, P = 0.55), whereas the relative position of the hive affected both the absolute and relative differences in the number of marked workers in each colony (P = 0.008 and P = 0.012, respectively). In both fields the increase of standard errors at D0 + 9 d (Table 4) was a consequence of drifting that occurred within the two rows of colonies (Fig. 1).

Table 2. Visit duration of bumblebees on control and treated sunflower heads in greenhouse

Visit duration, s	No workers		Chi-square value
	Control	Treated	
1–50	30	20	3.2
>50	10	16	(P = 0.07)

Table 3. Presence of sunflower pollen in pollen loads of 241 foragers in field

Field	No loads with sunflower as pure or major species	
	Nectar gatherers	Pollen gatherers
Control	45 (46)	17 (70)
Treated	61 (62)	16 (63)

Sample size in parentheses.

The mean population increase, checked 26 d after the introduction of hives to fields, was 3.3 and 3.0 workers/d/colony in control and treated field, respectively. The difference was not significant (Table 5).

New queens were produced by eight colonies out of 10 in each field. There were 17 and 24 queens/colony in hives of the control and treated field, respectively, but the difference was not significant (Student t = 1.6; P = 0.12). Mating ability of these new queens was similar (71% and 74%).

Discussion

In the greenhouse, observations on foraging revealed no difference in the presence of workers on blooming heads and the duration of their visits between treated and control plants. This is not in accordance with Koppert's statement (1996) reporting side effects of several commercial products containing imidacloprid (Confidor, Admire, Bayer AG, Leverkusen, Germany) and used as a drench for aphid control in greenhouse on sweetpepper and tomato. Such treatments resulted in a severe reduction of bumblebee activity and high larval mortality. These negative consequences occurred 1–7 wk after the last application and varied according to the season and the substrate (soil, perlite, or rockwool). Soil produced the shortest residual effects, which may be due to the quicker degradation of imidacloprid and its metabolites in this substrate compared with the two artificial root media. Other factors may be involved in the translocation of residues to pollen and nectar and exposure of bumblebees such as the rapidity of insecticide absorption presumably enhanced by drench application. Furthermore, growth rate as well as nutrients and water transport by xylem and phloem may differ in vegetables and

Table 4. Loss of workers during 9 d of foraging in field

Field	No marked workers/colony (means ± SE)			Chi-square value[a]
	D0 (A)	D0 + 9 d (B)	Loss = (A) − (B)	
Control	46.3 ± 1.9	35.6 ± 5.6	10.7 ± 6.0	0.27
Treated	53.6 ± 2.5	35.6 ± 3.9	18.0 ± 5.3	(P = 0.6)

D0, day of introduction to field.
[a] Performed on the total numbers of workers per field and per date.

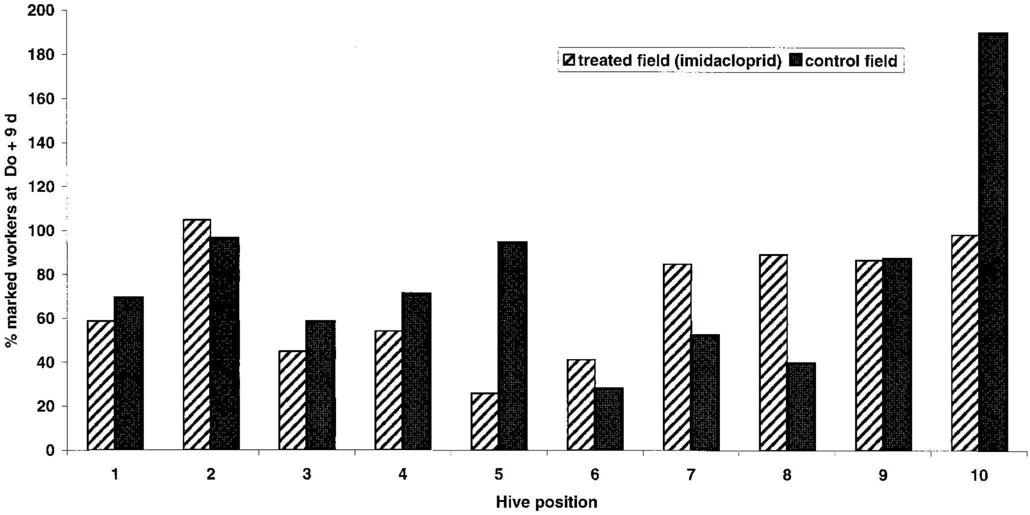

Fig. 1. Homing rates of workers in 20 hives of *B. terrestris* placed in a row after a 9-d foraging period in two sunflower fields. The x-axis indicates the position of colonies in each row of 10 hives. Number 1 was attributed to the position close to the aisle entrance.

sunflowers and consequently account for discrepancies between hazards reported in both kinds of plants treated with imidacloprid. In five samples of honey collected by bumblebee workers from sunflowers grown in our greenhouse, imidacloprid was detected but not above the threshold of 0.01 mg/kg (J.N.T., unpublished data). These analyses indicate that nectar contained low amounts of imidacloprid resulting in no negative effect on forager behavior. Mayer and Lunden (1997) reported that honey bees reduced their visits to a syrup feeder by only 7% when it was contaminated by 2 mg/kg imidacloprid, i.e., 200 times more than the determination threshold. Their results strengthen the assumption that seed coating of sunflower by imidacloprid does not affect bee foraging behavior significantly when nectar contamination is below the determination threshold.

No residues of imidacloprid or metabolites were detected in the soil of our experimental treated field (limit of detection = 5 μg/kg), whereas residues were detected in samples of pollen and honey collected by honey bee colonies placed in the same field and even determined in leaves and dead bees (Pham-Delègue and Cluzeau 1999). These chemical analyses demonstrate that bumblebees visiting the treated field have been exposed to the active substance. The proportion of experimental individuals foraging on sunflowers was estimated by the percentage of nectar and pollen gatherers returning to their hive with loads containing a majority of sunflower pollen. Our data revealed similar visitation patterns in both fields for the two kinds of foragers, the exposure to residues in nectar being about four times higher than to those in pollen (98% and 25% of foragers, respectively). The detection of residues in treated plants and the identification of pollen on worker bodies provide grounds for validating our field experiment.

The use of colored marks on workers showed that foragers drifted in both fields from central hives to lateral ones, which is a phenomenon frequently reported in the honey bee when hives are in a row (Free 1958). These homing errors, similar in the treated and control fields, increased the heterogeneity of the figures of marked workers recorded after 9 d of foraging in field. The difference between worker losses in the two fields was not significant. When the colonies were taken back to the laboratory and checked at D0 + 26 d, no significant difference in population growth was noted between the two series, 3.3 and 3.0 workers per day and per colony in control and treated colonies, respectively. Our observations on colony growth is consistent with previous results showing that bumblebee longevity and fecundity were little or not affected under laboratory conditions by sugar solutions contaminated with 0.01 mg/kg and 0.025 mg/kg imidacloprid, supplied during a 3-mo long-term toxicity test (Tasei et al. 2000).

We conclude that applying Gaucho at the registered dose, as a seed coating treatment to sunflowers cultivated either in greenhouse or in the field, affects neither the foraging and homing behavior of worker bumblebees nor the development of colonies, including their reproductive capacity.

Table 5. Population increase of 20 bumblebee hives, 26 d after their introduction to field

Field	No workers/colony (mean ± SE)			t-test value
	D0 (A)	D0 + 26 d (B)	Increase = (B) − (A)	
Control	46.3 ± 1.9	132.9 ± 19.3	86.5 ± 17.4	0.44
Treated	53.6 ± 2.5	131.7 ± 14.0	78.1 ± 11.5	(P = 0.67) (df = 18)

D0, day of introduction to field.

Acknowledgments

The authors are grateful to Y. Loublier (LNCI, Bures-sur-Yvette, INRA, France) for his help in pollen identification. Thanks are also due to B. Vaissière (Station de Zoologie et Apidologie, Avignon, INRA, France) for his advice and help in statistical analysis, and Biotec Center (Orléans, France) for analyzing imidacloprid residues in bumblebee honey.

References Cited

Davis, A. R., K. R. Solomon, and R. W. Shuel. 1988. Laboratory studies of honeybee larval growth and development as affected by systemic insecticides at adult-sublethal levels. J. Apic. Res. 27: 146–161.

Faegri, K., and J. Iversen. 1964. Textbook of pollen analysis. Oxford University, Oxford, UK.

Free, J. B. 1958. The drifting of honeybees. J. Agric. Sci. 51: 294–306.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33: 149–168.

Jaycox, E. R. 1964. Effect on honeybees of nectar from systemic insecticide-treated plants. J. Econ. Entomol. 57: 31–35.

Koppert. 1996. Newsletter n°18. Koppert B.V. Berkel en Rodenrijs, The Netherlands.

Maurizio, A., and J. Louveaux. 1965. Pollens de plantes mellifères d'Europe. UGAPF 38 Bd. Sébastopol, Paris.

Mayer, D. F., K. D. Patten, R. P. Macfarlane, and C. H. Shanks. 1994. Differences between susceptibility of four pollinator species (Hymenoptera: Apoidea) to field weathered insecticide residues. Melanderia 50: 24–28.

Mayer, D. F., and J. D. Lunden. 1997. Effects of imidacloprid insecticide on three bee pollinators. Hortic. Sci. 29: 93–97.

Minitab. 1998. Minitab user's guide, release 12. Minitab Inc., State College, PA.

Pham-Delègue, M. H., and S. Cluzeau. 1999. Effets des produits phytosanitaires sur l'Abeille. Incidence du traitement des semences de tournesol par Gaucho sur la disparition des butineuses. Rapport final, Mars 1999. Association de Coordination Technique Agricole, Paris, France.

Schmidt, H. W. 1996. The reaction of bees under influence of the insecticide imidacloprid. Appendice 12. *In* Proceedings of the 6th International Symposium on Hazards of Pesticides to Bees, 17–19 September, Braunschweig, Germany. D. Brasse, Braunschweig, Germany.

Stevenson, J. H., and P. A. Racey. 1967. Toxicity of insecticides to bumblebees, p. 176. *In* Report of Rothamsted Experimental Station for 1966. AFRC Institute of Arable Crops Research, Rothamsted Experimantal Station, Harpenden, UK.

Tasei, J. N., J. Lerin, and G. Ripault. 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. Pest Manag. Sci. 56: 784–788.

Vandame, R., M. Meled, M. E. Colin, and L. P. Belzunces. 1995. Alteration of the homing flight in the honeybee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. Environ. Toxicol. Chem 14: 855–860.

Waller, G. D., and R. J. Barker. 1979. Effects of dimethoate on honeybee colonies. J. Econ. Entomol. 72: 549–551.

Waller, G. D., B. J. Erickson, J. Harvey, and J. H. Martin. 1984. Effects of dimethoate on honeybees (Hym:Apidae) when applied to flowering lemons. J. Econ. Entomol. 77: 70–74.

Wilkinson, L. 1989. Systat: the system for statistics. Systat, Evanston, IL.

Received for publication 11 May 2000; accepted 1 December 2000.