Does Imidacloprid Seed-Treated Maize Have an Impact on Honey Bee Mortality?

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ABSTRACT Beekeepers suspected maize, *Zea mays* L., treated with imidacloprid to result in substantial loss of honey bee (Hymenoptera: Apidae) colonies in Belgium. The objective of this study was to investigate the potential impact of maize grown from imidacloprid-treated seeds on honey bee mortality. A survey of 16 apiaries was carried out, and all maize fields treated or not with imidacloprid were located within a radius of 3,000 m around the observed apiaries. Samples of honey, beeswax, and bees were collected in three colonies per apiary and analyzed for pesticide contain by liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. We first found a significant correlation between the number of colonies per apiary and the mortality rates in an apiary. In addition, this mortality rate was inversely correlated with the surface of maize fields treated and not with imidacloprid, suggesting that this pesticide do not interact with bees' fitness. Moreover, a very large number of our samples contained acaricides either prohibited or ineffective against *Varroa destructor* (Anderson & Trueman) (Acari: Varroidae), suggesting that the treatment methods used by the beekeepers to be inadequate for mite control. Our results support the hypothesis that imidacloprid seed-treated maize has no negative impact on honey bees.

KEY WORDS honey bees, mortality bees, seed-treated maize, pesticides, imidacloprid

Domesticated and wild bees are key components of terrestrial ecosystems. Most phanerograms would be unable to complete their development cycle without the intervention of pollinators (Michener 2000, Klein et al. 2007). In the European Union, 84% of the plant crop species are directly or indirectly influenced by pollinators' activity (Williams 1996). Their economic contribution to world agriculture is valued at US$117 billion (Costanza et al. 1997). In addition, honey bees (Apidae) have other positive impacts, including the producing honey, propolis, and royal jelly; maintaining genetic diversity in plant populations; and acting as bioindicators in natural and managed ecosystems (Free 1993, Kevan 1999).

Since 1999, in Europe, Belgian beekeepers have observed abnormal increases in overwintering mortality in honey bee colonies (Lefebvre and Bruneau 2005, Haubruge et al. 2006). Similar observations have been made in other European and North American countries (Faucon et al. 2002, Otten 2003, Vanengelsdorp et al. 2007).

French beekeepers believe that the collapse of honey bee colonies since 1995 (and especially in 1997) has been caused by the use of seeds coated with the systemic insecticide imidacloprid (Gaucho, Bayer, Leverkusen, Germany), which is registered for protection of many cultivated plants such as cereals, maize (*Zea mays* L.), canola (*Brassica* spp.), and sunflowers (*Helianthus* spp.) against arthropod pests. This insecticide, which belongs to the neonicotinoid family, has been extensively used in France since 1993. The toxicity of this pesticide to honey bees has been investigated in several studies, and published results are variable. Naunen et al. (2001) demonstrated an acute oral toxicity (LD_{50} at 48 h) for imidacloprid at 41–81 ng per bee and an acute contact toxicity of 49–102 ng per bee. In contrast, Schmuck et al. (2001) found an oral LD_{50} of only 3.7–40.9 ng per bee but a contact LD_{50} of 59.7–242.6 ng per bee. Decourtye et al. (2003) examined chronic exposure of honey bees to imidacloprid and reported lethality to caged worker bees at level of 48–96 μg/kg sucrose solutions for 11 d. Suchail et al. (2001) reported 50% mortality after ingestion of imidacloprid at concentrations between 0.1 and 10 μg/kg sucrose solutions for 8–10 d. According to Bonmatin et al. (2003, 2005), imidacloprid is found in Gaucho-treated maize (stems and leaves, 1.1 μg/kg; flowers, 6.6 μg/kg; and pollen, 2.1 μg/kg) and in Gau-

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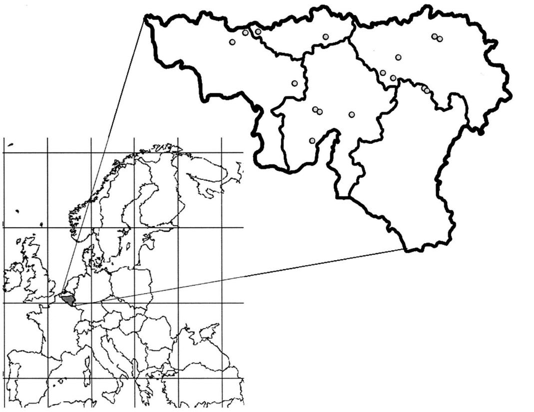

Fig. 1. Localization of the apiaries that were randomly selected in the southern part of Belgium (n = 16 apiaries mentioned as circles).

cho-treated sunflower (flowers, 5–10 μg/kg; and pollen, 3 μg/kg) at an average concentration sufficient to induce mortality and/or to alter honey bee behavior. Although Rortais et al. (2005) and Halm et al. (2006) suggest that honey bees are exposed to lethal and sublethal concentrations in fields that regularly used imidacloprid, the majority of studies report imidacloprid exposures below either acute or chronic toxicity levels and conclude that honey bee field exposure is negligible (Schmuck et al. 2001, Maus et al. 2003, Faucon et al. 2005, Alix and Vergnet 2007).

Concerns about adverse effects of Gaucho seed treatments on honey bees have been the subject of much debate. The French government has chosen to apply the precautionnary principle, and the use of Gaucho seed treatment has been suspended in France for sunflower since 1999 (Ministèrede-l'Agriculture-et-de-la-Pêche 1999) and for maize since 2004 (Ministèrede-l'Agriculture-et-de-la-Pêche 2004).

Sunflower is not grown commercially in Belgium; thus, Belgian beekeepers have suspected for many years that imidacloprid treatments applied to maize seeds are the local cause of excessive colony mortality (Bruneau 2001). The main goal of this field study was to investigate the potential impact of maize imidacloprid treatment on honey bees in Belgium.

Materials and Methods

Apiary Selection, Environmental Characterization, and Mortality Assessment. Sixteen apiaries were randomly selected in the southern region of Belgium (Fig. 1). In each apiary, three hives were randomly selected and visited every 2 mo between March 2004 and March 2005. Beekeepers were asked to follow their usual apicultural methods. However, for practical reasons, they were asked to leave the surveyed colonies in the same location year-round (no migratory beekeeping was allowed). Beekeepers were interviewed regarding their apicultural practices and any problems they encountered. Particular attention was paid to honey bee colony mortality. During the year 2004, all maize fields within a radius of 3,000 m around the observed apiaries were located and all crops flowering at the same time as maize flowering were catalogued. Each field was characterized by its surface (S) expressed in hectares and its lower (L) and upper (U) limit of distance to the apiary expressed in meters. All maize fields treated with imidacloprid were noted. Data on imidacloprid use were collected from four sources: the Agricultural Headquarter of the Walloon Region, the farmers, the plant protection products vendors, and field observations.

In social insects, a host population consists of a number of groups such as colonies, each containing a number of host individuals (Schmidt-Hempel 1998). A colony is therefore considered as a superorganism due to its complexity and to the coordination that exists among individuals. Because the colony behaves as a unit (Winston 1972), we considered the colony to be the sampling unit for mortality calculations. The mortality rate in an apiary was defined as the number of dead colonies (i.e., no live bees) divided by the total number of colonies in the apiary multiplied by 100.

Sample Collection and Residue Analysis. Honey, beeswax, and bee samples were collected from the three previously chosen colonies per apiary between 20 August and 20 October 2004 and analyzed for pesticide residues, including imidacloprid. Maize flowering occurred in August. Pollen was collected from cells in the hives, and presence of maize pollen was confirmed by microscopy.

Two grams of honey from each colony was collected from randomly selected capped cells on honey super frames by using disposable plastic spatulas and were stored in a brown glass jar. Randomly selected samples of food-free beeswax (25 cm^2) were collected from honey super frames of each colony by using disposable spatulas and placed in sealable freezer bags. Twenty honey bees from each colony also were collected. Ten workers were collected in the hives and 10 at the entrance. All samples were stored at −20°C before residue analysis. Half of the samples was used for gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS) analysis and the other half for liquid chromatography analysis (LC-MS/MS). On-column liquid-liquid extraction (OCLLE) followed by LC-MS/MS was described previously for pesticide analysis in honey, wax, and bee samples (Pirard et al. 2007). Calibration curves have been produced for quantification. The method has been slightly adapted for the GC-amenable compounds. In the case of honey and bee sample analysis, ethanol was used instead of acetone in the 5-ml ChemElut cartridge (Varian, Sint-Katelijne-Waver, Belgium), and a 50/50 (vol:vol) mixture of n-hexane and dichloromethane was used as the eluting solvent instead of ethyl acetate. For wax sample analysis, water (1.5 ml) and ethanol (2.5 ml) were added to 0.5 g of ground wax previously frozen with liquid nitrogen. After 20 min of centrifugation, the supernatant was loaded on a ChemElut cartridge humidified with 1 ml of 20% NaCl. Analytes were eluted after a waiting period of 15 min with two by 10 ml of

Table 1. Mortality rate in an apiary as a function of the maize field description in the area of a 3,000-m radius circle (2,826 ha) around apiaries (*n* = 16) and the imidacloprid detection (presence, 1; absence, 0)

Apiary		Fields of maize treated or untreated with imidacloprid							Hives	
No.	Detection of imidacloprid	In ha				In % circle area around apiaries			Maximum no.	Mortality rate (%)
		Treated [A]	Untreated	Total [B]	Proportion [A]/[B]	Treated	Untreated	Total		
1	0	0	50.79	50.79	0	0	1.80	1.80	8	25.00
2	0	0	136.91	136.91	0	0	4.84	4.84	27	51.85
3	0	13.29	207.78	221.07	0.060	0.47	7.35	7.82	24	4.17
4	0	4.89	370.39	375.28	0.013	0.17	13.11	13.28	12	0
5	1	70.15	101.26	171.41	0.409	2.48	3.58	6.07	3	0
6	0	63.64	105.23	168.87	0.377	2.25	3.72	5.98	11	0
7	0	1.66	67.05	68.71	0.024	0.06	2.37	2.43	12	16.67
8	0	0	137.78	137.78	0	0	4.88	4.88	42	54.76
9	0	11.09	68.4	79.49	0.140	0.39	2.42	2.81	19	84.21
10	0	1.28	63.94	65.22	0.020	0.05	2.26	2.31	21	47.62
11	1	42.21	52.3	94.51	0.447	1.49	1.85	3.34	4	0
12	0	0	147.5	147.5	0	0	5.22	5.22	6	0
13	0	0	115.82	115.82	0	0	4.1	4.1	10	30.00
14	0	6.31	168.05	174.36	0.036	0.22	5.95	6.17	15	0
15	0	0	97.34	97.34	0	0	3.44	3.44	14	50.00
16	0	11.47	118.7	130.17	0.088	0.41	4.2	4.61	5	0

n-hexane-dichloromethane 50/50 (vol:vol). Extracts were then evaporated at 30°C to obtain ≈1 ml, under a gentle stream of nitrogen, and frozen at −20°C overnight. Supernatant was then evaporated at 30°C under a gentle stream of nitrogen, and reconstituted with 200 μl of *n*-hexane before being filtered and transferred to a GC vial.

Statistical Analyses. Mean areas with unequal variances between maize fields treated and untreated with imidacloprid were compared using Welch's test (Dagnelie 1998). The correlation between the lower (L) and the upper (U) limits of distance for fields to each apiary was tested by Pearson's correlation coefficient (R^2).

We have raised two hypotheses, i.e., the relationship between the mortality rates in an apiary was correlated with the surface of the maize fields treated by imidacloprid (first hypothesis) or with the total area of the maize fields (second hypothesis) in a considered radius circle around apiaries. We included the second hypothesis in the analysis because if the pollen resource in the environment is low, maize pollen may represent a stock of nutrients and amino acids that allow the development and survival of honey bees during the winter period. Moreover, we analyzed the relationship between the mortality rate and the proportion of treated maize surface/total maize surface. The relationship between the mortality rates of hives and the three variables investigated was assessed using the Spearman's rank correlation coefficient, a nonparametric statistical method. It measures the association (not necessarily linear) between two variables which may be ordinal. The limits of the Spearman's rank correlation coefficient are −1 (inversely proportionality) and +1 (proportionality). The formula for the calculation of the Spearman's rank correlation coefficient with a 95% confidence interval is detailed in Dagnelie (1998). The limit of statistical significance of all the tests was defined as $P \leq 0.05$.

To take into account the distance and the surface of fields in our analyses, six scenarios were considered. They were selected on the basis of the results of Steffan-Dewenter and Kuhn (2003), who showed a mean ± SEM foraging distances of pollen-collecting bees equal to 1,743 ± 95.6 m in simple and 1,543.4 ± 71 m in complex landscapes. Scenarios 1–6 corresponded, respectively, to foraging distances of pollen-collecting bees of ≈1,000, 1,500, 1,750, 2,000, 2,500, and 3,000 m.

Results

Field Description. Maize fields treated with imidacloprid represented 13.2% of the total maize area, which is significantly smaller than the fields area untreated with imidacloprid (Welch's test; df = 135, P = 0.02). The imidacloprid treated fields covered between 0.05% (1.28 ha) and 2.48% (70.15 ha) of the maximum foraging area studied. The proportion of treated maize varied greatly between apiaries (proportion treated maize field/total maize field: 0–0.44). The surface coverage of untreated maize ranges from 50.79 to 370.39 ha (Table 1). No beneficial crop flowered at the same time as maize. The alternative crops with the biggest largest surface coverage around apiaries were winter wheat (mean = 392.22 ha), beet (mean = 150.98 ha), and meadow for breeding cows (mean = 714.56 ha).

Mortality Rates. ***Determination of Mortality Rate.*** The mortality rate ranged from 0 to 84.2%. All colonies that died did so during the winter period 2004–2005 (between November 2004 and March 2005), except in one apiary. The honey bee colonies that died in apiary 7 died in August. We observed that the majority of the hives with a zero mortality rate were in areas with treated maize fields. In contrast, some of the highest mortality occurred in areas where only untreated maize was encountered (Table 1).

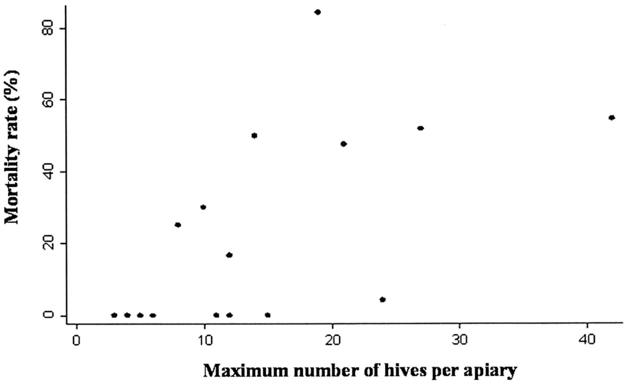

Fig. 2. Relation between the mortality rate and the maximum number of hives per apiary.

Relationship between Number of Colonies and the Mortality Rate in an Apiary. The number of colonies per apiary ranged between 3 and 42, and the mean was 14.56 colonies per apiary in our study. There was a significant correlation between the number of colonies per apiary and mortality rate ($r_s = 0.68$; ddl = 14; $P = 0.0035$) (Fig. 2).

Relationship between Surface of Maize Fields Treated or Not with Imidacloprid and the Mortality Rate in an Apiary. The relationship between the area of imidacloprid-treated maize fields or the total area of maize fields around 16 apiaries and the mortality rate in an apiary was analyzed according to six scenarios (taking into account the distance of fields to each apiary) (Table 2). Only the minimum limit of the distance to each field was considered because a strong correlation was observed between the minimum and maximum limits of distance between fields and each apiary (n = 1,027 fields; $R^2 = 0.98$; $P < 0.001$). Six scenarios were therefore considered, and all the data analysis showed a significant correlation ($r_s \leq -0.57$ ddl = 14; $P \leq 0.02$) between the maize areas treated with imidacloprid and the mortality rates in an apiary (Table 2). This relationship was inversely proportional (Fig. 3). In addition, an inversely proportional correlation (according to the scenario: $r_s \leq -0.50$ ddl = 14; $P < 0.05$) was observed between the total maize area and the mortality rate in the apiary excepted for the first (distance of pollen-collecting <1,000 m; $r_s = -0.47$; ddl = 14; $P = 0.08$) and the third (distance of pollen-collecting <1,750 m: $r_s = -0.49$, ddl = 14; $P = 0.052$) scenarios. Finally, an inversely significant correlation also was observed between the mortality rate and the proportion of treated maize surface/total maize surface ($r_s \leq -0.52$ ddl = 14; $P < 0.05$).

Residue Analysis. The detection of pesticides in 48 samples (16 apiaries, three hives per apiary) from each matrix (i.e., honey, beeswax and bees) is presented in Table 3. Nine pesticides were detected in honey, and imidacloprid was detected in four samples. All concentrations of the active ingredient ranged between the limit of detection (0.05 ppb) and the limit of quantification (0.5 ppb). The most commonly occurring pesticide was rotenone (31.3% of honey samples), which is a banned acaricide used to control *Varroa destructor* (Anderson & Trueman) (Acari: Varroidae). Only a single compound, the organochlorine lindane, was detected in honey bees, and 11 pesticides were present in beeswax. Among these latter, four can be attributed to beekeeping practices (bromopropylate, coumaphos, rotenone, and tau-fluvalinate) and seven to agricultural practices. Triazole flusilazole was the most often present in beeswax (31.3% of samples), and imidacloprid was not detected in the samples from honey bees or beeswax.

Discussion

In France, maize is an important pollen source for honey bees. It was established that 20–40% of the honey bee harvest during the whole flowering period is composed of maize pollen, when available (Doucet-Personemi et al. 2003). Pollen loads are stocked by bees within the colony in the form of beebread, which is a mixture of honey, pollen, and several enzymes. When honey bees forage on maize flowers, there is therefore a risk of pesticide ingestion (Halm et al. 2006). Moreover, bees are often covered with pollen, and hivemates could be contaminated by topical contact (Bonmatin et al. 2005). In hives, bees and larvae feed on pollen and beebread. They could therefore be exposed directly to pesticides when they handle pollen or beebread, or indirectly when honey bees use the stocked pollen, especially during winter and spring (Bonmatin et al. 2005).

Since the use of chemicals for insect pest management in the 1940s and 1950s, high honey bee colony mortalities have been attributed to the application of insecticide to the maize crops. Indeed, Johansen and Brown (1972) detected carbaryl residues in maize pollen within hives that apparently led to high colony mortalities in the United States.

The systemic insecticide imidacloprid applied to maize and sunflower by seed coating has been thought to be responsible for honey bee colony winter losses by many beekeepers (Bruneau 2001, Haubruge et al. 2006, Olroyd 2007).

Nevertheless, several researchers show that chronic exposure to imidacloprid concentrations equivalent to those found in seed treatments pose negligible risks to

Table 2. Relationship between the mortality rates in an apiary[a] and the total or treated maize areas with imidacloprid as a function of the six radius circles around apiariesb (scenarii 1–6)

Scenario	Radius circle (m)	No. fields (area of maize in ha)			Parameter	Spearman's rank correlation (ddf = 14)	
		Treated [A]	Total [B]	Proportion [A]/[B]		Value (95% CI)	P
1	<1,000	40 (58)			Surface of maize fields treated around apiaries	−0.76 (−0.92 to −0.41)	0.0009*[c]
			188 (412)		Total surface of maize fields around apiaries	−0.47 (−0.79 to −0.05)	0.08
				0.213 (0.141)	Proportion treated maize surface total maize surface	−0.76 (−0.91 to −0.43)	0.0006*
2	<1,500	61 (98)			Surface of maize fields treated around apiaries	−0.73 (−0.93 to −0.47)	0.0013*
			344 (732)		Total surface of maize fields around apiaries	−0.50 (−0.81 to −0.01)	0.06*
				0.177 (0.134)	Proportion treated maize surface total maize surface	−0.73 (−0.90 to −0.37)	0.0013*
3	<1,750	70 (122)			Surface of maize fields treated around apiaries	−0.75 (−0.91 to −0.38)	0.0009*
			441 (936)		Total surface of maize fields around apiaries	−0.49 (−0.80 to −0.02)	0.052
				0.159 (0.130)	Proportion treated maize surface total maize surface	−0.74 (−0.91 to −0.39)	0.001*
4	<2,000	82 (129)			Surface of maize fields treated around apiaries	−0.75 (−0.91 to −0.38)	0.0009*
			555 (1134)		Total surface of maize fields around apiaries	−0.55 (−0.83 to −0.06)	0.03*
				0.148 (0.114)	Proportion treated maize surface total maize surface	−0.74 (−0.91 to −0.39)	0.001*
5	<2,500	109 (193)			Surface of maize fields treated around apiaries	−0.61 (−0.86 to −0.14)	0.0121*
			791 (1699)		Total surface of maize fields around apiaries	−0.53 (−0.82 to −0.02)	0.036*
				0.138 (0.114)	Proportion treated maize surface total maize surface	−0.56 (−0.83 to −0.09)	0.024*
6	<3,000	122 (226)			Surface of maize fields treated around apiaries	−0.57 (−0.84 to −0.08)	0.02*
			1027 (2235)		Total surface of maize fields around apiaries	−0.50 (−0.81 to −0.01)	0.046*
				0.119 (0.101)	Proportion treated maize surface total maize surface	−0.522 (−0.81 to −0.04)	0.038*

CI, confidence interval (for details, see Materials and Methods).
[a] For the mortality rates of each apiary, see the last column of the Table 1).
[b] Distance of pollen collection.
[c] Statistically significant at $P = 0.05$.

honey bees (Schmuck et al. 2001, Maus et al. 2003, Alix and Vergnet 2007). In laboratory studies, when individual honey bees are exposed to sublethal doses of imidacloprid (1–10 μg/kg), their performance in olfactory learning (Decourtye et al. 2003), associative learning and memory tests (Decourtye et al. 2004) are impaired.

In the field, honey bees may be at risk via contaminated pollen and nectar due to exposure to plants treated with the systemic insecticide imidacloprid (Rortais et al. 2005, Halm et al. 2006). However, when imidacloprid is fed to colonies in syrup or pollen at amounts likely to be found in the fields, development and survival of colonies are equivalent in treated and control colonies (Faucon et al. 2005). Schmidt (1996) showed that imidacloprid has no impact on sunflower visitation by honey bees. In a field survey initiated in 2002 in French apiaries, residues of imidacloprid were reported in 49% of pollen loads from traps, but no honey bee colony mortality was observed (Chauzat et al. 2006).

Despite these results, the French government suspended the Gaucho registration for sunflower in 1999 and for maize in 2004. In Belgium, there is no commercial sunflower production, so Belgian beekeepers have suspected imidacloprid maize treatments are responsible for losses of honey bee colonies. The typical mortality rate of honey bee colonies in Belgium is <10% (Haubruge et al. 2006). The rate was exceeded in 50% of the apiaries observed in this study; mortality ranged from 0 to 84.2% (Table 1). The analysis of our data confirms previous studies showing that in the southern part of Belgium the average is 14 colonies per apiary (Lefebvre and Bruneau 2003).

Maize provides bees only with pollen, and we confirmed the presence of maize pollen in bee hives. Chauzat et al. (2006) studied only the pesticide residues in pollen loads and found imidacloprid in analyzed samples but did not observe high honey bee mortality. We examined other matrices in the bee hive, and we conducted multiresidue method to

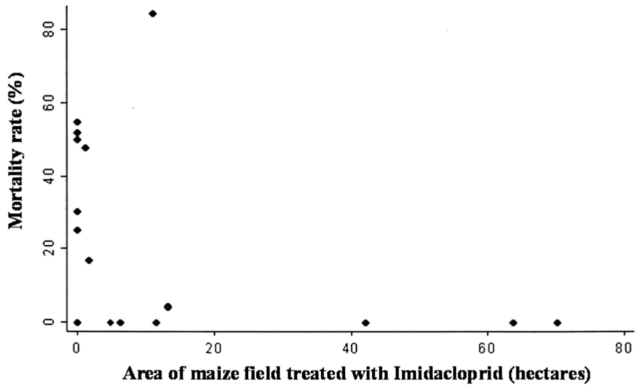

Fig. 3. Mortality rate in an apiary in function of the area of maize field treated with imidacloprid in a radius of 3,000 m (n = 16 apiaries).

analyze residues of pesticides in honey, bees, and wax (Pirard et al. 2007). Imidacloprid was detected in samples collected in two different apiaries, but none of these colonies died. Our honey residue data were between the limit of detection (0.05 ppb) and the limit of quantification (0.5 ppb). This result is closed to the results obtained by Suchail et al. (2001); in this study, mortality reaching 50% occurs after a chronic ingestion of imidacloprid at levels of 0.1 μg/kg.

Moreover, we showed an inverse relationship between mortality rate and the surface of imidacloprid-treated maize fields and the noninvolvement of the imidacloprid-treated maize in the mortality was confirmed by the inverse relationship between the mortality rate and the proportion of treated maize surface/total maize surface. We observed also an inverse correlation between the mortality rate and the total surface of maize around the studied apiaries. The lack of beneficial crop for the honey bees that flowered at the same time as maize may be responsible of this result. Agricultural crops such as maize, white and red clover (*Trifolium repens* L. and *T. pratense* L.), rape (*Brassica napus* L.), and sunflowers (*Helianthus* sp.) are known as pollen sources for honey bees (Keller et al. 2005). In Belgium, during maize flowering, pollen resources in the environment are low. Although the crude protein of maize is low (Roulston et al. 2000 cited by Chauzat and Pierre 2005), without other pollen sources in the environment, maize pollen is a stock of nutrients and amino acids that could allow the development and survival of honey bees.

The relationship between mortality rates and the number of colonies per apiary may be explained by the difficulty of managing many colonies or by the

Table 3. Pesticide residue concentrations in honey, bees and wax (n = 48 samples)

Matrix	Pesticide	No. samples		Residue concn		
		Positive	%	Min. (μg/kg)	Mean (μg/kg)	Max (μg/kg)
Honey	Rotenone	15	31.3	>LOD	15.2	69
	Flusilazole	7	14.6	>LOD	0.0275	<LOQ
	Methiocarb sulfoxide	6	12.5	>LOD	2.75	<LOQ
	Triflxystrobin	6	12.5	>LOD	0.275	<LOQ
	Imidacloprid	4	8.4	>LOD	0.275	<LOQ
	Coumaphos	3	6.3	128	576	800
	Bitertanol	1	2.1	0.12	0.12	0.12
	Bromopropylate	1	2.1	>LOD	27.5	<LOQ
	Carbufuran	1	2.1	0.6	0.6	0.6
Bees	Lindane	1	2.1	>LOD		
	Imidacloprid	0	0	0		
Wax	Flusilazole	15	31.3	>LOD		
	Bromopropylate	12	25.0	>LOD		
	Coumaphos	12	25.0	>LOD		
	Rotenone	10	20.9	>LOD		
	Tau-fluvalinate	6	12.5	>LOD		
	Trifloxystrobin	4	8.4	>LOD		
	Pirimicarb	2	4.2	>LOD		
	Lindane	2	4.2	>LOD		
	Bitertanol	2	4.2	>LOD		
	Atrazine	1	2.1	>LOD		
	Chlorpyrifos	1	2.1	>LOD		
	Imidacloprid	0	0	0		

LOD, limit of detection; LOQ, limit of quantification.

lack of nutrients available for a large number of colonies.

In conclusion, our study does not support the involvement of maize treated with imidacloprid in the observed mortality-related problems that affect honey bees in Belgium. However, nutritive scarcity in the environment must be further studied. The very large number of samples containing acaricides, especially ineffective (tau-fluvalinate) or prohibited (rotenone, bromopropylate) materials, to control *V. destructor* along with apicultural practices highlighted during beekeeper interviews suggest the inadequacy of the methods used for mite control. These parasites, along with the protozoan *Nosema ceranae* (Higes et al. 2006) and various viruses (Cox-Foster et al. 2007) have recently been identified as potential risk factors for honey bees mortality, which is described by many experts as multifactorial (Haubruge et al. 2006).

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References Cited

Alix, A., and C. Vergnet. 2007. Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds. Pest Manag. Sci. 63: 1069–1080.

Bonmatin, J. M., I. Moineau, R. Charvet, C. Fleche, M. E. Colin, and E. R. Bengsch. 2003. A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. Anal. Chem. 75: 2027–2033.

Bonmatin, J. M., P. A. Marchand, R. Charvet, I. Moineau, E. R. Bengsch, and M. E. Colin. 2005. Quantification of imidacloprid uptake in maize crops. J. Agric. Food Chem. 53: 5336–5341.

Bruneau, E. 2001. Printemps 2001, inquiétudes. Abeilles Cie 82: 6–7.

Chauzat, M. P., J. P. Faucon, A. C. Martel, J. Lachaize, N. Cougoule, and M. Aubert. 2006. A survey on pesticide residues in pollen loads collected by honey-bees (*Apis mellifera*) in France. J. Econ. Entomol. 99: 253–262.

Chauzat, M. P., and J. Pierre. 2005. L'importance du pollen pour l'abeille domestique: le pollen et ses composants. Bull. Tech. Apicole 32: 11–28.

Costanza, R., R. dArge, R. deGroot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. ONeill, J. Paruelo, et al. 1997. The value of the world's ecosystem services and natural capital. Nature (Lond.) 387: 253–260.

Cox-Foster, D. L., S. Conlan, E. C. Holmes, G. Palacios, J. D. Evans, N. A. Moran, P. L. Quan, T. Briese, M. Hornig, D. M. Geiser, et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science (Wash., D.C.) 318: 283–287.

Dagnelie, P. 1998. Statistique théorique et appliquée. Tome 2. Inférence statistique à une et à deux dimensions. De Boeck & Larcier, Paris, France.

Decourtye, A., E. Lacassie, and M. Pham-Delègue. 2003. Learning performance of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to season. Pest Manag. Sci. 59: 269–278.

Decourtye, A., J. Devillers, S. Cluzeau, M. Charreton, and M. Pham-Delègue. 2004. Effects of imidacloprid an deltamethrin on associative learning in honeybees under semifield and laboratory conditions. Ecotoxicol. Environ. Saf. 57: 410–419.

Doucet-Personemi, C., M. P. Halm, F. Touffet, A. Rortais, and G. Arnold. 2003. Imidacloprid utilisé en enrobage de semences (Gaucho) et troubles des abeilles–Rapport final. Comité scientifique et technique de l'étude multifactorielle des troubles des abeilles, Caen, France.

Faucon, J. P., L. Mathieu, M. Ribière, A.-C. Martel, P. Drajnudel, S. Zeggane, C. Aurières, and M.F.A. Aubert. 2002. Honey bee winter mortality in France in 1999 and 2000. Bee World 83: 14–23.

Faucon, J. P., C. Aurières, P. Drajnudel, L. Mathieu, M. Ribière, A. C. Martel, S. Zeggane, M. P. Chauzat, and MFA. Aubert. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. Pest Manag. Sci. 61: 111–125.

Free, J. B. 1993. Insect pollination of crops. Academic, London, United Kingdom.

Halm, M. P., A. Rortais, G. Arnold, J. N. Tasei, and S. Rault. 2006. New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho). Environ. Sci. Technol. 40: 2448–2454.

Haubruge, E., B. K. Nguyen, J. Widart, J.-P. Thomé, P. Fickers, and E. Depauw. 2006. Le dépérissement de l'abeille domestique, *Apis mellifera* L., 1758 (Hymenoptera: Apidae): faits et causes probables. Notes Fauniques Gembloux 59: 3–21.

Higes, M., R. Martin, and A. Meana. 2006. Nosema ceranae, a new microsporidian parasite in honeybees in Europe. J. Invertebr. Pathol. 92: 93–95.

Johansen, C. A., and C. Brown. 1972. Toxicity of carbaryl contaminated pollen collected by honey bees. Environ. Entomol. 3: 385–396.

Keller, I., P. Fluri, and A. Imdorf. 2005. Pollen nutrition and colony development in honey bees: part I. Bee World 86: 3–10.

Kevan, P. G. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agric. Ecosyst. Environ. 74: 373–393.

Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274: 303–313.

Lefebvre, M., and Bruneau E. 2003. Suivi sanitaire d'urgence de ruchers présentant des symptômes de dépérissement. Rapport final. Projet FF 02/15 (414), Fonds budgétaire des Matières premières, Bruxelles, Belgium.

Lefebvre, M., and E. Bruneau. 2005. Etat des lieux du phénomène de dépérissement des ruchers en Région wallonne. Rapport final, convention DGNRE/CARI, Namur, Belgium.

Maus, C., G. Cure, and R. Schmuck. 2003. Safety of imidacloprid seed dressing to honey bees: a comprehensive overview and compilation of the current state of knowledge. Bull. Insectol. 56: 51–57.

Michener, C. D. 2000. The bees of the world. John Hopkins University Press, Baltimore, MD.

Ministère-de-l'Agriculture-et-de-la-Pêche. 1999. Avis aux détenteurs et aux utilisateurs de semences de tournesol. J. Off. Repub. Fr. 38: 2413.

Ministère-de-l'Agriculture-et-de-la-Pêche. 2004. Avis aux détenteurs d'autorisation de mise sur le marché, aux distributeurs et aux utilisateurs du produit phytopharmaceutique Gaucho. J. Off. Repub. Fr. 126: 79.

Naunen, R., U. Ebbinghaus-Kintscher, and R. Schmuck. 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Manag. Sci. 57: 577–586.

Olroyd, B. P. 2007. What's killing American honey bees? PLoS 5(6): e168.

Otten, C. 2003. Daten und Fakten zu den Völkerverlusten. ADIZ 8: 6–8.

Pirard, C., J. Widart, B. K. Nguyen, C. Deleuze, L. Heudt, E. Haubruge, E. De Pauw and J.-F. Focant. 2007. Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid–liquid extraction and liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1152: 116–123.

Rortais, A., G. Arnold, M. P. Halm, and F. Touffet-Briens. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36: 71–83.

Roulston, T. H., J. H. Cane, S. L. Buchmann. 2000. What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? Ecol. Monogr. 70: 617–643.

Schmidt, H. W. 1996. The reaction of bees under influence of the insecticid imidacoprid. Appendice 12, Proceedings of the 6th International Symposium on Hazards of Pesticides to Bees, 17–18 September, Braunschweig, Germany. D. Brasse, Braunschweig, Germany.

Schmidt-Hempel, P. 1998. Parasites in Socials insects. Princeton University Press, Princeton, NJ.

Schmuck, R., R. Schoning, A. Stork, and O. Schramel. 2001. Risk posed to honeybees (*Apis mellifera* L., Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manag. Sci. 57: 225–238.

Steffan-Dewenter, I., and A. Kuhn. 2003. Honeybee foraging in differentially structured landscapes. Proc. R. Soc. Lond. B 270: 569–575.

Suchail, S., D. Guez, and L. Belzunces. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera.* Environ. Toxicol. Chem. 20: 2482–2486.

Vanengelsdorp, D., R. Underwood, D. Caron, and J. Hayes. 2007. An estimate of managed colony losses in the winter of 2006–2007: a report commissioned by the apiary inspectors of America. Am. Bee J. 147: 599–603.

Williams, I. H. 1996. Aspects of bee diversity and crop pollination in the European Union, pp. 63–80. *In* H. Williams [ed.], The conservation of bees. Linnean Society of London and the International Bee Research Association, Academic, London, United Kingdom.

Winston, O. E. 1972. Social homeostasis and the superorganism. In The insect societies. The Belkup Press of Harvard University, Cambridge, MA.

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