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Potential Exposure of Bees, *Apis mellifera* L., to Particulate Matter and Pesticides Derived from Seed Dressing During Maize Sowing

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Abstract This paper assessed the potential exposure of bees (*Apis mellifera* L.) to pesticides during maize (*Zea mays* L.) sowing with pneumatic drills. Data were derived from tests carried out in field tests, comparing two configurations of a pneumatic precision drill: conventional drill; drill with air deflectors. In addition, static tests simulating the sowing under controlled conditions, were performed on the drill equipped with an innovative system developed at CRA-ING. During the field tests, the concentrations in the air of the active ingredients of four insecticides used in maize seed dressing (imidacloprid, clothianidin, thiamethoxam and fipronil) were recorded. The concentrations of active ingredients in the air were used for assessing the quantities of active ingredients that a bee might intercept as it flies in a sort of virtual tunnel, the dimensions of which were dependent upon the bee body cross-section and the length of flight. The results of the field tests show that the air deflectors were not completely effective in reducing the amount of active ingredients dispersed in the air. The results of the static tests with drill equipped with the prototype indicated reductions of the active ingredient air concentrations ranging from 72 % up to 95 %, with reference to the conventional drill. Such ratios were applied to the amounts of active ingredients intercepted by the bees in the virtual tunnel contributing to a consistent reduction of the probability that sub-lethal effects can occur.

Keywords Pesticides · Neonicotinoids · Bee · Dust drift · Seed coating

The seed dressing (or coating) with neonicotinoid insecticides (Elbert et al. 2008) and fipronil is a pest control technique against a broad range of species harmful to maize (*Zea mays* L.), and it allows pest control with a reduced amount of insecticide in comparison with other pesticides requiring whole-soil or furrow applications.

The seed dressing with such active ingredients (a.i.), may lead to exposure of the bee (*Apis mellifera* L.) and other pollinating insects during sowing, due to losses of a.i. from the outflow air fan of the pneumatic sowing machines (Greatti et al. 2003, 2006; Tremolada et al. 2010). This may cause direct poisoning (Pistorius et al. 2009) or sub-lethal effects (Bortolotti et al. 2003; Colin et al. 2004; Bonmatin et al. 2005). In recent years, the pesticides employed in maize seed dressing have been claimed to play a role in bee decline (Kindemba 2009; Maini et al. 2010; Bortolotti et al. 2009a, b). Bees exposure can also occur through the consume of leaf guttation drops containing insecticides (Girolami et al. 2009), feeding activities and contact with aerosols containing insecticide residues during their flight. The direct exposure during flight is enhanced by the bee's anatomy, characterized by thick hairs on the body, that work as an efficient trap for airborne particulates (Prier et al. 2001; Tremolada et al. 2010).

According to Iwasa et al. (2004), the acute toxicity by contact (LD_{50} values) of some commonly used neonicotinoid insecticides were 18 ng bee^{-1} for imidacloprid, 22 ng for clothianidin and 30 ng for thiamethoxam. An LD_{50} value of 4 ng bee^{-1} was obtained for fipronil (Tingle et al. 2003).

Regarding sub-lethal effects the definition of threshold values is a complicated topic particularly in social insects

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(Desneux et al. 2007). It was reported that a low dose of fipronil (0.5 ng bee^{-1} applied topically) impaired the olfactory learning of bees (El Hassani et al. 2005). For fipronil, sub-lethal effects have been observed with application rates of 0.075 up to 0.15 ng bee^{-1} day^{-1}, respectively representing 1/80 and 1/40 of the LD_{50} according to Chauzat et al. (2006).

Bortolotti et al. (2003) investigated the sub-lethal effects caused by the ingestion of a solution with 100 ppb of imidacloprid. It was demonstrated that a single administration of clothianidin at a dose of 0.7 ng bee^{-1}, compromised the ability of the bees to return to the hive. At a dose of 0.47 ng bee^{-1}, the treated bees returned to the hive, but were unable to adequately perform the function of loot for several hours (Apenet 2011).

To date, there have been few field scale investigations to establish the potential contribution of sowing dust to the exposure risk for bees. This paper proposes a theoretical approach for determining the contact exposure level for a bee flying during the sowing of maize, based on air concentration data collected in the framework of the Apenet project (Apenet 2009; Biocca et al. 2009, 2011; Bortolotti et al. 2009a, b; Pochi et al. 2011a). The experiments included field tests and fixed point tests, using conventional pneumatic drills and modified drills equipped with common air deflector pipes. The obtained data were also compared to the potential drift reduction provided by an innovative prototype developed at CRA-ING observed in tests at fixed point. We calculated the potential exposure of bees to pesticides during maize sowing with pneumatic drills.

Materials and Methods

The trials were carried out using commercial maize seed (Pioneer Hybreed PR32G44) dressed with four insecticides (Gaucho™, a.i.: imidacloprid; Poncho™, a.i.: clothianidin; Cruiser™, a.i.: thiamethoxam, Regent™, a.i.: fipronil) and a fungicide (Celest™, a.i.: fludioxonil and metalaxyl). The fungicide was not considered in this study due to its high LD_{50} value for bees. According to the manufacturers, the quantities of a.i. were respectively equal to 1.000 mg seed^{-1} for imidacloprid, 1.250 mg seed^{-1} for clothianidin, 0.600 mg seed^{-1} for thiamethoxam and 0.500 mg seed^{-1} for fipronil. The seed was packed in sacks (25,000 seeds sack^{-1}). Samples of the seed used in the 2009 tests underwent chemical analysis that showed the correspondence between the detected a.i. amounts and the doses declared by the manufacturers (Apenet 2011).

The seed predisposition to produce abrasion powder was assessed by means of the standard Heubach test (JKI 2008) for both 2009 and 2010 seed lots. According to the Heubach test method, the reference limit of powder produced is 3 g (100 kg)$^{-1}$. With the purpose of observing the behaviour of the dressing treatment and its stability after the manipulations, the data declared by the manufacturer were compared with the data of the test repeated at CRA-ING after the delivery of the seed. Moreover, since the Heubach method measures only the quantity of fine powder, in order to assess its total amount, all the powder deposited in the vessels of the Heubach cylinder was collected and weighed, by means of a precision balance (KERN mod. ABJ120/4M, full scale range: 110 g; sensibility: 0.1 mg). In the hypothesis that the seed undergoes similar abrasion process during the Heubach tests and into the drill during the sowing, the a.i. powder contents (g (100 kg)$^{-1}$) and the a.i. dispersion per unit of sowed surface (g ha^{-1}) have been provided.

A six-row precision pneumatic drill "Gaspardo Magica" was employed, equipped with an air deflector system applied at the fan opening with the aim of directing the expelled air in the furrows opened for the seed deposition. The deflector system consists of a steel frame, applied at the fan opening, from which the air is directed into the furrows opened by the two central sowing units by means of four flexible plastic pipes. This device can be removed, restoring the "conventional drill" conditions and allowing the comparison between conventional and modified machine.

The tests compared the levels of active ingredients in the air determined by the use of the "conventional drill" (thesis A), of the drill with the air deflector system (thesis B) and of a new drill configuration in which the deflector system (thesis C) was implemented with a prototype, developed at CRA-ING, consisting of an innovative air-recycling/filtering system (Apenet 2011; Pochi et al. 2011b). The prototype works by partially re-circulating the air that operates the seed distribution, channeling it through the properly modified drill pneumatic system into the hoppers. In order to avoid that the air flow could affect the regularity of seed distribution, the air in excess is forced outward through an opening fitted with an activated charcoal filter for automotive use.

For all theses, the following adjustments were adopted: distance in the row of 0.75 m; seed distance on the row of 0.18 m; sowing density of 75,000 seeds ha^{-1}; vacuum pressure of 45 mbar. As to the working speed, the same value of 1.67 m s^{-1} was adopted both for field tests (real forward speed) and static tests (virtual forward speed).

The field tests, performed in 2010, regarded theses A and B.

The trials were carried out on the experimental farms of CRA-ING and CRA-PCM (around 42°5′51.26″N; 12°37′3.52″E; 24 m a.s.l.) from April to June, 2010. Rectangular plots (150 × 200 m) of about 3 ha were sowed. During the trials, the main micrometeorological parameters were monitored (Table 1). A single trial has been made for each thesis and for each a.i. Three air samplers were used to collect samples of the powder present in the air during the sowing. They were placed leeward, considering each time the prevailing wind direction, at 5, 10 and 20 m from the edge of the field. They were calibrated with a constant flow of 4.5 L min^{-1}. The time needed for the sowing was approximately 80 min. After the sowing, the samplers were maintained in use for an additional time of 20 min in order to allow the dispersion and the deposition of most of the dust, so a total volume of about 450 L of air was sampled.

In the static tests, theses A and C have been compared during 2011 activity. The tests were carried out in the workshop's porch of CRA-ING, according to the settings described in Biocca et al. (2011). The drill, suitably placed in the test area, operated the seed distribution "*sur place*" by means of a system allowing to adjust the peripheral speed of the drill's driving wheel to obtain the same working conditions of the field tests. In the test site artificial wind conditions were produced by means of an axial fan (0.735 m diameter) operating at a speed of 1,358 min^{-1}. The obtained average wind speed in the sampling area was 1.3 m s^{-1} measured at 2.0 m from the soil (min. 0.8, max. 2.0 m s^{-1}). With the aim of capturing the depositing dust and providing the a.i. concentration in the air a 22.5 m long sampling area, leeward with respect to the drill position, was identified. Along the sampling area, five air samplers were used. The five sampling distances from the drill side were multiples of its working width: 4.5, 9.0, 13.5, 18 and 22.5 m. The pumps of the air samplers were set for sucking 100 L of air with a constant flow of 5 L min^{-1}, in order to complete the air sampling 5 min after the end of the seed distribution. Each trial was replicated three times. Each replication consisted of the distribution of two sacks of seed, corresponding to a 0.67 ha surface. The seeds released by the drill were collected at ground in vessels shielded from the wind action.

To detect the a.i. air concentration, low volume portable samplers (TCR Tecora model "Bravo") were employed. The samplers were equipped with 0.45 μm PTFE Millipore diskette filters (diameter 47 mm), without any sampling head. The air sampling height was 2.0 m, for a better adherence to the bees' flying conditions.

The a.i. determination in the samples (seed and filters) was made at CRA-PAV. Active substances were extracted from the samples with acetonitrile. Solutions were sonicated in an ultrasonic bath for 10 min, then filtered with HPLC 0.45 μm filters. The analytical determinations were carried out by means of HPLC–ESI–MS–MS and relative methods were validated in compliance with GLP procedures. The instrumentation consisted of Waters Alliance 2695 Separation Module and 2695 Autosampler, Micromass 4 micro Triple Quadrupole Mass Spectrometer with Electrospray Ionisation (ESI) probe, Waters X-Terra MS column C18, 5 μm 150 × 4.6 mm, flow 0.3 mL min^{-1}, gradient elution with water (0.1 % acetic acid) and 10 % acetonitrile (0.1 % acetic acid) up to 90 %, in MRM mode. The mass spectrometer detector was tuned in the MRM mode at the maximum sensitivity for each of the parent ions m/z and polarity, and two product ion fragmentations for each were followed and detected.

Tremolada et al. (2010) suggested the following approach to assess the exposure of a bee to the a.i. present in the air, during flight. The volume of air intercepted can be calculated by multiplying the flight distance over the treated fields by the area of its front section (approximately 12.56 mm^2, deriving from the approximation of the bee body to a cylinder 4 mm of diameter). In the case of a flight 500 m long, the resulting volume of such a virtual tube, "$v.t._{(1)}$", is 0.0063 m^3. If the a.i. concentration (μg m^{-3}) is known, multiplying it by 0.0063 m^3 will provide the amount of a.i. intercepted by the insect. Such a method was applied by the authors to the case in which a bee flies across the sowed field, in the hypothesis of calm air and, consequently, of a quite constant concentration in the air.

Table 1 Micrometeorological conditions during the 2010 field tests

Active ingredient	Thesis	Date	Average air temp. (°C)	R. H. (%)	Atmospheric. pressure [mbar]	Average wind speed (m s^{-1})	Prevailing wind direction	Notes about wind
Imidacloprid	A	June 14	29.7	56	1,014	2.05	S, SW	Direction and speed enough stable
	B	June 9	27.9	63	1,012	1.47	E, N, W	Highly variable direction
Clothiadinin	A	April 20	21.7	75	1,015	1.6	NE	Direction and speed enough stable
	B	April 19	20.8	85	1,012	1.4	NW	Direction and speed enough stable
Thiamethoxam	A	April 21	21.4	65	1,017	2.24	S, SE	Direction and speed enough stable
	B	June 8	24.3	67	1,013	1.03	W	Direction enough stable
Fipronil	A	June 15	25.3	80	1,013	2.4	SW	Direction and speed stable
	B	June 16	28.4	60	1,013	1	SW	Direction and speed stable

A similar approach was adopted in another study on the absorption of bacterial spores on flying bees (Prier et al. 2001). In this case electrostatic charges (also incited by wings movements) were called into question and it was supposed the radius of this second virtual tube, "*v.t.*$_{(2)}$", was equal to 10 mm (length of a wing). The area of such a section resulted in 314.16 mm^2. Referring to a 500 m long flight, the air volume intercepted by a bee will be 0.157 m^3.

The method based on the two described virtual tunnels was applied to the sampling area contiguous to the sowed field and exposed to the abrasion dust drift. The behaviors of the a.i. concentrations in this area depend on the distance from the sowed field. For both theses A and B, a regression on the series of concentration values was calculated, in order to describe the trend of the concentrations for distances even greater than 20 m (maximum sampled distance) from the initial sowing line. For each test condition, the process provided an exponential equation and the relative coefficient R^2. Such regression functions have been used for integrating the a.i. concentrations (μg m^{-3}) at a distance of 50 m from the sowing area, obtaining, for each a.i., the amounts of a.i. contained in a third, virtual tube, "*v.t.*$_{(calc)}$" based on the described calculation procedure. The "*v.t.*$_{(calc)}$" is localized at the height of 2 m, having a section of 1 m^2, a length of 50 m and passing through the three air samplers, perpendicular to the leeward side of the sowed area. Basing on the a.i. amounts into "*v.t.*$_{(calc)}$", the a.i. theoretically intercepted by a bee that flies in the centre of "*v.t.*$_{(calc)}$", should be proportional to the bee sections defined for "*v.t.*$_{(1)}$", and "*v.t.*$_{(2)}$" and can be calculated multiplying the a.i. amounts in "*v.t.*$_{(calc)}$", respectively, by 12.56 mm^2 and by 314.16 mm^2. The obtained values can be multiplied by 10 (in the hypothesis of five 50 m return flights) in order to get the a.i. quantity intercepted along the 500 m distance proposed above, enabling comparison between the data.

Results and Discussion

Table 2 shows the results of the Heubach test declared by seed manufacturer and of the CRA-ING determinations on the seed after the delivery. Some differences can be noticed between the results from the two test conditions, but all values are well below the threshold of 3 g (100 kg)$^{-1}$ and testify the good quality and the stability of the seed dressing treatment, even after the manipulations occurring during transport and delivery.

As to the amounts of fine powder, it would be interesting and useful to verify any existing correspondence between the Heubach test conditions and the quantity of powder (and a.i.) actually dispersed by the drills during the sowing. For this purpose, in a previous study (Tremolada et al.

Table 2 Results of the Heubach tests reported by the seed manufacturer, compared with the data obtained at CRA-ING

Year	a.i. in seed dressing	Heubach test results		Coarse powder	Total powder	A.i. powder contents[a]	A.i. in the fine fraction[a]		A.i. in total powder[a]	
		Manufact. g (100 kg)$^{-1}$	CRA-ING g (100 kg)$^{-1}$	g (100 kg)$^{-1}$	g (100 kg)$^{-1}$	%	Mass g (100 kg)$^{-1}$	Surface mg ha^{-1}	Mass g (100 kg)$^{-1}$	Surface *mg ha*$^{-1}$
2009	Imidacloprid	0.96	1.67	15.00	16.66	31.1	0.52	124.4	5.18	1,243.8
	Clothianidin	1.77	2.17	33.34	35.50	33.0	0.72	171.6	11.72	2,811.8
	Thiamethoxam	1.33	2.50	16.67	19.17	33.5	0.84	201.0	6.42	1,541.0
	Fipronil	1.11	1.67	18.33	20.00	32.0	0.53	127.9	6.40	1,535.8
2010	Imidacloprid	1.10	0.88	10.83	11.71	31.1	0.27	65.3	3.64	874.1
	Clothianidin	2.43	1.83	19.16	20.99	33.0	0.60	145.2	6.93	1,662.5
	Thiamethoxam	1.20	0.95	5.00	5.95	33.5	0.32	76.4	1.99	478.3
	Fipronil	1.78	0.72	9.08	9.81	32.0	0.23	55.5	3.14	753.4

Here, the amounts of coarse powder deposited during the Heubach tests were also determined and used for calculating the corresponding a.i. quantities per 100 kg of seed and for hectare

[a] The a.i. contents has been analytically determined only in 2009 samples. The same a.i. percentage have been applied in 2010 samples to quantify the hypothetical a.i. amounts in the powder and per surface unit

2010) consisting of the sowing of a 7 ha field with maize dressed with thiamethoxam, it was hypothesized that the total amount of a.i. dispersed was 1 % of the a.i. globally employed in seed dressing, corresponding to 514.5 mg and to 73.5 mg ha^{-1}. The data reported in Table 2 for thiamethoxam (from 0.950 g $(100\ kg)^{-1}$ up to 2.500 g $(100\ kg)^{-1}$ of fine powder) show that applying the percentage values of a.i. content in the powder indicated in the same Table, the corresponding amounts of a.i. per surface unit, are, respectively, 76.4 and 201.0 mg ha^{-1}. Beyond the variability determined by the dressing treatment, the data for thiamethoxam appears of the same order of magnitude in the two cases.

Considering also the coarse fraction of the powder, the total powder amount (sum of fine fraction and coarse fraction) resulted about ten times higher than the fine fraction only. It is noteworthy that the coarse powder could contribute to the contamination of the areas surroundings the sowed field, with marked effects with increasing wind speed.

The results of air concentrations of a.i. sampled during the 2010 field tests are shown in Table 3.

The regression of the observed values provide exponential equations used for calculating, the quantity of each a.i., intercepted by a bee during the flight, according to the previously described method. The exposure levels calculated on the basis of the field test results, assuming that the insect repeats ten times a 50 m long flight in the area concerned by the drift, are shown in Table 4, where the a.i. contact LD_{50} values (Iwasa et al. 2004) are reported as well.

The concentrations do not seem to always follow a spatial gradient, probably because of the long persistence of the finest fraction of powder in the air. Nevertheless, a regression of the observed values was made in order to obtain equations that describe the pattern of the concentrations better than the means (Table 3). These equations were used for calculating the quantity of each a.i. intercepted by a bee during the flight, according to the previously described method.

Considering the "$v.t._{(1)}$", the calculated values are always lower than the contact LD_{50} but, in some cases, they reach levels that do not allow for the exclusion of the possibility of sub-lethal effects on the bees. As regards the "$v.t._{(2)}$", if the criterion of proportionality based on the area of the section is respected, the calculated values dramatically increase, far exceeding the LD_{50} for clothianidin and fipronil. The section of "$v.t._{(2)}$", in this case aims at defining the portion of space in which the wings' movements can affect the electrostatic charge of the particles suspended in the air and their tendency to adhere to bee's body and hairs. At the moment, no data are available about the effect of electrostatic charge on the a.i. behaviors. The adoption of the "$v.t._{(1)}$" seems more correct in the present study, even if the amounts obtained basing on it probably underestimate the actual quantity intercepted and retained by the bee, as electrostatic phenomena due to wings movement are not considered in their calculation.

Regarding the action of the air deflectors, in previous studies aimed at determining the a.i. depositions at ground ($\mu g\ m^{-2}$) by means of series of Petri dishes containing a water-acetonitrile solution, they appeared to be effective in the reduction of the a.i. deposition at ground level (Biocca et al. 2011; Pochi et al. 2011a, b). However, a similar response was not evident for the a.i. residues measured in the air. The air deflectors probably work better with the coarse fraction of powder, as the fine fraction escapes their action and spreads all around due to the presence of wind. This effect is expected to be amplified by increasing wind speed.

Among the data reported in Table 4, the "$v.t._{(1)}$" values for thiamethoxam were 0.16 ng bee^{-1} (thesis A) and 0.14 ng bee^{-1} (thesis B), corresponding to approximately 2 % of the 9.2 ng bee^{-1} concentration intercepted by a bee when flying over a field sown with thiamethoxam-treated seed, as proposed by Tremolada et al. (2010). The differences depend on several factors such as the a.i. application rates, the adopted seed dressing processes and, mainly, the environmental conditions. In fact, the cited study referred to calm air conditions with bees flying into the sowing area; in the present paper there was wind and the insects were

Table 3 Air concentrations ($\mu g\ m^{-3}$) values during 2010 field tests and related regression equations, using the conventional drill (thesis A) and the drill with deflectors (thesis B)

Distance (m)	Clothianidin		Fipronil		Imidacloprid		Thiametoxan	
	Th. A	Th. B	Th. A	Th. B	Th. A	Th. B	Th. A	Th. B
5	0.5455	0.4091	0.0027	0.0182	0.0614	0.0614	0.0239	0.0364
10	0.1364	0.2500	0.0023	0.0227	0.0273	0.0455	0.0227	0.0341
20	0.2273	0.2273	0.0030	0.0409	0.0159	0.0341	0.0227	0.0227
Average	0.3030	0.2955	0.0027	0.0273	0.0348	0.0470	0.0231	0.0311
Regression	$y = 7.57x^{-0.42}$ $R^2 = 0.86$	$y = 10.98x^{-0.63}$ $R^2 = 0.38$	$y = 0.07x^{0.58}$ $R^2 = 0.94$	$y = 0.02x^{0.06}$ $R^2 = 0.09$	$y = 1.21x^{-0.42}$ $R^2 = 1.0$	$y = 2.81x^{-0.97}$ $R^2 = 0.98$	$y = 0.66x^{-0.34}$ $R^2 = 0.85$	$y = 0.25x^{-0.035}$ $R^2 = 0.75$

Table 4 Levels of exposure assessed on the basis of the field test results, assuming that the bee repeats ten times a 50 m long flight in the area leeward the sowed field and perpendicular to its side

	LD 50 contact[a]	u.m. ng bee^{-1}	Imidacloprid 18.00	Clothiadinin 22.00	Thiamethoxam 30.00	Fipronil 4.00
Thesis A (conventional drill)	a.i. in $v.t._{(calc)}$; length: 50 m	μg m^{-2} (50 m)$^{-1}$	2.07	12.49	1.33	2.07
	a.i. per bee ref. 500 m[b]	ng bee^{-1}	0.24	1.02	0.16	0.25
	a.i. per bee ref. 500 m[c]	ng bee^{-1}	6.00	25.6	4.0	6.25
Thesis B (drill with air deflectors)	a.i. in $v.t._{(calc)}$; length: 50 m	μg m^{-2} (50 m)$^{-1}$	2.93	12.37	1.13	0.14
	a.i. per bee ref. 500 m[b]	ng bee^{-1}	0.35	1.48	0.14	0.02
	a.i. per bee ref. 500 m[c]	ng bee^{-1}	8.81	37.13	3.40	0.41

[a] Iwasa et al. (2004); [b] 10 flights of 50 m into $v.t._{(1)}$; [c] 10 flights of 50 m into $v.t._{(2)}$

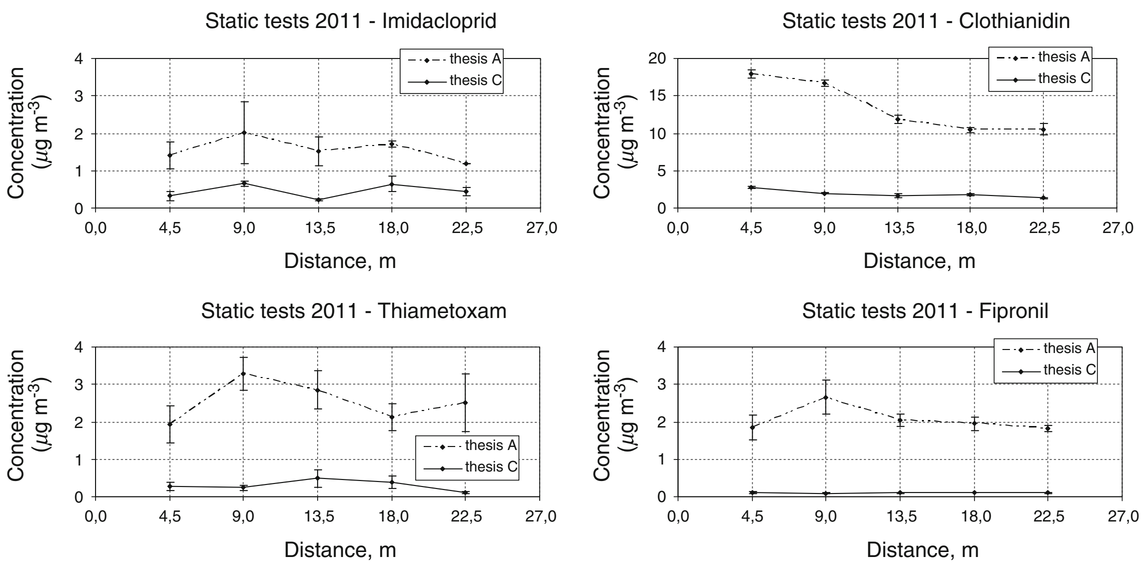

Fig. 1 Pattern of the concentrations in the air (μg m^{-3} ± SE) in the static tests for the four active ingredients

supposed flying in the leeward area that was subject to the drift and to the dispersion operated by the wind itself at the same time.

The results of the static tests are shown in the Fig. 1. For each sampling distance, the diagrams report the average values obtained from three repetitions with their standard errors. The reduction of the concentrations is evident for all active ingredients.

The series of data underwent exponential regression providing the functions reported in Table 5. Integrating them for a given distance, as previously described, provided the amount of a.i. in a virtual tube similar to $v.t._{(calc)}$. In Table 5, a 50 m distance has been considered and the amounts of the four active ingredients in a $v.t._{(calc)}$ 50 m long (μg m^{-2}(50 m)$^{-1}$) are shown, together with the percentage reduction determined by the use of the CRA-ING prototype (thesis C).

A comparison of thesis A values in Table 5 with field test results in Table 4 clearly shows that the former are considerably higher than the latter, as might be expected, since the static tests were performed on a small surface area that received a quantity of powder which would have been distributed over a large area in an open field. The usefulness of the virtual tube in the static test lies in the possibility of reliably assessing the a.i amounts and the reductions resulting from the use of the prototype.

Lastly the a.i. amounts intercepted by a bee flying for 500 m in $v.t._{(1)}$ and $v.t._{(2)}$ (Table 4) in the thesis A have been multiplied by the percent reduction values reported in the Table 5, provide a prediction of the amounts theoretically intercepted in the field in the presence of the CRA-ING prototype (theoretical thesis C). The results of such calculation are presented in Table 6. The values obtained for the $v.t._{(1)}$ are well below both the test field results of air deflectors (thesis B, Table 4) and the LD_{50}, indicating that the use of the prototype would effectively reduce the probability of occurrence of sub-lethal effects on the bees.

In conclusion, the Heubach test carried out on the seed confirmed the high quality of the dressing treatment and its stability, with values of the fine powder always below the limit

Table 5 The exponential functions used for integrating the concentrations on the 0–50 m distance

a.i.	Thesis A			Thesis C			Reduction
	Equation	R^2	a.i. in *v.t.*[a]	Equation	R^2	a.i. in *v.t.*[a]	%
Imidacloprid	$y = 1.87e^{-0.014x}$	0.24	67.0	$y = 0.36e^{0.0133x}$	0.04	25.7	61.7
Clothiadinin	$y = 20.81e^{-0.0341x}$	0.88	499.1	$y = 2.95e^{-0.0353x}$	0.84	69.3	86.1
Thiamethoxam	$y = 12.42e^{0.0023x}$	0.01	104.7	$y = 0.39e^{-0.0281x}$	0.13	8.6	91.7
Fipronil	$y = 3.09e^{-0.0179x}$	0.47	102.0	$y = 0.13e^{-0.0126x}$	0.40	4.7	95.3

[a] a.i. in the virtual tube with 1 m^2 section and 50 m long (from 0 m to 50 m), µg $m^{-2}(50\ m)^{-1}$

Table 6 A.i. amounts intercepted by the bees in a theoretical thesis C in field

	u.m.	Imidacloprid	Clothiadinin	Thiamethoxam	Fipronil
DL_{50} contact[a]	ng bee^{-1}	18	22	30	4
A.i. per bee ref. 500 m[b]	ng bee^{-1}	0.09	0.14	0.01	0.01
A.i. per bee ref. 500 m[c]	ng bee^{-1}	2.28	3.56	0.33	0.29

The values were obtained by applying the reductions indicated in Table 5 to the amounts reported in Table 4 for thesis A

[a] Iwasa et al. (2004); [b] 10 flights of 50 m into $v.t._{(1)}$; [c] 10 flights of 50 m into $v.t._{(2)}$

of 3 g $(100\ kg)^{-1}$. However in this work also the coarse fraction has been determined, revealing that it represents about 90 % of the total powder produced under test conditions. The coarse fraction could be responsible for ground and air contamination, in the sowing field and in the surrounding areas, in different manners, depending on the environmental conditions, mostly on wind speed and direction.

The effectiveness of drill modifications must be evaluated with the purpose of assessing the possible risks for the bees represented by residual powder dispersion. Such indications can be obtained both in field tests and static test. In the former, the test conditions coincide with real sowing and the results could represent a benchmark. Nevertheless, their reliability is reduced by the impossibility of controlling the test conditions variability. The static tests offer the reproducibility of the test conditions and the possibility of reliably assessing powder emissions and the reductions provided by different drill configurations.

Combining field and static tests experiences, it is possible to use the a.i. air concentrations of a defined area for assessing the a.i. amounts intercept by a bee flying in the same area, comparing its flight to a virtual tunnel dug in the air. The virtual tunnel section can be represented by the section of the insect body or by a larger section having a radius equal to the length of the wings, the movement of which is supposed to induce electrostatic charges in the powder. If the presence of electrostatic effects is confirmed in the future for the seed dressing powder, the data obtained by adopting only the bee body cross-section will underestimate the a.i. amounts to which the bee is exposed. At the moment, no data are available on the a.i. quantity actually retained by the bees during the flight, with reference to the a.i. concentration in the air.

The method based on the virtual tunnel provided the amounts (ng bee^{-1}) intercepted during the sowing under different drill configurations and gave the possibility to compare them with reference values such as the LD_{50}. The a.i. amounts retained by the bee, obtained for the conventional drill and for the drill with air deflector were lower than the LD_{50}, but not enough to exclude in some cases the possibility of sub-lethal effects. It is important to notice that these values, like the a.i. air concentrations from which they originate, do not seem to significantly differ in the two drill configurations. This probably means that the air deflectors, which in other studies have resulted in reductions of the air concentrations at ground level from 30 % to 70 % (Biocca et al. 2011; Pochi et al. 2011a, b), are not effective towards the a.i. fraction dispersed in the air at 2 m above ground level, probably represented by the fine fraction detected in the Heubach test.

The application to the drill of the prototype proposed by CRA-ING determined substantial reductions of the a.i. concentrations in the air (ranging from 62 % to 95 %), in comparison with the conventional drill. This clearly resulted from static tests under controlled conditions. Such reductions have been applied to the field values obtained for the conventional drill, in order to assess the theoretical in field effect of the prototype on the reduction of the a.i. intercepted by the bees. With particular reference to the virtual tunnel based on the bee body section, the results seem encouraging. The lower air concentrations of active ingredients with this prototype system were 0.5, 0.6, 0.04 and 0.3 % of the LD_{50} values for imidacloprid, clothianidin, thiamethoxam and fipronil respectively, suggesting a consistent decrease in the probability of the occurrence of sub-lethal effects. However, this probability cannot be excluded, due to the high sensitivity of bees to these insecticides. Further reductions of the a.i. intercepted by the bees will be possible by improving the efficiency of this prototype. For this purpose, several technical solutions are being studied.

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