Bulletin of Insectology **65** (2): 273-280, 2012
ISSN 1721-8861

Effects of neonicotinoid dust from maize seed-dressing on honey bees

Fabio Sgolastra[1], Teresa Renzi[1], Stefano Draghetti[1], Piotr Medrzycki[2], Marco Lodesani[2], Stefano Maini[1], Claudio Porrini[1]
[1]*Dipartimento di Scienze Agrarie - Entomologia, Università di Bologna, Italy*
[2]*Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di Ricerca in Apicoltura e Bachicoltura, Bologna, Italy*

Abstract

In Northern Italy from 2000 to 2008, many spring bee mortalities were clearly linked to sowing of maize seeds dressed with insecticides. In the present study, we investigated the effects on honey bees of clothianidin derived from maize seed-dressing (Poncho®) in laboratory (test by indirect contact) and in semi-field conditions. Despite the reduction of dust dispersion due to the application of the best available sowing techniques (pneumatic seeder equipped with deflector, improvement of seed-dressing quality) our results showed negative effects on honey bees at individual level. In semi-field study, no effect was observed at the colony level despite the high bee mortality rate for 2-3 days after dust application. However, we can expect a colony decline and low honey production if this high forager mortality rate lasts for longer than 10 days. Such a situation is possible if the sowing period lasts several days as in the Po Valley, where the landscape is characterized by extended maize cultivation.

Specific methodologies to assess the effects of dust have never been included in the official guidelines for the evaluation of side-effects of plant protection products on honey bees. For this reason, suitable and standardized methods for testing in laboratory and in semi-field conditions the effects on honey bees of contaminated dust dispersed during sowing were evaluated.

Key words: *Apis mellifera*, ecotoxicology, clothianidin, bee mortality, colony losses, dust, maize seed-dressing.

Introduction

In the last years, bee and colony losses have been reported in numerous countries worldwide and many factors, acting singularly or simultaneously, were taken into account to explain these phenomena (Neumann and Carrek, 2010; Alaux *et al.*, 2010; Pettis *et al.*, 2012). Factors contributing to the bee decline include: viruses (Berthoud *et al.*, 2010; Martin *et al.*, 2010; 2012; Nazzi *et al.*, 2012); *Nosema ceranae* (Microspora Nosematidae) (Higes *et al.*, 2007; Paxton, 2010; Santrac *et al.*, 2010); *Varroa destructor* Anderson et Trueman (Dahle, 2010; Martin *et al.*, 2010; 2012; Nazzi *et al.*, 2012); agrochemicals (Maini *et al.*, 2010; Chauzat *et al.*, 2010; Medrzycki *et al.*, 2010; Mullin *et al.*, 2010; Lu *et al.*, 2012); acaricides (Harz *et al.*, 2010); loss of genetic diversity (Meixner *et al.*, 2010) and habitat loss and fragmentation (Potts *et al.*, 2010). Many scientists agree that bee decline is a multifactorial process in which a particular mechanism seems to be more important in a given period of the year than in another, and different mechanism may predominate in another period or in other environmental conditions. For these reasons, a time-space differentiation of bee mortality factors needs to be considered (Maini *et al.*, 2010). In Italy, the bee mortality follows a clear seasonal pattern: a) during spring and summer colonies loose many foragers due to agrochemicals (bee losses); b) from late summer to winter, the impact of pests and pathogens becomes more important (colony losses). In Northern Italy from 2000 to 2008, many spring bee mortalities were clearly linked to sowing of maize seeds dressed with insecticides (Bortolotti *et al.*, 2009). In 2008, over 700 beekeepers with around 12,000 hives in the Rhine Valley, Germany, were affected by contaminated dust during sowing of maize and similar incidents were observed also in France, Slovenia and US (Pistorius *et al.*, 2009; Alix *et al.*, 2009; Krupke *et al.*, 2012). Greatti *et al.* (2003; 2006) showed that pesticides used in maize seed coating may be dispersed as dust from the pneumatic drilling machine and drift to surrounding areas. Subsequently bees may enter in contact with these contaminated dusts in several ways. The first way of exposure occurs during sowing when the bees are flying over the maize field to reach a foraging site and all around when dusts are dispersed by wind. In this case, bees enter in direct contact with the dusts dispersed into the air from the pneumatic machine (Marzaro *et al.*, 2011; Girolami *et al.*, 2011). Another way of exposure occurs within few days after sowing operation when forager bees collect pollen, nectar or dew from the vegetation surrounding the sown field (Greatti *et al.*, 2003; 2006). In this case, bees are exposed both by ingestion (pollen, nectar and dew) and by indirect contact (walking on contaminated vegetation). In Italy, the high bee mortality during the sowing of coated seeds resulted in the suspension of use of three neonicotinoids (imidacloprid, clothianidin, thiamethoxam) and one fenylpirazol (fipronil) for seed coating (Ministerial Decree 17/09/2008). At the same time a research project "ApeNet monitoring and research in apiculture" was financed in order to establish the causes of bee mortality (external and internal to the hive) and the possible ways of mitigation. In particular, a specific objective within ApeNet project was to investigate whether the application of the best available sowing techniques (pneumatic seeder equipped with deflector, improvement of seed-dressing quality) can reduce the dust dispersion below a negligible effect to bees.

The pesticides used for maize seed-dressing (clothianidin, imidacloprid, thiametoxam and fipronil) are extremely toxic for bees with lethal and sublehtal effects even at very low doses. Effects on orientation and foraging activity were observed in foraging bees fed *ad libitum* with 50-100 ppb of imidacloprid (Bortolotti *et al.*, 2003; Yang *et al.*, 2008), 1.34 ng/bee of thiametoxam (Henry *et al.*, 2012) and 0.3 ng/bee of fipronil (Decourtye *et al.*, 2011). In laboratory conditions, bees fed with low concentrations (100-500 ppb) of imidacloprid showed a reduction in the activity (Medrzycki *et al.*, 2003) and in olfactory learning performances (with 12 ng/bee) (Decourtye *et al.*, 2004). Similar effects on learning performance were observed in honey bees exposed by contact at low doses (0.5 ng/bee) of fipronil (Bernadou *et al.*, 2009). In the ApeNet project, the amount of active ingredients (a.i.) deposited on the ground during sowing at 5, 10, 20 m distances from the field edge was measured and a decline in pesticide concentration was observed as distance increased (ApeNet, 2009; 2010). However, it was shown that during the maize sowing operation bees can be exposed to variable pesticide contamination levels. This exposure depends on many factors, as: way of contact with the a.i., time from the sowing operation, size of the sown area, quality and quantity of vegetation in the margin of the field, meteorological conditions, and of course seed-dressing quality and the application of deflector in the pneumatic seeder.

In the present study, within the framework of the ApeNet project, we investigated the effects on honey bees of clothianidin derived from maize seed-dressing (Poncho®). The study was carried out in laboratory (test by indirect contact) and in semi-field conditions. We decided to consider not the a.i. but the commercial compound, in order to simulate field conditions. Thus in our trials we applied the contaminated dust extracted by abrasion from dressed maize seeds.

We address the following questions: 1) Is the amount of contaminated dust dispersed at 5 meters from a maize field harmful for forager bees? 2) Is the dust containing Poncho® more toxic than the liquid formulation of the same active substance (Dantop®)? 3) Can the contaminated dust affect the colony at medium and long terms, including its sociophysiological parameters?

Despite the recent implication of contaminated dust in bee mortality phenomena in several countries around the world, no particular indication on how to assess the effects of dust to bees is taken into account in the official guidelines (OEPP/EPPO, 2010; OECD, 1998a; 1998b; 2007). For this reason, the aim of this study was also to develop suitable and standardized methods for testing in laboratory and in semi-field conditions the effects on honey bees of contaminated dust dispersed during sowing.

Materials and methods

Contaminated dust was extracted from maize seed dressed with Poncho® using Heubach cylinder, the dust was sieved and the fraction <45 µm was used. The choice of the particle dimension was made in order to reflect field conditions where the major part of the dispersed particles during sowing operation was smaller than 45 µm (ApeNet, 2011). The dust was analyzed to assess the percentage of clothianidin and the tested dose (5.12 $\mu g/m^2$) was chosen based on the previous results of field studies (ApeNet, 2010). In fact, this quantity reflects the amount deposited on the ground at 5 m distance from the edge of the field during maize sowing using a Gaspardo Magica six row-precision pneumatic seeder (75,000 seeds/ha) with dual pipe deflector. The seeds (Hybrid employed PR32G44; Pioneer Hi-Bred) were supplied in 2010 by the Italian Seed Association and the quantity of dust abrasion resulted below 2 g/100 kg of seeds. Contaminated dust was mixed with an appropriate quantity of talc (used as a dispersing agent) in order to reach the desired concentration. Four samples of the talc-Poncho® mixture used for the treatments, were analysed to assess the real concentration of active ingredient. The same concentration of a.i. (5.12 $\mu g/m^2$) was used in laboratory and semi-field study. We chose talc as dispersing agent because it is a common mineral material, not toxic to bees and it is usually added to seed boxes to reduce friction and stickiness and ensure smooth flow of seeds during planting. In a recent study it was shown that waste talc expelled during and after sowing represents another route of pesticide exposure for bees (Krupke *et al.*, 2012).

Laboratory study

The indirect contact toxicity of dust contaminated by the clothianidin-based product Poncho® was compared, in laboratory conditions, to that of spray formulation of the same active substance (Dantop®) and at the same dose. In both treatments, forager bees (10 bees per cage) were exposed to clothianidin by walking for 3 h on treated apple leaves, placed on the bottom of plexiglass hoarding cage (13 × 6 × 11 cm - surface of contaminate area = 57.2 cm^2). The exposure time of 3 hours was chosen in according to the protocol developed by Arzone and Vidano (1980). Bees were kept in darkness at 25 °C during the test. For the liquid formulation, the leaves were sprayed with 200 µl of test solution (water only in the control) and for the dust treatment, 0.01 g of Poncho® dust mixed with talc was applied (talc only in the control). The forager bees were transferred onto treated apple leaves immediately after treatment application or soon as the spray had dried (for the liquid formulation). During the trial, bees were fed with 50% (w/w) sugar solution. Five groups of 10 bees were used for each treatment. Mortality data was corrected for control mortality with Schneider-Orelli's formula and the effects of dust and liquid formulation were compared using Student t-test for each assessment hour. Before processing the mortality rate was arcsine transformed to normalize the data.

Semi-field study

In 2010, a semi-field cage test was conducted following the EPPO 1/170 (4) guidelines (OEPP/EPPO, 2010) adapted to seed treatment. The study was carried out in an oilseed rape (*Brassica napus* L.) field of 2000 m^2 in the Experimental Farm of the University of Bologna. Six cages (three for each treatment) of 40 m^2 each covered with white anti-aphid net were set up before oilseed rape blooming. On May 31st, with 50% blooming, in each cage, one nuke containing a healthy queen dated 2009 and bees arranged in three frames (about 5000 adult bees, two frames containing all brood stages and one with 20-25% of nectar and pollen stores) was introduced. All nukes were prepared at the same time with sister queens to guarantee uniform bee colonies. A trap for dead bee collection (type "underbasket") was placed in front each nuke.

The treatment was applied on June 7th at noon, when the crop was in full flowering and the bees were actively foraging. In each cage, 200 g of talc (pure in the control cages and containing 204.77 µg of clothianidin in the treated cages) was distributed uniformly with a mechanic pulverizer (Cifarelli® M3; Dusts-out: 0-6 Kg/min; Speed air: 125 m/sec; Volume air: 20 m^3/min). The dose of clothianidin was calculated in order to assure the same concentration (per m^2 of soil) as that applied in the laboratory study.

During the semi-field test, the following parameters were assessed:

1 - Daily mortality: the daily number of dead bees in "underbasket" traps;
2 - Strength of the colony: the number of adult bees and the brood extension assessed with the Liebefeld method (Imdorf *et al.*, 1987);
3 - Flight activity: the number of bees exiting the nuke in 30";
4 - Foraging activity: the instant number of bees in three fixed plots of 0.25 m^2 each (total surface = 0.75 m^2).
5 - Foraging behaviour: the abnormal behaviour of the bees in each plot was recorded using a standardised approach by Giffard and Mamet (2009). The abnormal foraging behaviour was classified in three groups related to increasing levels of intoxication: a) motionless bees on plants, b) bees in cleaning activity, c) hanging-knocked out bees;
6 - Bee behaviour in front of the nuke;
7 - Socio-physiological status of the colony: a) thermoregulation capacity - temperature inside the nuke (between the two brood frames) was recorded by data logger iButton DS1923; b) Comb construction capacity - an empty frame was introduced in the nuke the day of treatment and the percentage of frame surface covered by built comb was subsequently measured. Both the thermoregulation and the comb construction capacities are considered two important physiological parameters to assess the vitality of a colony (Tautz, 2008).

Mortality and behavioural assessments were conducted before and at several moments after treatment: on days –3, –1, 0, 1, 2, 3, 5 and 7. Foraging and flight activities were assessed every two hours in the middle part of the daytime (10.00-12.00-14.00-16.00) except for day –3 when the data was collected only in the afternoon. The strength of the colonies was assessed once before (on day –4) and 7 and 15 days after the treatment.

The comb construction capacity was recorded 7 and 15 days after treatment. The internal temperature was recorded constantly from the day –3 till the day 5.

After 8 days from the treatment, the screening net was removed in order to allow the free foraging activity of bees. On June 30th the colonies were moved to another site, about 6 Km away from the experimental field. On July 15th other two frames were added in each nuke and in mid summer (August) and before wintering, anti-varroa treatments were applied, respectively with Api-Var® (a.i. Amitraz) stripes and with oxalic acid. The colony strength was assessed every two months until wintering and once after wintering (February 2011) in order to assess potential delayed effects.

We used repeated-measures ANOVA to analyse differences in daily bee mortality and colony strength between treatments and among the different observation days. To address normality and homoscedasticity, the daily mortality values were log(x+1)-transformed. The percentage (arcsine-transformed) of built comb in the two treatments was compared using t-test. The differences between treatments and days of the mean daily in-hive temperature were analysed with repeated-measures ANOVA. The flight activity and foraging activity were compared between the two treatments, separately before and after application, with Wilcoxon test. Since the current guideline (OEPP/EPPO, 2010) gives no particular indication about the method of elaboration and interpretation of semi-field and field data, in order to evaluate the level of bee mortality, we used the index proposed by Schmidt *et al.* (2003). This index is based on the ratio of daily bee mortality between and after treatment calculated for the treated colonies and divided by the same ratio calculated for the control colonies. Thus we obtain the deviation of the mortality in the treated-cages from the control-cages.

Results

The analysis of the four samples of talc mixed with contaminated dust showed an a.i. concentration 10.0% ± 4.7 lower than the estimated values.

Laboratory study

Despite the real a.i. concentration in dust was slightly lower than the expected one, no significant differences were found in the indirect toxicity test between the liquid and the dust formulation. Our laboratory results showed that, up to 24th hour, mortality induced by the two products was comparable and below 15%. During the subsequent hours, the number of dead bees increased similarly in both treatments (figure 1).

Semi-field study

We found no significant differences in bee mortality between treated- and control-cages ($F = 0.95$; $df = 1, 4$; $p = 0.38$) and among the days of the trial ($F = 1.99$; $df = 4, 24$; $p = 0.11$). However, we found a significant interaction between the two factors ($F = 4.10$; $df = 4, 24$; $p = 0.006$). In the treated-cages, the daily bee mortality increased in the first 2-3 days after the dust application,

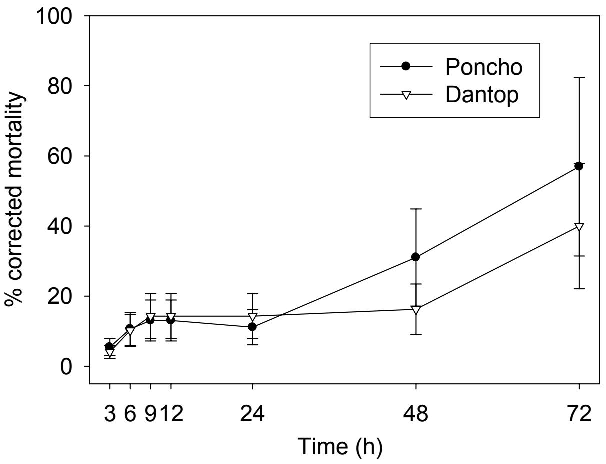

Figure 1. Corrected bee mortality (± SE) in dust (Poncho®) and liquid (Dantop®) formulation treatments (No statistical differences (p > 0.05) were observed between treatments).

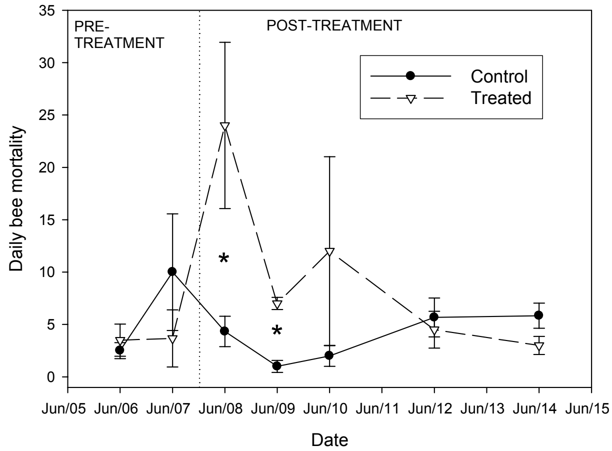

Figure 2. Mean ± SE daily bee mortality in control and treated cages. * Statistically significant differences between control and treated within the same day (p < 0.05).

whereas it was stable in the controls. Bee mortality in treated cages was significantly higher than in control cages the first two days. A similar trend was observed also after 3 days, but differences failed significance (figure 2). The index proposed by Schmidt *et al.* (2003) was calculated based on mortality data collected between day –3 and day 5. The relative bee mortality was then ~10 times higher in treated than in control cages (table 1). The colony strength (number of adult bees and brood) significantly changed during the trial but with similar trend in both treatments (table 2). The number of adult bees and brood cells decreased after 7 days from treatment due to the confined condition, but then rapidly increased during summer. Later, at the beginning of wintering the brood decreased as the mean environmental temperatures dropped to 10 °C. In February 2011, treated and control colonies showed adequate number of adult bees and brood to assure good colony growth during spring (figure 3). In April, all the colonies were transferred from the nukes to the 10-frames hives.

The comb constructions started in all colonies after 7 days from the treatment and after 15 days the percentage of comb constructed was similar between treatments (control: 20.6 ± 2.4%; treated: 22.2 ± 14.7%) ($t = 0.29$; $p = 0.78$).

The mean in-hive temperature was 35.3 ± 0.1 and 35.0 ± 0.1 °C in control and treated-cages respectively, thus we conclude that the thermoregulation capacity was not affected by treatment ($F = 0.69$; df = 1, 4; $p = 0.45$). In both treatments, the temperature decreased and showed large fluctuations during the confinement period whereas it became stable after the removing of the screening net ($F = 6.20$; df = 17, 68; $p < 0.001$). The treatment-days interaction was not significant ($F = 0.85$; df = 17, 68; $p = 0.63$).

Table 1. Bee mortality before and after treatment, comparison of daily bee mortality in treated and control colonies using the Index proposed by Schmidt *et al.* (2003).

# Colony	Treatment	Mean daily bee mortality before treatment (3 days)	Mean daily bee mortality after treatment (5 days)	Ratio post-treatment/ pre-treatment
1	Control	9.0	4.8	
3	Control	3.3	1.8	0.9
5	Control	2.7	4.6	
2	Treated	0.3	7.8	
4	Treated	6.7	6.4	9.7
6	Treated	3.7	17.0	
Index treated/control				10.7

Table 2. Repeated measures ANOVA test for colony strength. *Statistically significant differences (p < 0.05).

Effect	Adult bees			Brood		
	F	df	p	F	df	P
Treatment	0.23	1, 4	0.66	0.09	1, 4	0.78
Days	7.85	6, 24	< 0.01 *	107.26	6, 24	< 0.01 *
Interactions	0.76	6, 24	0.61	1.12	6, 24	0.35

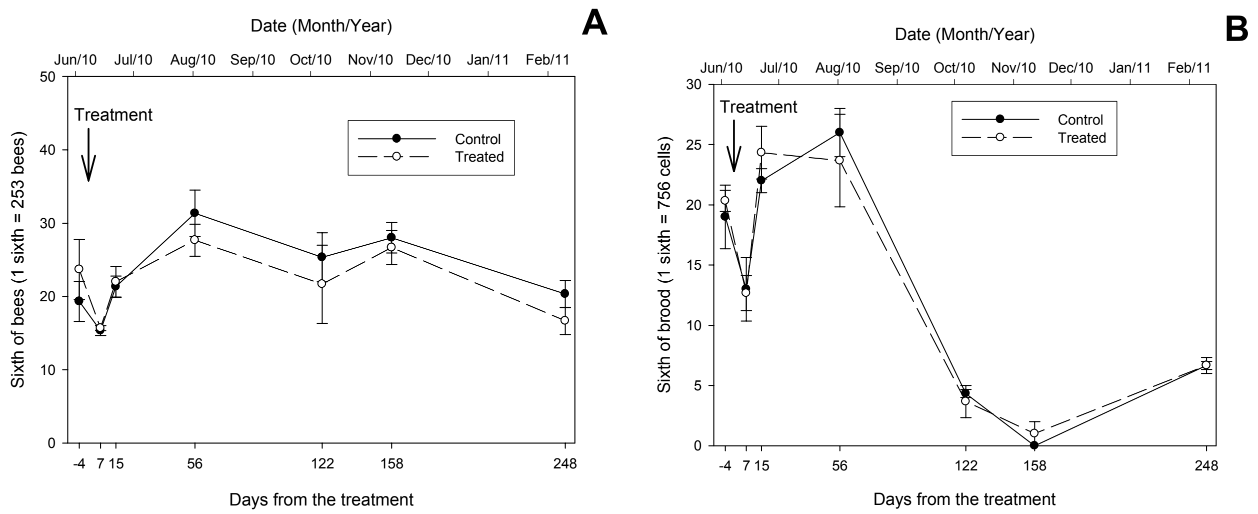

Figure 3. Mean ± SE of number of sixths of adult bees (A) and brood (B) in control and treated cages.

We found no significant differences in flight activity between treatments before application (control: 8.1 bees; treated: 9.5 bees; $p = 0.26$). But after application, the flight activity in treated cages was significantly higher than in control ones (8.7 bees and 7.3 bees respectively; $p < 0.01$). The foraging activity (the total number of bees in the three plots) was similar between treatments, both before (control: 23.0 bees; treated: 22.6 bees; $p = 0.57$) and after application (control: 16.9 bees; treated: 16.2 bees; $p = 0.50$). In addition, the foraging behaviour observed on the plots showed no obvious symptoms of poisoning. This was demonstrated by the low frequency of abnormal behaviours observed in both groups (table 3). However, in the treated cages, the day after the dust application, we noted many agitated bees and some bees (~10 per cage), showing abnormal behaviours (cleaning behaviour and uncoordinated body movements) at the entrance of the hive.

Discussion and conclusions

The laboratory indirect toxicity test showed that bee mortality caused by the dust contaminated with clothianidin-based product Poncho® was not significantly different from that caused by liquid formulation (Dantop®), even if in our study the test concentration of the former was slightly lower than in the latter. Both application ways caused significant mortality rates, even if delayed in time. This demonstrates that bees can get intoxicated after exposure to quantities of a.i. dispersed during sowing of treated maize seeds and deposited on wild vegetation. In previous studies (ApeNet, 2010) sub-lethal effects were also observed in several bees exposed to the dust at the concentration found at 5 meters from edge of the sowing field.

Various studies have reported the sub-lethal and lethal effects of neonicotinoids on the individual bees (Bortolotti *et al*., 2003; Medrzycki *et al*., 2003; Decourtye *et al*., 2004; Yang *et al*., 2008). Information on these effects on colony is scarce, but recently Henry *et al*. (2012) and Lu *et al*. (2012) have found potential negative neonicotinoid effects on the colony. Semi-field and field studies are suitable to study the effects on colonies, including assessment of behaviour, bee mortality and the interaction among bees, exposed to the compound under realistic conditions. Compared to field studies, semi-field studies are easier to control and allow higher numbers of replicates which facilitates statistical evaluations. However, until now the available standardized test methods (OEPP/EPPO, 2010) do not consider the possibility to study bee exposure to dust and do not give any particular indication in order to study long period effects and specific behaviours.

In this study we propose a new method to test in laboratory and in semi-field (cage) the effects of the dust dispersed during sowing operations on honey bees, knowing the exact exposure concentration of the active ingredient.

Only few methods have been proposed to assess in standardized way the impact of dust from coated seeds on bees. In a combined field to laboratory study, Giffard and Dupont (2009) test mortality of bees on *Tibouchina* spp. foliage following the methodology based on EPA

Table 3. Total number of bees observed on oilseed rape plots exhibiting abnormal behaviour. Values between parentheses refer to pre-treatment. N - absolute number of bees observed in the plots.

	Bees immobile on leaves or flowers	Bees engaged in cleaning activity	Hanging-knocked out bees
Control (N = 1669)	3 (0)	0 (0)	0 (0)
Treated (N = 1614)	10 (0)	2 (4)	0 (0)

guideline relative to residues on foliage (EPA, 1996). The foliage of *Tibouchina* spp. planted in the edge of the field, was exposed to dust dispersed during sowing of treated seeds. Assessments were conducted in the laboratory under controlled conditions and bees were introduced in containers with foliage collected 2 and 24 hours after sowing. Bees were exposed to treated leaves for 4, 24 and 48 hours. Similarly, Georgiadis *et al.* (2011) proposed to assess the impact of dust to bees in semi-field studies simulating the sowing process carried out in a maize field surrounded by areas with flowering oilseed rape. In both studies, bees are exposed to the dust, simulating the field scenario but it is not possible to know the pesticide exposure concentration *a priori*. In our laboratory and semi-field method it is possible to apply the desired concentration estimated with specific sowing studies. In the present study we used the mean a.i. concentration deposited on the ground at 5 meters distance from the field's edge, during sowing with a drilling machine equipped with dual pipe deflector. Our results showed that this concentration is toxic to bees despite the deflector pipe modification reduced the quantity of dispersed a.i. by an average of 50% compared with the unmodified seed drill (ApeNet, 2010).

After dust application, the mortality level observed in the semi-field study increased about 10-11 times compared to the control. The mortality was significantly higher than in control during the first 2 days and was still ecologically relevant during the 3rd day. Similar results were observed in a field study with thiametoxam. In this study, the bee mortality increased on the day of sowing and the number of foraging bees decreased on the day after sowing (Tremolada *et al.*, 2010).

In our semi-field test, sub-lethal effects (cleaning behaviours and agitation) were observed only in few bees in front of the treated hives and no effect was evident during foraging activity. Despite the peak of mortality observed after dust application, no significant differences emerged with regard to colony strength (figures 2 and 3). Colony development decreased during confined period but increased from day 7 to day 15, i.e. after removal of the net that covered the cage. In fact, confined conditions resulted in a natural reduction of egg laying in control and in treated cages.

Comparing the treated cages with the control ones, the lethal effects on individual bees did affect neither the colony development nor the socio-physiological parameters (thermoregulation and comb construction capacity) and did not show long-term effects. Probably the homeostatic capacity of the colony avoided the colony decline despite the high bee mortality rate for 2-3 days.

According to Khoury's model (Khoury *et al.*, 2011), colonies are able to survive at a forager mortality rate 3 times higher than that of control colonies, if this mortality lasts for a few days. A colony decline can be expected if this high forager mortality rate lasts for more than 10 days. If this forager mortality lasts for a period of 30 days, instead, colony survival may be impaired (Henry *et al.*, 2012). Such a situation is possible if the sowing period lasts several days as in the Po Valley, where the landscape is characterized by extended maize cultivation. However, even if the mortality peak did not affect the colony development and survival, the forager loss may result in a decline of honey production and pollination service. This is particularly important in spring, in coincidence with maize sowing operations, when many crops and wild plants are in bloom. Due to limitation of the semi-field tests to measure the effects on honey production and colony development (because small colonies are used), specific field tests, as recently proposed in the EFSA Opinion (EFSA, 2012), should be carried out in order to confirm this hypothesis. Moreover, due to the short distance between the hive and the foraging source, in semi-field conditions, disorientation (one of the most important sublethal effects) does not occur with the same probability and intensity as it may happen in the open field (EFSA, 2012).

In conclusion, the a.i. concentration dispersed at the edge and over the field from the pneumatic seeder equipped with deflector, used as mitigation action, cannot be considered sufficiently safe for bees and higher tier tests are required.

Acknowledgements

We are grateful to P. Pulcini (CRA-PAV), M. Boi, G. Serra and R. Colombo (CRA-API) for the chemical analysis. Thanks are due to C. Sciò, L. Mattioli (Bologna) for their help during the semi-field study. We very much appreciate the constructive and helpful comments of three anonymous reviewers. The study was supported by Project "ApeNet: monitoring and research in apiculture", funded by the Italian Ministry of Agricultural Food and Forestry Policies.

References

ALAUX C., BRUNET J. L., DUSSAUBAT C., MONDET F., TCHAMITCHAN S., COUSIN M., BRILLARD J., BALDY A., BELZUNCES L. P., LE CONTE Y., 2010.- Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). -*Environmental Microbiology*, 12: 774-782.

ALIX A., VERGNET C., MERCIER T., 2009.- Risks to bees from dusts emitted at sowing of coated seeds: concerns, risk assessment and risk management.- *Julius-Kuhn Archive*, 423: 131-132.

APENET, 2009.- *"Effects of coated maize seed on honey bees". Report based on results obtained from the first year of activity of the ApeNet project*.- CRA-API, Bologna, Italy. [online] URL: http://www.reterurale.it/apenet

APENET, 2010.- *"Effects of coated maize seed on honey bees". Report based on results obtained from the second year of activity of the ApeNet project*.- CRA-API, Bologna, Italy. [online] URL: http://www.reterurale.it/apenet

APENET, 2011.- *"Effects of coated maize seed on honey bees". Report based on results obtained from the third year of activity of the ApeNet project*.- CRA-API, Bologna, Italy. [online] URL: http://www.reterurale.it/apenet

ARZONE A., VIDANO C., 1980.- Methods of testing pesticide toxicity to honey bees.- *Bollettino del Laboratorio di Entomologia Agraria "Filippo Silvestri"*, 37: 161-165.

BERNADOU A., DAMARES F., COURET-FAUVEL T., SANDOZ J. C., GAUTHIER M., 2009.- Effect of fipronil on side-specific antennal tactile learning in the honeybee.- *Journal of Insect Physiology*, 55 (12): 1099-1106.

BERTHOUD H., IMDORF A., HAUETER M., RADLOFF S., NEUMANN P., 2010.- Virus infections and winter losses of honey bee colonies (*Apis mellifera*).- *Journal of Apicultural Research*, 49 (1): 60-65.

BORTOLOTTI L., MONTANARI R., MARCELINO J., MEDRZYKI P., MAINI S., PORRINI C., 2003.- Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees.- *Bulletin of Insectology*, 56 (1): 63-67.

BORTOLOTTI L., SABATINI A. G., MUTINELLI F., ASTUTI M., LAVAZZA A., PIRO R., TESORIERO D., MEDRZYCKI P., SGOLASTRA F., PORRINI C., 2009.- Spring honey bee losses in Italy.- *Julius-Kühn-Archiv*, 423: 148-145.

CHAUZAT M. P., MARTEL A. C., BLANCHARD P., CLÈMENT M. C., SHURR F., LAIR C., RIBIÈRE M., WALLNER K., ROSENKRANZ P., FAUCON J. P., 2010.- A case report of a honey bee colony poisoning incident in France.- *Journal of Apicultural Research*, 49 (1): 113-115.

DAHLE B., 2010.- The role of *Varroa destructor* for honey bee colonies losses in Norway.- *Journal of Apicultural Research*, 49 (1): 124-125.

DECOURTYE A., ARMENGAUD C., RENOU M., DEVILLERS J., CLUZEAU S., GAUTHIER M., PHAM-DELEGUE M. H., 2004.- Imidacloprid impairs memory and brain metabolism in the honey bee (*Apis mellifera L.*). *Pesticide Biochemistry and Physiology* 78: 83-92. DOI:10.1016/j.pestbp.2003.10.001.

DECOURTYE A., DEVILLERS J., AUPINEL P., BRUN F., BAGNIS C., FOURRIER J., GAUTHIER M., 2011.- Honeybee tracking with microchips: a new methodology to measure the effects of pesticides.- *Ecotoxicology*, 20: 429-437.

EFSA, 2012.- Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees).- *EFSA Journal 2012*, 10 (5): 2668.

EPA, 1996.- *Honey bee toxicity of residues on foliage*.- Ecological effects test guidelines OPPTS 850.3030.

GEORGIADIS P.-TH., PISTORIUS J., HEIMBACH U., STÄHLER M., SCHWABE K., 2011.- Dust drift during sowing of maize-effects on honey bees, p. 42. In: *Programme and abstracts of the 11th international symposium hazards of pesticides to bees*, 2-4 November 2011, Wageningen, The Netherlands.

GIFFARD H., DUPONT T., 2009.- A methodology to assess the impact on bees of dust from coated seeds.- *Julius-Kühn-Archiv*, 423: 73-75.

GIFFARD H., MAMET O., 2009.- Behavior of honey bees: a guideline to assess troubles in bee foraging activity under insect-proof tunnels.- *Julius-Kühn-Archiv*, 423: 71-73.

GIROLAMI V., MARZARO M., VIVAN L., MAZZON L., GREATTI M., GIORIO C., MARTON D., TAPPARO A., 2011.- Fatal powdering of bees in flight with particulates of neonicotinoidi seed coating and humidity implication.- *Journal of Applied Entomology*, 136: 17-26.

GREATTI M., SABATINI A. G., BARBATTINI R., ROSSI S., STRAVISI A., 2003.- Risk of environmental contamination by active ingredient imidacloprid used for corn seed dressing. Preliminary results.- *Bulletin of Insectology*, 59 (1): 69-72.

GREATTI M., BARBATTINI R., STRAVISI A., SABATINI A. G., ROSSI S., 2006.- Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds.- *Bulletin of Insectology*, 59 (2): 99-103.

HARZ M., MÜLLER F., RADEMACHER E., 2010.- Organic acids: acute toxicity on *Apis mellifera* and recovery in the haemolymph.- *Journal of Apicultural Research*, 49 (1): 95-96.

HENRY M., BEGUIN M., REQUIER F., ROLLINE O., ODOUX J.-F., AUPINEL P., APTEL J., TCHAMITCHIAN S., DECOURTYE A., 2012.- A common pesticide decreases foraging success and survival in honey bees.- *Science*, 336 (6079): 348-350.

HIGES M., GARCI-PALENCIA P., MARTIN-HERNANDEZ R., MEANA A., 2007.- Experimental infection of *Apis mellifera* honey bees with the Microsporidia *Nosema ceranae*.- *Journal of Invertebrate Pathology*, 94: 211-217.

IMDORF A., BUEHLMANN G., GERIG L., KILCHENMANN V., WILLE H., 1987.- Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern.- *Apidologie*, 18: 137-146.

KHOURY D. S., MYERSCOUGH M. R., BARRON A. B., 2011.- A quantitative model of honey bee colony population dynamics.- *PLoS ONE*, 6 (4): e18491.

KRUPKE C. H., HUNT G. J., EITZER B. D., ANDINO G., GIVEN K., 2012.- Multiple routes of pesticide exposure for honey bees linving near agricultural fields.- *PloS ONE*, 7 (1): e29268.

LU C., WARCHOL K. M., CALLAHAN R. A., 2012.- *In situ* replication of honey bee colony collapse disorder.- *Bulletin of Insectology*, 65 (5): 99-106.

MAINI S., MEDRZYCKI P., PORRINI C., 2010.- The puzzle of honey bee losses: a brief review.- *Bulletin of Insectology*, 63 (1): 153-160.

MARTIN S. J., BALL B. V., CARRECK N. L., 2010.- Prevalence and persistence of deformed wing virus (DWV) in untreated or acaricide treated *Varroa destructor* infested honey bee (*Apis mellifera*) colonies.- *Journal of Apicultural Research*, 49 (1): 72-79.

MARTIN S. J., HIGHFIELD A. C, BRETTELL L., VILLALOBOS E. M., BUDGE G. E., POWELL M., NIKAIDO S., SCHROEDER D. C., 2012.- Global honey bee viral landscape altered by a parasitic mite.- *Science*, 336 (6086): 1304-1306.

MARZARO M., VIVAN L., TARGA A., MAZZON L., MORI N., GREATTI M., PETRUCCO TOFFOLO E., DI BERNARDO A., GIORIO C., MARTON D., TAPPARO A., GIROLAMI V., 2011.- Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat.- *Bulletin of Insectology*, 64: 119-126.

MEDRZYCKI P., MONTANARI R., BORTOLOTTI L., SABATINI A. G., MAINI S., PORRINI C., 2003.- Effects of imidacloprid administered in sub-lethal doses on honey bees behaviour. Laboratory tests.- *Bulletin of Insectology*, 59 (1): 59-62.

MEDRZYCKI P., SGOLASTRA F., BORTOLOTTI L., BOGO G., TOSI S., PADOVANI E., PORRINI C., SABATINI A. G., 2010.- Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides.- *Journal of Apicultural Research*, 49 (1): 52-59.

MEIXNER M. D., COSTA C., KRYGER P., HATJINA F., BOUGA M., IVANOVA E., BÜCHLER R., 2010.- Conserving diversity and vitality for honey bee breeding.- *Journal of Apicultural Research*, 49 (1): 85-92.

MULLIN C. A., FRAZIER M., FRAZIER J. L., ASHCRAFT S., SIMONDS R., VANENGELSDORP D., PETTIS J. S., 2010.- High levels of miticides and agrochemicals in north American apiaries, implications for honey bee health.- *PLoS One*, 5 (3): e9754.

NAZZI F., BROWN S. P., ANNOSCIA D., DEL PICCOLO F., DI PRISCO G., VARRICCHIO P., DELLA VEDOVA G., CATTONARO F., CAPRIO E., PENNACCHIO F., 2012.- Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies.- *PLoS Pathogens*, 8 (6): e1002735.

NEUMANN P., CARRECK N. L., 2010.- Honey bee colony losses.- *Journal of Apicultural Research*, 49 (1): 1-6.

OECD, 1998a.- Honeybees, acute oral toxicity test.- *OECD guidelines for the testing of chemicals. Section 2: Effects on biotic systems*, Guideline 213: 1-8

OECD, 1998b.- Honeybees, acute contact toxicity test.- *OECD guidelines for the testing of chemicals. Section 2: Effects on biotic systems*, Guideline 214: 1-7.

OECD, 2007.- Guidance document on the Honey bee (*Apis mellifera* L.) brood test under semi-field conditions.- *OECD Environment, Health and Safety Publications, Series on testing and assessment*, Number 75. ENV/JM/MONO(2007): 22.

OEPP/EPPO, 2010.- PP 1/170 (4): Side-effects on honeybees.- *EPPO Bulletin*, 40: 313-319.

PAXTON R. J., 2010.- Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)?- *Journal of Apicultural Research*, 49 (1): 80-84.

PETTIS J. S., VANENGELSDORP D., JOHNSON J., DIVELY G., 2012.- Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*.- *Naturwissenschaften*, 99: 153-158.

PISTORIUS J., BISCHOFF G., HEIMBACH U., STÄHLER M., 2009.- Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize.- *Julius-Kühn-Archiv*, 423: 118-126.

POTTS S. G., BIESMEIJER J. C., KREMEN C., NEUMANN P., SCHWEIGER O., KUNIN W. E., 2010.- Global pollinator declines: trends, impacts and drivers.- *Trends in Ecology and Evolution*, 25: 345-353.

SANTRAC V., GRANATO A., MUTINELLI F., 2010.- Detection of *Nosema ceranae* in *Apis mellifera* from Bosnia and Herzegovina.- *Journal of Apicultural Research*, 49 (1): 100-101.

SCHMIDT H.-W., BRASSE D., KÜNAST C., MÜHLEN W., VON DER OHE W., TORNIER I., WALLNER K., 2003.- Introduction of indices for the evaluation of tent tests and field tests with honeybees.- *Bulletin of Insectology*, 56 (1): 111-118.

TAUTZ J., 2008.- *The buzz about bees*.- Springer Verlag, Berlin, Germany.

TREMOLADA P., MAZZOLENI M., SALIU F., COLOMBO M., VIGHI M., 2010.- Field trial for evaluating the effects on honeybees of corn sown using Cruiser ® and Celest xl ® treated seeds.- *Bulletin of Environmental Contamination and Toxicology*, 85: 229-234.

YANG E. C., CHUANG Y. C., CHEN Y. L., CHANG L. H., 2008.- Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae).- *Journal of Economic Entomology*, 101: 1743-1748.

Authors' addresses: Fabio SGOLASTRA (corresponding author, fabio.sgolastra2@unibo.it), Teresa RENZI, Stefano DRAGHETTI, Stefano MAINI, Claudio PORRINI, Dipartimento di Scienze Agrarie - Entomologia, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy; Piotr MEDRZYCKI, Marco LODESANI, CRA-API, Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di Ricerca in Apicoltura e Bachicoltura, via di Saliceto 80, 40128 Bologna, Italy.

Received June 29, 2012. Accepted September 28, 2012.