JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication

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Keywords

Apis mellifera, clothianidin, colony losses, corn dressing, imidacloprid

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Received: February 23, 2011; accepted: May 19, 2011.

doi: 10.1111/j.1439-0418.2011.01648.x

Abstract

Losses of honeybees have been reported in Italy concurrent with the sowing of corn coated with neonicotinoids using a pneumatic drilling machine. Being unconvinced that solid particles containing systemic insecticide, falling on the vegetation surrounding the sown area, could poison bees foraging on contaminated nectar and pollen, the effect of direct aerial powdering was tested on foragers in free flight near the drilling machine. Bees were conditioned to visit a dispenser of sugar solution whilst a drilling machine was sowing corn along the flight path. Samples of bees were captured on the dispenser, caged and held in the laboratory. Chemical analysis showed some hundred nanograms of insecticide per bee. Nevertheless, caged bees, previously contaminated in flight, died only if kept in conditions of high humidity. After the sowing, an increase in bee mortality in front of the hives was also observed. Spring bee losses, which corresponded with the sowing of corn-coated seed, seemed to be related to the casual encountering of drilling machine during foraging flight across the ploughed fields.

Introduction

In the last few years, honeybee colonies throughout the world have been subject to rapid losses (Underwood and vanEngelsdorp 2007; vanEngelsdorp et al. 2009), in particular in South Europe in the order of 40% (Mutinelli et al. 2009; Neumann and Carreck 2010). The beehive heritage in Europe decreased from over 22.5 million in 1990 to about 15.8 million in 2009 [Food and Agriculture Organization of United Nations (FAO) 2011]. From the 1970s to the 1980s, the parasitic bee mite, *Varroa destructor*, appeared in Europe and United States, (Thompson et al. 2002), passed from the East Asiatic *Apis cerana* Fabricious to the African and European *Apis mellifera* L (Anderson and Truman 2000), causing losses to apiaries (Neumann and Carreck 2010; Ratnieks and Carreck 2010). Colonies infected by varroa die within 1–3 years without chemical interventions (vanEngelsdorp et al. 2008; Rosenkranz et al. 2010), which are effective in reducing the losses that are observed in autumn and the end of winter (Kraus and Page 1995; Fries and Perez-Escala 2001).

The sowing of maize (*Zea mais* L) from mid-March to May, in the corn-growing regions of Italy and Europe, was often accompanied by a rapid disappearance of foraging bees, sometimes with accumulations of dead bees in front of the hives. These spring time deaths of colonies are chronologically distinguishable from those caused by varroa; the latter is efficiently controlled by professional beekeepers, who, on the other hand, do not know how to avoid the deaths that occur at the time of the maize sowing. During the last decade, a close relationship was observed between the deaths of bees and the use of pneumatic drilling machines for the sowing of maize seeds coated with neonicotinoid insecticides (Greatti et al. 2003).

At the end of the 1990s, soil insecticides were replaced with the coating of maize seeds (Taylor and Harman 1990) with fipronil (Colliot et al. 1992; Turnblad 1998) and the widespread neonicotinoids (Elbert et al. 2008) which, being systemic, penetrate the seedlings, protecting them from wireworms beetles (*Agriotes* spp.), cutworms (*Agrotis* spp.) and the rootworm, *Diabrotica virgifera* LeConte (Stamm et al. 1985; Altmann 2003; Van Rozen and Ester 2010) that are the most dangerous insect pests of maize seedlings in spring. Furthermore, these insecticides also control aphids (*Rhopalosiphum* spp., *Sitobion* spp. and *Metopolophium* spp.) and leafhoppers (*Laodelphax striatellus* Fallen) that are virus vectors.

In pneumatic sowing machines, the seed is sucked in, causing the erosion of fragments of the insecticide shell that are forcefully expelled with a current of air. How the insecticide comes into contact with the bees is the subject of this paper. The premiss (Greatti et al. 2003) was that the bees die by collecting contaminated pollen and nectar, because solid fragments of the neonicotinoid seed coating fall on the vegetation surrounding the seeded areas. This hypothesis is up to now widely accepted (Pistorius et al. 2009). Neonicotinoid concentrations in the vegetation at the margins of the seeded areas were shown to be about 50 ppb (Greatti et al. 2006; Maini et al. 2010), not sufficient to cause acute toxicity in foraging honeybees (Yang et al. 2008). Being unconvinced that bees were poisoned by the contact with the surrounding vegetation, other lethal sources in the fields were sought to justify such rapid mortality during the spring maize sowing.

A cause of acute contamination was attributed to the high concentration of neonicotinoids in the guttation drops of coated maize seedlings; these contain lethal doses of insecticide (Tapparo et al. 2011), sufficient to kill a bee within minutes of contact (Girolami et al. 2009; Riebe 2009). However, these bee deaths may not occur at the time of sowing, but after the emergence of plants when guttation drops are produced; therefore, further sources of poisoning were hypothesized. In the first instance, the possibility that bees could be poisoned by drinking dew and guttation drops on nearby vegetation directly contaminated by particulates during the sowing was considered but without finding acute toxicity. On the other hand, an aerial contamination of caged bees near the sowing machine in action was observed, with lethal effect if the relative humidity is high (Marzaro et al. 2011).

In this work, attention is focused on the possibility that bees can be directly dusted in flight with fragments of shell coating emitted by a drilling machine in action, whilst flying on the usual route between the hive and food sources. High humidity was further studied as a possible key factor increasing the lethal effects of powdering.

Material and Methods

Experimental site and insect origin

Field trials took place at the experimental farm of the Agricultural Faculty (University of Padova) located in Legnaro (Padova). The plot was 50 m wide by 70 m long (coordinates: 45°20′41.19″N–11°57′16.22″E). The Padova Beekeeping Association (A.P.A. Pad) supplied four hives.

Seed employed and sowing

Two batches of seed were used for the trials: one produced in 2009 and the second in 2010 hereafter called ‘2009 or 2010 coating’, respectively. The coatings (hybrid employed X1180D 964890 in 2009 and PR32G44 in 2010; Pioneer Hi-bred, Johnston, IA, Italy) were: Celest XL®, containing only fungicides (Fludioxonil 2.4% and Metalaxyl-M 0.93%; Syngenta, Basel, Switzerland), Poncho® (clothianidin 1.25 mg per seed; Bayer Cropscience AG., Leverkusen, Germany) (Altmann 2003; Andersch and Schwarz 2003) and Gaucho 350FS® (imidacloprid, 0.5 mg per seed; Bayer Cropscience AG.) (Elbert et al. 1990) (table 1). The seeds were supplied by A.I.S. (Italian seed association) courtesy of MiPAAF (Ministry of Agriculture, Food and Forestry) for the research project Apenet. The 2009 and 2010 seed batches have a quantity of dust abrasion under the limit of 3 g per 100 kg seeds. The quantity was tested with the Heubach test, considered the method that best allows standardization of dust abrasion measurements within the seed industry (Apenet, 2009, 2010; Nikolakis et al. 2009). A Monosem NG Plus (Monosem, Largeasse-France) drilling machine was used for all the sowing operations. Normally, 73 000–74 000 seeds per hectare were sown (75 cm between rows, 18 cm between seeds in the row). The drill moves at 6–7 km/h with a seeding width of 3 m and requires a minimum of 30 min to sow 1 ha. The air waste pipe is situated on the right hand side of the machine and expels air (and dust) at about150 l/s, at a height of 1.8 m and an angle of 45° to the horizontal. A deflector for direct air

Table 1 Free flight field trials

No	Date	Starting time, length (min)	Active ingredient and coating year	Meteorological conditions				No. bees tested	
						Wind		Relative humidity	
				t (°C)	RH (%)	Direction	Speed (m/s)	High	Laboratory
1	14/7/09	9.30, 60	Clothianidin – 2009	28	65	ENE	2.5	60	60
2	23/7/09	9.00, 60	Imidacloprid – 2009	28	69	E	2.4	60	60
3	15/10/09	11.00, 60	Imidacloprid – 2009	13	29	ENE	3.9	60 + 60*	60
4a	02/09/10	10.30, 60	Fludioxonil+Metalaxil-M-2010	21	50	NNE	2.8	60	60
4b	02/09/10	12.00, 60	Clothianidin – 2010	24	46	ENE	2.7	60	60

*Bees collected in the front of the hive.

stream directed to the soil is not reported in this paper.

Conditions after exposure and influence of relative humidity

Once the bees were exposed to the insecticide powder in the field and captured (as reported in the following paragraph), they were singly transferred in small cubic (5 × 5 × 5 cm) tulle cages in a room at a controlled temperature (22 ± 1.5°C). Half of the cages were kept at a relative humidity lower than 70% with the use of de-humidifier if needed, hereafter designated as laboratory humidity; the other half of the cages were kept at a relative humidity close to saturation (>95%), hereafter designated as high humidity. To obtain conditions of high humidity, singly caged bees were held in plastic boxes with plexiglass on top that was sprayed with water. Moistened absorbent paper was placed under the cages that were raised above the paper to prevent the bees getting wet. The humidity was repeatedly checked with an electronic hygrometer and also with a traditional hygrometer (with dry and wet bulb). All the bees were fed with drops of honey on the top of the cage, which was replaced when necessary.

Dusting trials

The progressive number, the date, the duration and the insecticides or fungicides used in the different trials are reported in table 1 along with the meteorological conditions and the number of bees tested. For the trials, bees were conditioned to visit a dispenser containing a 50% (wt : vol) water solution of sucrose, placed to the north of the hives. Initially, the dispenser was put close to the landing board and then moved progressively further from the apiary. The dispenser was an earth-coloured plate ø 0.25 m to avoid the possible attraction of bees from other apiaries.

The bees leaving from the hives, to reach the dispenser, had to fly over a screen house, a small vineyard for 25 m, and over a 70-m ploughed area of the plot for a total of 100 m. The sowing was carried out on the plot keeping a minimum distance of 35 m from the hives and from the dispenser. In all the trials (table 1), samples of 24 bees were collected, the first before the beginning of the sowing and four others at intervals of 15 min. The bees were captured at the dispenser with vials and placed singly in tulle cages, then kept, half in high humidity and half in laboratory humidity, for a total of 120 bees per trial. In all the trials (table 1), dead bees were counted in front of the hives 2 h after the end of the sowing and in the evening of the same day, as well as the morning and the evening of the following days. In trial 3 (table 1), in addition to the five samples taken from the sugar dispenser, five other samples of 12 bees, employing the same timetable, were captured in front of the hives using an entomological net, successively caged and all held in high humidity (table 1). At the end of this trial, samples of the sucrose solution from the dispenser were fed to six single caged bees in the laboratory to verify the possibility of acute poisoning of bees. At the end of the same experimentation, a non-woven net 8 m wide and 60 m long was stretched over the sowing area along the flight path of the bees.

Samples of dead bees

In trial 1, after 3 h from the end of the sowing and the day after, two samples of seven dead bees were collected from the ground in front of the apiary. Also, in trial 3, samples of eight dead bees were taken from the front of the hives at 3 and 4 h from the end of the experiment (tables 2 and 3).

Table 2 Number of dead bees (in groups of 12), exposed in free flight to the emissions of the drilling machine, captured at intervals of 15 min from the beginning of the sowing, caged and placed in varying RH conditions

			Time from start of sowing				
No. – date of trial	Insecticide	Condition after exposure	0 min	15 min	30 min	45 min	60 min
1–14/07/09	Clothianidin – 2009	Laboratory humidity	0	0***	0***	0***	0***
		High humidity	0	12	12	12	12
2–23/07/09	Imidacloprid – 2009	Laboratory humidity	0	2***	0***	1***	3***
		High humidity	0	12	11	12	12
3–15/10/09	Imidacloprid – 2009	Laboratory humidity	0	0***	0***	1***	4***
		High humidity	0	10	12	12	12
		High humidity	0†	0†	12†	10†	10†
4a–02/09/10	Fludioxonil + Metalaxil-M (Celest XL) – 2010	Laboratory humidity	0	0^{ns}	0^{ns}	1^{ns}	0^{ns}
		High humidity	0	0	1	0	1
4b–02/09/10	Clothianidin – 2010	Laboratory humidity	0	1**	1***	3***	5*
		High humidity	0	7	12	11	12

The asterisks indicate significant differences in the same trial, with respect to the successive number within the same column (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$).
†Bees collected in the front of the hive.

Table 3 Content of neonicotinoids in honeybee samples collected at different times from the starting of sowing, after their flight near the drilling machine

No – date of trial	Insecticide	Collecting site	Sampling time*	No. of bees analysed	Quantity of insecticide in ng/bee
1 – 14/07/09	Clothianidin – 2009	Dispenser	30 min†	7	674
		Hive	3 h‡	7	161
		Hive	Day after‡	7	118
3 – 15/10/09	Imidacloprid – 2009	Dispenser	30 min§	4	3661
		Dispenser	45 min†	8	442
		Hive	3 h‡	8	500
		Hive	4 h‡	8	53
		Non-woven net	Day after	4	29

*Time from start of sowing.
†Bees captured at the dispenser and dead in laboratory in high humidity.
‡Bees found dead on the ground in front of the apiary.
§Bees found dead on the ground near the dispenser.

Furthermore, samples of four bees found dead on the ground near the dispenser were collected at 30 min from the starting of the sowing in trial 3 and on the non-woven net the day after (table 3). Two other samples of apparently healthy bees that were collected at the dispenser and subsequently died inside the cage in the laboratory were taken for analysis (seven bees collected after 30 min in trial 1 and eight bees after 45 min in trial 3).

Neonicotinoid content in bee samples

Samples of honeybees that died during the trials were taken for analysis of the insecticide content to the Department of Chemical Sciences of the University of Padova. The samples of bees, pooled for analysis, were stored at +2°C for few days. The treatment of the samples started with a drying process; the bees were put in a thermostatic oven at 100°C for about 2 h. The samples were then ground with a metallic pestle, methanol was added and the samples were placed in an ultrasonic bath for 25 min. Samples were finally centrifuged, and the floatage was separated and filtered with Millex HV 0.45-μm syringe filter (Millipore, Billerica, MA). The analyses were performed in a UFLC instrument (Ultra Fast Liquid Chromatography, Shimadzu XR – Prominence) equipped with an UV-Vis diode array detector and a Shimadzu XR – ODS II (2.2 μm, 2 × 100 mm) analytical column and a Phenomenex Security Guard pre-column. The following instrumental procedure was optimized: eluent flow rate of 0.4 ml/min, gradient elution (0–0.5 min 70 : 30% water/acetonitrile; 0.5–1.5 min linear gradient to 100% acetonitrile; 1.5–3 min 100% acetonitrile), 45°C column temperature, multiwavelength acquisition of detector signal and analyte quantification at 269 nm for clothianidin and imidacloprid. Instrumental calibration (external) was performed by the analysis of standard solutions in the 0.1–100 mg/l concentration range of analytes, prepared in methanol–water solutions (50% vol : vol) from pure analytical standards (Pestanal, purity >99.7%; Sigma-Aldrich Group, Milan, Italy). Analysis of spiked samples (blank bees added with 0.5–1 μg per bee of clothianidin and imidacloprid) showed recovery factors in the range 75–105%. Methanol (VWR, International, Milan, Italy) and acetonitrile (Riedel de Haen; Sigma-Aldrich Group) were of HPLC grade. Pure water was produced by Milli-Q equipment (Millipore).

Statistical analysis

For each sample of 24 bees collected at a particular time interval, we tested the null hypothesis that the frequency of mortality occurred independently of humidity using a chi-squared goodness-of-fit test.

Results

Behavioural aspects

When the sucrose solution was poured into the dispenser, it was possible, after few minutes, to see the arrival of some hundreds of experienced foragers. It was easy to observe that bees usually flew at a height of about 2 m, and normally they do not change their direction in proximity of machine, only if they encountered the outline of the drilling machine they passed at a distance of few metres to the sides. Observing the bees in flight in sunny conditions, a minimum of 15–20 foragers per minute over the ploughed area was calculated approximately.

Free flight dusting and humidity

To assess the influence of toxic powder from the drilling machine on honeybees in free flight, the machine was placed on the flight path between the hive and the dispenser with sugar solution. The bees captured, in the trial 1 at the sugar dispenser, at the beginning of the sowing showed no symptoms of poisoning and none died when taken to the laboratory, either in the conditions of laboratory or high humidity. In the subsequent four samples, all the bees died in the conditions of high humidity and none in laboratory humidity (table 2). After 3 h from the end of this test, an accumulation of about 400 dead bees was observed in front of the four hives which, by the end of the day after, had reached the number of 1490. On the previous days, the number of dead bees in front of the hives was <50 in the apiary.

In trial 2, similar results to the previous test were obtained. None of the bees collected at the beginning of the sowing died. In the succeeding samples, only in high humidity conditions did high mortality emerge, whilst only six died (of a total of 60 bees) of those held in laboratory humidity. By the evening, and different from trial 1, the number of dead bees in front of the 4 hives did not significantly increase and was lower than 50 bees.

In trial 3, no substantial differences emerged in respect of the previous trials 1 and 2 when referring to the mortality of bees collected at the dispenser (table 2). In this trial, samples of flying bees were also collected in front of the hives, then caged and all held in high humidity. No mortality was observed at time 0 and also in the sample collected after 15 min; in the successive samples, high or total mortality occurred. In the evening, about 300 dead bees were present in front of the hives, the day after there were about 500. None of the six bees fed in laboratory with the sugar solution of the dispenser, collected at the end of the sowing, died.

In trial 4a, seeds coated just with fungicides were used and only three bees of 120 tested died; no indicative difference emerged amongst the samples collected at the beginning and during the sowing nor between the two humidity conditions (table 2).

In trial 4b, seeds treated with insecticide were sown immediately after the trial 4a. In the sample collected at 15 min, the mortality was relatively high in high humidity, and in the successive samples, almost all died (table 2). A discrete mortality was also observed in samples held in laboratory humidity, collected after 45 and 60 min from the beginning of the sowing.

In front of the hives, <100 dead bees were found on the succeeding day. A highlight of the four free flight trials, when seed treated with insecticides was employed, is shown in fig. 1.

Chemical analysis

The sample of bees captured in trial 1 at the dispenser, apparently in good health, caged, transported

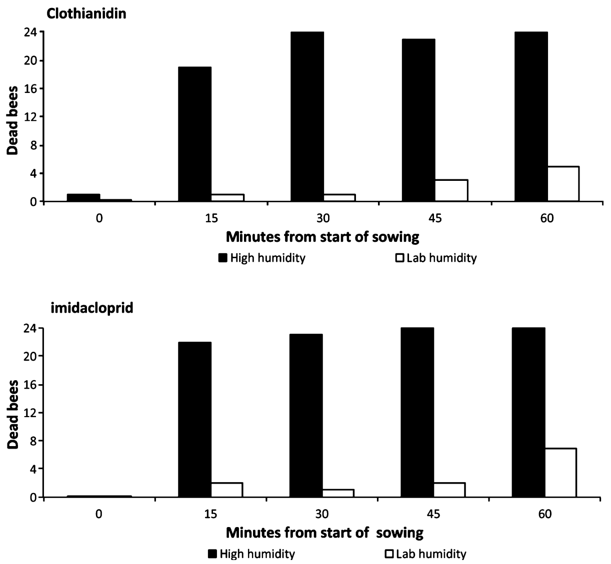

Fig. 1 The mortality of groups of 24 bees that flew in free flight near the drilling machine placed between the hives and the sucrose dispenser in four different trials with two insecticide coatings. The bees where captured, on the dispenser, every 15 min, from the starting up of the drilling machine, subsequently caged and placed in high or laboratory humidity. Each column shows the sum of bee deaths in two different experiments.

to the laboratory and subsequently dead after some hours, showed average concentrations of clothianidin of 674 ng/bee (table 3). The sample of bees found dead 3 h after the end of the sowing in front of the hives revealed an average of 161 ng/bee of active ingredient. In the sample of seven dead bees collected the day after, in front of the hives, concentrations of more than 100 ng/bee (table 3) were found. In trial 3, with seed coated with a.i. imidacloprid, a sample of four bees found expired on the ground near the dispenser, during the sowing, contained an average of 3661 ng/bee of imidacloprid. The sample of bees collected healthy at the dispenser during the sowing which died subsequently in laboratory showed a concentration of 442 ng/bee of a.i. The samples collected in front of the hives, in succeeding hours after the sowing, showed contents of insecticide of 500 and 53 ng/bee (table 3). The day after the sowing, the dead bees collected from the non-woven sheet revealed a content of about 30 ng/bee.

Discussion

Direct dusting in flight

The experimental results showed that the bees can be powdered with fragments of seed coat during their foraging activity when they are flying freely near the drilling machine in action. The trials reproduced the natural behaviour of bees that repeatedly visit a food source, flying along the same route. Chemical analysis of the bees captured alive during the sowing at the dispenser (which died subsequently in laboratory) showed a high content of the neonicotinoid insecticide employed in the trial, with values in the order of 500 ng/bee of active ingredient. This amount is potentially lethal as the topic LD_{50} for clothianidin is 21.8 ng/bee and for imidacloprid 17.9 ng/bee (Iwasa et al. 2004). These amounts make sense of the almost total mortality observed in bee samples, collected at the dispenser after their flight during the sowing and held in high humidity conditions. Furthermore, the use of single cages for holding bees minimizes the possibility of the contamination of bees after capture, as was observed when contaminated and uncontaminated bees are caged together (Greatti, unpublished data). The link between bee poisoning and the toxic emissions of the drilling machine is confirmed by the absence of mortality in foragers captured before the starting up of the machine (time 0; fig. 1) and also in all the samples when only fungicide coating was used (trial 4a; table 2). This allows us to disregard other hypothetical sources of acute toxicity, for example, warm air and exhaust emissions from the tractor, airborne particles of soil containing residues of pesticides that could also contaminate the sugar solution of the dispenser. In fact, when this solution was offered to bees, in trial 3, no acute toxicity was observed. Furthermore, the absence of toxicity at time 0, in all the trials reported, suggests that long-distance wind-borne powders and seed abrasion particles emanating from seed bags need further experimental approach to confirm their implication of lethal bee poisoning.

As bees are poisoned with neonicotinoid in flight, it no longer seems important to consider, as cause of massive bees death, the moot point of contact with contaminated vegetation (contaminated pollen, dew or leaf surfaces) hypothesized in relation to the dust emitted by the drilling machine. Note that, on the basis of new acquisitions, the presence of insecticides on pollen in the hive is not necessarily a consequence of plant contamination before collection, but may be due to the brushing of pollen from the bee body previously contaminated by insecticide during the flight.

The aerial powdering of bees with lethal consequences has been observed also where caged bees were forcibly exposed to the emission for half an hour (Marzaro et al. 2011). The research based on bees in free flight first adopted in this experimentation allows us to establish experimentally the correlation between emissions of particles from the drilling machines and bee poisoning during their foraging activity. It is noteworthy that bees collected during trial 3 in front of the hives, with an entomological net, presented a mortality similar to that of bees collected at the dispenser; this suggested that all the foragers in front of the hives had visited the dispenser; it is quite acceptable because, in October, when the trial was carried out, flowers or sugar sources were not available; furthermore, some contamination between bees cannot be excluded.

Data reported on poisoning in flight were obtained with an old unmodified drilling machine to understand the implications of neonicotinoids (before they were banned) in bee poisoning incidents during sowing of maize in Italy (Mutinelli et al. 2009), the Upper Rhine Valley and in parts of South Bavaria (Pistorius et al. 2009). The consequences on bee survival of the attachment to sowing machines of low-drift sowing equipment (Alix et al. 2009) are not considered in this paper. Nevertheless, it is now possible to experimentally induce poisoning of bees in the fields and therefore consider the beneficial effects not only on drift reduction but also on bees survival. Preliminary results of the effects of the exhaust air directed onto the soil (Forster 2009) seem unclear even if improved coating (2010) is adopted (table 2).

Influence of humidity

In the previous research, the influence of high relative humidity in bee deaths, after the dusting in the field with toxic emissions inside cages, was hypothesized (Marzaro et al. 2011). In this work, the influence of different relative humidity was further tested on bees powdered in free flight and subsequently caged. The results (table 2) confirmed that a high humidity condition is a determinant in the lethal poisoning of bees in the laboratory. The results are clear (fig. 1), both with bees previously powdered with clothianidin and imidacloprid. Furthermore, no differences in toxicity emerged between the two seed batches containing clothianidin (2009 and 2010). Research is in progress also for thiamethoxam and fipronil, the other corn coating insecticides that are banned in Italy (Mutinelli et al. 2009). The bee samples collected without insecticide contamination, before the starting up of the machine or when only fungicides were used, do not show mortality differences when held in different humidity in the laboratory. Therefore, the high humidity condition adopted cannot be considered *per se* the cause of bee death.

Coated corn sowing has caused losses in front of the hives in the open field in trial 3 whilst they were less remarkable in the others trials. The lethal influence of high humidity in laboratory is clear, whilst in the field, the relationship between high relative humidity and bee death in front of the hives is not always evident. For example, with similar values of temperature, relative humidity and wind (direction and speed), some thousands of dead bees were registered in front of the hive in trial 1 and no increase in bee death was observed in trial 2. Probably, in addition to the simple air humidity, other environmental parameters could be considered, for example, cloud movements can induce a rapid decrease in solar radiation with sudden thermal shock that can modify relative humidity both in the fields and inside the hive. The bees collected at the dispenser in free flight (trial 1) and dead in high humidity conditions were shown to be contaminated with an average quantity of 674 ng/bee of clothianidin (table 3). Being randomly divided, after capture on the dispenser, the same quantity was obviously present in insects held in laboratory humidity that all survived. This suggests that in dry condition, bees can tolerate a very high quantity of insecticide powder and can survive. Irrespective of all the parameters that can condition the lethal consequences to bees powdered with neonicotinoids, the possibility of dusting in the field of bees flying in proximity of the drilling machines with potentially lethal doses is demonstrated as highly probable.

Powdering and cleaning

The intense dusting of foragers in flight in the field, confirmed by chemical analysis, may be related to the characteristics of the integument of bees, which is adapted to harvest and retain pollens. The bees in flight could be particularly efficient in intercepting particulates, but their legs are equipped with small brushes that can be used to clean themselves. Probably, bees possess a hygienic instinct, also reported for varroa (Spivak and Reuter 1998), to rid the integument of undesired powder or fragments, maybe in flight, thus preventing the rapid contact of water-soluble insecticide with the body, which allows foragers to survive in dry conditions. The sudden death of bees in spring, during maize sowing, may be related to the possibility that in particular weather conditions, bees may become damp before they were able to rid the integument of the fragments.

Poisoning scenario

Corn is the most commonly grown crop in northern Italy. For example, in the province of Padova, of a total of 114 000 ha of arable land, more than 50 000 ha is cultivated with maize (Regione Veneto 2008; Istat 2009). Corn fields in the north of Italy are interspersed with other herbaceous crops, orchards and gardens, as is easy to check using, for example, grid references (Google Earth®) reported for the experimental plot.

The scenario of the deaths of bees at the time of the maize sowing could be linked to the normal repeated flights of foraging bees to meadow flowers such as dandelion (*Taraxacum officinalis* L.), herbaceous crops such as winter rape (*Brassica napus* L.), flowering trees in gardens, hedges and orchards (*Prunus* spp., *Malus* spp., *Crataegus* spp., etc.). In such flights, there is a probability that they will cross plots assigned for maize sowing. Taking in account that a drilling machine requires 45 min for seeding one ha, the probability of encountering the toxic cloud surrounding the drilling machine is high and will be the topic of another work.

When bees fly near the drilling machine at a height of about 2 m, they get powdered with a high quantity of insecticide with lethal consequences when the humidity is high. In the north of Italy, in spring, these weather conditions are frequently present in the first few hours of morning sun.

It should be noted that if extended monocultures of maize are present, with consequent lack of flowers in spring, bees would not normally cross these large areas and thus avoid contamination. This has probably happened in France where thiamethoxam maize coating is not banned, and neonicotinoid insecticides are not considered a serious problem for bees [Agence française de securitè sanitaire des aliments (Afssa) 2009]. In Germany, mortality was observed where 'many small sized corn fields are located in a diverse agricultural landscape with canola fields, orchards and other bee-attractive crops' (Nikolakis et al. 2009).

The reason why the powder emitted by the drilling machine, independently of the synergistic effects of humidity, had such a dramatic effect on bees may have a rather simple explanation. The neonicotinoids used by farmers are diluted in water in the order of 100 ppm of active ingredients for example Dantop® (clothianidin 50%) is used to control sucking insects (Uneme 2011), at 15 g/hl corresponding to 75 ppm. The fragments expelled during the sowing contain more than 20% of active ingredient that is a content of insecticide at least 2600 times more concentrated than that diluted in water for agricultural sprays.

Finally, it is probable that in the immediate future, the drilling machines will be improved to avoid, or drastically reduce, toxic emissions. Bees in the field seem to tolerate a relatively high powdering with neonicotinoids; this means that is not necessary to completely stop the powdering, instead it would be opportune to reduce the contamination below a probable level that incurs bee deaths. In any case, the trials reported on the bees powdered in the field with relation to the use of pneumatic drilling machines with corn seed treated with neonicotinoids give comparable results if the bees are successively held in laboratory, and this is the first clear demonstration of acute lethal poisoning, in free flight, in the field.

Acknowledgements

We thank the patient sowing machine driver Tarcisio Zanella. Simone Pastorello, Andrea Calgaro, Edoardo Petrucco Toffolo and Andrea Targa for their assistance. We also thank the ARPAV (Regional Agency for Environmental Prevention and Protection of Veneto – Meteorological Centre of Teolo, Italy) for the meteorological data reported in this paper and the Beekeepers Association of Padova (A.P.A. Pad). The research was in part supported by the project APENET: Monitoraggio e ricerca in Apicoltura, Consiglio per la Ricerca e la Sperimentazione in Agricoltura from the Ministry of Agriculture and Forestry (MiPAAF).

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