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ORIGINAL CONTRIBUTION

Performance of honeybee colonies located in neonicotinoid-treated and untreated cornfields in Quebec

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Abstract

Twenty-two honeybee (*Apis mellifera)* colonies were placed in four different cornfield areas in order to study the potential *in situ* effects of seed-coated systemic neonicotinoid pesticides used in cornfields (*Zea mays* spp) on honeybee health. Two apiaries were located in two independent neonicotinoid-treated cornfield areas and two others in two independent untreated cornfield areas used as controls. These experimental hives were extensively monitored for their performance and health traits over a period of one year. Trapped pollen was collected and microscopically identified to define the visited flowers and the amount of corn pollen collected by bees. Liquid chromatography–mass spectrometry was performed to detect pesticide residues in honeybee foragers and trapped pollen. Honeybee colonies located in neonicotinoid-treated cornfields expressed significantly higher varroa mite loads than those in untreated cornfields. However, brood production and colony weight were less disturbed by the treatment factor. Sublethal doses of neonicotinoids were detected in the trapped corn pollen and none in bee foragers. Overall, our results show that forager bees collected 20% of corn pollen containing variable concentrations of neonicotinoids. Colonies located in treated cornfields expressed higher varroa loads and long-term mortality than those in untreated cornfields. On the other hand, no significant differences were observed regarding the brood production and colony weight.

Introduction

Honeybee *Apis mellifera* is one of the most important pollinator insects for a large variety of plants and crops (Aizen et al. 2009; Klatt et al. 2014; Putra and Kinasih 2014; Wilson et al. 2015). The value of crops pollinated by bees was estimated at $14.6 billion US dollars in the United States alone in 2000 (Morse and Calderone 2000). In the last decade, the number of honeybee populations has significantly decreased around the world (Bacandritsos et al. 2010; Johnson et al. 2010). The decline or insufficient global growth of pollinators has significant implications for plant and crop pollination (Aizen et al. 2009). It has been predicted that the loss of the honeybee may cause a threat to global food security (Withgott 1999; Kremen and Ricketts 2000). In Canada, colony losses in 2009–2010 were ~23.8% (Van der Zee et al. 2012), while winter loss in 2015 was 16.4% with provincial losses of 10.4–37.8 according to the 2015 statement of the Canadian Association of the Professional Apiculturists CAPA.

Despite the unclear causes of honeybee decline, several studies highlight the impact of endemic and emergent pathogens (Fries 2010; Dainat et al. 2012), and others pointed on the excessive use of pesticides (Johnson et al. 2010; Van der Sluijs et al. 2013). Multiple chemical residues of synthetic origin have been

detected inside the bee hives, such as fluvalinate and coumaphos, which are pesticides used for varroa treatment (Vanbergen and Initiative 2013). However no individual factor, environment, pesticide or pathogen seems to act as the principal driver of global honeybee losses. Thus, the massive decline of honeybee populations in the world is widely considered as a multifactorial phenomenon (VanEngelsdorp et al. 2010; Wu et al. 2012).

Over the years, agricultural pesticides and their application methods have shifted substantially. In order to limit the risk of exposure to non-target plants and to protect pollinators, systemic insecticides such as neonicotinoids were made available for pest control on many crops (Elbert et al. 2008; Douglas and Tooker 2015). Neonicotinoids are the most used class of insecticide for the last two decades and their use is permitted in more than 120 countries and on more than 1000 different crops (EFSA (European Food Safety Authority) 2012). Despite the negative effects of the neonicotinoids on the environment, which are extremely persistent in soil, water and crops (Van Dijk et al. 2013), this class of insecticide is still considered to be the least harmful for pollinators and other non-target organisms, and so it is commonly applied to seed coatings worldwide. However, various studies have revealed that neonicotinoids are toxic to honeybees at relatively low concentrations (Williamson et al. 2014; Chakrabarti et al. 2015). In addition, the exposure of pollinators to these insecticides is chronic, due to their systemic properties; the active ingredients are taken up by a plant's root system and are translocated to all of its parts, including inflorescence (Jeschke et al. 2011), thus directly exposing pollinators during the whole temporal window of anthesis.

In the field, lethal pesticide toxicity among honeybees has been widely studied across multiple classes of synthetic agents (Davis 1989; Van der Sluijs et al. 2013). In such cases, lethal toxicity is easily confirmed via the presence of dead bees in front of the hives. However, few studies deal with the effects of sublethal doses of pesticides on honeybees (Williamson et al. 2014). It is known that the sublethal doses impair the essential activities of insects (Desneux et al. 2007; Gill et al. 2012; Schneider et al. 2012) even at concentrations below the detection limits of analytical chemistry (Leonardi et al. 1996). Sublethal doses significantly decrease honeybee performance and trigger disorders in colony dynamics and labour partition (Mackenzie and Winston 1989). Moreover, it has been shown that honeybee behaviour, orientation, communication dances and return flights, especially for foragers, are highly affected by sublethal insecticide doses (Vandame et al. 1995; Williamson et al. 2014). Sublethal doses of neonicotinoids in particular are known to impair the olfactory memory and learning capacity of honeybees (Decourtye et al. 2005; Aliouane et al. 2009; Williamson and Wright 2013; Williamson et al. 2014) and alter the flying behaviour and navigational capacity of forager bees (Henry et al. 2012).

Recent studies have demonstrated synergistic effect between neonicotinoid exposure and honeybee pathogens. Neonicotinoid insecticides, more specifically clothianidin and imidacloprid, negatively modulate the nuclear factor-kappa B (NF-kB) immune signalling in honeybees, which leads to reduce the honeybee immune defences and promote viral replication (Nazzi et al. 2012; Di Prisco et al. 2013).

In this study, we assessed the potential *in situ* effects of neonicotinoid-coated *Zea mays* (henceforth 'corn') seeds on honeybee colony health. This was carried out by testing how foraging in neonicotinoid-treated cornfields can impact honeybee health and survival.

Materials and Methods

Honeybee colonies and locations

This current study was conducted in 2013 on the 22 remaining colonies of an original 32 tested for the same parameters in 2012 (Alburaki et al. 2015). At the end of the indoor wintering, on 10 April 2013, 22 colonies survived and were divided into the same four apiaries of the previous year and redistributed to four different cornfields' clusters south-west of Quebec (fig. S1 and table S1). Colonies were placed as follows: 6 colonies in apiary 1, 7 colonies in apiary 2, 4 colonies in apiary 3 and 5 colonies in apiary 4 (fig. S1 and table S1). In total, 11 colonies were located in treated (TC) and untreated cornfields (UTC).

All of the 2012's locations remained the same except for apiary 2/Portneuf (Alburaki et al. 2015). This location was changed to a new one (Donnacona, fig. S1) as no intensive treated cornfields were available in Portneuf in 2013. Based upon communication from local farmers, both apiaries 1 and 4 were located in areas of untreated cornfields, while apiaries 2 and 3 were situated in cornfields seed treated with neonicotinoid insecticides, Cruiser (Syngenta) and Poncho (Bayer).

The four cornfield locations are located in geographical areas where the climate, environmental conditions and flora are similar. To the best of our knowledge, no additional chemical pesticides such as fungicides or herbicides were sprayed on the corn. We do not exclude the probable use of such pesticides on

other marginal cultures. Crop rotations in the four locations are mainly alternations between corn *Zea mays* and white clover *Trifolium* sp. A GIS study was performed to determine the percentage of the available agricultural area for bees at each location in a 5-km radius. This study was conducted using ArcGIS® software (http://www.esri.com/software/arcgis) by Esri under licence.

Varroa mite infestation

Each studied colony was equipped with a sticky base board for varroa mite count. Passive varroa mite counts were made from this board for 72 h at four time periods for each colony during the period of peak activity (July, August and September), which coincides with corn flowering, Table 1. Board counts were used to estimate variation in varroa abundance between colonies of TC and UTC. No chemical treatment for varroa was applied during this study.

Forager bee sampling

Approximately one hundred forager bees were sampled from each hive's entrance at three time points (12 July, 19 August and 2 September) and were stored at −20°C. Foragers sampled from colonies of each apiary, at each date, were pooled and treated as one sample for pesticide detection. Foragers were collected during their return-flight home using a mesh fixed to the hive entrance.

Honeybee hive development

Two parameters were evaluated to measure the biological development of each experimental hive: colony weight (kg) and the worker brood production (Louveaux et al. 1966; Louveaux 1973). During the period of honeybee activity (April to September 2013), a biweekly record was kept of the hive's weight as a measure of the colony performance. Brood production was evaluated by photographing all bee frames containing capped worker brood cells twice a month during the highest period of activity (June to August). Capped worker brood cells were manually counted from photographs using ImageJ software (Bertrand et al. 2015; Alburaki et al. 2015). This measurement was taken five times during our experiment (table 1).

Table 1 Mean comparison of varroa abundance, brood production and colony weight of colonies located in TC and UTC, exposed by date and treatment. P-values are calculated based on the analysis of variance (ANOVA)

Pathogen/ Parameter/2013	Apiary 1 (UTC) Porneuf (Mean/ 6 colonies)	Apiary 2 (TC) Donnacona (Mean/7 colonies)	Apiary 3 (TC) St-Marc des Carrières (Mean/4 colonies)	Apiary 4 (UTC) Ste-Anne de la Pérade (Mean/5 colonies)	Mean TC vs. UTC	
					F-value	P-value
Varroa 31-July	80.3	222.8	136.7	147.4	1.25	0.27
Varroa 06 August	172.1	593.4	420	202.4	6.44	0.019
Varroa 15 August	179.3	509.2	569.5	282.2	8.42	0.008
Varroa 27 August	155.6	401	309.7	181.2	3.81	0.06
Varroa/Overtime	146.8	431.6	359	203.3	13	0.0005
Brood 29 May	12 129	17 379	18575.2	19477.6	2.23	0.15
Brood 13 June	13130.8	14111	11264.7	14 527	0.008	0.93
Brood 04 July	4548	9762.4	0	6438.4	0.02	0.87
Brood 25 July	8338	3608.1	4355.7	4308.4	0.87	0.36
Brood 21 August	11778.6	3314.2	8130.2	2553.8	0.26	0.61
Brood/Overtime	9984.8	9634.9	8465.2	9461	0.049	0.82
Weight (kg)/Overtime	38.6	47.0	39.0	45.8	1.23	0.26

Pollen collection and analysis

Trapped pollen was collected from the hives at three different time points: before, during and after the corn flowering period (table 1). Pollen collected from each colony was desiccated at 37°C for 48 h and individually conserved at −20°C. A detailed palynological analysis was performed on the trapped pollen to define its origin and composition. Briefly, each dried sample was homogenized and 1 g of each sample was used to determine the botanical origin of the pollen loads with 4500 observed pollen grains. The taxonomic diversity of pollen samples for each colony at each sampled date was determined by observing the total surface of slides (Loublier et al. 2003). Trapped corn pollen balls collected by foragers of each apiary were visually identified, screened and pooled per location for further chemical analysis.

Chemical analysis

Pesticide residues were quantified in honeybee foragers, trapped pollen and screened corn pollen using the liquid chromatography–mass spectrometry method (Barnett et al. 2007; Walorczyk and Gnusowski 2009). Analyses were processed at the laboratory of the Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec (MAPAQ) in Quebec, Canada, as well as at the Centre de Biophysique Moléculaire, Centre National de la Recherche Scientifique, Orléans, France. In the latter laboratory, the neonicotinoid limit of detection ranges from 0.01 to 0.03 ng/g. Trapped pollen of each apiary at each date were pooled. In total, 12 pollen samples (4 apiaries at 3 dates), 4 corn pollen samples (4 apiaries) and 12 forager samples (4 apiaries at 3 dates) were analysed for pesticide residues. Five grams of each pollen and honeybee workers (~50 bees) were used for the pesticide detection.

Statistical analysis

Statistical analyses were carried out in the R environment (Team 2011). Each honeybee colony was considered an independent biological unit. Variables were brood production, varroa load, colony weight and the geographical percentage of the agricultural area available in a 5-km radius in each apiary's location. Each of these variables was tested for normality with the Shapiro–Wilk test (Shapiro and Wilk 1965). Variables not normally distributed were normalized for their distribution by log transformation. The factor 'date' was treated as a 'repeated measure' for each variable. Our data set were analysed at both unit and apiary levels. However, in order to assess the treatment factor, the data were also analysed by treatment categories: colonies located in the TC (7 + 4 colonies) and those located in the UTC (6 + 5 colonies). As each studied group had the same number of observations, analysis of variance ANOVA was used to study the difference between variables regarding the treatment factor at 95% confidence level. Principal component analyses (PCAs) were performed on our data using the package 'FactoMineR'. Correlation matrixes between variables and plots were generated using additional packages such as 'corrplot' and 'PerformanceAnalytics'.

Results

Varroa mite abundance

Significantly higher levels of varroa infestation were repeatedly recorded in honeybee hives located in the TC compared with the varroa loads in the hives placed in the UTC (fig. 1 and table 1). The greatest difference in varroa loads among colonies of the TC and UTC was recorded after the corn flowering period (15 August 2013) ($P = 0.008$). Varroa load was also greater in colonies of the TC compared with those of the UTC on 6 August ($P = 0.019$), (fig. 1d and table 1). Similarly, overtime varroa loads were greater in hives of the TC than those located in the UTC ($P < 0.001$). However, no significant differences were recorded on the first and last dates between both groups (fig. 1d and table 1). The first PCA axis expressed 48.64% of the variability in varroa data set and differentiated between two clusters of colonies indicating higher varroa loads in hives of the treated cornfields.

Colony weight and brood production

Differential mean values were calculated for the two colony groups: located in TC and UTC, as well as the means of the brood production (fig. 2b, c). The two colony groups were similar in weight at the beginning of our study until 6 May 2013 (fig. 2c). The weight divergence between colonies of the two groups began on 6 May-13 but did not show any significant differences at any time point. The last two recorded weights showed high decreases in colony weight of the TC group compared with the UTC group. At the end of our experiment on the 18 September 2013, 4 hives

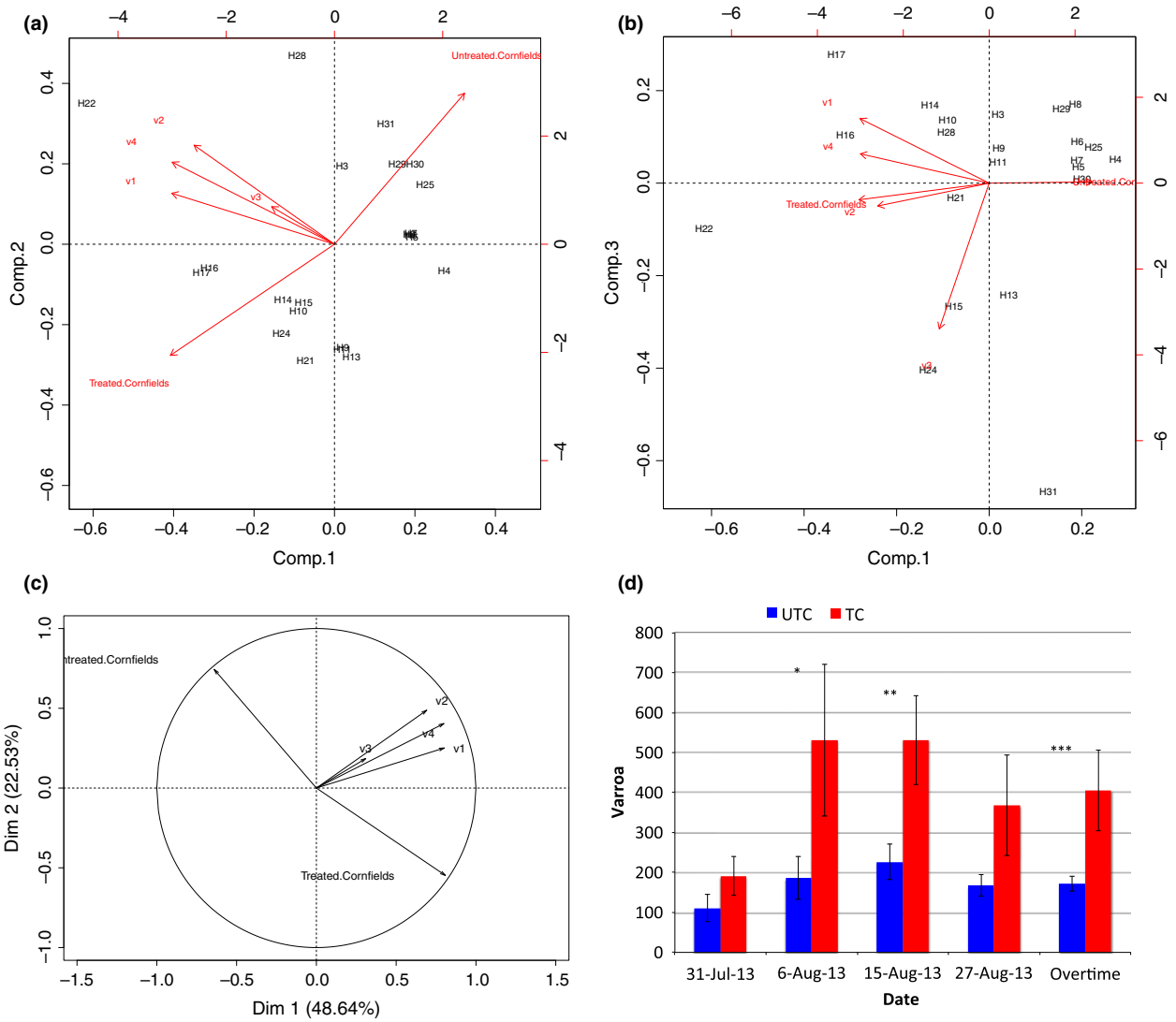

Fig. 1 (a, b) PCA of varroa mite abundance conducted on all the studied hives and dates. v1, v2, v3 and v4 are the varroa loads of the four studied dates. (c) Variables factor map of the PCA shows the percentages of the variables expressed on both axes 1 and 2. (d) Varroa loads of both treated and untreated colony groups. Error bars are the standard errors (SE) of each group, and P values are *P < 0.05, **P < 0.01 and ***P < 0.001.

were lost in the TC and 1 in the UTC. Overtime weight development showed no significant differences between the two groups (P = 0.26), (table 2).

In brood production, none of the studied dates or the overtime-brood production showed any significant differences in brooding of the TC and UTC colonies (fig. 2b and table 1). The correlation matrix showed various correlations between both colony weight and brood production. Regardless of the treatment factor, b5 is particularly interesting as it constantly expressed negative correlations with most of the weight dates. However, b4 positively correlated with w3 and w4 and b1 with w6 and w7 (fig 2d).

Chemical analysis

Detectable pesticide residues in forager bees, corn pollen and pollen of each apiary and date are summarized in Table 2. Neonicotinoids were not detected at any time points in forager bees. However, low concentrations of other pesticides such as phosmet, carbendazim, picoxystrobin, boscalid and diuron were identified at different periods in honeybee foragers and pollen (table 2). In the corn pollen collected by foragers, clothianidin was detected at 0.17 ng/g and 0.09 ng/g in apiaries 3 and 4, respectively, while the thiamethoxam was identified in corn pollen of apiary 2 at 0.07 ng/g.

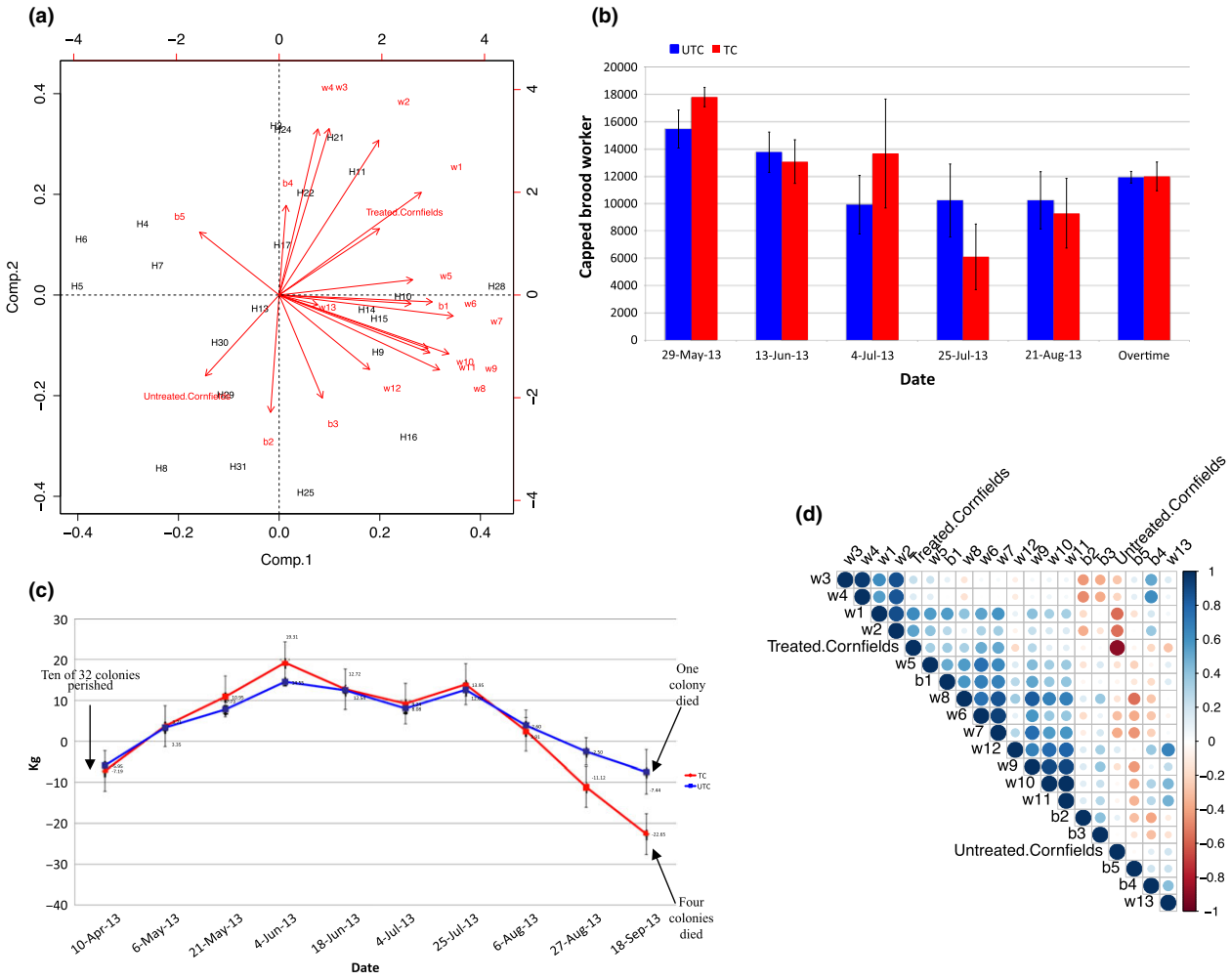

Fig. 2 (a) PCA of the brood and weigh development projected on axes 1 and 2 with percentage expression of 34.45% and 21.36%, respectively. (b) Colony brood production of the studied hives exposed by data and treatment. (c) Colony differential weight value of both studied groups during the 2013 season. (d) Correlation matrix of both weight and brood production, b1 to b5 are the five studied brood dates, while w1 to w13 are those of the colony weight.

Pollen identification

The identification of the pollen collected at three different dates revealed various types of pollen (table 3). Corn pollen (*Zea mays*, Poaceae) was identified at the three sampled dates (28 July, 15 August and 2 September, 2013) at percentages of 14.4%, 4.1% and 1.1% of the total pollen, respectively. At the colony level, 10 colonies collected corn pollen on the first date (28 July), 8 colonies on the second date (15 August) and 4 colonies on the third date (2 September). *Trifolium* sp. (Fabaceae) were the most visited flowers in the three sampled dates at percentages of 19.5% for 28 July, 25.7% for 15 August and 25.8% for 2 September. Pollen grains of several other vegetation and plants such as *Lythrum* sp. (Lythraceae) and *Lotus* sp. (Fabaceae) were also identified at different percentages (table 3).

Discussion

Our experiment was carried out to assess any potential *in situ* side effects of the neonicotinoid-coated corn seeds on honeybee health and survival. The four studied apiaries were located in the south-west of Quebec City in areas dominated by corn cultivation (fig. S1). This study performed in 2013 is a continuation of a previous study from 2012 applied to the 22 hives that survived the 2012 winter, from an initial 32 hives. The 2012 colony loss was equally distributed between treatment locations, 5 colonies in the TC and 5 others in the UTC.

Table 2 Chemical pesticide residues analysed by LC-MS for trapped corn pollen, honeybee foragers and trapped pollen collected from the four studied apiaries on different dates. LD_{50} is based on the data provided by Sanchez-Bayo and Goka (2014) and the toxicity databases ECOTOX of the US Environmental Protection Agency

Sample/2013	Apiary 1 (UTC) (ng/g)	Apiary 2 (TC) (ng/g)	Apiary 3 (TC) (ng/g)	Apiary 4 (UTC) (ng/g)	Detected molecule	Pesticide	Limit of detection LOD (ng/g)	LD_{50} Honeybee oral toxicity (ng/bee)
Trapped Corn	–	0.07	–	–	Thiamethoxam	Neonicotinoid/insecticide	0.01	20
pollen only	–	–	0.17	0.09	Clothianidin	Neonicotinoid/insecticide	0.01	40
All dates								
Trapped Pollen	2	101	3	1	Phosmet	Insecticide	1	620
28 July	1	1	–	–	Carbendazim	Fungicide	1	>50 000
	–	–	–	2	Boscalid	Fungicide	1	>166 000
Trapped Pollen	1	53	3	1	Picoxystrobin	Fungicide	1	>200 000
15 August	–	–	–	1	Phosmet	Insecticide	1	620
	1	–	–	–	Carbendazim	Fungicide	1	>50 000
	–	19	6	25	Allethrin	Insecticide	1	>10 000
Trapped Pollen	–	5	3	–	Picoxystrobin	Fungicide	1	>200 000
2 September	11	–	–	–	Phosmet	Insecticide	1	620
Honeybee forager	–	<1	<1	–	Diuron	Herbicide	1	>145 000
12 July	–	<1	<1	–	Carbendazim	Fungicide	1	>50 000
Honeybee forager	–	1	1	–	Picoxystrobin	Fungicide	1	>200 000
19 August	–	1	1	–	Carbendazim	Fungicide	1	>50 000
	–	1	1	–	Diuron	Herbicide	1	>145 000
Honeybee forager	–	1	–	–	Carbendazim	Fungicide	1	>50 000
2 September	–	1	–	–	Diuron	Herbicide	1	>145 000

(–) means chemical compound not found or below the level of detection (LOD).

The aim of this additional experimental year was to continuously monitor for a second year and on the same hives, the probable effect of the neonicotinoid-treated cornfields on honeybee health. We followed classical biological measurements on our hives and assessed for potential chronic toxicity, as those hives had been previously exposed to neonicotinoid-treated cornfields in 2012 (Alburaki et al. 2015).

In 2013, 10 of 22 hives had collected a total of 19.6% corn pollen (table 3), while in the previous studied year, only 5 hives had collected ~ 1% corn pollen in which no neonicotinoids were detected (Alburaki et al. 2015). For 2013, sublethal doses of thiamethoxam and clothianidin (0.07 and 0.17) ng/g, respectively, were detected in the trapped corn pollen collected by foragers of the TC. No neonicotinoids were detected in apiary 1 (UTC) and 0.09 ng/g of clothianidin in apiary 4 (UTC) (table 2). Although these low concentrations are below the LD_{50} of honeybee oral toxicity (3.7 ng/g) (EFSA (European Food Safety Authority) 2012), sublethal doses of neonicotinoids were demonstrated to impair honeybee functions, including immunity (Nazzi et al. 2012; Di Prisco et al. 2013) and foraging behaviour (Decourtye et al. 2005; Henry et al. 2012; Palmer et al. 2013; Williamson and Wright 2013; Williamson et al. 2014). The detection of 0.09 ng/g of clothianidin in one UTC location was unexpected. It suggests that some foragers of apiary 4 were able to get into treated cornfields far from the studied location or that contamination occurred from non-corn sources. Besides corn, various flowers of the genus *Trifolium* such as the White, Red and Alsike clovers were intensively visited by foragers (table 3).

Interestingly, the level of varroa infestation for 2013 is significantly greater than what had been reported in the previous year with similar patterns. Variance analysis with PCA confirmed on axes 1 and 2 that foraging in TC highly elevates the risk of varroa infestation than foraging in UTC (fig. 1a, b). This conclusion is further confirmed on axes 1 and 3. It is known that elevated varroa mite *Varroa destructor* infestation is usually combined with higher viral infections in bees, as varroa is a very efficient vector of bee viruses and many other honeybee pathogens (Chen et al. 2006; Rosenkranz et al. 2010; Nazzi et al. 2012). This alteration in honeybee defence against varroa mite, seen for a second consecutive year in the colonies of the TC, might not be totally due to the treatment factor. However, in the light of our results, sublethal levels of neonicotinoids found in the corn pollen cannot be neglected either and could lead to a chronic toxicity by accumulation over years. Besides that, other routes of pesticide exposure were not

Table 3 Overview of the trapped pollen composition collected by forager bees on three different dates. Percentage calculated for each visited plant is based on the total number of pollen found for each plant compared with the total number of the counted pollen grain in the slide (Loublier et al. 2003). Total amount of the corn pollen collected by bees on the three studied dates is 19.6%

Common name	Latin name/family	28 July 2013 %	15 August 2013 %	2 September 2013 %
Corn	*Zea mays*	14.4	4.1	1.1
White clover	*Trifolium repens*	19.5	12.5	13.1
Alsike clover	*Trifolium hybridum*	7.0	25.7	25.8
Red clover	*Trifolium pratense*	–	9.5	22.6
Crucifers	*Brassicaceae*	14	5.8	5.2
Purple lythrum	*Lythrum salicaria*	11.2	17.7	2.6
Bird's-foot Trefoil	*Lotus corniculatus*	18.3	15.3	1.4
Buckwheat	*Fagopyrum esculentum*	5.8	–	1.4
Burdock	*Arctium* sp.	2.7	0.9	–
Lucerne	*Medicago sativa*	1.9	1.2	1.9
Common melilot	*Melilotus* sp.	1.6	3.4	–
Blackweed	*Medicago lupulina*	1.6	–	–
Parsnip	*Pastinaca sativa*	0.8	–	–
Canada goldenrod	*Solidago canadensis*	0.4	2.7	11.2
Dewberries	*Rubus* sp.	0.4	–	1.9
Fleaworts	*Plantago* sp.	0.4	–	–
Yellow Toadflax	*Linaria vulgaris*	–	–	10.8

(–) means pollen grain not found.

tested in our study, which could have contributed in impairing bee pathogen resistance. The analysis of variance conducted on our data did not show any significant differences for both brood production and colony weight between colonies of TC and UTC (table 1). In other words, these two variables, in the circumstances of our study, do not seem to be affected by the treatment factor. This result is similar to that reported in 2012 on the same set of colonies. Data of 2012 indicated that colonies of both groups (TC and UTC) had no significant differences in brood production and colony weight, except for two dates in October 2012 in which the TC group showed greater weight development than the UTC one (Alburaki et al. 2015). The slightly greater colony weight recorded in the TC is most easily noted in the PCA plot of fig. 2. Most weight data (except for w12) are biased towards the TC. The correlation between the weight and brood regarding the treatment factor indicated no statistically significant results for these two variables when treated independently. However, regardless of neonicotinoid exposure, colony weight correlates significantly with the brood production with the exception for b5. Such a correlation is not unexpected.

This experimental year started on the 10 April 2013 with 22 honeybee colonies and ended in September with 17 colonies. Four colonies perished at the TC locations and one at the UTC area (fig. 2c). Overall the two studied years (2012–2013), honeybee mortality was 28% in the colonies placed in treated cornfields (TC) and 15% in colonies of the untreated cornfields (UTC).

In conclusion, our data show that honeybee colonies foraging in neonicotinoid-treated cornfields experienced significantly higher varroa loads, particularly at the corn flowering period, than those foraging in untreated cornfields. Colony mortality over the two experimental years was higher in the treated cornfields. The identification of sublethal clothianidin and thiamethoxam doses in the corn pollen makes from the corn flowering period a critical period for colony health. It would be, therefore, recommended for beekeepers to remove their beehives from cornfields during this period.

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Data Accessibility

All our data concerning this study (sampling, locations, GIS study, brood and varroa counting, statistical R outputs and plots) are available on LabArchives website under the DOI: http://dx.doi.org/10.6070/H4MW2F6T.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

MA, ND and MC conceived and designed the experiment; MA, PLM and LQ performed the experiment

and collected the data. MA, BC and ND analysed the data, interpreted it and wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 The geographical locations of the four honeybee apiaries in the southwest of Quebec City.

Table S1. Timetable of the procedures carried out in this study.