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A field study examining the effects of exposure to neonicotinoid seed-treated corn on commercial bumble bee colonies

G. Christopher Cutler · Cynthia D. Scott-Dupree

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Abstract Neonicotinoid insecticides have been studied as possible contributors to bumble bee declines in North America and Europe. This has potential significance in corn agro-ecosystems since this crop is frequently treated with neonicotinoids and dominates much of the agricultural landscape in North America and Europe where bumble bees and other pollinators are commonplace. We conducted an experiment where commercial bumble bee (*Bombus impatiens*) hives were placed during pollen shed next to corn (*Zea mays*) fields that were grown from "conventional" seed that was treated with neonicotinoids, or "organic" seed that was not treated with pesticides. Samples of pollen were collected from corn plants for neonicotinoid residue analysis, pollen types carried by worker bees returning to hives were determined, and in autumn hives were dissected to measure various endpoints that serve as markers of colony vigor. Clothianidin was detected (0.1–0.8 ng/g) in pollen collected from all conventional fields, but was not detected in pollen from organic fields. Corn pollen was only rarely collected from bumble bee foragers and the vast majority of pollen was from wild plants around the corn fields. All hives appeared healthy and neonicotinoid seed treatments had no effect on any hive endpoints measured, except the number of workers, where significantly fewer workers were recovered from hives placed next to conventional fields (96 ± 15 workers per hive) compared to organic fields (127 ± 17 workers per hive). The results suggest that exposure during pollen shed to corn grown from neonicotinoid-treated shed poses low risk to *B. impatiens.*

Keywords *Bombus impatiens* · Colony development · Neonicotinoids · Seed-treatment · Corn · Bees

G. Christopher Cutler (✉)
Department of Environmental Sciences, Dalhousie University Agricultural Campus, Truro, NS, Canada
e-mail: chris.cutler@dal.ca

C. D. Scott-Dupree
School of Environmental Sciences, University of Guelph, Guelph, ON, Canada

Introduction

There is widespread concern over reports of declines in pollinator communities. Much attention has been given to challenges facing honey bees (*Apis mellifera* L.) (van Engelsdorp et al. 2009; van Engelsdorp and Meixner 2010), but problems confronting non-*Apis* pollinators are being increasingly studied and debated (Colla and Packer 2008; Winfree et al. 2009; Potts et al. 2010; Cameron et al. 2011; Oliver 2012; Gonzalez-Varo et al. 2013; Vanbergen et al. 2013). Pesticides are a potential risk to pollinator survival and development. The vast majority of pesticide toxicology studies with pollinators have been done with honey bees, owing mainly to their importance as pollinators and the convenience of being able to obtain with relative ease large numbers of individuals or colonies for experiments. In general, honey bees are a useful surrogate for other bees in risk assessments (Porrini et al. 2003). However, it is also recognized that non-*Apis* bees encompass a large diversity of taxa, and have morphological, physiological, and life history traits that may result in exposure and susceptibility profiles that are quite different from that of honey bees. Thus, there is a move among scientists and regulators to incorporate more ecotoxicological testing of pesticides on non-*Apis* pollinators (Fischer and Moriarty 2011).

Bumble bees (*Bombus* spp. Latreille) are primitively eusocial bees. The genus is represented by at least 250 species, most of which are Holarctic in distribution (Williams 1998). Long recognized as important pollinators in natural ecosystems, improved and large-scale production of commercial bumble bee hives has resulted in greatly increased use of bumble bees in agricultural and horticultural crop pollination (Velthuis and van Doorn 2006). The availability of commercial colonies has also resulted in increased pesticide toxicological testing with *Bombus* spp. The majority of *Bombus*-pesticide studies conducted of late have focused on the neonicotinoid class of insecticide, which has been a lightning rod for debate among scientists, policy makers, beekeepers, and the general public as a possible cause of pollinator declines. Results from a large number of studies give contrasting results. Recent laboratory-based studies have shown that constant feeding upon field-relevant concentrations of neonicotinoids can detrimentally affect bumble bee colony development (Laycock et al. 2012; Whitehorn et al. 2012), foraging ability (Feltham et al. 2014), and that bumble bees may inherently be more susceptible than honey bees to these pesticides (Cresswell et al. 2012, 2013). On the other hand, several other laboratory, semi-field and field studies indicate that bumble bee colonies are unaffected by exposure to concentrations of neonicotinoid insecticide that may be encountered in the field (Tasei et al. 2000, 2001; Morandin and Winston 2003; Franklin et al. 2004; Thompson et al. 2013). A study into causes of bumble bee declines in North America found that pesticide use and habitat loss are unlikely to be a major cause of declines (Szabo et al. 2012; Colla et al. 2013).

Bumble bees are common in many agroecosystems across North America, including regions such as southern Ontario, Canada, that produce large amounts of corn. Here we present the results of a field study in which commercial *B. impatiens* colonies were placed adjacent to corn (*Zea mays* L.) fields grown from 'certified organic' pesticide-free and non-genetically modified seed, or 'conventional' genetically modified seed treated with neonicotinoids. Colonies were exposed to corn during pollen shed and thereafter allowed to develop in a natural ecosystem away from agricultural land. We predicted that although pesticide may be present in corn pollen: (1) residues will be below levels thought to be of concern to bumble bees (Cresswell et al. 2012; Laycock et al. 2012; Whitehorn et al. 2012); (2) bumble bees will minimally collect corn pollen since it often has poor nutritional value for bees (Somerville 2001) and has been shown to compromise bumble bee colony development (Malone et al. 2007); and therefore, (3) colony development will not be affected by placement next to corn fields grown from seed treated with neonicotinoids.

Materials and methods

Corn fields and seed treatments

Four conventional forage corn fields used in the experiment were near Elora (2 fields) and Guelph (2 fields), Ontario, which were 8, 10, 31, and 44 ha. Four organic forage corn fields near Teviotdale (2 fields) and St. Mary's (2 fields), Ontario, were 7, 6, 17, and 18 ha. All fields were within 100 km of each other and the minimum distance between fields was 9 km.

Pioneer P9675 corn seed containing no pesticide treatment or genetic modification was grown at all organic sites. At the conventional sites, all planted seed was genetically modified for expression of *Bacillus thuringiensis* (Bt) endotoxin and was treated with thiamethoxam or its primary metabolite, clothianidin. Because we were working with independent farmers, seed types and seed-treatment specifications varied. A summary of seed details at conventional (CV) sites follows:

- CV1. Pride Seeds A5909G2 RIB: Round-Up Ready®; Bt traits; seed-treatment with clothianidin (Poncho® 250, 0.25 mg AI/kernel; Bayer Crop Science, Calgary, Alberta) and the fungicides ipconazole, metalaxyl, and trifloxystrobin.
- CV2. Pride Seeds A5120G2 RIB: Round-Up Ready; Bt traits; seed-treatment with clothianidin (Poncho 250) and the fungicides fludioxinil, metalaxyl-M, and azoxystrobin.
- CV3. A combination of the following were planted at this site:
  - Dekalb DKC38-03 RIB: seed-treatment with clothianidin (Poncho 250) and ipconazole.
  - Pioneer P8906R: seed-treatment with thiamethoxam (Cruiser® 5FS, 0.25 mg AI/kernel; Syngenta Canada Inc., Guelph, ON), and the fungicides fludioxinil, mefenoxam, thiabendazole, and azoxystrobin.
  - Pioneer 39B23: Liberty Link®, Round-Up Ready, Bt traits; seed-treatment with thiamethoxam (Cruiser 5FS).
  - Pioneer 38B11: Liberty Link; seed-treatment with thiamethoxam (Cruiser 5FS), fludioxinil, metalaxyl-M, and azoxystrobin.
- CV4. A combination of the following were planted at this site:
  - Pickseed 2751GX Rib: Liberty Link, Round-Up Ready, Bt traits; seed-treatment with clothianidin (Poncho 250);
  - Pioneer 38B14: Liberty Link; Bt traits; seed-treatment with thiamethoxam (Cruiser 5FS).

The only field that received any additional pesticide while the bumble bees were in placed was CV1 which received a foliar application of the fungicide pyraclostrobin (Headline® EC, BASF) on 23 July.

Bumble bee colonies

Commercial *B. impatiens* multi-hive colonies, each consisting of three bumble bee colonies (22 × 27 × 15 cm) housed within a weather resistant Styrofoam box (72 × 32 × 21 cm), were obtained from Biobest (Leamington, ON). Each unit was provided with Biogluc® (Bobest, Leamington, ON) as a carbohydrate source, but no pollen supplement was provided. One multi-hive colony was placed directly adjacent to each field (Day 0) when 25–35 % of anthers were dehiscing and silks were visible on over 50 % of 75 randomly selected corn plants. Each multi-hive was elevated 1.25 m on a wooden platform in an area protected from prevailing winds and intense sunlight. Colonies remained in fields for 5–6 days of exposure during corn pollen shed. Since fields were planted with different corn hybrids and at slightly different times, completion of the pollen shed period varied and ranged from 27 July to 9 August across all sites.

On the night of the final day of pollen shed at each field, colony entrances were shut and colonies transported after 21:30 h to a site near Meaford, ON (N44.66354; W80.666839) that was approximately 165 km northeast Guelph and, so far as we are aware, isolated from any crops grown from seeds treated with neonicotinoids by approximately 10 km. At this site bumble bees foraged on a variety of wildflowers. Colonies remained at the Meaford site for 30–35 days and were then returned to the University of Guelph after 21:30 h, where they were placed in a −20 °C freezer and killed.

Data collection

Forager activity

Four times during corn pollen dehiscence (Days 1, 2, 3 and 4), pollinator foraging activity was recorded in corn fields in areas near the bumble bee hives. On each observation period, a stepladder was positioned in four different locations and over a 5 min interval at each location insect activity on nearby tassels (≤2 m away) was recorded, giving a total of 20 min of observation at each site per day. These observations were made between 12:50 and 13:15 h during good foraging conditions. Foraging activity by *B. impatiens,* other wild *Bombus* spp., honey bees, other bees (e.g. Andrenidae, Halictidae), and other insects (e.g., flies, beetles and butterflies) was quantified based on incidence of landing and sustained activity/movement of at least 2 s on corn tassels.

Following exposure in corn fields, on 27 Aug when hives were in the Meaford site the number of *B. impatiens* foragers entering and exiting each hives (three per multi-hive) was recorded over a 5 min period.

Pollen analysis

During the middle of the corn pollen dehiscence period at each site, a total of 18 bees returning with pollen loads were collected at the entrances of each multi-hive at each of the eight corn field locations. Bees were captured individually in a glass jar, labeled, placed in a cooler, returned to the laboratory, and placed in a −20 °C freezer to kill them. Pollen loads (mg) per individual bee were measured, and pollen pellets from each site were thereafter pooled into a sample for subsequent analysis of pollen types. For each pollen sample, a 25:1 suspension in distilled water was prepared in a 10 ml centrifuge tube based on weight (25 parts distilled water, 1 part pollen). Each tube was capped and shaken for 2 min on a vortex mixer, and then left for 2–12 h, depending on the rate of disintegration of the pollen pellets, with occasional shaking by hand. When dissolution of pollen pellets was completed in each sample (visual inspection), the contents were mixed with vortex mixer for an additional 1–2 min to achieve a homogeneous mixture, and a small drop of the pollen preparation was pipetted on to a glass slide. The slide was then warmed on a hot plate (not over 65 °C). A small cube of basic fuchsin stained glycerin jelly was placed on the almost dry pollen preparation and stirred delicately with a needle until the cube is completely melted. A cover glass was placed over the entire preparation, and a drop of melted paraffin and thereafter clear nail polish was used to seal the slide. Five hundred pollen grains per slide were analyzed at 1,000× magnification. Each pollen grain was identified to species, genus, family or pollen type.

Colony assessment

Individual colonies (24 total) were examined separately in each multi-hive. A random number generator was used to determine the order in which colonies were assessed. Each colony was weighed. Workers, drones, and queens were then counted and removed from each colony. Bees were placed on an aluminum tray in a drying room maintained at 60 °C for 48 h and then the total dry weight of all individuals of each caste per hive was measured. The number of honey pots, pollen pots, and brood cells (i.e. cells containing eggs, larvae, or pupae) was also recorded (Heinrich 2004).

Corn pollen collection and residue analysis

To collect corn pollen, a minimum of 15 randomly selected dehiscing tassels were removed from corn plants within a 50 m^2 area near hives at each site. Tassels were place in a cooler and brought to the lab where they were placed in a glass beaker partially filled with tap water. Each beaker with tassels was placed on a piece of white card stock paper, and left on a bench-top at room temperature. Each day tassels were shaken so that pollen would fall on the paper. Pollen was then transferred to a labeled brown glass jar. This process was repeated each day up to approximately until all pollen was released or 3 g of pollen had been collected. Tassels from different fields were placed in different rooms to eliminate the possibility of cross-contamination of samples. Pollen was then sieved to remove debris before residue analysis.

For residue analyses, the samples of pollen were homogenized and fortified with deuterated neonicotinoid insecticides and then extracted with acetonitrile. The resulting acetonitrile extract was subjected to liquid–liquid partitioning with hexane to remove bulk nonpolar co-extracted components. The acetonitrile extract was then further cleaned by performing dispersive solid phase extraction with C18 and PSA (primary–secondary amine) adsorbents. The acetonitrile was evaporated and the residue was reconstituted with a mixture of methanol and water. The final extract was analyzed using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Quantitation was performed using internal standardization with matrix-matched (bee pollen) calibration standards over a range from 0.5 to 25 ng/g (ppb). The limit of detection (LOD) was 0.1 ng/g and the limit of quantification was 0.5 ng/g.

Data analysis

Field site was considered the replicate in the experiment and data from the three colonies in each multi-hive (subsamples) were averaged (Hurlbert 1984; Whitlock and Schluter 2009). There were inadequate degrees of freedom to do a multivariate analysis of variance with the $k = 10$ variables we measured over $n = 4$ replicates, so *t*-tests were conducted on hive endpoints to compare effects of exposure to conventional corn grown from neonicotinoid-treated, to effects of exposure to organic corn grown from untreated seed. Assumptions of normal distribution of the error term (Shapiro–Wilk test) and homogeneity of variance (O'Brien test) were met for these data. A Wilcoxon test was used to analyze data on bee observations in the field (20 min counts on tassels with day as a blocking factor) or post-exposure hive entry and exit counts (hive as a blocking factor) since residual and variance assumptions for these data could not be fulfilled. Values are presented as mean ± standard deviation. All data analyses were done using JMP software (SAS 2012) at the level of $\alpha = 0.05$.

Results

Exposure of bumble bee colonies during pollen shed to organic vs. conventional corn plants had no significant effect on any of the endpoints measured, except the number of workers per colony, where there were significantly more workers recovered from colonies placed next to organic corn fields (Table 1). Overall, all colonies appeared healthy in the field and in the post-exposure site (e.g. workers were regularly and frequently observed exiting and returning to all hives), and upon inspection during dissection, all hives

Table 1 Effects (mean ± SD) on commercial *Bombus impatiens* colonies when exposed during pollen shed to corn (*Zea mays*) grown from conventional seed treated with neonicotinoid insecticide or certified organic seed, Ontario 2013

Endpoint measure (per hive)	Corn seed type		*t* test statistics
	Conventional	Organic	
Hive weight (g)	883.3 (156.2)	843.2 (80.4)	$t_6 = -0.46$, $P = 0.66$
No. honey pots	331.3 (127.8)	270.2 (55.7)	$t_6 = -0.88$, $P = 0.41$
No. pollen pots	32.6 (21.9)	19.2 (5.0)	$t_6 = -1.19$, $P = 0.28$
No. brood cells	554.8 (93.9)	505.0 (54.7)	$t_6 = -0.91$, $P = 0.39$
No. workers	96.0 (15.1)	127.9 (17.2)	$t_6 = 2.80$, $P = 0.032$
Worker weight (g)[a]	6.7 (1.9)	9.1 (1.2)	$t_6 = 2.12$, $P = 0.078$
No. drones	99.5 (41.0)	112.1 (10.6)	$t_6 = 0.59$, $P = 0.57$
Drone weight (g)[a]	7.3 (3.4)	11.1 (1.9)	$t_6 = 1.90$, $P = 0.10$
No. queens	9.2 (2.1)	7.5 (1.2)	$t_6 = -1.41$, $P = 0.21$
Queen weight (g)[a]	3.1 (0.9)	2.2 (0.4)	$t_6 = -1.82$, $P = 0.12$

[a] Total dry weight of all bees

Table 2 Number of insects (mean ± SD) on tassels of dehiscing corn (*Zea mays*) grown from conventional seed treated with neonicotinoid insecticide or certified organic seed, Ontario 2013

Insect	Number insects counted (20 min)		Wilcoxon statistics
	Conventional	Organic	
Bombus impatiens	0.37 (0.76)	0.06 (0.36)	$Z = -1.10$, $P = 0.27$
Other *Bombus*	0.31 (0.54)	0.13 (0.37)	$Z = -1.05$, $P = 0.29$
Apis mellifera	0.06 (0.27)	0.44 (0.82)	$Z = 1.23$, $P = 0.22$
Solitary bees	4.25 (3.61)	1.44 (1.63)	$Z = -2.70$, $P = 0.007$
Other insects	9.00 (4.62)	8.50 (4.18)	$Z = 0.13$, $P = 0.89$

Table 3 Residues of thiamethoxam or clothianidin in pollen collected from corn (*Zea mays*) plants grown from conventional seed treated with neonicotinoid insecticide or certified organic seed, Ontario 2013

Field	Clothianidin (ng/g)	Thiamethoxam (ng/g)
Conventional 1	0.8	<0.1[a]
Conventional 2	0.4	<0.1
Conventional 3	0.1	<0.1
Conventional 4	0.3	<0.1
Organic 1	<0.1	<0.1
Organic 2	<0.1	<0.1
Organic 3	<0.1	<0.1
Organic 4	<0.1	<0.1

[a] Limit of detection = 0.1 ng/g

contained a healthy complement of food stores, brood, and adults.

In our observations of pollinators on corn plants, there was no difference among conventional or organic fields in the number of *B. impatiens*, other *Bombus*, or other insects (beetles, flies) on corn tassels. However, 3-fold more solitary bees (Andrenidae, Halictidae) were counted on dehiscing tassels at conventional fields (Table 2). Following the post-exposure period in corn, there was no difference ($Z = 0.29$, $P = 0.77$) among hives in the number of entries and exits of *B. impatiens* foragers over five minutes, whether hives were from conventional fields (9.4 ± 4.0 entries and exits) or organic fields (11.7 ± 10.3 entries and exits).

We did not detect thiamethoxam in any pollen samples and detected clothianidin in four of the eight samples, ranging from 0.1 to 0.8 ng/g (Table 3). All samples with positive detections were from conventional fields that contained neonicotinoid seed treatments. Pollen samples from organic fields contained no detectable thiamethoxam or clothianidin residues.

Table 4 Floral sources (species, genus, or family) used by commercial *Bombus impatiens* colonies when exposed during pollen shed to corn (*Zea mays*) grown from conventional seed treated with neonicotinoid insecticide or certified organic seed, Ontario 2013

Field	Pollen type[a]	Percentage total pollen
Conventional 1	*Lotus*	42.6
	cf. *Solanum dulcamara*	35.2
	Coronilla	9.6
	Z. mays	0.8
	Other (10)	11.8
Conventional 2	cf. *S. dulcamara*	38.4
	Lotus	34.0
	Type *Trifolium hybridum*[b]	11.2
	Z. mays	1.8
	Other (8)	14.6
Conventional 3	cf. *S. dulcamara*	96.4
	cf. *Hypericum*	1.2
	Type *Taraxacum*[c]	1.0
	Z. mays	0.0
	Other (4)	1.4
Conventional 4	cf. *S. dulcamara*	89.0
	cf. *Medicago sativa*	6.0
	Type *T. hybridum*	1.8
	Z. mays	0.0
	Other (7)	3.2
Organic 1	*Lotus*	31.4
	cf. *S. dulcamara*	23.4
	Type *Taraxacum*	12.4
	Z. mays	0.0
	Other (12)	32.8
Organic 2	cf. *S. dulcamara*	70.8
	Type *Taraxacum*	11.8
	Arctium	7.6
	Z. mays	0.0
	Other (4)	9.8
Organic 3	cf. *S. dulcamara*	67.6
	Type *Taraxacum*	25.6
	Cirsium or *Carduus*	2.2
	Z. mays	0.0
	Other (4)	4.6
Organic 4	cf. *S. dulcamara*	67.2
	Type *Taraxacum*	23.2
	Z. mays	2.6
	cf. *M. sativa*	2.2
	Other (6)	4.8

[a] For brevity, only the top-three floral sources and portion of corn pollen detected in pollen samples are listed. Values in parentheses indicate the number of other pollen types found

[b] May include (share the same palynological features) *T. hybridum, T. agrarium, T. arvense, T. repens,* and *Medicago lupulina*

[c] May include (share the same palynological features) *Taraxacum, Arnoseris, Cichorium, Crepis, Hieracium, Hypochoeris, Lactuca, Lapsana, Leontodon, Picris, Sonchus,* and *Tragopogon*

Analysis of pollen types recovered from worker bees returning to hives found that a very low portion of corn pollen was collected. Corn pollen was recovered from bees at only two conventional sites and a single organic site, and never constituted more than 2.6 % of the total pollen collected (Table 4). Pollen samples recovered from bees at most sites were dominated by *Solanum dulcamara* (bittersweet nightshade), although 10–40 % of some samples consisted of Type *Taraxacum, Lotus* (e.g. bird's-foot trefoil, deervetches), Type *Trifolium hybridum,* or *Coronilla* (Table 4). Depending on the site, pollen from 4 to 12 other floral resources was found in lower amounts.

Discussion

Our field study suggests that exposure to corn grown from neonicotinoid-treated seed during pollen shed poses low risk to *B. impatiens*. This is significant given that neonicotinoid insecticides have been suggested as possible culprits in ongoing bumble bee declines, as they have been suggested as a cause of failing honey bee colonies. Several recent laboratory-based studies have indeed shown that feeding bumble bees food contaminated with neonicotinoids can adversely affect individual bees and colony development (Mommaerts et al. 2010; Cresswell et al. 2012; Gill et al. 2012; Laycock et al. 2012; Whitehorn et al. 2012; Feltham et al. 2014). These controlled experiments are important for the risk assessment process and highlight the potential hazard (but not risk) of neonicotinoids to bumble bees and other pollinators.

However, just as field studies have limitations, laboratory-based experiments have inherent uncertainties that limit their use in risk assessment (OCSPP 2012). Perhaps most important is the uncertainty in the accuracy of feeding in the laboratory to reflect foraging in the field. Even if it is readily accessible in the field, a bee may not choose to forage upon a particular crop depending on characteristics or constraints of floral anatomy, or if the pollen is of poor nutritional value (Winston 1987; Somerville 2001; Heinrich 2004; Willmer 2011). On the other hand, a crop may be an adequate source of forage for a pollinator, but some other floral resource in close proximity may be more favored, or competition with other pollinators could change foraging patterns (Heinrich 2004). Either scenario could reduce the exposure of bees to crop pollen and nectar.

Our results showed that although our bumble bee hives were directly next to corn fields during pollen shed that provided easy access to an abundance of pollen, very little (0–2.6 %; mean of 8 samples = 0.6 %) of the pollen collected off returning forager bees was corn pollen. This indicates that even if corn pollen does contain pesticide residues, as it did in our study, there is a low probability that foragers or bees in the nest (queens, drones, workers and brood) will be exposed to the pesticide via this route. Foragers we collected returning to hives carried pollen from a large number floral sources that were in the landscape. Although corn is often considered not a nutritious pollen source for bees (Somerville 2001), some varieties of maize can produce pollen with relatively high levels of protein (one of the best indicators of nutritional quality) with a good spectrum of essential amino acids (Hoecherl et al. 2012), and corn/maize pollen is used by honey bees (Somerville 2001; Nguyen et al. 2009; Hoecherl et al. 2012; Krupke et al. 2012). It is possible that where there is a dearth of alternative pollen, bumble bees would increase their use of corn pollen, which would increase exposure to pesticides if they were present in the pollen.

Measured concentrations of neonicotinoids in pollen and nectar from crops grown with treated seeds have recently been summarized by the US EPA (OCSPP 2012) and show that mean concentrations of clothianidin in corn pollen range from 2.9 to 3.9 ng/g, whereas residues of thiamethoxam in corn pollen average 1.7 ng/g (Krupke et al. 2012). The levels of clothianidin we detected in corn pollen from conventional sites (0.1–0.8 ng/g; mean of 4 samples = 0.4 ng/g) were similar to, albeit several-fold lower than, levels previously reported from corn grown from treated seed. It is not unusual for there to be high variability in levels of neonicotinoid detected in pollen of a particular crop. For example, Bonmatin et al. (2005) collected samples throughout France from 2000 to 2003 and reported an average concentration of imidacloprid in corn pollen of 2.1 μg/kg (ng/g), with a range of less than 0.1 μg/kg up to 33.6 μg/kg. We did not detect thiamethoxam in pollen samples from conventional sites CV3 and CV4, which were planted with seeds treated with both clothianidin and thiamethoxam. It is probable that the tassels we collected in the field were from those plants treated with clothianidin rather than thiamethoxam. It is also possible that thiamethoxam on treated seed was metabolized to clothianidin, as may occur with foliar sprays or irrigation treatments of thiamethoxam (Dively and Kamel 2012) or on corn seeds treated with thiamethoxam (Pilling et al. 2013). This second possibility seems less likely because in a previous study where thiamethoxam was used as the corn seed treatment the metabolite (clothianidin) was detected in lower levels than the parent compound (Pilling et al. 2013). As expected, we did not detect clothianidin or thiamethoxam in pollen from tassels of organic corn that contained no pesticide treatments.

During the study, all hives qualitatively appeared active and healthy, and the quantitative data support this assessment. The only statistically significant effects we found in our study were that: (1) more solitary bees were observed on tassels in conventional than organic fields; and (2) fewer

workers were recovered from hives placed next to conventional fields. It is unclear what caused these differences. Because so little corn pollen was recovered from foragers returning to our hives and that relatively low amounts of clothianidin were in the corn pollen, we suspect that the difference in number of workers per hive was not due to field treatment. Development of corn plants at organic sites was slower than that at conventional sites, and hives were therefore placed in organic fields approximately a week later than in conventional corn fields. This staggered placement of hives in conventional vs. organic fields might have resulted in hives that differed in worker production. In addition, it rained (~9.6–19.5 mm) on both evenings when bees were collected (21:00–22:00) from conventional sites, whereas it rained (~18.6 mm) only one of two evenings during collections at organic fields (Anonymous 2014). Given that bumble bees may seek shelter under foliage if it is raining and may not immediately not return to their nest (Benton 2006), the additional rain at conventional sites may have meant fewer foraging workers had returned to their hives when they were collected. Whatever the cause, multi-hives from both conventional and organic sites appeared healthy, having approximately 200 workers and drones, multiple queens, and 500 brood cells (eggs, larvae, pupae), suggesting that the lower number of workers detected at conventional fields was not biologically significant.

Because we were working with independent growers, our study lacked strict treatment designations, particularly in our conventional fields. Conventional fields were all consistent in that corn was grown from seeds that express Bt Cry toxins, and were treated with neonicotinoids and fungicides, but specific pesticide treatments used were not identical. Our study therefore might be considered more along the lines of a monitoring study or "quasi-experiment". This admittedly results in uncertainty about conclusions. Nonetheless, information that is useful to pollinator risk assessment can be gleaned from our study. It is important to remember that the ecotoxicological risk assessment process is iterative and involves multiple lines of evidence; laboratory, semi-field, field, monitoring, and modeling studies all have value and all come with different uncertainties. Studies like ours conducted in real agricultural settings provide important exposure and effects data, while being an economical complement to well-controlled laboratory or semi-field studies.

It is also important to emphasize that the result of our study do not necessarily transfer to other cropping systems. For example, bumble bees would likely forage more heavily on canola (oil seed rape) (Turnock et al. 2007; Stanley et al. 2013) than corn, for which neonicotinoids are also used widely as a seed treatment. This would result in increased exposure to neonicotinoids in pollen and nectar, and therefore potentially greater risk, although previous field studies with neonicotinoid seed-treated oil seed rape and sunflower suggests that dietary exposure to these crops is of low risk to bumble bees (Tasei et al. 2001; Thompson et al. 2013). A key component of our study was to quantify exposure of bumble bees to corn pollen. This is significant given the ubiquity of bumble bees throughout temperate holarctic regions (Michener 2007), the dominance of corn (maize) in many of those same landscapes across North America and Europe (FAO 2014), and the widespread use of neonicotinoid seed treatments on corn/maize seed (Jeschke et al. 2011). Thus, despite the potential for bumble bees nesting around corn fields to be heavily exposed to pollen from corn containing neonicotinoid insecticides, we have shown that exposure to corn pollen is probably low in landscapes where other forage is available, resulting in low risk to bumble bees.

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Conflict of interest The authors declare that they have no conflicts of interest.

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