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