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*