

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 (Felltham 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 place 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[®] (Biobest, 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).