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

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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

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).

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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).