

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.

## References

- Anonymous (2014) Historical climate data. Environment Canada. <http://climate.weather.gc.ca/>. Accessed 14 March 2014
- Benton T (2006) Bumblebees. Harper Collins, London
- Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME (2005) Quantification of imidacloprid uptake in maize crops. *J Agric Food Chem* 53:5336–5341
- Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL (2011) Patterns of widespread decline in North American bumble bees. *Proc Natl Acad Sci* 108:662–667
- Colla SR, Packer L (2008) Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. *Biodivers Conserv* 17: 1379–1391
- Colla SR, Szabo ND, Wagner DL, Gall LF, Kerr JT (2013) Response to Stevens and Jenkins’ pesticide impacts on bumblebees: a missing piece. *Cons Letters* 6:215–216
- Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, de Ibarra NH, Smirnoff N, Tyler CR (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology* 115:365–371
- Cresswell JE, Robert F-XL, Florance H, Smirnoff N (2013) Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). *Pest Manag Sci* 70:332–337