

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.

Acknowledgments We are grateful to the various corn growers in Ontario who allowed us to use their fields, and to Drew Mochrie, Melissa Eisen, Alexandra Kruger, Cam Menzies, and Kelly Greig for technical assistance in field and lab. We thank Johanne Parent (Laboratoire BSL, Rimouski, QC) for conducting the floral source (pollen) analysis and Tom Thompson (Residue Chemist, Alberta Agriculture and Rural Development, Agri-Food Laboratories Branch) for performing residue analysis. Funding for this study was through the Natural Sciences and Engineering Research Council Canadian Pollination Initiative (NSERC-CANPOLIN). This is publication no. 118 of NSERC-CANPOLIN.

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

- Dively GP, Kamel A (2012) Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *J Agric Food Chem* 60:4449–4456
- FAO (2014) FAO STAT. Food and Agriculture Organization of the United Nations. <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>. Accessed 7 March 2014
- Feltham H, Park K, Goulson D (2014) Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 23:317–323
- Fischer D, Moriarty T (2011) Pesticide risk assessment for pollinators: summary of a SETAC Pellston workshop. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola
- Franklin MT, Winston ML, Morandin LA (2004) Effects of clothianidin on *Bombus impatiens* (Hymenoptera: Apidae) colony health and foraging ability. *J Econ Entomol* 97:369–373
- Gill RJ, Ramos-Rodriguez O, Raine NE (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491:105–108
- Gonzalez-Varo JP, Biesmeijer JC, Bommarco R, Potts SG, Schweiger O, Smith HG, Steffan-Dewenter I, Szentgyorgyi H, Woyciechowski M, Vila M (2013) Combined effects of global change pressures on animal-mediated pollination. *Trends Ecol Evol* 28:524–530
- Heinrich B (2004) Bumblebee economics. Harvard University Press, Cambridge
- Hoecherl N, Siede R, Illies I, Gaetschenberger H, Tautz J (2012) Evaluation of the nutritive value of maize for honey bees. *J Insect Physiol* 58:278–285
- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol Monogr* 54:187–211
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59:2897–2908
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7:e29268
- Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21:1937–1945
- Malone LA, Scott-Dupree CD, Todd JH, Ramankutty P (2007) No sub-lethal toxicity to bumblebees, *Bombus terrestris*, exposed to Bt-corn pollen, captan and novaluron. *NZ J Crop Hortic Sci* 35:435–439
- Michener CD (2007) The bees of the world. John Hopkins University Press, Baltimore
- Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G (2010) Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* 19:207–215
- Morandin LA, Winston ML (2003) Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ Entomol* 32:555–563
- Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J, Tuirionet B, Verheggen FJ, Berkvens D, De Pauw E, Haubruge E (2009) Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J Econ Entomol* 102:616–623
- OCSPP (2012) White Paper in Support of the Proposed Risk Assessment Process for Bees. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division, Environmental Protection Agency, Washington
- Oliver R (2012) The extinction of the honey bee? *Am Bee J* 152:697–704
- Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I (2013) A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. *PLoS One* 8:e77193
- Porrini C, Sabatini AG, Girotti S, Fini F, Monaco L, Celli G, Bortolotti L, Ghini S (2003) The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. *Bull Insectol* 56:147–152
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25:345–353
- SAS (2012) JMP 10.0. SAS Institute, Cary
- Somerville D (2001) Nutritional value of bee collected pollen. Rural Industries Research and Development Corporation. NSW Agriculture, 176 pp. http://www.nbba.ca/wp-content/uploads/2013/12/Nutritional_Value_of_Bee_Collected_Pollens.pdf. Accessed 10 March 2014
- Stanley DA, Gunning D, Stout JC (2013) Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland: ecological and economic incentives for pollinator conservation. *J Insect Conserv* 17:1181–1189
- Szabo ND, Colla SR, Wagner DL, Gall LF, Kerr JT (2012) Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? *Conserv Lett* 5:232–239
- Tasei JN, Lerin J, Ripault G (2000) Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag Sci* 56:784–788
- Tasei JN, Ripault G, Rivault E (2001) Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J Econ Entomol* 94:623–627
- Thompson H, Harrington P, Wilkins W, Pietravalle S, Sweet D, Jones A (2013) Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions. Food and Environment Research Agency report. <http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371Mar13.pdf>. Accessed 25 March 2014
- Turnock WJ, Kevan PG, Lavery TM, Dumouchel L (2007) Abundance and species of bumble bees (Hymenoptera: Apoidea: Bombinae) in fields of canola, *Brassica rapa* L., in Manitoba: an 8-year record. *J Entomol Soc Ont* 137:31–40
- Vanbergen AJ, Baude M, Biesmeijer JC, Britton NF, Brown MJF, Brown M, Bryden J, Budge GE, Bull JC, Carvel C, Challinor AJ, Connolly CN, Evans DJ, Feil EJ, Garratt MP, Greco MK, Heard MS, Jansen VAA, Keeling MJ, Kunis WE, Marris GC, Memmott J, Murray JT, Nicolson SW, Osborne JL, Paxton RJ, Pirk CW, Polce C, Potts SG, Priest NK, Raine NE, Roberts S, Ryabov EV, Shafir S, Shirley MDF, Simpson SJ, Stevenson PC, Stone GN, Tormans M, Wright GA (2013) Threats to an ecosystem service: pressures on pollinators. *Front Ecol Environ* 11:251–259
- vanEngelsdorp D, Meixner MD (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J Invert Pathol* 103:S80–S95
- vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen YP, Underwood R, Tarpy DR, Pettis JS (2009) Colony collapse disorder: a descriptive study. *PLoS One* 4:e6481
- Velthuis HHW, van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37:421–451
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–352
- Whitlock MC, Schluter D (2009) The analysis of biological data. Roberts and Company Publishers, Greenwood Village
- Williams PH (1998) An annotated checklist of bumble bees with an analysis of patterns of description. *Bull Natl Hist Mus Lond (Ent)* 67:79–152