NEONICOTINOIDS

Chronic exposure to neonicotinoids reduces honey bee health near corn crops

N. Tsvetkov,[1] O. Samson-Robert,[2] K. Sood,[1] H. S. Patel,[1] D. A. Malena,[1] P. H. Gajiwala,[1] P. Maciukiewicz,[1] V. Fournier,[2] A. Zayed[1]*

Experiments linking neonicotinoids and declining bee health have been criticized for not simulating realistic exposure. Here we quantified the duration and magnitude of neonicotinoid exposure in Canada's corn-growing regions and used these data to design realistic experiments to investigate the effect of such insecticides on honey bees. Colonies near corn were naturally exposed to neonicotinoids for up to 4 months—the majority of the honey bee's active season. Realistic experiments showed that neonicotinoids increased worker mortality and were associated with declines in social immunity and increased queenlessness over time. We also discovered that the acute toxicity of neonicotinoids to honey bees doubles in the presence of a commonly encountered fungicide. Our work demonstrates that field-realistic exposure to neonicotinoids can reduce honey bee health in corn-growing regions.

Neonicotinoid insecticides (NNIs) are highly toxic to insects (*1*) and have been implicated in the decline of pollinators (*2*, *3*) and other wildlife (*4*). Many studies that experimentally treated bees with sublethal doses of NNIs documented negative effects on bee health (*5*–*8*). However, these studies have been criticized for using unrealistic doses and duration of exposure (*9*). Although recent surveys have quantified agrochemical residues in several environments (*10*–*12*), they have done so during one or two time periods in the season. We thus lack knowledge of the typical duration that pollinators are exposed to NNIs—a fundamental parameter in ecotoxicology and one that is central to the current debate regarding the safety of NNIs. Addressing this knowledge gap is essential for developing evidenced-based policy on the use of NNIs.

Honey bees (*Apis mellifera*) experienced high colony mortality in Indiana, Ontario, and Québec's corn-growing regions early this decade (*11*, *13*). Corn production represents the largest use of arable land in North America (*14*), and almost all corn is grown from NNI-treated seeds (*15*). The timing of honey bee deaths in Ontario, Québec, and Indiana, along with the presence of NNI residues in dead bees and hives in the spring (*11*, *13*), suggested that NNI-contaminated dust generated during seeding was the main route of acute exposure (*13*). However, in the absence of season-long data, it is impossible to rule out that honey bees are also chronically exposed to sublethal levels of NNIs after planting. Here, we present the findings of a 2-year study that quantified the duration and magnitude of NNI exposure in Canada's corn-growing regions and experimentally evaluated the influence of field-realistic NNI exposure on honey bee health.

We quantified agrochemicals in 55 bee colonies that were randomly allocated to five apiaries close to corn (exposed sites, <500 m) or six apiaries away from agriculture (unexposed sites, >3 km) in 2014. We conducted our study after Canada mandated the use of seed fluency agents (*16*) to reduce NNI-contaminated dust generated during corn planting. We detected 26 different agrochemicals that included miticides, fungicides, herbicides, NNIs, and other insecticides (tables S1 and S5). NNIs included clothianidin, thiamethoxam, imidacloprid, and acetamiprid. We detected agrochemicals in significantly more samples in exposed, relative to unexposed, sites (Welch's t test: $t_{7.92} = -3.48$, $P = 0.008$). NNIs were detected in significantly more time periods in exposed, relative to unexposed, sites ($t_{8.02} = 5.88$, $P < 0.001$); and the period of contiguous exposure to NNIs was longer in exposed (83.4 ± 13.47 SEM days), relative to unexposed, sites (22.7 ± 10.7; $t_{8.07} = 3.53$, $P = 0.007$) (Fig. 1 and fig. S1). Honey bee colonies near corn are thus chronically exposed to NNIs for a substantial proportion of the active season in temperate North America.

Agrochemicals and NNIs were most prevalent in pollen (fig. S2). However, pollen from seed-treated crops was rarely found in NNI-positive samples (1 in 21 for corn and 5 in 21 for soybean), and, when present, it constituted a minute proportion of the pollen grains (0.2% for corn and a mean of 0.6% ± 0.22 SEM for soybeans). Most pollen from NNI-positive samples originated from nontarget plants common in Ontario and Québec (table S2). Our findings are consistent with recent studies that documented NNIs in pollen from bee-attractive wildflowers in the United Kingdom and USA (*12*, *17*).

Although we detected many agrochemicals in 2014, the concentration of NNIs found in bee samples combined with their high toxicity (table S3) rendered them the most likely compounds to influence honey bee health (fig. S3). We carried out an experiment to investigate the effects of clothianidin exposure—the most common NNI found in our study—on honey bees by chronically treating colonies with an artificial pollen supplement containing clothianidin over a 12-week period in 2015. We approximated field-realistic exposure by treating colonies with progressively smaller concentrations of clothianidin, mirroring typical levels found in pollen collected from naturally exposed colonies in 2014 (fig. S4).

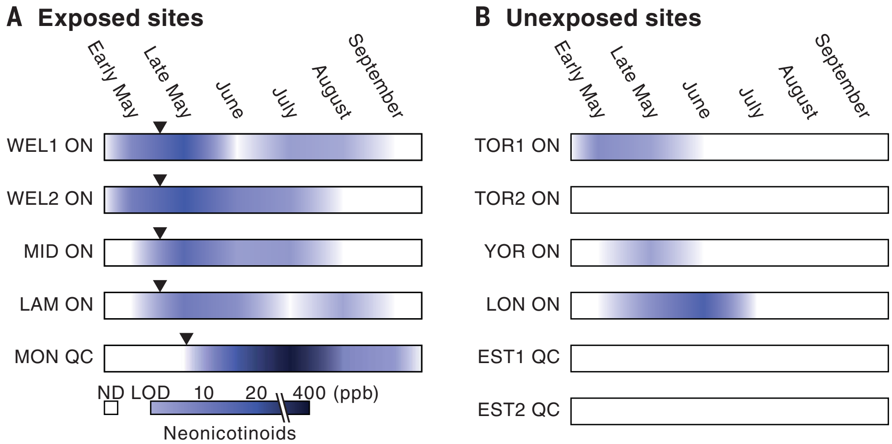

Fig. 1. Honey bees near corn are chronically exposed to neonicotinoids. A heat map showing total NNIs detected in bees and colony food stores in (**A**) exposed and (**B**) unexposed sites. Residues between sampling periods were extrapolated on the basis of adjacent measurements. White areas (ND) represent periods when NNIs were below the limit of detection (<0.4 to 1.1 ppb). Triangles represent corn planting. The NNI detected in Québec (QC) (acetamiprid, LD_{50} = 63,180 ppb) is considerably less toxic to bees than clothianidin and thiamethoxam, and the peak of exposure in Québec in July does not reflect acute exposure.

[1]Department of Biology, York University, 4700 Keele Street, Toronto, M3J 1P3, Ontario, Canada. [2]Centre de Recherche en innovation sur les végétaux, Université Laval, 2480 boulevard Hochelaga, Québec, Québec, G1V 0A6, Canada.
*Corresponding author. Email: zayed@yorku.ca

We first investigated the effect of clothianidin exposure during larval development on adult traits by removing sealed brood from treated and control colonies after the first 3 weeks of exposure and tagging the emerging workers with radio frequency–identification chips before introducing them into a common untreated observation hive. We observed age by treatment differences in the number and duration of flights taken by experimental workers (fig. S5), consistent with previously documented effects of NNIs on navigation in honey bees (*18*). The treated workers, which were exposed to contaminated brood food during the first 9 days of their lives as larvae, had a 23% reduced life span relative to controls (Fig. 2A) [$F_{(1,7)} = 5.78$, $P = 0.047$, $n = 93$]. The presence of sublethal levels of NNIs in colony pollen for 3 to 4 months is thus expected to shorten the life span of many cohorts of workers produced in the spring and summer. The high forager mortality brought upon by chronic sublethal NNI exposure can, in theory, lead to cycles of precocious foraging that reduce colony fitness and cause colony failure (*19*).

We quantified hygienic behavior and the presence of a laying queen in treated colonies and control colonies over the course of our 12-week experiment. We hypothesized that phenotypic effects of exposure—if they exist—should manifest as a function of exposure time (*20*) (i.e., significant treatment by time interactions). We detected a significant treatment by time interaction on hygienic behavior [$F_{(1,23)} = 14.86$, $P = 0.001$, $N = 34$]; the average hygienic behavior of clothianidin-treated colonies decreased over time but that of control colonies did not (Fig. 2B). We observed a similar pattern in the field in 2014, where exposed colonies near corn had significantly lower hygienic behavior relative to unexposed colonies at the end of the season (Fig. 2C) [$F_{(1,48)} = 6.42$, $P = 0.015$, $N = 50$]. Our study is similar to a recent study that found an association between chronic exposure to imidacloprid and reduced hygienic behavior (*21*). Our findings indicate that NNIs impair the honey bee's social immune system.

We also observed a significant treatment by time interaction on queenlessness [generalized linear mixed model (GLMM), $z = 2.242$, $P = 0.025$, $N = 54$] whereby the presence of a laying queen declined over time in the clothianidin-treated group (Fig. 2D). Strong colonies, like many of our controls, typically become queenless in midsummer during swarming season, but then rapidly rear and sustain a replacement queen. However, that pattern of queen loss in treated colonies peaked well after Ontario's swarming period, and most treated colonies were not able to rear replacement queens by the end of our experiment. Our finding is consistent with a recent study (*22*) that documented NNI effects on queen mortality and reproductive physiology. The association between chronic clothianidin exposure and queenlessness is expected to have major consequences on colony fitness, because colonies that are unable to rear replacement queens eventually perish (*23*).

Finally, we studied possible interactions between NNIs and co-occurring agrochemicals on bee health. Clothianidin was most commonly found with herbicides (50%), of which linuron was the most common (31%). Thiamethoxam was commonly found with fungicides (79%), of which boscalid was the most common (45%). We investigated how field-realistic doses of boscalid (mean 497 ppb in pollen) and linuron (mean 7.3 ppb in pollen) influenced the 24-hour oral toxicity of NNIs to honey bee workers. Boscalid and linuron did not, on their own, cause mortality to honey bees at field-realistic doses (0% 24-hour mortality in triplicate trials). Linuron did not influence the median lethal dose (LD_{50}) of clothianidin [generalized linear model (GLM), $z = -0.700$, $P = 0.487$, $N = 45$] or thiamethoxam (GLM, $z = 0.611$, $P = 0.544$, $N = 45$) (Fig. 3). However, boscalid significantly reduced the LD_{50} of clothianidin (GLM, $z = 2.317$, $P = 0.026$, $N = 45$) and thiamethoxam (GLM, $z = 2.060$, $P = 0.046$, $N = 45$) (Fig. 3). Both NNIs became nearly twice as toxic to honey bees in the presence of field-realistic levels of boscalid.

Our study demonstrates that honey bees in corn-growing regions of Canada are exposed to

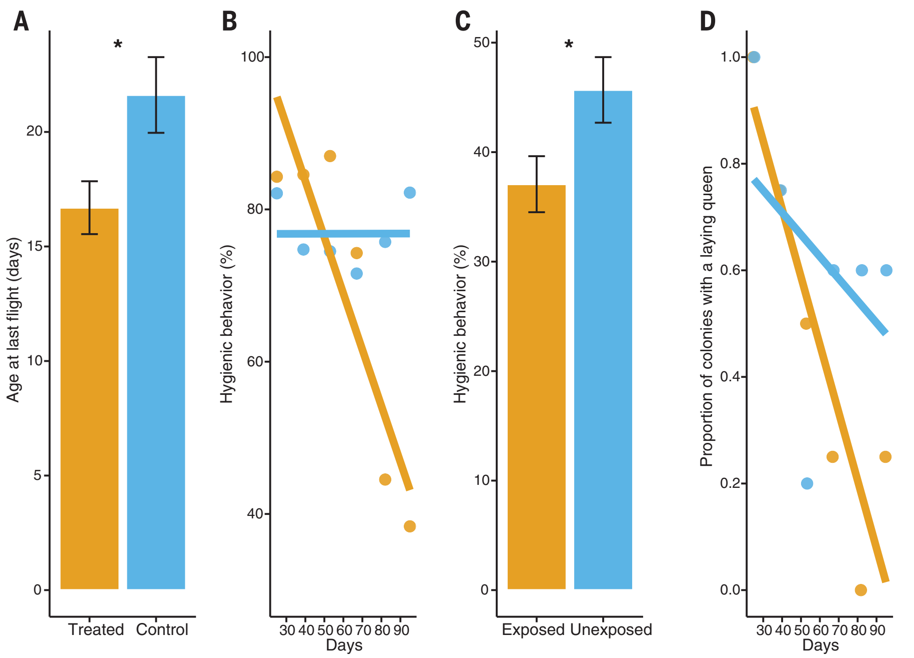

Fig. 2. Chronic clothianidin exposure reduces honey bee health. (**A**) Adults exposed to clothianidin as larvae ($n = 49$) were significantly younger during their final recorded flight relative to controls ($n = 44$). (**B**) We detected a significant treatment by time effect whereby the hygienic behavior of treated colonies ($N = 4$) decreased over time but that of control colonies ($N = 5$) did not. (**C**) Colonies near corn ($N = 25$) had significantly less hygienic behavior relative to colonies away from corn ($N = 25$) at the end of the 2014 season. (**D**) We detected a significant treatment by time effect whereby the number of colonies with a laying queen substantially declined over time in the treated group relative to the control group. Means and SEM. *$P < 0.05$ (see text for details). Yellow and blue indicate treated or exposed and control or unexposed workers or colonies, respectively.

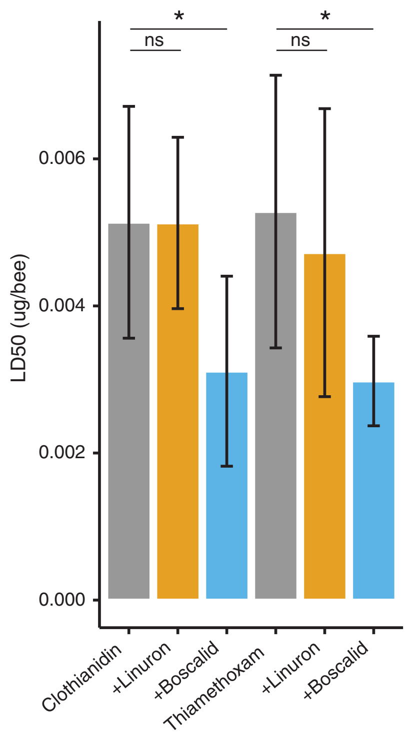

Fig. 3. NNIs are twice as toxic to honey bees in the presence of a common fungicide. The median oral lethal dose (LD_{50}) of the neonicotinoid clothianidin and thiamethoxam are significantly lower in the presence of field-realistic levels of boscalid. Field-realistic levels of the herbicide linuron did not influence NNI toxicity to honey bees. Means and SEM. ns, Not significant; *$P < 0.05$.

toxicologically significant levels of NNIs for the majority of the active bee season despite the mandated use of dust-reducing seed lubricants during planting. Pollen from nontarget plants represents the primary route of exposure to NNIs in our study. Like most bees, honey bees are diet generalists, and it is thus expected that native bees found in Canada's corn-growing regions would be similarly chronically exposed to NNIs. We carried out experiments that approximated field-realistic exposure and found biologically significant effects of clothianidin exposure on honeybee worker morality, hygienic behavior, and the abilities of colonies to sustain a laying queen over time. Finally, we uncovered that the acute toxicity of NNIs to honey bees increases in the presence of field-realistic levels of a common fungicide. Our findings indicate that chronic NNI exposure reduces the health of honey bee colonies near corn crops.

REFERENCES AND NOTES

1. M. Tomizawa, J. E. Casida, *Annu. Rev. Pharmacol. Toxicol.* **45**, 247–268 (2005).
2. D. Goulson, *J. Appl. Ecol.* **50**, 977–987 (2013).
3. M. Rundlöf *et al*., *Nature* **521**, 77–80 (2015).
4. C. A. Hallmann, R. P. Foppen, C. A. van Turnhout, H. de Kroon, E. Jongejans, *Nature* **511**, 341–343 (2014).
5. R. J. Gill, O. Ramos-Rodriguez, N. E. Raine, *Nature* **491**, 105–108 (2012).
6. R. Ramirez-Romero, J. Chaufaux, M. Pham-Delegue, *Apidologie (Celle)* **36**, 601–611 (2005).
7. G. Di Prisco *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 18466–18471 (2013).
8. C. Sandrock *et al*., *PLOS ONE* **9**, e103592 (2014).
9. N. L. Carreck, F. L. Ratnieks, *J. Apic. Res.* **53**, 607–614 (2014).
10. C. A. Mullin *et al*., *PLOS ONE* **5**, e9754 (2010).
11. C. H. Krupke, G. J. Hunt, B. D. Eitzer, G. Andino, K. Given, *PLOS ONE* **7**, e29268 (2012).
12. E. Y. Long, C. H. Krupke, *Nat. Commun.* **7**, 11629 (2016).
13. Pest Management Regulatory Agency (PMRA), Evaluation of Canadian Bee Mortalities in 2013 Related to Neonicotinoids Pesticides: Interim Report as of September 26, 2013 (Health Canada, 2013); www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/fact-sheets-other-resources/evaluation-canadian-mortalities-2013-related-neonicotinoid-pesticides-interim-report.html.
14. M.-A. Hamel, E. Dorff, Canadian Agriculture at a Glance (96-325-X) (Government of Canada, 2014); www5.statcan.gc.ca/olc-cel/olc.action?objId=96-325-X&ObjType=2&lang=en&limit=0.
15. G. Stewart, T. Baute, Neonicotinoids and Field Crop Production in Ontario (2013); www.omafra.gov.on.ca/english/about/beehealthpresentations/omafcrop.htm.
16. PMRA, Pollinator Protection and Responsible Use of Treated Seed–Best Management Practices (Health Canada, 2014); www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/fact-sheets-other-resources/pollinator-treated-seed/best-management-practices.html.
17. C. Botías *et al*., *Environ. Sci. Technol.* **49**, 12731–12740 (2015).
18. M. Henry *et al*., *Science* **336**, 348–350 (2012).
19. C. J. Perry, E. Søvik, M. R. Myerscough, A. B. Barron, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 3427–3432 (2015).
20. T. J. Cleophas, A. H. Zwinderman, *Statistics Applied to Clinical Studies* (Springer, ed. 5, 2012).
21. J. Wu-Smart, M. Spivak, *Sci. Rep.* **6**, 32108 (2016).
22. G. R. Williams *et al*., *Sci. Rep.* **5**, 14621 (2015).
23. H. Shimanuki, K. Flottum, A. Harman, *The ABC & XYZ of Bee Culture* (The A. I. Root Company, Medina, OH, ed. 41, 2006).

ACKNOWLEDGMENTS

This project was funded through Growing Forward 2 (GF2) and a New Directions grant (ND2013-2084) from the Ontario Ministry of Agriculture, Food and Rural Affairs to A.Z. and V.F. We thank G. Thompson and the Toronto Beekeeping Cooperative for help in locating unexposed sites, B. Harpur for blinding the experimenters, F. McCune and J. Parent for assistance. Data can be accessed at Dryad Digital Repository at doi:10.5061/dryad.p039j.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6345/1395/suppl/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 to S5
References (*24*–*50*)

12 January 2017; accepted 9 May 2017
10.1126/science.aam7470

Science

Chronic exposure to neonicotinoids reduces honey bee health near corn crops

N. Tsvetkov, O. Samson-Robert, K. Sood, H. S. Patel, D. A. Malena, P. H. Gajiwala, P. Maciukiewicz, V. Fournier and A. Zayed

Science **356** (6345), 1395-1397.
DOI: 10.1126/science.aam7470

Damage confirmed

Early studies of the impacts of neonicotinoid insecticides on insect pollinators indicated considerable harm. However, lingering criticism was that the studies did not represent field-realistic levels of the chemicals or prevailing environmental conditions. Two studies, conducted on different crops and on two continents, now substantiate that neonicotinoids diminish bee health (see the Perspective by Kerr). Tsvetkov *et al.* find that bees near corn crops are exposed to neonicotinoids for 3 to 4 months via nontarget pollen, resulting in decreased survival and immune responses, especially when coexposed to a commonly used agrochemical fungicide. Woodcock *et al.*, in a multicounty experiment on rapeseed in Europe, find that neonicotinoid exposure from several nontarget sources reduces overwintering success and colony reproduction in both honeybees and wild bees. These field results confirm that neonicotinoids negatively affect pollinator health under realistic agricultural conditions.

Science, this issue p. 1395, p. 1393; see also p. 1331