Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production

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Growing evidence for declines in bee populations has caused great concern due to the valuable ecosystem services they provide. Neonicotinoid insecticides have been implicated in these declines as they occur at trace levels in the nectar and pollen of crop plants. We exposed colonies of the bumble bee *Bombus terrestris* in the lab to field-realistic levels of the neonicotinoid imidacloprid, then allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered an 85% reduction in production of new queens compared to control colonies. Given the scale of use of neonicotinoids, we suggest that they may be having a considerable negative impact on wild bumble bee populations across the developed world.

Bees in agroecosystems survive by feeding on wildflowers growing in field margins and patches of semi-natural habitat, supplemented by the brief gluts of flowers provided by mass flowering crops such as oilseed rape and sunflower (*1*, *2*). Many crops are now routinely treated with neonicotinoid insecticides as a seed dressing; these compounds are systemic, migrating in the sap to all parts of the plant and providing protection against insect herbivores. The most widely used of these compounds in imidacloprid, which is routinely used on most major crops including cereals, oilseed rape, corn, cotton, sunflower and sugar beets (*3*). Being systemic, imidacloprid spreads to the nectar and pollen of flowering crops, typically at concentrations ranging from 0.7-10μg kg^{-1} (*4*, *5*). Thus bee colonies in agroecosystems will be exposed to 2-4 week pulses of exposure to neonicotinoids during the flowering period of crops (*6*).

It is unclear what impact this exposure has on bee colonies under field conditions. A recent meta-analysis based on 13 studies on honey bees found that consumption of realistic doses of imidacloprid under laboratory and semi-field conditions reduced their expected performance by 6 to 20% (*7*), but had no lethal effects. Fewer studies have been carried out on bumble bees and results are conflicting (*8–11*). There is some evidence that low doses of neonicotinoids may reduce foraging ability (*12*), which is likely to have significant impacts under natural conditions, but little effect in cage studies. Although recent studies (*11*) have shown some evidence that neonicotinoids reduced forager success under field conditions, no studies have examined their impacts on colonies foraging naturally in the field. Here we present an experiment, using 75 *Bombus terrestris* colonies, designed to simulate the likely effect

of exposure of a wild bumble bee colony to neonicotinoids present on the flowers of a nearby crop. The colonies were randomly allocated to one of three treatments. Control colonies received *ad lib* pollen and nectar over a period of 14 days in the laboratory. Over the same period, colonies in the 'low' treatment were fed pollen and sugar water containing 6μg kg^{-1} and 0.7μg kg^{-1} imidacloprid respectively, representing the level found in seed-treated rape (*13*). The 'high' treatment colonies received double these doses, still close to the field-realistic range. After two weeks, all colonies were then placed in the field where they were left to forage independently for a period of six weeks while their performance was monitored.

All colonies experienced initial weight gain followed by a decline as they switched from their growth phase to producing new reproductives. Colonies in both the low and high treatments gained less weight over the course of the experiment compared to the control colonies [(Fig. 1); linear mixed effect model; t (568) = -4.03, $P < 0.001$ and t (568) = -5.39, $P < 0.001$ respectively]. By the end of the experiment the low and high treatment colonies were on average 8% and 12% smaller respectively than the control colonies. The weight change in the high treatment colonies was not significantly different from the low treatment colonies [(Fig. 1); linear mixed effect model; t (568) = -1.44, $P = 0.151$]. The rate of colony growth was also dependant on the number of workers present at week 0 [(Table 1); linear mixed effect model; t (568) = 2.61, $P = 0.009$], reflecting the importance of a large workforce for optimal development. No significant differences between treatments were found in the numbers of males, workers, pupae or empty pupal cells at the end of the experiment, although the number of empty pupal cells was 18% and 30% lower, respectively, in low and high treatments compared to controls.

The mean number of queens produced by colonies in the control treatment was 13.72 (5.70), whilst in the low and high treatments it was only 2.00 (1.13) and 1.4 (0.53) respectively [(Fig. 2); Kruskall-Wallis test: H (2) = 9.57, $P = 0.008$]. The drop in queen production is dispro-

Table 1. Linear mixed effect model for colony weight. Parameter estimates are with reference to the control treatment. Degrees of freedom are given in parentheses.

| Fixed effect	Value	SE	t value	P
(Intercept)	564.21	39.59	14.24 (568)	<0.001
Treatment (high)	13.62	27.80	0.490 (71)	0.626
Treatment (low)	13.62	27.11	0.502 (71)	0.617
Week	89.21	5.50	16.22 (568)	<0.001
Week^2	−6.68	0.430	−15.51 (568)	<0.001
No. workers at week = 0	0.759	1.92	0.396 (71)	0.694
Treatment (high)*Week	−13.42	2.49	−5.39 (568)	<0.001
Treatment (low)*Week	−9.95	2.47	−4.03 (568)	<0.001
Week*No. workers at week = 0	0.448	0.172	2.61 (568)	0.009
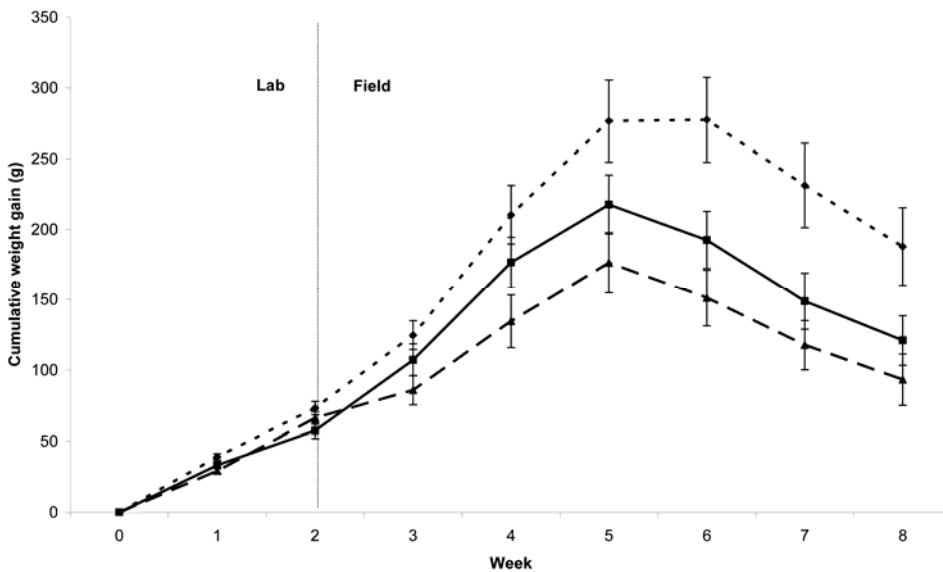
Fig. 1. Mean observed colony weight for the control (short dashed line), low (solid line) and high (long dashed line) treatments at weekly intervals. The change in weight over time was significantly smaller ($P < 0.001$) in the low and high treatment colonies compared to control colonies. The number of colonies per treatment was 25 in weeks 0 to 3. In the following weeks the numbers of colonies in the control, low and high treatments respectively were as follows: week 4 (25, 24, 25), week 5 (25, 24, 25), week 6 (23, 23, 25), week 7 (22, 23, 25) and week 8 (20, 18, 21). Points represent cumulative weight increase since week 0 (and their standard errors); weight includes all accumulated biological material (wax, brood, food stores, adult bees).

portionately large compared to the impact of imidacloprid on colony growth. However, there is evidence that only the very largest bumble bee colonies succeed in producing queens (*15*). For example in field studies of reproduction of 36 colonies of the closely related *Bombus lucorum*, all queen production came from the largest 6 nests (*15*). Thus even a small drop in colony size may bring it below the threshold for queen production. Bumble bees have an annual life cycle and it is only new queens that survive the winter to found colonies in the spring. Our results suggest that trace levels of neonicotinoid pesticides can have strong negative consequence for queen production by bumble bee colonies under realistic field conditions, and this is likely to have a substantial population-level impact.

Our colonies received *ad lib* treated food which could result in them gathering more food and thus receiving higher exposure than they would in the wild. However, bumble bee colonies do not store substantial food reserves in the way that honey bees do, and the period of exposure (2 weeks) is substantially less than the flowering period of crops such as oilseed rape (3-4 weeks), so our experiment is conservative in this respect

We did not study the mechanism underlying the observed effects, but previous lab studies suggest that workers treated with neonicotinoids have reduced foraging efficiency (*12*, *14*). Such effects are likely to be stronger when foragers have to navigate through a natural landscape, and could readily explain reduced colony growth and queen production. Flowering crops such as oilseed rape attract numerous honey bees and a range of species of bumble bee (*16*). Bumble bee and honey bee workers travel a kilometer or more to collect food (*17*, *18*) and in a recent study of a 10 x 20 km rectangle of lowland England, 100% of the land area in a 2007 snapshot was within 1 km of an oilseed rape crop, with rape providing the large majority of all floral resources in the landscape when flowering (*19*). Recent studies describe levels of neonicotinoid up to 88 µg kg^{-1} in pollen collected by honey bees foraging on treated corn (14 times our field-realistic dose), and also demonstrate the presence of up to 9 µg kg^{-1} in wildflowers growing near treated crops, so that exposure is not limited to bees feeding on the crop (*20*). Hence we predict that impacts of imidacloprid on reproduction of wild bumble bee colonies are likely to be widespread and significant, particularly as this chemical is registered for use on over 140 crops in over 120 countries (*3*). As bumble bees are valuable pollinators of crops and wild flowers and vital components of ecosystems, we suggest that there is an urgent need to develop alternatives to the widespread use of neonicotinoid pesticides on flowering crops wherever possible.
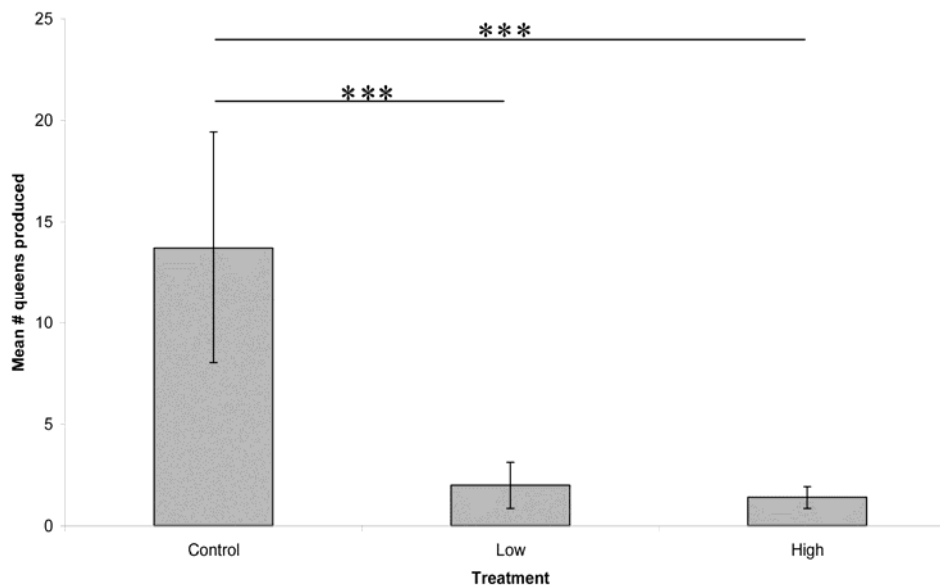
References and Notes
1. T. Diekoetter *et al*., Oilseed rape crops distort plant-pollinator interactions. *J. Appl. Ecol.* **47**, 209 (2010). doi:10.1111/j.1365-2664.2009.01759.x
2. R. D. Fell, Foraging behaviors of *Apis mellifera* L and *Bombus* spp on oilseed sunflower (*Helianthus annuus* L). *J. Kans. Entomol. Soc.* **59**, 72 (1986).
3. A. Elbert, M. Haas, B. Springer, W. Thielert, R. Nauen, Applied aspects of neonicotinoid uses in crop protection. *Pest Manag. Sci.* **64**, 1099 (2008). doi:10.1002/ps.1616 Medline

Fig. 2. The number of new queens produced by the control colonies was greater than the number produced in both the low and high treatment colonies. Bars represent the mean number of queens and their standard errors.

4. C. Brittain, S. G. Potts, The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. *Basic Appl. Ecol.* **12**, 321 (2011). doi:10.1016/j.baae.2010.12.004

5. J. M. Bonmatin *et al.*, A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Anal. Chem.* **75**, 2027 (2003). doi:10.1021/ac020600b Medline

6. R. Schmuck, R. Schöning, A. Stork, O. Schramel, Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag. Sci.* **57**, 225 (2001). doi:10.1002/ps.270 Medline

7. J. E. Cresswell, A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* **20**, 149 (2011). doi:10.1007/s10646-010-0566-0 Medline

8. J. N. Tasei, J. Lerin, G. Ripault, Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag. Sci.* **56**, 784 (2000). doi:10.1002/1526-4998(200009)56:9<784::AID-PS208>3.0.CO;2-T

9. J. A. Gels, D. W. Held, D. A. Potter, Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J. Econ. Entomol.* **95**, 722 (2002). doi:10.1603/0022-0493-95.4.722 Medline

10. L. A. Morandin, M. L. Winston, Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ. Entomol.* **32**, 555 (2003). doi:10.1603/0046-225X-32.3.555

11. J. N. Tasei, G. Ripault, E. Rivault, Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J. Econ. Entomol.* **94**, 623 (2001). doi:10.1603/0022-0493-94.3.623 Medline

12. V. Mommaerts *et al.*, Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* **19**, 207 (2010). doi:10.1007/s10646-009-0406-2 Medline

13. J. M. Bonmatin *et al.*, Quantification of imidacloprid uptake in maize crops. *J. Agric. Food Chem.* **53**, 5336 (2005). doi:10.1021/jf0479362 Medline

14. R. Ramirez-Romero, J. Chaufaux, M. H. Pham-Delegue, Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie (Celle)* **36**, 601 (2005). doi:10.1051/apido:2005039

15. C. B. Müller, P. Schmid-Hempel, Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecol. Entomol.* **17**, 343 (1992). doi:10.1111/j.1365-2311.1992.tb01068.x

16. K. E. Hayter, J. E. Cresswell, The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: implications for landscape-scale gene dispersal. *J. Appl. Ecol.* **43**, 1196 (2006). doi:10.1111/j.1365-2664.2006.01219.x

17. M. E. Knight *et al.*, An interspecific comparison of foraging range and nest density of four bumblebee (Bombus) species. *Mol. Ecol.* **14**, 1811 (2005). doi:10.1111/j.1365-294X.2005.02540.x Medline

18. J. L. Osborne *et al.*, Bumblebee flight distances in relation to the forage landscape. *J. Anim. Ecol.* **77**, 406 (2008). doi:10.1111/j.1365-2656.2007.01333.x Medline

19. D. Goulson *et al.*, Effects of land use at a landscape scale on bumblebee nest density and survival. *J. Appl. Ecol.* **47**, 1207 (2010). doi:10.1111/j.1365-2664.2010.01872.x

20. C. H. Krupke, G. J. Hunt, B. D. Eitzer, G. Andino, K. Given, Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* **7**, e29268 (2012). doi:10.1371/journal.pone.0029268 Medline

Supplementary Materials

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