

# 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 because of the valuable ecosystem services they provide. Neonicotinoid insecticides have been implicated in these declines because they occur at trace levels in the nectar and pollen of crop plants. We exposed colonies of the bumble bee *Bombus terrestris* in the laboratory 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 with 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 seminatural 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 is 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 to 10  $\mu\text{g kg}^{-1}$  (4, 5). Thus bee colonies in agroecosystems will be exposed to 2- to 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 of honey bees found that consumption of realistic doses of imidacloprid under laboratory and semifield 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 substantial impacts under natural conditions but little effect in cage studies. Although recent studies (11)

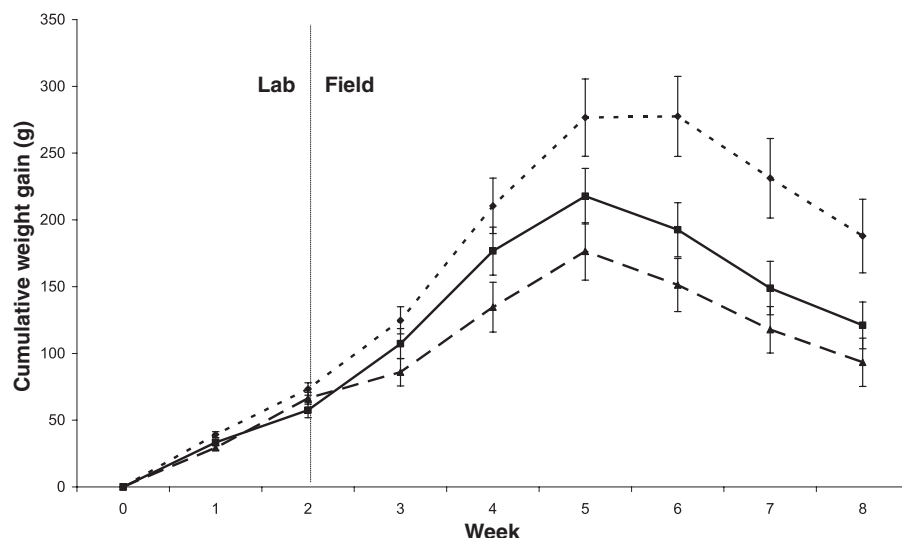
have shown some evidence that neonicotinoids reduced forager success under field conditions, no studies have examined their impacts on colonies foraging naturally in the field. Here, we present an experiment, using 75 *Bombus terrestris* colonies, designed to simulate the likely effect of exposure of 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 libitum (ad lib) pollen and sugar water 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 6  $\mu\text{g kg}^{-1}$  and 0.7  $\mu\text{g kg}^{-1}$  imidacloprid, respectively, representing the levels found in seed-treated rape (13). The “high”-treatment colonies received double these doses, still close to the field-realistic range. After 2 weeks, all colonies were then placed in the field, where they were left to forage independently for a period of 6 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 low and high treatments gained less weight over the course of the experiment compared with the control colonies (Fig. 1) [linear mixed-effect model;  $t(568) = -4.03$  (where the number in parentheses indicates the degrees of freedom),  $P < 0.001$  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 that of the low-treatment colonies (Fig. 1) [linear mixed-effect model;  $t(568) = -1.44$ ,  $P = 0.151$ ]. The rate of colony growth was also dependent on the number of workers present

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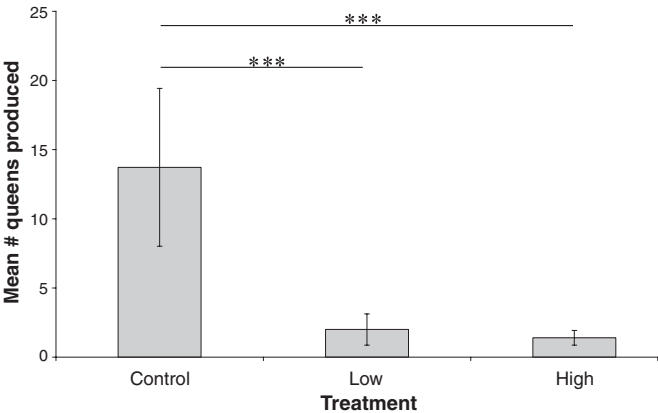
**Fig. 1.** Mean observed colony weight for control (short-dash line), low (solid line), and high (long-dash line) treatments at weekly intervals. The change in weight over time was significantly smaller ( $P < 0.001$ ) in low- and high-treatment colonies compared with control colonies. The number of 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, and 25), week 5 (25, 24, and 25), week 6 (23, 23, and 25), week 7 (22, 23, and 25), and week 8 (20, 18, and 21). Points represent cumulative weight increase since week 0 (and their standard errors); weight includes all accumulated biological material (wax, brood, food stores, and adult bees).



**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	Parameter estimate	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 <sup>2</sup>	−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. 2.** The number of new queens produced by the control colonies was greater than the number produced in both low- and high-treatment colonies. Bars represent the mean number of queens and their standard errors. Asterisks indicate significant differences.



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 19% and 33% lower, respectively, in low and high treatments compared with controls.

The mean number of queens produced by colonies in the control treatment was 13.72 (SE = 5.70), whereas in low and high treatments it was only 2.00 (1.13) and 1.4 (0.53), respectively [Kruskal-Wallis test:  $H(2) = 9.57$ ,  $P = 0.008$ ] (Fig. 2). The drop in queen production is disproportionately large compared with the impact of imidacloprid on colony growth. However, there is evidence that only the very largest bumble bee colonies succeed in producing queens (14). For example, in field studies of reproduction of 36 colonies of the closely related *Bombus lucorum*, all queen production came from the largest six nests (14). 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 consequences for queen production by bumble bee colonies under realistic field conditions and that 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 to 4 weeks), so our experiment is conservative in this respect.

We did not study the mechanism underlying the observed effects, but previous lab studies suggested that workers treated with neonicotinoids have reduced foraging efficiency (12, 15). 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-km-by-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 described levels of neonicotinoid up to 88  $\mu\text{g kg}^{-1}$  in pollen collected by honey bees foraging on treated corn (14 times our field-realistic dose) and also demonstrated the presence of up to 9  $\mu\text{g kg}^{-1}$  in wildflowers growing near treated crops, so 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 important, particularly because this chemical is registered for use on over 140 crops in over 120 countries (3). Because bumble bees are valuable pollinators of crops and wildflowers 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.

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### Bad News for Bees

Neonicotinoid insecticides were introduced in the early 1990s and have become one of the most widely used crop pesticides in the world. These compounds act on the insect central nervous system, and they have been shown to be persistent in the environment and in plant tissues. Recently, there have been controversial connections made between neonicotinoids and pollinator deaths, but the mechanisms underlying these potential deaths have remained unknown. **Whitehorn et al.** (p. 351, published online 29 March) exposed developing colonies of bumble bees to low levels of the neonicotinoid imidacloprid and then released them to forage under natural conditions. Treated colonies displayed reduced colony growth and less reproductive success, and they produced significantly fewer queens to found subsequent generations. **Henry et al.** (p. 348, published online 29 March) documented the effects of low-dose, nonlethal intoxication of another widely used neonicotinoid, thiamethoxam, on wild foraging honey bees. Radio-frequency identification tags were used to determine navigation success of treated foragers, which suggested that their homing success was much reduced relative to untreated foragers.

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