Supplementary Materials for

Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production

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This PDF file includes:

Supporting Online Material

Materials & Methods

Experimental protocol. Commercial *Bombus terrestris audax* colonies (Biobest N.V., Belgium) were randomly assigned to three treatments (control, low and high), with 25 colonies in each treatment. There was no difference in initial colony weight between treatments. The number of worker bees present within the colony at the start of the experiment was recorded to control for variations in initial size (mean: 15.44, range: 5 to 34). Pure imidacloprid (Sigma-Aldrich, UK) was dissolved in a known volume of distilled water and used to dose pollen and sugar water. The low treatment pollen and sugar water contained 6μg kg^{-1} and 0.7μg kg^{-1} imidacloprid respectively. The high treatment pollen and sugar water contained twice these concentrations (12μg kg^{-1} and 1.4μg kg^{-1} imidacloprid respectively). Equivalent volumes of distilled water were added to the control pollen and sugar water. All colonies remained in the laboratory for 14 days, where they were provided with the treated food *ad libitum*, before being transferred to the field where the workers could forage under natural conditions for a further six weeks. The experiment was timed to correspond to wild colony development, with colonies being placed out in the field on 11th July. The field site was situated on the edge of Stirling University campus and close to ornamental gardens, deciduous woodland and mixed farmland, so that scattered patches of wild and ornamental flowers were available within foraging range. Colonies were randomly allocated to locations and evenly distributed across the site. There were no flowering crops within 2 km. The doors on the nestboxes were designed to ensure any new queens produced were not able to leave the colony. The fresh weight of all colonies was recorded at the start of the experiment and weekly thereafter. After field placement the colonies were weighed after dark to minimise disturbance. These nests are housed within a plastic box, in turn placed within a cardboard box. It is not possible to remove the nest material from the inner plastic box without causing severe disturbance. We weighed the inner box and all biological material within, which includes bees, wax, brood, honey pots etc. Any colonies that died during the course of the experiment were collected and stored at -20^{o}C. At the end of the

experiment all colonies were freeze-killed and then dissected and the number of new queens, males, workers, pupae and empty pupal cells present was recorded.

Data analysis. Data were analysed in R, version 2.12.0 (2010 The R Foundation for Statistical Computing). A linear mixed effect model was used to analyse determinants of colony weight. Treatment, week, week^2 (to account for the curved relationship of weight over time) and the number of workers present at week = 0 were entered as fixed effects. The interactions between week and both treatment and the number of workers were also fixed effects and the individual colonies were entered as a random effect. The difference between the number of new queens, males, workers, pupae and empty pupal cells in colonies in each treatment was analysed with Kruskall-Wallis tests.

Results supplement. Colonies in all treatments were in their reproductive, senescent phase by the end of the experiment, (as demonstrated by weight loss in Fig. 1), so it is clear that treated colonies were not delaying queen production to a later date. There were very few queen pupae remaining in nests (3.1 ± 1.38, 0.08 ± 0.08 and 2.5 ± 1.54, means ± SE for control, low and high treatments, respectively, Kruskall-Wallis test, H = 5.09, d.f. = 2, n.s.)