**Supporting Information**

**Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure**

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**Supporting Methods**

***Experimental setup***

A detailed description of the colony setup can be found in Gill et al. ([2012](#_ENREF_4)). This study involved two replicate experiments; 20 colonies arrived on 30th June (WB01-20) and 20 more on 1st September 2011 (WB21-40: supplied by Syngenta Bioline Bees, Weert, the Netherlands). The split block design we employed accounted for any potential seasonal variation between replicates. The day after arrival, colonies were provided with 11ml of untreated 50% sucrose/distilled water solution (volume/volume) in a vertical gravity feeder in the food chamber and 2g of pollen (Koppert Biological Systems) placed in the brood chamber.

Treatment applications were staggered by one day between blocks: treatment started a day earlier in blocks 1-3 than for blocks 4-5. The reason for staggering of applications was because: 1) close monitoring of 20 colonies in the same day was practically problematic; 2) the number of RFID readers available limited the number of colonies we could record at any one time; 3) this ensured that observations and measurements were always taken the same period of time after treatment application for all colonies. Twenty-four RFID readers connected to three hosts (data loggers) monitored the foraging activity of 12 colonies from three blocks at any one time (i.e., eight readers and one host per block). Therefore, reader attachment to colonies was staggered in accordance with the offset of treatment application date between blocks, whereby RFID readers were attached immediately after treatment renewal in each colony. RFID readers remained in place for 24 hours (or 48 hours over a Sunday, when the second 24 hour period was excluded from analyses), after which each pair of readers were attached to a colony from the same treatment group in another. Each pair of readers was always moved between the same two colonies (i.e. readers attached to a control colony in block 1 would be moved to the control colony in block 4, and similarly between blocks 2 and 5). Each colony had its own transparent Perspex tubing to which readers could be easily connected, and the RFID readers were located 10cm (inner reader) and 26cm (outer reader) from the nest box. Outside the window (to which the outlet tube was connected) was a landing platform (below the exit hole) and a unique coloured pattern (above the exit hole) to assist returning workers to orientate back to their nest-box.

**Pesticide treatment**

For the sucrose treatment, we dissolved 100mg of imidacloprid (C9H10CIN5O2 powder; *grade*: PESTANAL®, analytical standard; *brand*: Fluka) in 100ml of acetone to produce a primary stock solution (1mg/ml). An aliquot of the primary stock solution was added to a 40/60% sucrose/water (volume/volume) solution to produce a 10μg/L (10ppb) imidacloprid solution. A control solution was made by repeating this process but using an acetone stock solution (without imidacloprid). For the spray treatment, we dissolved 100mg of λ-cyhalothrin (C23H19CIF3NO3 powder; *grade*: PESTANAL®, analytical standard; *brand*: Fluka) in 100ml of acetone to produce a primary stock solution (1mg/ml). An aliquot of this stock solution was diluted with distilled water to produce a 37.5mg/L (37.5ppm) solution. A control solution was made by repeating this process but using an acetone stock solution (without λ-cyhalothrin).

**Observations and measurements**

RFID tags (Microsensys GmbH: mic3-Tag 64bit read only transponder; *carrier frequency*: 13.56 MHz; *measuring*: 2 x 1.6 x 0.5mm; *mass*: 4mg - so do not affect normal behaviour ([Streit et al. 2003](#_ENREF_9), [Molet et al. 2008](#_ENREF_6), [Decourtye et al. 2011](#_ENREF_1))) were applied with Superglue gel to the thorax of bees immobilised inside a marking cage (E.H. Thornes, UK). Superglue gel allows reliable and rapid attachment of tags, and the speed of adhesion minimises the period during which each bee is immobilised reducing the stress to the workers and colony ([Molet et al. 2008](#_ENREF_6), [Stelzer & Chittka 2010](#_ENREF_8)). Superglued tags very rarely become detached even when using a very small amount of adhesive, minimising loss of RFID activity pattern data (Gill et al. 2012). During the marking stage each tag ID code was recorded using a handheld USB pen reader (iID® PEN mini USB, Microsensys GmbH) allowing us to track the progress of each worker from this point on. The RFID readers were Maja IV reader modules with optimized antenna for mic3 transponders (Microsensys GmbH).

Due to some observed forager movement between colonies, but high consistency within treatment groups ([Gill et al. 2012](#_ENREF_4)), pollen loads were initially assigned to the individual forager that collected it, with this load subsequently being attributed to the forager’s ‘majority colony’ (see below) rather than to the specific colony to which it was brought. Pollen load size was scored relative to the size of the worker that collected it.

During the experiments, local weather conditions (including hourly temperature and daily rainfall data) were recorded at Silwood Park meteorological station.

All queens were free from parasites at the start of the experiment (see Gill *et al.* 2012). Two colonies, both from the M treatment group, failed during the experiment: the queen and three workers from colony WB28 went outside and did not return and the two remaining workers died in the colony on day-8. In colony WB32 the queen died before any worker pupae eclosed (day-3).

Data Analysis

Nineteen workers lost their tag over the 28 days of the experiment. These 19 individuals were found in 15 colonies (no more than 2 individuals lost their tag per colony), and included three foragers (from 3 different colonies). For these three foragers we were unable to definitively match the previous worker tag ID number with the new tag ID number due to worker losses occurring outside the nest. Given there were so few re-tagged foragers (1.2% of all foragers; 0.4% of all tagged workers), we included the three newly tagged workers as separate individuals in the analysis for the foraging patterns. However, we did not include the three retagged foragers in the analysis of the relationship between foraging performance and forager age/experience.

**Supporting Results**

Analysis of the RFID data showed that foragers moved between colonies, a common scenario in social insects, such as bumblebees ([Lopez-Vaamonde et al. 2004](#_ENREF_5)), honeybees ([Pfeiffer & Crailsheim 1998](#_ENREF_7)), and wasps ([Sumner et al. 2007](#_ENREF_10)). In order to deal with this issue, we assigned each forager to the colony it visited during the majority of its foraging bouts (the ‘majority colony’). Using the majority colony as a reference, we then excluded any foragers that had visited colonies from the same treatment group as their majority colony in fewer than 75% of all their foraging bouts (only 3% of all foragers; see Gill et al. 2012). Subsequently, our analysis included only foragers that remained consistent to the same colony treatment group for an average of 97% of their foraging bouts (median (inter-quartiles) = 100% (99-100)). Furthermore, we found no significant difference between treatments in the proportion of orientation mistakes when using the majority colony as a reference (see Gill et al. 2012).

When analysing foraging bout duration we included only those bouts leaving and returning to the same colony. This allowed us to ensure that we were assessing the time bees spent foraging, rather than visiting or inspecting other colonies (trips that could have lasted less than five minutes). We also excluded any foraging bouts when the worker spent the night outside.

Foraging bout duration increased across all treatments as the experiment progressed, possibly because suitable pollen sources were harder to find. Another possible explanation for the increase in bout duration as the experiment progressed could be that as colonies grew, and produced more foragers, individuals became more willing to forage further away from the nest, search for longer to find more rewarding flowers, or simply that they are more willing to continue foraging until they collected enough pollen to completely fill their corbiculae (pollen sacs) as they became more experienced.

**Supplementary Figures and Tables**



**Figure S1.** Forager number as a function of effective colony size. Daily mean colony size (i.e., the cumulative number of workers eclosed, minus the cumulative number of workers found dead) across all colonies over all four treatments (n = 40 colonies) was plotted against the mean number of foragers per colony (n = 28 data points). The graph illustrates that as colonies grew they also sent out more foragers (linear regression: F(1,27) = 73.4, p < 0.001).



**Figure S2**. Forager activity. Daily measures of foraging activity per colony per treatment (left to right; control = 10 colonies; I = 10; LC = 10); M = days 1-3: n = 10; days 4-8: n = 9; days 9-28: n = 8), with a linear regression line plotted with 95% confidence surrounding. Row **A**: number of foragers per colony. Row **B:** number of foraging bouts carried out per forager. Row **C:** mean duration (seconds) of foraging bouts carried out per forager.



**Figure S3**. Each data point indicates the daily mean duration of successful pollen foraging bouts conducted by a single forager. A linear regression line is plotted surrounded by a 95% confidence interval envelope.



**Figure S4**. Weekly analysis (weeks 2, 3 and 4) of pollen collected by foragers from different plant types represented as proportions of all observed successful pollen foraging bouts. Pie charts consist of 18 different types (although a 19th different type was collected by M foragers in week 1, but not presented in this figure) with the numbers linking the pollen type with Table S2. *NB*: the colours in the pie charts are not the actual colours of the pollen brought back.



**Figure S5**. Relationship between pollen load size brought back by foragers and previous forager experience per treatment. The x axis represents a worker’s experience showing the number of days that a worker has been foraging for (i.e. the number of days since the worker first went out foraging). This is plotted against the mean (± s.e.m.) pollen load size brought back by foragers (y axis), to examine whether pollen load size changes as foragers become more experienced.



**Figure S6**. Box and whisker plots showing thorax width of workers that were present before pesticide treatment(s) started (pre-workers), and workers that eclosed during weeks 1, 2, 3 and 4 of the experiment (eclosed workers). The horizontal line in the box represents the median, the box the lower and upper quartiles, the whiskers the 5% and 95% confidence limits, and filled dots indicate outliers.

**Table S1:** Weekly analyses: statistical outputs from a Linear Mixed Effects model (LMER) are comparisons with control colonies (‘intercept’). ‘Queenloss’ indicates loss of the queen within the first two weeks, and ‘days’ accounts for the daily variation found within each week. Z values indicate that the analysis was carried out considering a poisson distribution due to count data. Above the statistic is stated the number of data entries (*n*) and the number of colonies the data is from (*blocks*). Significant effects are shown in bold.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Factors** | **Week1** | | **Week 2** | | **Week 3** | | **Week 4** | |
| A) No. of foragers |  | Z | p | Z | p | Z | p | Z | p |
|  | (n = 276, blocks = 10) | | (n = 267, blocks = 10) | | (n = 266, blocks = 10) | | (n = 266, blocks = 10) | |
| I | 4.932 | **<0.001** | 5.57 | **<0.001** | 7.503 | **<0.001** | 9.678 | **<0.001** |
| LC | 3.443 | **<0.001** | -0.033 | 0.97 | -1.015 | 0.31 | 2.158 | **0.03** |
| M | 6.702 | **<0.001** | 3.675 | **<0.001** | 2.081 | **0.038** | 5.574 | **<0.001** |
| queenloss | -0.18 | 0.86 | 1.733 | **0.083** | 4.976 | **<0.001** | 6.346 | **<0.001** |
| days | 4.24 | **<0.001** | 1.334 | 0.18 | 0.2104 | 0.21 | -3.657 | **<0.001** |
|  |  |  |  |  |  |  |  |  |  |
| B) Foraging bouts |  | Z | p | Z | p | Z | p | Z | p |
|  | (n = 201, blocks = 8) | | (n = 344, blocks = 8) | | (n = 612, blocks = 10) | | (n = 745, blocks = 9) | |
| I | -1.155 | 0.25 | -6.618 | **<0.001** | -0.828 | 0.41 | 1.958 | 0.0502 |
| LC | 1.565 | 0.12 | -2.52 | **0.012** | -1.316 | 0.19 | -4.1 | **<0.001** |
| M | -1.087 | 0.28 | -3.87 | **<0.001** | -6.851 | **<0.001** | -4.408 | **<0.001** |
| queenloss | 1.778 | 0.075 | 0.567 | 0.57 | -3.338 | **<0.001** | -0.893 | 0.37 |
| days | -0.748 | 0.45 | -1.125 | 0.26 | 5.675 | **<0.001** | 3.725 | **<0.001** |
|  |  |  |  |  |  |  |  |  |  |
| C) Foraging bout duration |  | t | p | t | p | t | p | t | P |
|  | (n = 143, blocks = 8) | | (n = 264, blocks = 8) | | (n = 511, blocks = 10) | | (n = 633, blocks = 9) | |
| I | 0.765 | 0.45 | -1.075 | 0.28 | -0.814 | 0.42 | -1.049 | 0.29 |
| LC | 2.988 | **0.0034** | 0.208 | 0.84 | 0.179 | 0.86 | 0.303 | 0.76 |
| M | 2.403 | **0.018** | -0.21 | 0.83 | -1.962 | 0.0503 | -2.373 | **0.02** |
| queenloss | 0.797 | 0.43 | 1.773 | 0.078 | -0.06 | 0.95 | -1.082 | 0.28 |
| days | 2.058 | **0.042** | 0.352 | 0.73 | 5.057 | **<0.001** | 0.011 | 0.99 |
|  |  |  |  |  |  |  |  |  |  |
| D) Pollen load size brought back from all pollen foraging bouts |  |  |  | t | p | t | p | t | p |
|  |  |  | (n = 154, blocks = 8) | | (n = 275, blocks = 9) | | (n = 301, blocks = 9) | |
| I |  |  | -1.928 | 0.056 | -4.967 | **<0.001** | -4.997 | **<0.001** |
| LC |  |  | -0.278 | 0.78 | -0.923 | 0.36 | -0.949 | 0.34 |
| M |  |  | -2.901 | **0.0043** | -2.19 | **0.029** | -5.226 | **<0.001** |
| queenloss |  |  | 0.424 | 0.67 | -0.561 | 0.58 | -0.041 | 0.97 |
| days |  |  | -1.373 | 0.172 | 2.846 | **0.0048** | 0.707 | 0.48 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| E) Pollen load size brought back from successful pollen foraging bouts |  |  |  | t | p | t | p | t | p |
|  |  |  | (n = 43, blocks = 7) | | (n = 82, blocks = 9) | | (n = 78, blocks = 9) | |
| I |  |  | -1.772 | 0.088 | -0.685 | 0.5 | -0.722 | 0.47 |
| LC |  |  | -1.037 | 0.31 | -0.345 | 0.73 | -1.745 | 0.09 |
| M |  |  | -2.641 | **0.014** | -0.96 | 0.34 | -1.011 | 0.32 |
| queenloss |  |  | -0.264 | 0.79 | 1.241 | 0.22 | -0.817 | 0.42 |
| days |  |  | -1.013 | 0.32 | 0.465 | 0.64 | 0.075 | 0.94 |
|  |  |  |  |  |  |  |  |  |  |
| F) Successful pollen foraging bout duration |  |  |  | t | p | t | p | t | P |
|  |  |  | (n = 43, blocks = 7) | | (n = 82, blocks = 9) | | (n = 78, blocks = 9) | |
| I |  |  | 1.383 | 0.18 | 1.118 | 0.27 | 2.332 | **0.02** |
| LC |  |  | 1.64 | 0.11 | -0.333 | 0.74 | 0.786 | 0.44 |
| M |  |  | 1.865 | 0.073 | 1.024 | 0.31 | 2.94 | **<0.01** |
| queenloss |  |  | 0.189 | 0.85 | 1.636 | 0.11 | -0.004 | 0.99 |
| days |  |  | 0.114 | 0.91 | -0.798 | 0.43 | 2.762 | **<0.01** |

**Table S2.** Weekly analyses: statistical outputs from pairwise chi-square tests (χ2) comparing control against each treatment (I, LC and M) groups in terms of the proportion of foraging bouts in which (A) no pollen (unsuccessful) or (B) large pollen loads (size = 3) were collected. Significant treatment group differences are shown in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Comparison** | **Week 2** | | **Week 3** | | **Week 4** | |
|  |  | χ2 | p | χ2 | p | χ2 | p |
| A) Unsuccessful | control vs I | 4.53 | **0.033** | 18.1 | **<0.001** | 44.5 | **<0.001** |
| control vs LC | 0.404 | 0.53 | 0.082 | 0.77 | 0.544 | 0.46 |
| control vs M | 0.823 | 0.36 | 1.21 | 0.27 | 32.2 | **<0.001** |
|  |  |  |  |  |  |  |  |
| B) Large pollen loads | control vs I | 16.3 | **<0.001** | 26.4 | **<0.001** | 62.6 | **<0.001** |
| control vs LC | 0.5 | 0.48 | 1.54 | 0.21 | 11.2 | **0.001** |
| control vs M | 29.4 | **<0.001** | 2.97 | 0.085 | 49.5 | **<0.001** |

**Table S3.** Total number of bouts observed during pollen foraging observations for bees returning with pollen loads. Pie chart no. refers to the numbers given in the pie charts of Figure S4, and is linked with the plant type that matched the colour of pollen collected.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Pie Chart No.*** | ***Plant type (colour)*** | **Control** | **I** | **LC** | **M** | **Total bouts** |
| 1 | Blackberry | 3 | 3 | 2 | 1 | **9** |
| 12 | Blackberry/Raspberry | 0 | 2 | 0 | 0 | **2** |
| 10 | Dandelion | 0 | 1 | 1 | 0 | **2** |
| 2 | Dahlia | 8 | 77 | 10 | 49 | **144** |
| 3 | Himalayan Balsam | 24 | 53 | 23 | 35 | **135** |
| n/a | Himalayan Balsam/Dahlia | 0 | 0 | 0 | 1 | **1** |
| 6 | Himalayan Balsam/Heather | 1 | 0 | 0 | 0 | **1** |
| 17 | Himalayan Balsam/Heather/Raspberry | 0 | 0 | 0 | 1 | **1** |
| 18 | Himalayan Balsam/Michaelmas Daisy | 0 | 1 | 2 | 3 | **6** |
| 8 | Himalayan Balsam/Raspberry | 1 | 4 | 1 | 1 | **7** |
| 11 | Heather | 0 | 1 | 1 | 0 | **2** |
| 9 | Heather/Raspberry | 1 | 0 | 0 | 0 | **1** |
| 4 | Michaelmas Daisy | 28 | 49 | 27 | 39 | **143** |
| 13 | Michaelmas Daisy/Heather | 0 | 1 | 0 | 0 | **1** |
| 5 | Oilseed Rape | 1 | 3 | 15 | 0 | **19** |
| 7 | Raspberry | 3 | 11 | 8 | 5 | **27** |
| 14 | White Clover | 0 | 3 | 2 | 2 | **7** |
| 15 | White Clover/Blackberry | 0 | 0 | 1 | 2 | **3** |
| 16 | Unidentified (white pollen) | 0 | 0 | 1 | 1 | **2** |

**Table S4.** Statistical outputs from chi-square tests (χ2) showing comparisons between control and treatment (I, LC and M) colonies with significant differences shown in bold. Differences in the proportion of foraging bouts bringing back pollen of a colour that matches A) Himalayan Balsam (HB), B) Dahlia (DH) and C) Michaelmas Daisy (MD) are shown.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Comparison** | **All weeks** | |
|  |  | χ2 | p |
| A) HB pollen | control vs I | 2.21 | 0.14 |
| control vs LC | 1.12 | 0.33 |
| control vs M | 2.22 | 0.14 |
|  |  |  |  |
| B) DH pollen | control vs I | 17.1 | **<0.001** |
| control vs LC | 0.0014 | 0.97 |
| control vs M | 13.6 | **<0.001** |
|  |  |  |  |
| C) MD pollen | control vs I | 7.60 | **0.01** |
| control vs LC | 1.37 | 0.24 |
| control vs M | 3.55 | 0.06 |

**Supporting References**

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