**Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting**

Andres N. Arce, Thomas I. David, Emma Randall, Ana Ramos Rodrigues, Thomas J. Colgan, Yannick Wurm, Richard J. Gill

**Supporting Information**

**Supplementary Methods**

*Silwood Park land type*

Silwood Park is 110 ha of non-agricultural parkland consisting of grassland, scrubland, mature woodland, three streams and a small lake. The area surrounding Silwood Park consists of mainly woodland, gardens and a small amount of pasture land. To our knowledge no areas within at least a 5km radius experience intensive farming practices.

*Field colonies*

Twenty *B. t. audax* colonies were provided by a commercial supplier (Agralan Ltd, UK). Within 24 hours of arrival, each colony was transferred from the plastic shipping container to a respective wooden nest box under red light. Each wooden nest box (26 × 16 × 11cm) consists of two equally sized chambers; a front feeding chamber and rear brood chamber, connected by a 15mm diameter hole. The roof of the nest box was made-of two separate Perspex lids, one per chamber, which could be opened to gain access. On arrival from the supplier, colonies spent 48hrs in an environmentally controlled room under red light at 23°C and 65% humidity to settle. Each nest box was then placed inside a separate larger 110L plastic (opaque) box (with lid) to provide protection against weathering and predation. An exit hole (25mm diameter) was present in the front of the feeding chamber which was connected to a corresponding exit hole at the front of the plastic box by a transparent Perspex tube (OD = 25mm; ID = 19mm; see Fig. S1). The tube was flush with the inside wall of the feeding chamber, but extended 200mm from the outside of the plastic box to allow observation of workers leaving and returning. To aid the bees in orienteering and to prevent accidental drifting of bees between next boxes we used brightly coloured blue and yellow tape to mark each box with a unique pattern of horizontal and vertical stripes (Pfeiffer & Crailsheim 1998; Gill, Ramos-Rodriguez & Raine 2012).

Colonies were given two hours after being moved from the laboratory to the field site to settle after which the rubber bung preventing workers leaving the nest was removed. In the feeding chamber each colony was provided with a gravity feeder containing 20ml of 40% sucrose solution. The field location of colonies was first determined by finding 10 locations that were: i) typically protected from the wind; ii) at the interface of highly vegetative habitat and open grassland; iii) facing south east to receive the sun in the morning, but shaded from the midday and afternoon sun.

*Ranking of colony size*

While transferring the colonies to a nest box, each colony was given a rank based on the number of workers and a separate rank based on the number of pupae; the sum of the two ranks was used as a proxy for colony size and used to create matched pairs of equally sized colonies.

*Feeding*

Each colony was assigned two gravity feeders to allow immediate replacement of feeders when replenishing sucrose provisions during the experiment. Therefore, we could then take the substituted feeder from the field and back to the laboratory where we could measure any remaining volume of sucrose solution. Afterwards we thoroughly rinsed the feeders with water before being refilled and placed back in the assigned colony when the next replenishment was due and the other feeder substituted. To calculate the daily volume of provisioned sucrose solution to each colony we estimated that one forager would bring back 1600mg of nectar per day, based on the assumption that foragers would forage for eight hours per day (Gill, Ramos-Rodriguez & Raine 2012), and bring back 200mg nectar per hour (Peat & Goulson 2005). We divided 1600 by 1166 (the weight of 1ml sucrose solution in mg) and multiplied that value by colony size (number of adult workers) estimated for that week (at the start of week 1 this was known to be a mean of 44 workers). We then divided the calculated value by four, because we first made the assumption that approximately half the workers would forage (personal observation), and that our objective was to feed the colony half of their expected sucrose intake to encourage nectar foraging and take into consideration that not all floral resources in a rural landscape would be treated with a pesticide (i.e. presumed that workers would forage 50:50 on treated plants (e.g. crops and horticulture) and untreated plants (e.g. wild flowers)). As in-situ monitoring of colony size was not possible in the field, we used the growth-rate of laboratory colonies (Clarke *et al.* unpublished data) to predict colony size growth to inform the volume of sucrose we should provide during the experiment (see Table S3). However, by the end of week-3 of the experiment, it became apparent that some feeders had sucrose solution remaining, so we did not increase the volume during weeks 4 and 5.

*General observations*

Throughout the observation period (Friday 6th June to Thursday 11th July 2013) the ambient day time temperature remained mild (15.91-28.23 ℃) and well within the range of other studies foraging rate for *B. terrestris* (Peat *et al.* 2005; Peat & Goulson 2005), and the wind speeds remained low (mean wind speed 1.14m s-1, range 0-3.17 m s-1). We found signs of wax moth in all but four colonies, however, equal numbers of control and treated colonies contained wax moth (n = 8 control, 8 treatment) so the presence was not included as a factor in the analysis.

**Supplementary Figures and Tables**

**Figure S1**: Wooden nest box placed inside the plastic 110L box (390 × 685 × 440 mm) for protection in the field. Nest box was connected to the outside of the plastic box by a clear Perspex tube to allow foragers to leave and enter and preventing colony members from getting into the plastic box compartment.

****

**Figure S2:** Image taken by *observer 2* (Thomas I. David) of a returning bee (forager) entering the colony through the transparent Perspex entrance tube. The orange pollen ball, outlined by a red dotted line, in the corbiculae of the hind right leg can be clearly observed.

****

**Table S1:** Selection of studies reporting mean levels and ranges of Clothianidin residues (in ppb) across a range of agricultural settings. OSR=Oilseed Rape, WW = winter wheat, (-) = missing value.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source |  | Mean | Range | Study |
| Plant | Field border flowering plants (<2 days after sowing) | 1.2 | <LOD-5.9 | Rundlöf *et al.* (2015) |
| Field border flowering plants (2 weeks after sowing) | 1 | 0-6.5 | Rundlöf *et al.* (2015) |
| Nectar | Bumblebee collected | 0.08 | 0-0.283 | Thompson *et al.* (2013) |
| Bumblebee collected | 5.4 | 1.4-14 | Rundlöf *et al.* (2015) |
| Honeybee collected | 10.3 | 6.7-16 | Rundlöf *et al.* (2015) |
| Honeybee collected | (-) | 0-2.24 | Cutler and Scott-Dupree (2007) |
| OSR flowers | 2.18 | 0.17-13.24 | Botías *et al.* (2015) |
| OSR nectar and HB honey | 2.3 | <LOD-10.1 | Pohorecka *et al.* (2012) |
| Pollen | Honeybee collected | 13.9 | 6.6-23 | Rundlöf *et al.* (2015) |
| Honeybee collected | 18.33 | 1.1-88 | Krupke *et al.* (2012) |
| Maize pollen | 3.9 | (-) | Krupke *et al.* (2012) |
| OSR flowers | 3.6 | <LOD-11 | David *et al.* (2016) |
| OSR flowers | 2.27 | 0.12-14.5 | Botías *et al.* (2015) |
| OSR pollen and HB pollen bread | 1.8 | <LOD-3.7 | Pohorecka *et al.* (2012) |
| WW margins, wildflowers | 0.5 | <LOD-5 | David *et al.* (2016) |
| Soil | Field margin | 6.57 | 2.25-13.33 | Botías *et al.* (2015) |
| OSR cropland | 13.28 | 5.1-28.6 | Botías *et al.* (2015) |
| WW field margin | 7.71 | 0.41-19.12 | Botías *et al.* (2015) |

**Table S2: a)** Census for each experimental colony prior to the start of the experiment, and **b)** census at the end of the experiment after five weeks in the field. Colonies were assigned into ten pairs based on colony size (assessed by the number of workers and the number of pupae), and each pair was assigned to one of two observers using either a pollen removal method (removal of one pollen load), or photographic method (photograph taken of pollen load). One colony within each pair was randomly assigned to either a treatment or control group.

a)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Observer | Treatment | Colony No. | Pair | Size rank | Worker No. | Pupae No. | Brood weight (g) |
| Pollen removal | Control | 1 | 1 | 8 | 51 | 50 | 44.57 |
| Pollen removal | Control | 3 | 2 | 3 | 36 | 54 | 38.60 |
| Pollen removal | Control | 5 | 3 | 1 | 36 | 38 | 51.04 |
| Pollen removal | Control | 7 | 4 | 2 | 39 | 37 | 28.52 |
| Pollen removal | Control | 9 | 5 | 10 | 58 | 64 | 57.10 |
| Photographic | Control | 11 | 6 | 6 | 42 | 53 | 56.05 |
| Photographic | Control | 13 | 7 | 5 | 57 | 17 | 32.04 |
| Photographic | Control | 15 | 8 | 9 | 45 | 67 | 47.65 |
| Photographic | Control | 17 | 9 | 4 | 46 | 42 | 45.85 |
| Photographic | Control | 19 | 10 | 7 | 45 | 54 | 68.50 |
|  |  |  |  | Mean | 45.50 | 47.60 | 46.99 |
|  |  |  |  | SEM | 2.48 | 4.64 | 3.81 |
|  |  |  |  |  |  |  |  |
| Observer | Treatment | Colony No. | Pair | Size rank | Worker No. | Pupae No. | Brood weight (g) |
| Pollen removal | Treatment | 2 | 1 | 8 | 48 | 55 | 48.40 |
| Pollen removal | Treatment | 4 | 2 | 3 | 37 | 50 | 52.40 |
| Pollen removal | Treatment | 6 | 3 | 1 | 35 | 36 | 39.26 |
| Pollen removal | Treatment | 8 | 4 | 2 | 40 | 47 | 51.56 |
| Pollen removal | Treatment | 10 | 5 | 10 | 51 | 99 | 55.05 |
| Photographic | Treatment | 12 | 6 | 6 | 54 | 41 | 45.82 |
| Photographic | Treatment | 14 | 7 | 5 | 35 | 65 | 36.45 |
| Photographic | Treatment | 16 | 8 | 9 | 45 | 72 | 45.20 |
| Photographic | Treatment | 18 | 9 | 4 | 37 | 53 | 44.43 |
| Photographic | Treatment | 20 | 10 | 7 | 51 | 48 | 41.85 |
|  |  |  |  | Mean | 43.30 | 56.60 | 46.04 |
|  |  |  |  | SEM | 2.32 | 5.77 | 1.88 |

b) After the experiment, each frozen colony was subject to a full census, counting the number of Eggs, Larvae, Pupae and all Workers and Sexuals (gynes and males) in the colony. We also recorded the total weight of the brood (wax structures, Eggs, stored food), Larvae, Pupae, Workers, Sexuals and the original queen (if present).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Brood | Eggs | Larvae | | Pupae | | Workers | | Males | | Gynes | | Original queen | |
| Observer | Treatment | Colony | weight (g) | No. | No. | weight (g) | No. | weight (g) | No. | weight (g) | No. | weight (g) | No. | weight (g) | Presence | weight (g) |
| Pollen removal | Control | 1 | 154.69 | 192 | 235 | 28.04 | 135 | 63.02 | 121 | 30.09 | 19 | 5.93 | 2 | 1.51 | Present | 0.88 |
| Pollen removal | Control | 3 | 151.37 | 103 | 111 | 14.05 | 65 | 20.56 | 56 | 10.12 | 2 | 0.58 | 18 | 15.01 | Present | 0.79 |
| Pollen removal | Control | 5 | 104.36 | 10 | 7 | 0.92 | 38 | 16.32 | 16 | 3.36 | 1 | 0.24 | 0 | 0.00 | Absent | NA |
| Pollen removal | Control | 7 | 84.50 | 99 | 58 | 2.92 | 89 | 25.96 | 34 | 5.25 | 34 | 9.61 | 0 | 0.00 | Absent | NA |
| Pollen removal | Control | 9 | 191.07 | 262 | 50 | 9.05 | 140 | 53.19 | 76 | 14.44 | 14 | 4.66 | 20 | 16.11 | Present | 0.86 |
| Photographic | Control | 11 | 211.45 | 272 | 165 | 30.09 | 186 | 78.03 | 153 | 30.49 | 33 | 10.46 | 28 | 22.21 | Absent | NA |
| Photographic | Control | 13 | 115.18 | 50 | 108 | 4.30 | 101 | 30.88 | 44 | 7.45 | 36 | 10.48 | 1 | 0.88 | Absent | NA |
| Photographic | Control | 15 | 216.00 | 298 | 5 | 1.61 | 92 | 31.46 | 142 | 37.29 | 1 | 0.31 | 2 | 1.11 | Absent | NA |
| Photographic | Control | 17 | 186.28 | 476 | 17 | 5.28 | 134 | 47.97 | 67 | 15.57 | 75 | 23.24 | 0 | 0.00 | Absent | 1.01 |
| Photographic | Control | 19 | 219.97 | 269 | 233 | 32.26 | 179 | 75.29 | 109 | 26.64 | 44 | 14.95 | 1 | 0.63 | Absent | 0.95 |
| Pollen removal | Treatment | 2 | 151.10 | 191 | 345 | 22.17 | 198 | 73.99 | 115 | 22.08 | 2 | 0.54 | 14 | 9.54 | Present | 0.67 |
| Pollen removal | Treatment | 4 | 111.02 | 239 | 80 | 8.31 | 103 | 41.55 | 58 | 12.38 | 22 | 6.89 | 2 | 1.76 | Absent | NA |
| Pollen removal | Treatment | 6 | 54.85 | 0 | 41 | 7.52 | 39 | 16.39 | 23 | 4.72 | 0 | 0.00 | 5 | 3.92 | Absent | NA |
| Pollen removal | Treatment | 8 | 200.07 | 86 | 159 | 15.35 | 154 | 49.84 | 91 | 17.23 | 56 | 16.51 | 8 | 6.19 | Present | 1.02 |
| Pollen removal | Treatment | 10 | 165.32 | 336 | 115 | 13.40 | 224 | 84.60 | 78 | 15.77 | 7 | 2.17 | 0 | 0.00 | Absent | NA |
| Photographic | Treatment | 12 | 42.91 | 132 | 88 | 8.36 | 28 | 9.14 | 41 | 7.42 | 9 | 2.83 | 0 | 0.00 | Present | 0.98 |
| Photographic | Treatment | 14 | 87.50 | 159 | 62 | 8.75 | 17 | 6.20 | 50 | 10.45 | 0 | 0.00 | 0 | 0.00 | Absent | NA |
| Photographic | Treatment | 16 | 163.10 | 272 | 101 | 12.88 | 171 | 54.54 | 67 | 13.27 | 39 | 11.23 | 4 | 3.91 | Absent | 0.93 |
| Photographic | Treatment | 18 | 202.92 | 140 | 124 | 3.10 | 198 | 68.77 | 80 | 13.29 | 58 | 16.94 | 13 | 10.94 | Absent | 0.75 |
| Photographic | Treatment | 20 | 157.18 | 214 | 149 | 18.18 | 197 | 101.26 | 85 | 17.53 | 13 | 3.81 | 1 | 0.75 | Absent | 0.92 |
|  | Control | Mean | 163.49 | 203.10 | 98.90 | 12.85 | 115.90 | 44.27 | 81.80 | 18.07 | 25.90 | 8.04 | 7.20 | 5.75 | - | 0.90 |
|  |  | SEM | 15.57 | 44.44 | 27.72 | 3.97 | 15.04 | 7.12 | 14.88 | 3.83 | 7.42 | 2.32 | 3.33 | 2.69 | - | 0.04 |
|  | Treatment | Mean | 133.60 | 176.90 | 126.40 | 11.80 | 132.90 | 50.63 | 68.80 | 13.41 | 20.60 | 6.09 | 4.70 | 3.70 | - | 0.88 |
|  |  | SEM | 17.96 | 30.38 | 26.92 | 1.80 | 25.15 | 10.31 | 8.46 | 1.61 | 7.12 | 2.08 | 1.68 | 1.27 | - | 0.06 |
|  | Observer 1 | Mean | 136.84 | 151.80 | 120.10 | 12.17 | 118.50 | 44.54 | 66.80 | 13.54 | 15.70 | 4.71 | 6.90 | 5.40 | - | 0.84 |
|  |  | SEM | 14.78 | 34.88 | 32.46 | 2.64 | 20.06 | 7.77 | 11.46 | 2.64 | 5.70 | 1.68 | 2.45 | 1.95 | - | 0.06 |
|  | Observer 2 | Mean | 160.25 | 228.20 | 105.20 | 12.48 | 130.30 | 50.35 | 83.80 | 17.94 | 30.80 | 9.42 | 5.00 | 4.04 | - | 0.92 |
|  |  | SEM | 19.53 | 36.57 | 16.27 | 2.62 | 22.86 | 8.89 | 12.86 | 2.59 | 6.14 | 1.81 | 3.14 | 2.50 | - | 0.05 |

**Table S3:** Volume of provisioned sucrose consumed (to the nearest 0.5ml) at the time of feeder replenishment (the volume of sucrose provisioned shows the volume provided two or three days prior to collection of the feeder).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Days after start of experiment** | | | | | | | | | | | | | | | | |
|  | |  | **3** | **5** | **7** | **10** | **12** | **14** | **17** | **19** | **21** | **24** | **26** | **28** | **31** | **33** | **35** | |
|  | |  | **Volume of sucrose provisioned (ml)** | | | | | | | | | | | | |  |  | |
|  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |
| **Method** | | **Colony** | **45** | **30** | **30** | **67.5** | **45** | **45** | **87** | **58** | **58** | **87** | **58** | **58** | **87** | **58** | **58** | |
|  | | Control | | | | | | | | | | | | | | | | |
| **Pollen removal** | | 1 | 45 | 30 | 30 | 67.5 | 45 | 42 | 25.5 | 30 | 34 | 53 | 20 | 40 | 61 | 58 | 58 | |
| 3 | 45 | 30 | 30 | 67.5 | 45 | 45 | 14 | 14 | 28 | 30 | 18 | 26 | 38 | 45 | 22 | |
| 5 | 45 | 30 | 30 | 67.5 | 45 | 44 | 26 | 7 | 8 | 11 | 14 | 6 | 8 | 14 | 25 | |
| 7 | 45 | 30 | 30 | 67.5 | 31.5 | 12 | 14 | 13 | 12 | 17 | 17 | 13 | 40 | 40 | 28 | |
| 9 | 45 | 30 | 30 | 67.5 | 31 | 39 | 21 | 31 | 31 | 14 | 39 | 28 | 37 | 56.5 | 29 | |
| **Photographic** | | 11 | 45 | 30 | 30 | 67.5 | 45 | 32.5 | 24 | 18 | 21 | 41 | 33 | 58 | 87 | 58 | 58 | |
| 13 | 45 | 30 | 30 | 67.5 | 45 | 39 | 14 | 6 | 6 | 4 | 5 | 6 | 3 | 23 | 58 | |
| 15 | 45 | 30 | 30 | 67.5 | 45 | 45 | 60 | 50 | 56 | 82 | 58 | 58 | 69 | 58 | 58 | |
| 17 | 45 | 30 | 30 | 67.5 | 45 | 45 | 44 | 50 | 54 | 81 | 58 | 56 | 55 | 53.5 | 56 | |
| 19 | 45 | 30 | 30 | 67.5 | 11 | 12 | 23 | 9 | 9 | 10 | 12 | 18 | 51 | 58 | 22 | |
|  | | Treatment | | | | | | | | | | | | | | | | |
| **Pollen removal** | | 2 | 45 | 30 | 30 | 67.5 | 45 | 45 | 61 | 50 | 54 | 61 | 55 | 58 | 87 | 58 | 58 | |
| 4 | 45 | 30 | 30 | 67.5 | 45 | 45 | 77 | 58 | 58 | 40 | 33 | 56 | 46 | 42 | 46 | |
| 6 | 45 | 30 | 30 | 67.5 | 45 | 41.5 | 18 | 6 | 9 | 2 | 7 | 10 | 12 | 27 | 25 | |
| 8 | 45 | 30 | 30 | 67.5 | 43 | 44 | 55 | 48 | 31 | 27 | 19 | 30 | 25 | 15 | 10 | |
| 10 | 45 | 30 | 30 | 67.5 | 36 | 34 | 10 | 21 | 14 | 10 | 39 | 21 | 41 | 35 | 16 | |
| **Photographic** | | 12 | 45 | 30 | 30 | 67.5 | 45 | 45 | 18 | 8 | 17 | 21 | 8 | 12 | 19 | 22 | 58 | |
| 14 | 45 | 30 | 30 | 67.5 | 41 | 45 | 34 | 10 | 13 | 19 | 17 | 22 | 27 | 58 | 58 | |
| 16 | 45 | 30 | 30 | 67.5 | 45 | 45 | 35 | 48 | 53 | 47 | 38 | 55 | 50 | 42 | 58 | |
| 18 | 45 | 30 | 30 | 67.5 | 45 | 43 | 63 | 33 | 18 | 11 | 24 | 22 | 63 | 54 | 17 | |
| 20 | 45 | 30 | 30 | 67.5 | 37 | 17 | 9 | 31 | 46 | 34 | 19 | 43 | 77 | 45 | 22 | |
|  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |
| **Control** | | Mean | 45 | 30 | 30 | 67.5 | 38.9 | 35.6 | 26.6 | 22.8 | 25.9 | 34.3 | 27.4 | 30.9 | 44.9 | 46.4 | 41.4 | |
|  | | SEM | 0.00 | 0.00 | 0.00 | 0.00 | 3.58 | 4.11 | 4.65 | 5.29 | 5.78 | 9.21 | 5.97 | 6.62 | 8.17 | 5.09 | 5.45 | |
| **Treatment** | | Mean | 45 | 30 | 30 | 67.5 | 42.7 | 40.5 | 38 | 31.3 | 31.3 | 27.2 | 25.9 | 32.9 | 44.7 | 39.8 | 36.8 | |
|  | | SEM | 0.00 | 0.00 | 0.00 | 0.00 | 1.12 | 2.82 | 7.76 | 6.12 | 6.17 | 5.82 | 4.80 | 5.87 | 7.92 | 4.74 | 6.48 | |
|  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |
| **Pollen removal** | | Mean | 45 | 30 | 30 | 67.5 | 41.15 | 39.15 | 32.15 | 27.8 | 27.9 | 26.5 | 26.1 | 28.8 | 39.5 | 39.05 | 31.7 | |
|  | | SEM | 0.00 | 0.00 | 0.00 | 0.00 | 1.87 | 3.21 | 7.40 | 5.96 | 5.62 | 6.19 | 4.67 | 5.69 | 7.25 | 5.20 | 5.28 | |
| **Photographic** | | Mean | 45 | 30 | 30 | 67.5 | 40.4 | 36.85 | 32.4 | 26.3 | 29.3 | 35 | 27.2 | 35 | 50.1 | 47.15 | 46.5 | |
|  | | SEM | 0.00 | 0.00 | 0.00 | 0.00 | 3.37 | 3.95 | 5.86 | 5.81 | 6.44 | 8.89 | 6.08 | 6.63 | 8.40 | 4.48 | 5.73 | |

**Table S4**: Example of an observer’s timetable for monitoring foraging behaviour for their 10 assigned colonies. Paired colonies were assigned an observation period randomly, with colonies from one pair always watched consecutively, but the order was also assigned randomly. The first job was to provision the colonies with sucrose, and whilst letting them settle any remaining sucrose in the previously provided feeder was measured. Two spare time slots were left in case previous observations had to be abandoned due to bad weather or for unforeseen circumstances.



***Bumblebee foraging behaviour, model outputs***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S5:** Model outputs for LMER or GLMER for foraging during the day: a) forager activity; b) proportion of foragers bringing back pollen; c) mean weight of pollen; d) mean area of pollen; e) total weight of pollen and; f) total area of pollen. The model and distribution used for each analysis are described in each sub-heading. Variables in the model include; Treatment (Colonies provided with sucrose containing 5 ppb Clothianidin), Observation hour (one of six one hour slots), Wind (Wind speed m/s ), Temperature (⁰C) and the interaction between treatment and observation hour. | | | | | | | | | |
| a) Forager activity – GLMER, Poisson distribution | | | | | | | | | |
|  | Estimate | | S.E. | z value | P | | |  |  |
| (Intercept) | 3.425 | | 0.200 | 17.113 | <0.001 | | |  |  |
| Treatment | -0.258 | | 0.101 | -2.542 | 0.011 | | |  |  |
| Observation hour | -0.112 | | 0.018 | -6.346 | <0.001 | | |  |  |
| Wind | 0.084 | | 0.027 | 3.117 | 0.002 | | |  |  |
| Temperature | -0.022 | | 0.007 | -3.067 | 0.002 | | |  |  |
| Treatment:observation hour | 0.0140 | | 0.028 | 0.510 | 0.610 | | |  |  |
|  |  | |  |  |  | | |  |  |
| b) Proportion of pollen carriers per hour – GLMER, binomial distribution | | | | | | | | | |
|  | Estimate | | S.E. | z value | P | | |  |  |
| (Intercept) | 0.560 | | 0.397 | 1.41 | 0.159 | | |  |  |
| Treatment | -0.191 | | 0.242 | -0.79 | 0.430 | | |  |  |
| Observation hour | 0.170 | | 0.038 | 4.508 | <0.001 | | |  |  |
| Wind | 0.302 | | 0.061 | 4.979 | <0.001 | | |  |  |
| Temperature | -0.038 | | 0.015 | -2.549 | 0.011 | | |  |  |
| Treatment:observation hour | 0.092 | | 0.061 | 1.501 | 0.133 | | |  |  |
|  |  | |  |  |  | | |  |  |
| c) Average weight of pollen per hour – LMER, normal distribution | | | | | | | | | |
|  | Estimate | | S.E. | t | x2 | | | df | P |
| (Intercept) | 3.293 | | 2.405 | 1.369 | 36.49 | | | 4 | <0.001 |
| Treatment | 2.566 | | 2.020 | 1.270 | 2.055 | | | 2 | 0.358 |
| Observation hour | 0.738 | | 0.307 | 2.404 | 11.52 | | | 2 | 0.003 |
| Temperature | 0.495 | | 0.105 | 4.727 | 21.93 | | | 1 | <0.001 |
| Treatment:observation hour | 0.222 | | 0.503 | 0.441 | 0.083 | | | 1 | 0.773 |
|  |  | |  |  |  | | |  |  |
| d) Average area of pollen per hour – LMER, normal distribution | | | | | | | | | |
|  | Estimate | S.E. | | t | | x2 | df | | p |
| (Intercept) | 0.092 | 0.005 | | 16.994 | | 29.425 | 3 | | <0.001 |
| Treatment | 0.011 | 0.008 | | 1.393 | | 18.507 | 2 | | <0.001 |
| observation hour | 0.007 | 0.001 | | 4.914 | | 23.247 | 2 | | <0.001 |
| Treatment:observation hour | -0.007 | 0.002 | | -3.629 | | 11.956 | 1 | | <0.001 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| e) Sum of weight of pollen per hour – LMER, normal distribution | | | | | | |
|  | Estimate | S.E. | t | x2 | df | P |
| (Intercept) | 104.146 | 39.965 | 2.606 | 0.1854 | 3 | 0.980 |
| Treatment | 20.939 | 56.222 | 0.372 | 0.1665 | 1 | 0.683 |
| observation hour | 2.704 | 9.752 | 0.277 | 0.2138 | 2 | 0.899 |
| Treatment:observation hour | -5.618 | 14.717 | -0.382 | 0.4846 | 2 | 0.785 |
|  |  |  |  |  |  |  |
| f) Sum of area of pollen per hour – LMER, normal distribution | | | | | | |
|  | Estimate | S.E. | T | x2 | df | P |
| (Intercept) | 1.033 | 0.207 | 4.979 | 4.067 | 3 | 0.254 |
| Treatment | -0.251 | 0.284 | -0.885 | 3.610 | 2 | 0.165 |
| observation hour | -0.029 | 0.056 | -0.526 | 0.513 | 2 | 0.774 |
| Treatment:observation hour | 0.004 | 0.082 | 0.046 | 0.002 | 1 | 0.962 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S6:** Model outputs for LMER or GLMER for foraging over the five weeks: **a)** forager activity; b) proportion of foragers bringing back pollen; c) average weight of pollen; d)average area of pollen; e)total weight of pollen and ;f)total area of pollen. The model and distribution used for each analysis are described in each sub-heading. Variables in the model include; Treatment (Colonies provided with sucrose containing 5 ppb Clothianidin), Wind (Wind speed m ∙ s-1), Temperature (⁰C), Day (Days since start of experiment), Day2 (A quadratic term applied to days since start of experiment to account for any curved relationship in the data), and the interaction between treatment and Day or Day2. | | | | | | | |
| a) Forager activity per day – GLMER, Poisson distribution | | | | | | | |
|  | Estimate | S.E. | | z value | P |  |  |
| (Intercept) | 1.532349 | 0.287307 | | 5.333 | <0.001 |  |  |
| Treatment | -0.57072 | 0.344289 | | -1.658 | 0.0974 |  |  |
| Day | 0.105955 | 0.013686 | | 7.742 | <0.001 |  |  |
| Day2 | -0.96699 | 0.113594 | | -8.513 | <0.001 |  |  |
| Temperature | -0.03262 | 0.006666 | | -4.894 | <0.001 |  |  |
| Treatment:Day | 0.024734 | 0.01971 | | 1.255 | 0.2095 |  |  |
| Treatment:Day2 | -0.10642 | 0.166313 | | -0.64 | 0.5223 |  |  |
|  |  |  | |  |  |  |  |
| b) Proportion of pollen carriers per day - GLMER, binomial distribution | | | | | | | |
|  | Estimate | S.E. | z value | | P |  |  |
| (Intercept) | -2.58056 | 0.65332 | -3.95 | | <0.001 |  |  |
| Treatment | 1.903 | 0.78477 | 2.425 | | 0.0153 |  |  |
| Day | 0.17989 | 0.02756 | 6.527 | | <0.001 |  |  |
| Day2 | -1.63769 | 0.25815 | -6.344 | | <0.001 |  |  |
| Wind | 0.25376 | 0.06107 | 4.155 | | <0.001 |  |  |
| Temperature | -0.03022 | 0.01477 | -2.046 | | 0.0408 |  |  |
| Treatment:Day | -0.09759 | 0.0412 | -2.368 | | 0.0179 |  |  |
| Treatment:Day2 | 0.90903 | 0.38568 | 2.357 | | 0.0184 |  |  |
|  |  |  |  | |  |  |  |
| c) Average weight of pollen per day – LMER, normal distribution | | | | | | | |
|  | Estimate | S.E. | t value | | X2 | DF | P |
| (Intercept) | 8.573524 | 2.617449 | 3.276 | | 30.898 | 5 | <0.001 |
| Treatment | 4.109516 | 3.449492 | 1.191 | | 27.633 | 4 | <0.001 |
| Day | 0.71196 | 0.282754 | 2.518 | | 28.721 | 4 | <0.001 |
| Day2 | -0.01221 | 0.0072 | -1.696 | | 25.73 | 3 | <0.001 |
| Treatment:Day | 0.131706 | 0.378853 | 0.348 | | 10.646 | 2 | 0.005 |
| Treatment:Day2 | -0.01015 | 0.009556 | -1.063 | | 1.162 | 1 | 0.281 |
|  |  |  |  | |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| d) Average area of pollen per day – LMER, normal distribution | | | | | | |
|  | Estimate | S.E. | t value | X2 | DF | P |
| (Intercept) | 0.0599 | 0.0150 | 3.988 | 20.230 | 4 | <0.001 |
| Treatment | -0.0003 | 0.0075 | -0.034 | 7.193 | 1 | 0.007 |
| Day | 0.0005 | 0.0004 | 1.479 | 3.571 | 2 | 0.168 |
| Temperature | 0.0021 | 0.0006 | 3.399 | 11.094 | 1 | <0.001 |
| Treatment:Day | -0.0007 | 0.0004 | -1.851 | 3.024 | 1 | 0.082 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| e) Sum of weight of pollen per day – LMER, normal distribution | | | | | | |
|  | Estimate | S.E. | t value | X2 | DF | P |
| (Intercept) | -87.262 | 48.526 | -1.798 | 145.7 | 6 | <0.001 |
| Treatment | 57.512 | 63.319 | 0.908 | 2.6795 | 3 | 0.444 |
| Day | 28.902 | 6.092 | 4.744 | 27.952 | 4 | <0.001 |
| Day2 | -0.713 | 0.158 | -4.521 | 23.889 | 2 | <0.001 |
| Wind | 16.994 | 13.484 | 1.26 | 109.16 | 1 | <0.001 |
| Treatment:Day | -10.081 | 8.426 | -1.196 | 2.6659 | 2 | 0.264 |
| Treatment:Day2 | 0.320 | 0.223 | 1.432 | 2.2482 | 1 | 0.134 |
|  |  |  |  |  |  |  |
| f) Sum of area of pollen per day – LMER, normal distribution | | | | | | |
|  | Estimate | S.E. | t value | X2 | DF | P |
| (Intercept) | -0.082 | 0.278 | -0.294 | 58.732 | 6 | <0.001 |
| Treatment | -0.094 | 0.375 | -0.249 | 4.167 | 3 | 0.244 |
| Day | 0.132 | 0.035 | 3.707 | 21.502 | 4 | <0.001 |
| Day2 | -0.003 | 0.0009 | -3.377 | 17.859 | 2 | <0.001 |
| Wind | 0.121 | 0.081 | 1.494 | 29.475 | 1 | <0.001 |
| Treatment:Day | -0.022 | 0.050 | -0.438 | 0.2304 | 2 | 0.891 |
| Treatment:Day2 | 0.0006 | 0.001 | 0.463 | 0.2266 | 1 | 0.634 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S7:** Model output for colony census. All models were LMER or GLMER, using a Gaussian or Poisson distribution | | | | | | | | |
| a) change in colony weight at the end of experiment – LMER, normal distribution | | | | | | | | |
|  | Estimate | S.E. | t value | X2 | DF | | P | |
| (Intercept) | 8.163 | 51.993 | 0.157 | 6.933 | 2 | | 0.031 | |
| Treatment | -26.75 | 17.504 | -1.528 | 2.463 | 1 | | 0.117 | |
| Starting weight | 2.305 | 1.06 | 2.176 | 4.811 | 1 | | 0.028 | |
|  |  |  |  |  |  | |  | |
| b) Number of eggs – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P |  | |  | |
| (Intercept) | 4.117672 | 0.370236 | 11.122 | <0.001 |  | |  | |
| Treatment | -0.09571 | 0.033259 | -2.878 | 0.004 |  | |  | |
| Starting weight | 0.018178 | 0.002774 | 6.553 | <0.001 |  | |  | |
|  |  |  |  |  |  | |  | |
| c) Number of larvae – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P |  | |  | |
| (Intercept) | 3.408253 | 0.245692 | 13.872 | <0.001 |  | |  | |
| Treatment | 0.282645 | 0.043278 | 6.531 | <0.001 |  | |  | |
| Starting weight | 0.020716 | 0.003011 | 6.879 | <0.001 |  | |  | |
|  |  |  |  |  |  | |  | |
| d) Number of pupae – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P |  | |  | |
| (Intercept) | 4.00945 | 0.202987 | 19.752 | <0.001 |  | |  | |
| Treatment | 0.166217 | 0.040875 | 4.067 | <0.001 |  | |  | |
| Starting weight | 0.013385 | 0.002972 | 4.504 | <0.001 |  | |  | |
|  |  |  |  |  |  | |  | |
| e) Number of workers – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P | |  | |  |
| (Intercept) | 3.146467 | 0.24206 | 12.999 | <0.001 | |  | |  |
| Treatment | -0.13047 | 0.052646 | -2.478 | 0.013 | |  | |  |
| Starting weight | 0.024338 | 0.004059 | 5.996 | <0.001 | |  | |  |
|  |  |  |  |  | |  | |  |
| f) Number of drones – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P | |  | |  |
| (Intercept) | 1.259346 | 0.495692 | 2.541 | 0.011 | |  | |  |
| Treatment | -0.26614 | 0.096151 | -2.768 | 0.006 | |  | |  |
| Starting weight | 0.03456 | 0.006872 | 5.029 | <0.001 | |  | |  |
|  |  |  |  |  | |  | |  |
| g) Number of gynes – GLMER, Poisson distribution | | | | | | | | |
|  | Estimate | S.E. | z value | P | |  | |  |
| (Intercept) | -0.14047 | 0.82036 | -0.171 | 0.864 | |  | |  |
| Treatment | -0.43752 | 0.18866 | -2.319 | 0.020 | |  | |  |
| Starting weight | 0.03875 | 0.01588 | 2.44 | 0.015 | |  | |  |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S8:** Model output for colony census including collection method as a fixed effect. | | | | | | | | | | |
| a) Forager activity – GLMER, Poisson distribution | | | | | | | | | | |
|  | Estimate | S.E. | z value | | | P | |  |  | |
| (Intercept) | 3.064867 | 0.126754 | 24.18 | | | < 2e-16 | |  |  | |
| Treatment | -0.25622 | 0.101202 | -2.532 | | | 0.01135 | |  |  | |
| Observation hour | -0.11261 | 0.017672 | -6.372 | | | 1.86E-10 | |  |  | |
| Wind | 0.061489 | 0.020001 | 3.074 | | | 0.00211 | |  |  | |
| Temperature | -0.07083 | 0.023176 | -3.056 | | | 0.00224 | |  |  | |
| Collection method | -0.00985 | 0.151976 | -0.065 | | | 0.94834 | |  |  | |
| Treatment:observation hour | 0.013825 | 0.027535 | 0.502 | | | 0.6156 | |  |  | |
| b) Proportion of pollen carriers per hour – GLMER, binomial distribution | | | | | | | | | | |
|  | Estimate | S.E. | z value | | | P | |  |  | |
| (Intercept) | 0.63253 | 0.40025 | 0.585 | | | 0.5582 | |  |  | |
| Treatment | -0.17196 | 0.24089 | -0.714 | | | 0.4753 | |  |  | |
| Observation hour | 0.17496 | 0.0378 | 4.629 | | | 3.67E-06 | |  |  | |
| Wind | 0.29815 | 0.06064 | 4.917 | | | 8.80E-07 | |  |  | |
| Temperature | -0.03711 | 0.01478 | -2.51 | | | 0.0121 | |  |  | |
| Collection method | -0.39813 | 0.2399 | 1.66 | | | 0.097 | |  |  | |
| Treatment:observation hour | 0.08775 | 0.06116 | 1.435 | | | 0.1513 | |  |  | |
|  |  |  |  | | |  | |  |  | |
| c) Forager activity per day – GLMER, Poisson distribution | | | | | | | | | | | |
|  | Estimate | S.E. | | | z value | | P |  | |  | |
| (Intercept) | 1.536031 | 0.300751 | | | 5.107 | | 3.27E-07 |  | |  | |
| Treatment | -0.57045 | 0.344305 | | | -1.657 | | 0.0976 |  | |  | |
| Day | 0.105962 | 0.013686 | | | 7.742 | | 9.77E-15 |  | |  | |
| Day2 | -0.96705 | 0.113597 | | | -8.513 | | < 2e-16 |  | |  | |
| Temperature | -0.03262 | 0.006666 | | | -4.893 | | 9.94E-07 |  | |  | |
| Collection method | -0.00785 | 0.183423 | | | -0.043 | | 0.9659 |  | |  | |
| Treatment:Day | 0.024719 | 0.019711 | | | 1.254 | | 0.2098 |  | |  | |
| Treatment:Day2 | -0.10631 | 0.166319 | | | -0.639 | | 0.5227 |  | |  | |
|  |  |  | | |  | |  |  | |  | |
| d) Proportion of pollen carriers per day - GLMER, binomial distribution | | | | | | | | | | | |
|  | Estimate | S.E. | | z value | | | P |  | |  | |
| (Intercept) | -2.86665 | 0.62381 | | -4.595 | | | 4.32E-06 |  | |  | |
| Treatment | 1.93999 | 0.78409 | | 2.474 | | | 0.0134 |  | |  | |
| Day | 0.18081 | 0.02726 | | 6.634 | | | 3.27E-11 |  | |  | |
| Day2 | -1.64916 | 0.25824 | | -6.386 | | | 1.70E-10 |  | |  | |
| Wind | 0.25378 | 0.06106 | | 4.156 | | | 3.24E-05 |  | |  | |
| Temperature | -0.03016 | 0.01479 | | -2.04 | | | 0.0414 |  | |  | |
| Collection method | 0.52809 | 0.30572 | | 1.727 | | | 0.0841 |  | |  | |
| Treatment:Day | -0.09943 | 0.0412 | | -2.413 | | | 0.0158 |  | |  | |
| Treatment:Day2 | 0.93676 | 0.38491 | | 2.434 | | | 0.0149 |  | |  | |

Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E. & Goulson, D. (2015) Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees. *Environmental Science & Technology,* **49,** 12731-12740.

Cutler, G.C. & Scott-Dupree, C.D. (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *Journal of Economic Entomology,* **100,** 765-772.

David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E.L., Hill, E.M. & Goulson, D. (2016) Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environment International,* **88,** 169-178.

Gill, R.J., Ramos-Rodriguez, O. & Raine, N.E. (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature,* **491,** 105-U119.

Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G. & Given, K. (2012) Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. *PLoS ONE,* **7,** e29268.

Peat, J., Darvill, B., Ellis, J. & Goulson, D. (2005) Effects of climate on intra- and interspecific size variation in bumble-bees. *Functional Ecology,* **19,** 145-151.

Peat, J. & Goulson, D. (2005) Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behavioral Ecology and Sociobiology,* **58,** 152-156.

Pfeiffer, K.J. & Crailsheim, K. (1998) Drifting of honeybees. *Insectes Sociaux,* **45,** 151-167.

Pohorecka, K., Skubida, P., Miszczak, A., Semkiw, P., Sikorski, P., Zagibajło, K., Teper, D., Kołtowski, Z., Skubida, M., Zdańska, D. & Bober, A. (2012) Residues of Neonicotinoid Insecticides in Bee Collected Plant Materials from Oilseed Rape Crops and their Effect on Bee Colonies. *Journal of Apicultural Science*, pp. 115.

Rundlöf, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederstrom, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J. & Smith, H.G. (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature,* **521,** 77-80.

Thompson, H., Harrington, P., Wilkins, W., Pietravalle, S., Sweet, D. & Jones, A. (2013) Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions. *EFSA Journal*.