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

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## Summary

**1.** Insect pollination is a vital ecosystem service that maintains biodiversity and sustains agricultural crop yields. Social bees are essential insect pollinators, so it is concerning that their populations are in global decline.

**2.** Although pesticide exposure has been implicated as a possible cause for bee declines, we currently have a limited understanding of the risk these chemicals pose. Whilst environmental exposure to pesticides typically has non-lethal effects on individual bees, recent reports suggest that sublethal exposure can affect important behavioural traits such as foraging. However, at present, we know comparatively little about how natural foraging behaviour is impaired and the relative impacts of acute and chronic effects.

**3.** Using Radio-Frequency Identification (RFID) tagging technology, we examined how the day-to-day foraging patterns of bumblebees (*Bombus terrestris*) were affected when exposed to either a neonicotinoid (imidacloprid) and/or a pyrethroid (λ-cyhalothrin) independently and in combination over a four-week period. This is the first study to provide data on the impacts of combined and individual pesticide exposure on the temporal dynamics of foraging behaviour in the field over a prolonged period of time.

**4.** Our results show that neonicotinoid exposure has both acute and chronic effects on overall foraging activity. Whilst foragers from control colonies improved their pollen foraging performance as they gained experience, the performance of bees exposed to imidacloprid became worse: chronic behavioural impairment. We also found evidence, suggesting that pesticide exposure can change forager preferences for the flower types from which they collect pollen.

**5.** Our findings highlight the importance of considering prolonged exposure (which happens in the field) when assessing the risk that pesticides pose to bees. The effects of chronic pesticide exposure could have serious detrimental consequences for both colony survival and also the pollination services provided by these essential insect pollinators.

**Key-words:** bumble bee colony, crop pollination, imidacloprid, insect pollinator, lambda-cyhalothrin, neonicotinoid, pyrethroid

## Introduction

Understanding and mitigating the causes of global insect pollinator declines has important consequences for food security and the global economy (Kremen & Ricketts 2000; Biesmeijer *et al.* 2006; Potts *et al.* 2010). Insect pollinators not only provide an essential ecosystem service for maintaining healthy and diverse wild plant populations (Ollerton, Winfree & Tarrant 2011), but also ensure effective pollination of *c.* 75% of agricultural crop species with an estimated global economic value of over $150 billion per annum (Gallai *et al.* 2009; Hein 2009). Social bees (honeybees, bumblebees and stingless bees) are key insect pollinators (Greenleaf & Kremen 2006; Winfree *et al.* 2007, 2008), so it is particularly worrying that populations have experienced significant declines in recent years (Oldroyd 2007; vanEngelsdorp *et al.* 2008; Goulson, Lye & Darvill 2008; Brown & Paxton 2009; Cameron *et al.* 2011; Burkle, Marlin & Knight 2013). Multiple factors have been implicated as causes of bee declines (Vanbergen *et al.* 2013) including habitat loss (e.g. Carvell *et al.* 2006; Kremen *et al.* 2007), pathogens and disease (e.g. Cox-Foster

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*et al*. 2007; Cameron *et al*. 2011; Meeus *et al*. 2011) and pesticides (e.g. Thompson 2001; Desneux, Decourtye & Delpuech 2007).

Although the risks posed by a 'pesticide exposure landscape' (Osborne 2012) have received a great deal of recent interest, we know comparatively little about the possible impacts that such chemicals may be having on individual bees, their colonies and populations (Godfray *et al*. 2014). As current agricultural practices rely heavily on pesticides to sustain high crop yields, insect pollinators can be exposed to multiple chemicals in the environment. Bees foraging on treated crops are exposed to pesticides both when they touch flowers (topical exposure) to extract nectar or pollen and when consuming these floral rewards (oral exposure). When these bees return to their nest, with pesticide residues on their cuticle and/or in the nectar or pollen they are carrying, other colony members (workers, males and the queen) and brood are also likely to be exposed. Indeed, recent studies report more than 30 different pesticides inside individual honeybee (*Apis mellifera*) colonies (Johnson *et al*. 2010; Mullin *et al*. 2010) and neonicotinoid residues in the nectar stores of bumblebee (*Bombus terrestris*) colonies placed in the field next to oilseed rape fields grown from untreated seed (Thompson *et al*. 2013). The level of pesticide to which bees are exposed depends on the amount applied to the target crop. Pesticide application guidelines are currently informed by a hazard quotient based on ecotoxicological tests assessing the lethal dosage ($LD_{50}$) for a range of indicator taxa (including *A. mellifera* as the only bee species). The objective is to provide application guidelines that kill target pests whilst avoiding lethal effects for essential insect pollinators, such as foraging bees. A growing criticism of this risk assessment procedure is that it does not consider potential sublethal effects (Thompson & Maus 2007), in spite of a growing body of evidence indicating that pesticide exposure, at levels found in treated crops, can lead to behavioural effects in bees (see Thompson 2003; Desneux, Decourtye & Delpuech 2007; Cresswell 2011; Blacquière *et al*. 2012; Gill, Ramos-Rodriguez & Raine 2012) and/or increase their susceptibility to parasites (Alaux *et al*. 2010; Vidau *et al*. 2011; Aufauvre *et al*. 2012; Pettis *et al*. 2012; Fauser-Misslin *et al*. 2014; but see Baron, Raine & Brown 2014).

Social bees rely on the cooperation of many individuals carrying out a multitude of tasks to ensure the colony functions efficiently. Foraging is a fundamental task because colony growth relies on a continuous food supply; therefore, any factors that impair foraging behaviour may have serious consequences for colony survival (Gill, Ramos-Rodriguez & Raine 2012; Bryden *et al*. 2013) and reproduction (Whitehorn *et al*. 2012). Laboratory and semi-field studies of honeybees indicate that exposure to field-realistic pesticide concentrations can cause neuronal inactivation (Palmer *et al*. 2013), affect motor function (Williamson *et al*. 2013), learning performance (e.g. Decourtye *et al*. 2004, 2005; Williamson & Wright 2013), communication (Eiri & Nieh 2012) and also impair homing ability and foraging behaviour (e.g. Yang *et al*. 2008; Mommaerts *et al*. 2010; Henry *et al*. 2012; Schneider *et al*. 2012; Fischer *et al*. 2014). However, the vast majority of studies to date have focused on the behavioural effects that follow acute exposure (i.e. within 48 hours), yet bees in the field are likely to be exposed to pesticide residues over extended periods of time (Garthwaite *et al*. 2012a,b). Therefore, it is important for us to increase our understanding of both the potential acute and chronic effects on individuals induced by prolonged exposure to field pesticide levels.

A recent study by Gill, Ramos-Rodriguez & Raine (2012) examined the effect of chronic exposure to two pesticides (a neonicotinoid and a pyrethroid) on bumblebee (*B. terrestris*) colonies. This study used pesticide exposure levels within the range found in the field, and bees were able to forage freely for pollen and nectar in the field. Using Radio-Frequency Identification (RFID) tagging technology, they collected detailed information on when individual foragers left and re-entered each colony and the amounts of pollen collected. These data showed that overall foraging performance was impaired after prolonged pesticide exposure (4 weeks) with knock-on effects for colony growth. Whilst this study was one of the first to quantify the impact of pesticides on natural foraging behaviour in insect pollinators, it did not report the temporal dynamics of behavioural impairment, nor did it discriminate between acute and chronic exposure effects. Such information is important because it (i) improves our understanding of how persistent sublethal pesticide exposure might affect the efficiency of beneficial pollinators; (ii) identifies whether subtle pesticide induced behavioural impairments might accumulate over time; and (iii) can be used to inform risk assessment protocols about the appropriate time period over which ecotoxicological testing should be conducted to detect sublethal effects and subsequently minimize the risks of pesticide exposure for foraging bees.

Here, we present a detailed analysis of the day-to-day foraging patterns of 259 *B. terrestris* foragers (Fig. 1) from 40 colonies over 28 days in the field. In this analysis, we examine how the temporal dynamics of foraging behaviour are affected following prolonged exposure to either a neonicotinoid (imidacloprid), a pyrethroid (λ-cyhalothrin), or the combination of both pesticides. Colonies were exposed to these two commonly used pesticides at levels approximating field exposure over a 4-week period (Gill, Ramos-Rodriguez & Raine 2012). Our results provide new insights, showing that prolonged pesticide exposure has both acute and chronic effects on fundamental aspects of forager behaviour and performance.

## Materials and methods

### EXPERIMENTAL SET-UP

The forty *B. terrestris* colonies used in the experiment each had a queen and an average of four workers (range = 0–10) at the start

**Fig. 1.** *Bombus terrestris* worker foraging on a Dahlia flower (photo: RJG).

of the experiment (day-0). These colony sizes reflect a realistic developmental stage of natural colonies when many agricultural crops come into flower in Europe (see Thompson 2001; Brittain & Potts 2011) and when the majority of pesticide treatments are applied (March to June: Garthwaite *et al.* 2012a,b). We used a split block design to control for variation in colony size. Before the experiment began, we ranked colonies by size according to the number of workers and pupae present, with the four highest ranked (largest) colonies being assigned to block 1, the next four highest ranked to block 2, and so on. Within each block, the four treatments [*n* colonies: control = 10; imidacloprid (*I*) = 10; λ-cyhalothrin (*LC*) = 10; imidacloprid and λ-cyhalothrin: mixed (*M*) = 10] were randomly assigned among the four colonies, and we confirmed there was no significant difference among treatments in colony size (Kruskal–Wallis: $H = 1{\cdot}79$, $P = 0{\cdot}62$).

Colonies were each housed in a two-chambered wooden nest box (28 × 16 × 11 cm). The rear chamber housed the nest (the 'brood chamber'), and a front chamber was used for pesticide exposure (the 'food chamber'). Colonies were kept at room temperature in a naturally lit laboratory throughout the experiment (although the brood chamber was covered when not being observed to mimic the darkness of a subterranean nest). Nest boxes were connected to the outside environment through an outlet tube leading to an exit hole in the laboratory window, allowing natural foraging behaviour. The laboratory is situated on the Royal Holloway University of London campus in Egham, Surrey (a 135 acre parkland site containing a diversity of wild and horticultural flowers), with further parkland areas, abundant privately owned gardens and some agricultural land adjacent to the campus within flight range of *B. terrestris*. Running the experiment from July onwards, however, minimized worker exposure to pesticides in the environment outside the laboratory as application to flowering crops visited by bees is low at this time of year (Garthwaite *et al.* 2012a,b), and the agricultural land within bumblebee flight range of campus did not contain a flowering crop during the experimental period.

Between the outlet tube and nest box were three sections of transparent Perspex tubing allowing us to observe the bees as they left or entered the nest box (setup described in Gill, Ramos-Rodriguez & Raine 2012). Between these three tube sections were two RFID readers that automatically monitored the passage of all tagged workers as they entered and left the colony. Two RFID readers were required per colony to establish whether the bee was entering or leaving the nest box, recording the tag (bee) ID number and exact time it passed underneath with at least 99% accuracy (Molet *et al.* 2008), with minimal disturbance to natural foraging patterns.

## PESTICIDE TREATMENT

In the food chamber was a gravity feeder (used for the sucrose treatment) placed on a petri dish (90 mm diameter) lined with filter paper (used for the spray treatment). Bees did not have to collect sucrose solution from the feeder as they had free access to collect nectar from flowers in the field; nor did bees have to walk over the filter paper lining the petri dish as they had enough room to walk around the dish. Thus, all bees could choose to ignore the filter paper and sucrose solution feeder.

The feeder contained either a control sucrose solution (control and *LC* colonies) or 10 ppb imidacloprid sucrose solution (*I* and *M* colonies). This concentration falls within the range found in the pollen and nectar of flowering crops visited by bees (also see Gill, Ramos-Rodriguez & Raine 2012). During the experiment, the sucrose treatment was applied every 2 days (or 3 days over the weekends) between 13:00 and 14:00 ($n = 12$ feeder replenishments per colony during the 28-day period). We provided 10 mL of sucrose treatment per application in week 1, with a 2 mL increment at the start of each subsequent week (week 2 = 12 mL, week 3 = 14 mL and week 4 = 16 mL) to reflect an increase in colony demand as they developed. Before sucrose feeders were refilled, they were thoroughly rinsed and dried to remove any remaining residues.

The spray treatment was applied using a hand sprayer following the E.P.A. OPPTS 850:3030 application guidelines (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0154-0017). The filter paper received $0{\cdot}69 \pm 0{\cdot}05$ mL of either a control solution (control and *I* colonies) or a 37·5 ppm λ-cyhalothrin solution (*LC* and *M* colonies), the maximum label-guidance concentration for spray application to oilseed rape in the UK. Spray treatments were applied once at the start of each experimental week using a new piece of filter paper for each application. This follows label guidance for the minimum time period between re-applications of λ-cyhalothrin to crops (i.e. at least 7 days between spraying events and a maximum of four applications within the flowering season).

## OBSERVATIONS AND MEASUREMENTS

### *Colony inspections, feeding and monitoring foraging performance*

Colonies were inspected once per day from Monday to Saturday to assess the number of newly eclosed (callow) workers, the number of dead workers (removed and frozen) and queen condition. All dead workers and newly eclosed males ($n = 4$ males) were removed and frozen (−20 °C). The volume of sucrose solution we provided colonies was *c.* 50% of the sugar that would be typically brought back by foragers (assuming colonies having an average of 9–10 foragers and each foraging for 8 h day$^{-1}$: Peat & Goulson 2005; Raine & Chittka 2008; Gill, Ramos-Rodriguez & Raine 2012). Additionally, colonies were not provided with any pollen during the experiment. Therefore, bees had to collect all their pollen and *c.* 50% of their nectar (sugar) from real flowers in the field.

All workers present at the start of the experiment (precise age unknown) were individually tagged with RFID transponders glued

to the dorsal part of the thorax (for details see Supporting Information). Similarly, during the experiment, all newly eclosed workers were tagged within 1–3 days of eclosion (precise age known). In total, 854 workers were tagged, with each tag providing a unique (16-digit) code for unambiguous identification. We used separate sets of equipment (forceps and marking cages) to tag and handle the bees from each treatment to prevent artificial cross-contamination. Any workers that eclosed between day 26 and day 28 ($n$ = 206) were not tagged, because they would be very unlikely to forage before the end of the experiment (Goulson 2010). Workers that lost their tag during the experiment ($n$ = 19 bees: 2·2% of tagged individuals) were re-tagged with a new tag as soon as tag loss was observed. We classified a foraging bout as a period of at least five minutes between a worker leaving and re-entering a colony and specified that workers must perform at least four foraging bouts during the 28-day experiment to be considered a forager (see Gill, Ramos-Rodriguez & Raine 2012). We set this threshold to ensure that our analyses only included motivated foragers (excluding workers that only explored the tube or the vicinity outside the laboratory window).

Pollen foraging was observed for 1 h day$^{-1}$ (5 days week$^{-1}$) for each colony. Observation periods were always two (*c*. 16:00) and 21 h (*c*. 10:00 the following day) after treatment application/renewal. We identified which individual workers brought back pollen loads by timing when they passed underneath the RFID readers (using a stopwatch synchronized with the RFID reader), and then matching this observed time with RFID records. We scored the amount of pollen in each forager's corbiculae (pollen baskets) as none (zero), small (score = 1), medium (score = 2) or large (score = 3) relative to the size of the worker. Scoring pollen loads using this method accounted for the fact that smaller workers are unable to carry as much pollen as larger workers because they have smaller corbiculae (Goulson *et al*. 2002; Spaethe & Weidenmüller 2002). In addition, we recorded the colour of all pollen loads collected using pollen identification cards (Kirk 2010) to help identify the source.

### *End of the experiment*

Nest box entrances were closed after dark on day 28 and the colonies frozen. Window exits remained open for a further 18 h with each outlet tube connected to an individual bottle trap to catch any returning foragers. All tagged workers present in the frozen colonies were identified using their RFID tag, and all recently eclosed (untagged) workers were assumed to have developed in the colony in which they were found. Untagged workers (those that eclosed on, or after, day 26) were assumed to have eclosed on day 26 when analysing worker size. Worker size was assessed by measuring thorax width three times per bee using digital callipers and then averaging these values.

## DATA ANALYSIS

Queen loss occurred in 14 colonies, either because the queen went outside and did not return (presumed to have got lost or died whilst out foraging) or the queen was found dead inside the nest box. In 11 colonies, queen loss occurred within the first 2 weeks (mean = day 6, range = day 2–day 9). We accounted for the effect of early queen loss by considering it as a potential explanatory factor in our statistical analyses, and previous analysis showed there was no effect of treatment on the loss of queens (Gill, Ramos-Rodriguez & Raine 2012). The remaining three colonies that experienced queen loss later in the experiment (mean = day 20, range = day 16–day 23) were pooled with queen-right colonies ($n$ = 3 + 26 = 29 colonies). Two colonies did not survive the full 28 days (WB28 and WB32, both in the $M$ treatment group) and were deemed to have failed (see Supporting information for details). Data from these two colonies were included in our analyses until the day they failed (from day 4, we included nine, and from day 9, we included eight $M$ colonies).

When assessing both the daily number of foragers and the number of foraging bouts each forager performed, we included all foragers that had completed at least one foraging bout on that day. For analyses of foraging bout duration, we excluded the lower and upper extreme values for each treatment group (i.e. the shortest and the longest foraging bout) for each treatment group per day to normalize the data. For the analysis of successful pollen foraging bouts (in which pollen was observed in corbiculae), we included only those bouts in which a forager returned to the same colony it left (without visiting any other in between). The rationale for this was to match the bout duration from RFID records to the size of pollen load collected. However, when analysing the colour of pollen loads, we included all successful foraging bouts. Body size could only be obtained from individuals either present in colonies at the end of the experiment or found dead during the experiment.

The first step of the data analysis was to examine trends in foraging behaviour within each treatment group over the course of the experiment. To do this, we examined the relationship between specific foraging performance measures (number of foragers, number of foraging bouts and bout duration per day) and the time since the start of the experiment for all colonies within a treatment. Trends across treatment groups were then explored by comparing their respective regression slopes (β) from a linear regression. For analysis of pollen foraging, we calculated the mean size of pollen loads each forager collected per day. As pollen load size was scored on a four-point scale (0, 1, 2, and 3), we used a Spearman's rank correlation for each treatment. These analyses were carried out in MINITAB (v.13; State College, PA, USA).

To investigate potential differences over time among treatments, we carried out a linear mixed effects model (LMER function; R Core Development Team) with treatment (categorical), queen loss (categorical) and day (integer) as fixed factors, and block as a random factor. This analysis focused on weekly time points (week 1 = days 1–7; week 2 = days 8–14; week 3 = days 15–21; week 4 = days 22–28) because daily analysis of foraging behaviour is susceptible to natural stochastic variation in the timing of worker eclosion and forager death and/or losses outside the colony. For count data (number of foragers and foraging bouts), we used a Poisson distribution, with the $P$-value calculated from a $Z$-value. For pollen score data, we calculated the value as a proportion of the maximum possible load the forager could have collected. As the minimum load was 0 (no pollen) and maximum was 3 (large): we divided the average pollen load score by the range [=3] to give a proportional value. For our analysis of successful foraging bouts the minimum load considered in the analysis was small (score = 1) and the maximum load was large (score = 3). To obtain proportional values we subtracted 1 from each score, yielding adjusted values of 0 for small loads, 1 for medium loads and 2 for large loads. Taking these adjusted values, we then calculated the mean pollen load size per worker and divided this average value by the range [=2]. These proportional data were arcsine square-root transformed, and $P$-values from the LMER analysis were calculated from a $t$-value and associated degrees of freedom. Our analysis considered days to be nested within each week. For each day, we provided either a single value per colony (i.e. number of foragers), or a value per forager nested within colony (i.e. number of foraging bouts, foraging duration or pollen score per forager). To provide values for foraging bout duration, we took the average time across all foraging bouts completed by each forager per day, and to provide a load score, we calculated the mean score for all pollen loads brought back each day per forager.

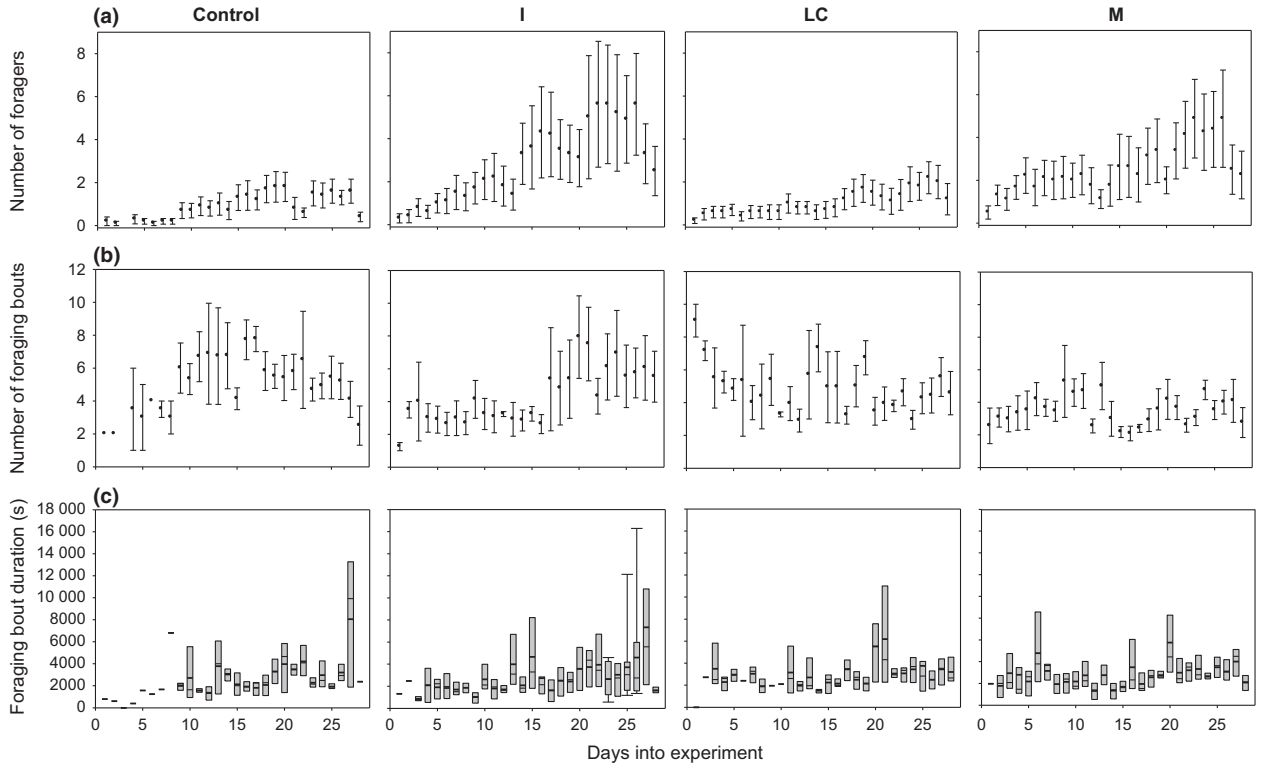


**Fig. 2.** Forager activity. Daily measure of foraging activity for all colonies in each treatment [left to right; control ($n$ = 10); $I$ = imidacloprid ($n$ = 10); $LC$ = λ-cyhalothrin ($n$ = 10); $M$ = mixed (days 1–3: $n$ = 10; days 4–8: $n$ = 9; days 9–28: $n$ = 8)]. Row (a): mean (±SEM) number of foragers per colony per day. Row (b): mean (±SEM) number of foraging bouts carried out per colony (the value for each colony is the average number of foraging bouts carried out by foragers per day). Row (c): box and whisker plots (the thick and thin horizontal lines represent the mean and median values, the box indicates lower and upper quartiles, and whiskers represent 5% and 95% confidence limits) showing foraging bout duration (values per colony per day were obtained by taking the daily average duration of all foraging bouts carried out per forager, and averaging across all foragers).

## Results

### FORAGING ACTIVITY

Daily records of foraging activity ($n$ = 259 foragers in total) showed a general increase in the average number of foragers per colony in all four treatments as the experiment progressed (linear regression: $F_{1,1074} = 64{\cdot}6$, $P < 0{\cdot}001$). We found that average colony size (defined as the cumulative number of workers eclosed minus those found dead) was positively correlated with the daily number of foragers as the experiment progressed ($n$ = 40 colonies; linear regression: $F_{1,27} = 73{\cdot}4$, $P < 0{\cdot}001$; Fig. S1, Supporting information). The rate at which the number of foragers increased over time varied among treatments (Fig. 2a), with a greater rate of increase in $I$ and $M$ compared with $LC$ and control colonies [linear regression with slopes (β): control: $\beta = 0{\cdot}053$, $F_{1,279} = 26{\cdot}6$, $P < 0{\cdot}001$; $I$: $\beta = 0{\cdot}180$, $F_{1,279} = 24{\cdot}9$, $P < 0{\cdot}001$; $LC$: $\beta = 0{\cdot}057$, $F_{1,279} = 25{\cdot}6$, $P < 0{\cdot}001$; $M$: $\beta = 0{\cdot}077$, $F_{1,234} = 11{\cdot}1$, $P = 0{\cdot}001$; Fig. S2a, Supporting information). There were already significantly higher numbers of foragers in $I$ and $M$ colonies compared with control colonies in week 1 of the experiment (LMER: $Z \geq 3{\cdot}44$, $P < 0{\cdot}001$; when excluding $LC$ colonies), and these treatment differences remained significant for $I$ and $M$ colonies for the rest of the experiment ($Z \geq 2{\cdot}08$, $P \leq 0{\cdot}04$). In contrast, there was no significant difference between $LC$ and control colonies in either weeks 2 or 3 ($Z \leq 1{\cdot}02$, $P \geq 0{\cdot}31$), but there was in week 4 ($Z = 2{\cdot}16$, $P = 0{\cdot}03$; see Table S1A for all analyses, Supporting information).

The number of foraging bouts carried out by foragers from control, $LC$ and $M$ colonies (Fig. 2b) remained relatively consistent throughout the experiment (linear regression: control: $\beta = -0{\cdot}0007$, $F_{1,110} = 0{\cdot}026$, $P = 0{\cdot}87$; $LC$: $\beta = -0{\cdot}004$, $F_{1,125} = 1{\cdot}50$, $P = 0{\cdot}22$; $M$: $\beta = 0{\cdot}0008$, $F_{1,155} = 0{\cdot}081$, $P = 0{\cdot}78$; Fig. S2b, Supporting information), whereas there was a steady increase in the daily number of foraging bouts carried out by $I$ colonies (markedly from day 17; linear regression: $\beta = 0{\cdot}012$, $F_{1,157} = 10{\cdot}03$, $P < 0{\cdot}01$). There were no differences across all treatments in the daily number of foraging bouts performed during week 1 (LMER: $Z \leq 1{\cdot}57$, $P \geq 0{\cdot}12$). However, $I$ foragers carried out significantly fewer foraging bouts than controls in week 2 ($I$: $Z = -6{\cdot}62$, $P < 0{\cdot}001$), $LC$ foragers significantly fewer in weeks 2 and 4 ($Z \geq 2{\cdot}52$, $P \leq 0{\cdot}01$) and $M$ foragers significantly fewer in weeks 2–4 ($Z \geq 3{\cdot}87$, $P < 0{\cdot}001$: Fig. 2b, Table S1B, Supporting information).

The average foraging bout duration increased significantly over time in all treatments (Fig. 2c; linear

regression: control: $\beta = 0{\cdot}014$, $F_{1,189} = 16{\cdot}4$, $P < 0{\cdot}001$; *I*: $\beta = 0{\cdot}013$, $F_{1,664} = 35{\cdot}1$, $P < 0{\cdot}001$; *LC*: $\beta = 0{\cdot}0050$, $F_{1,214} = 5{\cdot}3$, $P = 0{\cdot}02$; *M*: $\beta = 0{\cdot}0049$, $F_{1,155} = 7{\cdot}2$, $P < 0{\cdot}01$; Fig. S2c, Supporting information). When comparing across treatments we found that, on average, *LC* foragers carried out significantly longer foraging bouts than controls in week 1 ($t = 2{\cdot}99$, $P < 0{\cdot}01$) and *M* foragers carried out longer bouts than controls in weeks 1 and 4 ($t \geq 2{\cdot}37$, $P \leq 0{\cdot}02$: Table S1C, Supporting information).

## POLLEN FORAGING

Due to the small size of colonies, we observed only 57 pollen foraging bouts during week 1 of which only two were performed by control foragers. This low control sample size meant that we could not compare pollen loads between treatments during week 1 due to lack of statistical power. The average size of pollen loads brought back by foragers (including foraging bouts with no pollen; Fig. 3a) showed no significant trend within treatment (Spearman's rank: control: 0·095, d.f. = 114, $P = 0{\cdot}31$; *I*: −0·089, d.f. = 349, $P = 0{\cdot}10$; *LC*: 0·068, d.f. = 118, $P = 0{\cdot}46$; *M*: −0·112, d.f. = 202, $P = 0{\cdot}11$). However, comparing among treatments, we found that *I* foragers collected less pollen than control foragers: although this difference was not quite significant in week 2 (LMER: $t = 1{\cdot}93$, $P = 0{\cdot}06$), *I* foragers brought back significantly less pollen in weeks 3 and 4 ($t \geq 4{\cdot}97$, $P < 0{\cdot}001$). Similarly, *M* foragers brought back significantly less pollen than controls in weeks 2–4 ($t \geq 2{\cdot}19$, $P \leq 0{\cdot}03$; Table S1D, Supporting information).

When examining the average size of pollen loads collected by 'successful' foragers (i.e. excluding all foraging bouts resulting in no pollen being brought back to the colony; Fig. 3b), we found no significant trend within treatment (Spearman's rank: control: 0·004, $P = 0{\cdot}98$; *I*: 0·15, $P = 0{\cdot}18$; *LC*: −0·20, $P = 0{\cdot}18$; *M*: 0·08, $P = 0{\cdot}54$). The only noteworthy difference among treatments was that *M* foragers brought back significantly smaller pollen loads than control foragers in week 2 (LMER: $t = 2{\cdot}64$, $P = 0{\cdot}01$; Table S1E, Supporting information). On average, successful foragers in control, *I* and *M* colonies took longer to collect pollen as the experiment progressed (linear regression: control: $\beta = 0{\cdot}016$, $F_{1,36} = 5{\cdot}4$, $P = 0{\cdot}03$; *I*: $\beta = 0{\cdot}013$, $F_{1,78} = 12{\cdot}4$, $P < 0{\cdot}01$; *M*: $\beta = 0{\cdot}008$, $F_{1,59} = 6{\cdot}1$, $P = 0{\cdot}02$; Fig. S3, Supporting information), with no change in mean bout duration in *LC* colonies

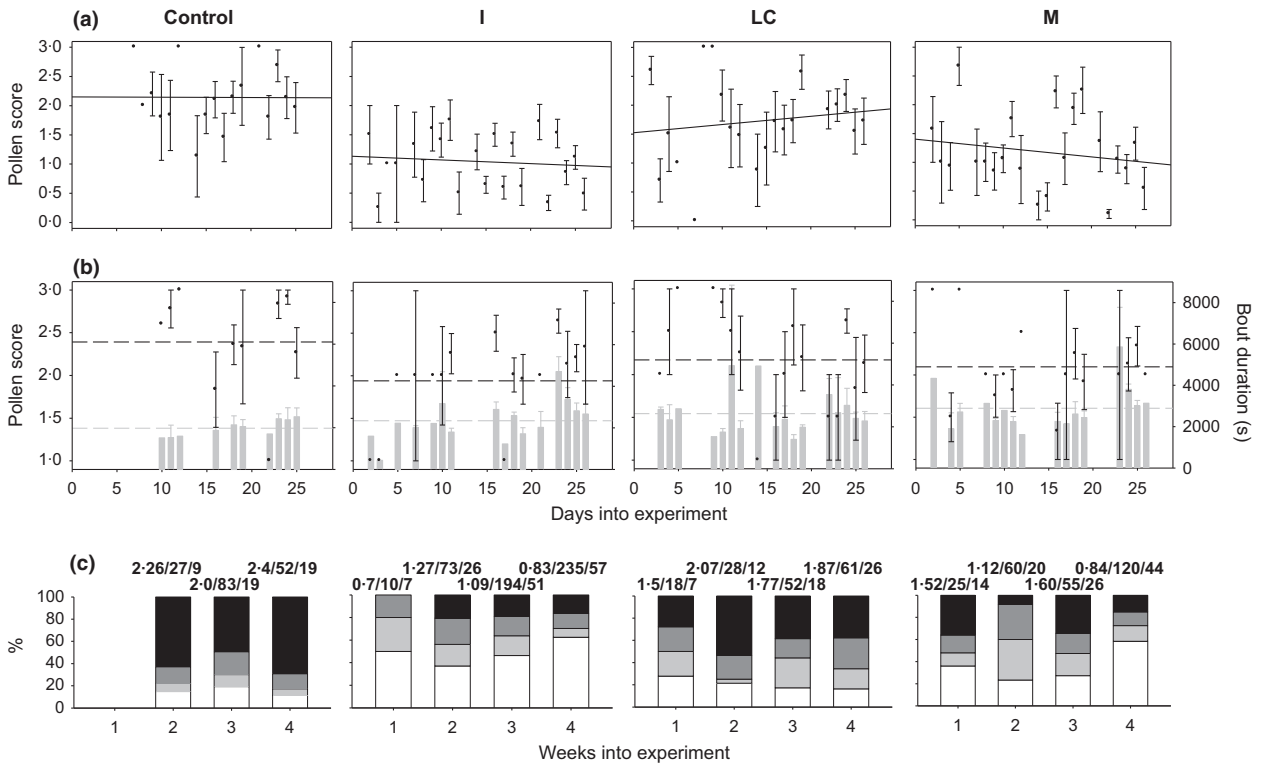


**Fig. 3.** Daily measures of pollen foraging performance by treatment. Row (a) Mean (±SEM) pollen load size brought back by all foragers per colony during foraging observations (total foragers/colonies: control = 30/7; *I* = 80/9; *LC* = 45/8; *M* = 75/7) with fitted regression line for comparison. Row (b) Mean (±SEM) pollen load size (scatter plot) brought back during successful pollen foraging bouts (i.e. all bouts from which bees returned with no pollen are excluded; total foraging bouts/foragers/colonies: control = 136/26/5; *I* = 243/66/8; *LC* = 129/40/6; *M* = 144/52/7). Columns represent mean (±SEM) duration of successful foraging bouts. Horizontal lines indicate mean pollen load size (black dashed line) and mean bout duration (grey dashed line) for all data points during the 4-week experiment. Rows (a) and (b): daily values for each colony were obtained by taking the mean score across all foraging bouts carried out by each forager, and then averaging across foragers. Row (c): The percentage of foraging bouts from which workers returned with either no pollen (score = zero; white), small pollen loads (score = 1; light grey), medium pollen loads (score = 2; dark grey) or large pollen loads (score = 3; black). Values above columns indicate the mean pollen score/total number of foraging bouts observed/number of foragers per week.

($\beta = 0{\cdot}00042$, $F_{1,44} = 0{\cdot}01$, $P = 0{\cdot}92$). Comparing bout durations across treatments, we found that *I* and *M* foragers did not differ from controls in either week 2 or 3 (*I*: $t \leq 1{\cdot}38$, $P \geq 0{\cdot}18$; *M*: $t \leq 1{\cdot}87$, $P \geq 0{\cdot}08$), but they took significantly longer to collect pollen in week 4 ($t \geq 2{\cdot}33$, $P \leq 0{\cdot}02$).

Compared with control, *I* and *M* foragers made 2·5 and 1·6 times more unsuccessful pollen foraging bouts in week 2, respectively, 2·4 and 1·4 times in week 3 and markedly increased to 5·4 and 5·1 more in week 4 (Fig. 3c; $\chi^2$ test: week 2: $P = 0{\cdot}03$ and $P = 0{\cdot}36$; week 3: $P < 0{\cdot}001$ and $P = 0{\cdot}27$; week 4: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; see Table S2A for all analyses, Supporting information). Furthermore, compared with control, *I* and *M* foragers brought back 3·1 and 7·6 times fewer large-sized pollen loads (score = 3) in week 2, 2·6 and 1·4 times fewer in week 3 and 4·3 and 4·6 times fewer in week 4 (Fig. 3c; $\chi^2$ test: week 2: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; week 3: $P < 0{\cdot}001$ and $P = 0{\cdot}09$; week 4: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; see Table S2B for all analyses, Supporting information).

We identified 19 different colours of pollen from the 1093 loads we observed being brought into colonies during the experiment (Fig. S4, Table S3, Supporting information). Four of these colours represented 86% of all pollen loads, indicating that foragers were probably concentrating on four plant species (Fig. 4). These four colours were consistent with pollen load colours collected by honeybees from Dahlia spp. (DH), Himalayan Balsam *Impatiens glandulifera* (HB), Michaelmas Daisies *Aster* spp. (MD) and Oilseed rape *Brassica napus* (OSR). Whilst we cannot confirm unequivocally that the pollen originated from these species, we know that HB, DH and MD were flowering on the university campus. However, it is very unlikely that pollen was collected from OSR as it was not flowering during the experiment, thus we presume it came from another species that has a similar pollen colour. The percentage of foraging bouts returning with HB pollen was similar across all treatments (see Table S4A for details of analysis, Supporting information). In contrast, there was a striking difference in the preference for DH pollen across treatments: whilst it was only collected in 11% of the foraging bouts in both control and *LC* colonies, it was collected in 37% and 35% of foraging bouts in *I* and *M* colonies (control vs. *I*: $\chi^2 = 17{\cdot}1$, $P < 0{\cdot}001$; control vs. *M*: $\chi^2 = 13{\cdot}6$, $P < 0{\cdot}001$; Table S4B, Supporting information). Our results also suggest that *I*, *LC* and *M* colonies had a less strong preference for MD pollen compared with control colonies (Fig. 4; Table S4C, Supporting information), and whilst OSR pollen was hardly collected by control, *I* and *M* colonies (0–1·4% of foraging bouts), it was collected in 16% of *LC* colony pollen foraging bouts.

### FORAGER AGE

Initially, we examined whether treatment affected the age at which workers first started foraging. Considering all workers that eclosed after the start of experiment, we found that the mean (±SEM) age to begin foraging across all treatments was $3{\cdot}8 \pm 0{\cdot}2$ days ($n = 214$ bees) after workers had been tagged (workers were tagged 1–3 days after eclosion) with no significant difference among treatments (mean ± SEM age in days: control = $3{\cdot}8 \pm 0{\cdot}4$; *I* = $3{\cdot}6 \pm 0{\cdot}3$; *LC* = $3{\cdot}6 \pm 0{\cdot}3$; *M* = $4{\cdot}2 \pm 0{\cdot}4$ LMER: $Z \leq 1{\cdot}32$, $P \geq 0{\cdot}19$). We then compared the total number of foraging bouts carried out by each worker and worker age when carrying out their last foraging bout to examine whether age was associated with level of foraging experience (considering only workers that eclosed after treatment started; $n = 212$ bees). We found a significant positive correlation between forager age and foraging experience either within each treatment (control: $\beta = 4{\cdot}8$, $F_{1,30} = 18{\cdot}0$, $P < 0{\cdot}001$; *I*: $\beta = 6{\cdot}0$, $F_{1,77} = 32{\cdot}6$, $P < 0{\cdot}001$; *LC*: $\beta = 2{\cdot}9$, $F_{1,39} = 14{\cdot}7$, $P < 0{\cdot}001$; *M*: $\beta = 2{\cdot}5$, $F_{1,65} = 18{\cdot}6$, $P < 0{\cdot}001$) or when analysing all foragers across treatments (linear regression: $F_{1,213} = 86{\cdot}7$, $P < 0{\cdot}001$). Subsequently, we examined whether the size of pollen load collected changed as foragers aged and whether this varied among treatments (Fig. 5). We found that older foragers from control colonies brought back significantly larger pollen loads (Spearman's rank coefficient: control: 0·19, d.f. = 112, $P = 0{\cdot}05$), whilst there was no change in load size with forager age in *LC* colonies (*LC*: 0·02, d.f. = 118, $P = 0{\cdot}81$). In contrast, we found a significant negative trend between pollen load size and forager age for both *I*

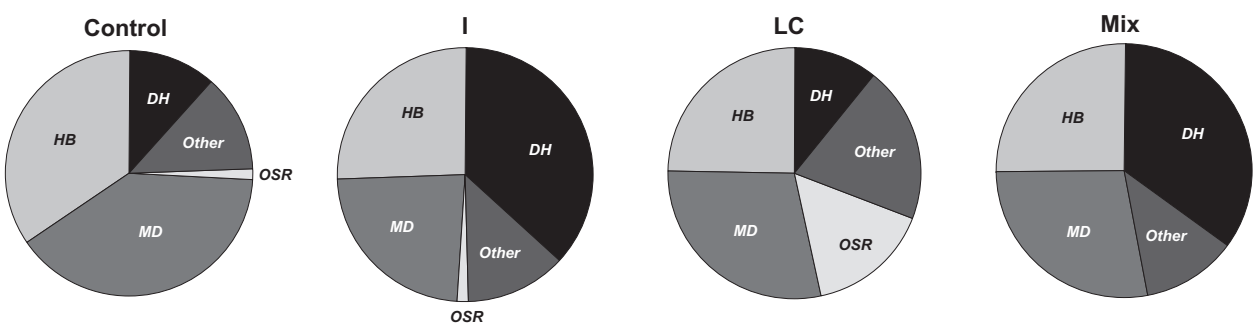


**Fig. 4.** Variation in pollen sources visited among treatment groups. Pie charts show the proportion of bouts in which bees visited each plant type based on pollen colour by treatment group: DH = Dahlia varieties; HB = Himalayan balsam; MD = Michaelmas daisies; OSR = Oilseed rape; Other = the 15 other identified pollen colours (see Supporting information for plant types representing 'Other').

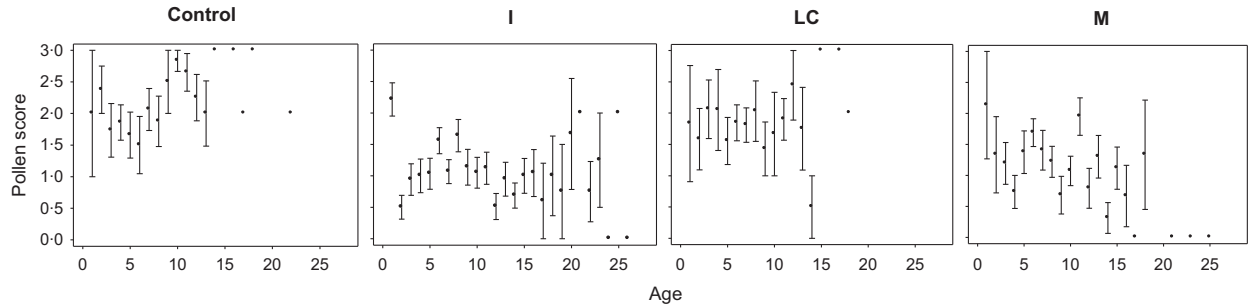


**Fig. 5.** Pollen foraging success as a function of forager age. Plots show the mean (±SEM) pollen score collected by foragers as they aged per treatment ['age' defined as the number of days after a worker had been Radio-Frequency Identification (RFID) tagged].

and *M* colonies (*I*: −0·12, d.f. = 343, $P = 0{\cdot}02$; *M* −0·16, d.f. = 200, $P = 0{\cdot}02$). We found a very similar pattern when we compared pollen load size with a measure of individual foraging experience (Fig. S5, Supporting information), defined by the number of days since each forager undertook its first foraging bout (Spearman's rank coefficient: control: 0·28, d.f. = 112, $P < 0{\cdot}01$; *I*: −0·09, d.f. = 343, $P = 0{\cdot}01$; *LC*: 0·046, d.f. = 118, $P = 0{\cdot}62$; *M*: −0·155, d.f. = 200, $P = 0{\cdot}03$).

### WORKER SIZE

At the end of the experiment, the 40 colonies had produced 1060 workers [152 workers were present before the start ('pre-workers'), and 908 workers eclosed during the experiment ('eclosed workers')] of which we measured thorax widths for 808 individuals (67 pre-workers and 741 eclosed workers; the remaining 252 workers were either lost outside when foraging or were too decayed to measure accurately). We found no significant difference in worker body size between pre-workers and eclosed workers for control, *I* and *LC* colonies (control = 4·25 ± 0·12 vs. 4·10 ± 0·04 mm; *I* = 4·33 ± 0·11 vs. 4·23 ± 0·05 mm; *LC* = 4·42 ± 0·15 vs. 4·22 ± 0·04 mm; GLM: $F \leq 3{\cdot}31$, $P \geq 0{\cdot}07$), but in *M* colonies pre-workers were significantly larger than eclosed workers (mean ± SEM thorax width: 4·58 ± 0·09 vs. 4·16 ± 0·05 mm; GLM: d.f. = 1, $n_1 = 19$, $n_2 = 156$, $F = 8{\cdot}44$, $P < 0{\cdot}01$). There was no difference in the size of either pre-workers (LMER: $t \leq 0{\cdot}94$, $P \geq 0{\cdot}35$) or eclosed workers (LMER: $t \leq 1{\cdot}61$, $P \geq 0{\cdot}11$) among treatments. Intriguingly, however, eclosed worker size was more variable towards the end of the experiment compared with the start (coefficient of variation for workers that eclosed between days 1–7 vs. days 22–28: control = 0·130 vs. 0·182; *I* = 0·102 vs. 0·156; *LC* = 0·105 vs. 0·159; *M* = 0·124 vs. 0·152; also see Fig. S6, Supporting information).

We also examined whether the size of workers that became foragers varied as the experiment progressed (considering only eclosed workers; $n = 144$ foragers available to measure after the experiment). We found that average forager size increased as the experiment progressed in *I* and *LC* colonies (linear regression: *I*: $\beta = 0{\cdot}034$, $F_{1,32} = 4{\cdot}48$, $P = 0{\cdot}042$, *LC*: $\beta = 0{\cdot}021$, $F_{1,34} = 8{\cdot}42$, $P < 0{\cdot}01$). Forager size showed a positive (though not significant) trend over time in control colonies ($\beta = 0{\cdot}030$, $F_{1,43} = 3{\cdot}29$, $P = 0{\cdot}08$), with no clear trend for *M* colonies ($\beta = 0{\cdot}011$, $F_{1,31} = 0{\cdot}75$, $P = 0{\cdot}39$). There was also no significant difference when comparing forager size across all treatments per week [LMER: week 2: $t \leq 1{\cdot}23$, $P \geq 0{\cdot}23$; week 3: $t \leq 0{\cdot}89$, $P \geq 0{\cdot}38$; *NB* foragers that eclosed during weeks 1 and 4 could not be compared due to low sample size ($n = 20$ and 12 foragers available)].

## Discussion

Our analyses provide valuable information about the acute and chronic effects of pesticide exposure on the temporal dynamics of bumblebee (*B. terrestris*) foraging in the field. Initial exposure to the neonicotinoid and pyrethroid pesticides (when colonies were at an early stage of development) had subtle, but detectable, effects on pollen foraging behaviour. However, prolonged exposure to these pesticides, particularly the neonicotinoid (imidacloprid), also resulted in significant chronic impairment of individual foraging performance.

We found that as colonies grew, the number of foragers per colony increased in all treatments; perhaps, an expected result given that colony growth increases both food demands (Pelletier & McNeil 2004; Lopez-Vaamonde *et al.* 2009) and the number of workers potentially available for foraging. However, colonies exposed to imidacloprid (*I* and *M*) had significantly higher numbers of foragers compared with control colonies in all 4 weeks of the experiment. A possible explanation could be that individual foragers were carrying out fewer foraging bouts, and subsequently colonies responded by recruiting more foragers to make up for this shortfall in food intake rate. Whilst *M* foragers did carry out significantly fewer foraging bouts than control foragers in weeks 2, 3 and 4, we found no such difference between *I* and control foragers throughout the whole experiment. These observations support the view that the increase in the number of workers going out to forage in *I* and *M* colonies during the early stages of this experiment is likely due to an acute effect of imidacloprid exposure on worker activity, rather than a colony response (increased worker recruitment). In other

words, it suggests that imidacloprid-exposed workers have a greater 'desire' to go out and forage. Imidacloprid is known to act as a neuronal partial agonist (Deglise, Grünewald & Gauthier 2002) that can acutely increase neuronal activity (Matsuda *et al*. 2001), which may explain why we observe increased forager activity (i.e. hyperactivity: Suchail, Guez & Belzunces 2001).

We also observed that the rate at which the number of foragers increased over time was greater in imidacloprid-exposed colonies (*I* and *M*) compared with control colonies, such that the number of foragers increasingly diverged from control levels during the experiment. Given that *I* and *M* colonies were similar in size to control colonies during the first two weeks and smaller during the latter 2 weeks (Gill, Ramos-Rodriguez & Raine 2012), this was not an effect due to differential colony size, showing that imidacloprid-treated colonies were allocating a higher proportion of workers to the task of foraging: an effect that became even more pronounced during the final 2 weeks of the experiment. Thus, in addition to the acute effect observed, prolonged exposure to imidacloprid appears to have a chronic effect on colony foraging activity. Given that the average age at which workers started foraging did not differ across treatments, this supports the view that imidacloprid-treated colonies were not selectively recruiting younger foragers, but recruiting a higher proportion of workers of all ages.

Our analysis also showed a decrease in pollen foraging efficiency of imidacloprid-exposed foragers (*I* and *M*), with their performance increasingly diverging away from that of control bees as the experiment progressed. On average, imidacloprid-exposed foragers brought back smaller pollen loads in week 4 than during the previous 3 weeks, suggesting this pesticide has a chronic effect on pollen foraging. Our findings support the hypothesis raised by Gill, Ramos-Rodriguez & Raine (2012), suggesting that increased forager recruitment and higher forager activity in imidacloprid-exposed colonies is a response to chronic impairment of individual pollen foraging ability.

The chronic effect on the size of pollen loads collected by foragers could be due to foraging performance of individual bees deteriorating with persistent pesticide exposure as adults, and/or that workers eclosing (and becoming foragers) later in the study were exposed for longer periods during brood development. In this study, we are unable to test the latter hypothesis because it was impossible to control the pesticide exposure for each individual (both during larval development or post-eclosion) or the age at which a forager first performed a foraging bout. However, our results provide support for the former hypothesis as prolonged adult pesticide exposure did significantly affect pollen foraging performance. In control colonies, foragers brought back larger pollen loads per bout as they got older, and more experienced (also see Fig. S5, Supporting information). These findings are consistent with a previous study showing that pollen collection rate increased with each subsequent bout for *B. terrestris* workers foraging on poppy flowers in a greenhouse (Raine & Chittka 2007a). However, our data go further to show longer-term individual improvement in pollen foraging efficiency over multiple days under field conditions. However, foragers exposed to pesticides did not show the same improvement in foraging performance. The pollen loads brought back by *LC* foragers did not increase in size as foragers got older, suggesting that prolonged exposure to λ-cyhalothrin may be preventing experience-dependent improvement in pollen foraging ability. Moreover, *I* and *M* foragers brought back smaller pollen loads as they gained experience, suggesting that exposure to imidacloprid results in deterioration of foraging performance with age and/or experience.

Analysing the colour of pollen loads collected by foragers (using pollen identification cards) revealed differences among treatments in the flowers visited. We found that imidacloprid-exposed colonies (*I* and *M*) had a significantly greater preference for Dahlia varieties, and a lower preference for Michaelmas Daisy and Himalayan Balsam than control foragers (Fig. 4). Whilst this study does not allow us to pinpoint the specific mechanism(s) underlying this differential preference, we suggest that imidacloprid could be affecting either individual forager's innate preference for specific flower types or colours (Raine & Chittka 2007b) and/or could be impairing their ability to find flowers, associate floral cues (as predictors of reward) or learn the motor skills required to handle specific flower types (Raine *et al*. 2006; Raine & Chittka 2008). For example, Dahlia varieties could be more abundant, easier to find and/or easier to extract pollen from than either Himalayan Balsam or Michaelmas Daisy. These hypotheses require further investigation, but previous research has reported that exposure to imidacloprid can affect bee learning performance (e.g. Decourtye *et al*. 2004; Williamson & Wright 2013) and flight ability and foraging behaviour (e.g. Yang *et al*. 2008; Mommaerts *et al*. 2010; Henry *et al*. 2012; Schneider *et al*. 2012; Fischer *et al*. 2014) all of which are important for successful foraging.

Whilst the size of workers was not affected by exposure to either imidacloprid or λ-cyhalothrin alone, the size of workers that eclosed in *M* colonies after the start of treatment with both pesticides were significantly smaller than workers present prior to pesticide application. These results suggest that multiple pesticide exposure can cause a decrease in the size of workers produced. In contrast, *B. terrestris* colonies chronically exposed to field-realistic levels of λ-cyhalothrin via spray-treated pollen, rather than walking across treated filter paper, show a significant reduction in worker body mass under *ad libitum* food conditions in the laboratory (Baron, Raine & Brown 2014). This suggests colony-level impacts of single pesticides could vary considerably depending on precise methods and profiles of exposure. Although we did not find an effect on the size of workers that became foragers during the 28 days of our experiment, the overall reduction in the size of workers eclosing in *M* colonies could eventually mean

smaller foragers being recruited later in the colony cycle. Large workers are more likely to forage, whereas smaller workers have a greater tendency to perform tasks within the nest (e.g. brood care Goulson *et al.* 2002; Jandt, Huang & Dornhaus 2009). This could in part be due to the fact that larger workers have greater visual acuity and antennal sensitivity which is important for foraging (Spaethe & Chittka 2003; Spaethe *et al.* 2007) and are able to carry much larger pollen loads per foraging trip (Goulson *et al.* 2002; Spaethe & Weidenmüller 2002). Taking this information together with our findings that *M* foragers carried out fewer foraging bouts and had chronically impaired pollen foraging ability, this suggests that multiple pesticide exposure can have a severe effect on the amount of pollen being brought into colonies after 3 or 4 weeks of exposure.

The acute and chronically impaired pollen foraging performance induced by neonicotinoid exposure shown in this study has implications for colony growth and survival. It is possible that colonies have sufficient redundancy in their worker force to be able to buffer the smaller acute effect of exposure that is either sporadic or lasts only a short time (i.e. 1–2 weeks) and/or if colonies are larger. But colonies are more likely to suffer significantly, and become more susceptible to colony failure, if exposure is persistent and/or colonies are smaller (Bryden *et al.* 2013). The increased number of foragers recruited in neonicotinoid-exposed colonies (*I* and *M*) seems to be a response to chronic impairment of the pollen foraging ability of individual bees, yet it is interesting that the rate at which forager numbers increased over time in *M* colonies was lower than for *I* colonies. A possible explanation for this is that *M* colonies were less able to recruit additional foragers compared with *I* colonies because of the additional effect(s) of λ-cyhalothrin exposure, such as a lower number of available workers due to increased mortality (Gill, Ramos-Rodriguez & Raine 2012).

In this experiment, we used early-stage colonies (containing an average of four workers) because this is the approximate size *B. terrestris* colonies are likely to be when a substantial amount of pesticides are applied to crops attractive to bees (Thompson 2001; Brittain & Potts 2011; Garthwaite *et al.* 2012a,b). Unlike perennial honeybee colonies, that overwinter as a colony and can start the spring with a work force of several thousand individuals, bumblebees have an annual life cycle in which newly produced gynes (unmated queens) emerge in the summer, mate and then hibernate alone overwinter. The following spring, these same queens must individually establish a new colony, requiring them to locate a suitable nest site, to produce and incubate at least their first batch of workers, and to forage extensively for nectar and pollen to feed themselves and their hungry offspring (Sladen 1912; Goulson 2010). In our experiment, we found that the queen from 11 of our 40 colonies went out to forage and subsequently did not return (even though workers were present in their colony nest box). In the earliest stages of nest searching and founding, queens will be flying around the landscape and are therefore likely to come into contact with pesticides when collecting nectar and pollen from treated crops. Such pesticide exposure could affect the queen's ability to return to the colony (e.g. Henry *et al.* 2012; Fischer *et al.* 2014), affect fecundity (e.g. Laycock *et al.* 2012; Elston, Thompson & Walters 2013) or impair nesting, brood rearing and/or foraging behaviour. Taking these possible impacts into consideration we might consider a lone queen performing this wide variety of tasks (without any workers to help) would be less able to buffer any detrimental effects of pesticide exposure with potentially serious consequences for future colony fitness (either through early queen loss or significant behavioural impairment).

Social bee colony (i.e. brood) development is reliant on a steady income of food from foraging workers. Pollen is the essential protein source required for brood development, in particular the rearing of gynes critical for the fitness of the colony (Sladen 1912; Free & Butler 1959). It is therefore concerning that we found a significant impact on pollen foraging performance. Indeed, just 2 weeks of imidacloprid exposure at a relatively early stage of colony development appears to be sufficient to significantly reduce the total number of gynes that were successfully reared by *B. terrestris* colonies 6 weeks later (Whitehorn *et al.* 2012). Our results provide a potential mechanism to explain these findings, and we also show that whilst imidacloprid exposure does not stop the flow of pollen into the colony, the rate at which it can be collected becomes reduced following a period of chronic exposure. Our findings also support the idea that even if colonies were able to continue recruiting foragers to compensate for impaired individual foraging efficiency, then other essential tasks may be affected. Therefore, it may not just be a lack of pollen but the knock-on effects to colony functioning as a whole, that cause reduced growth, survivorship and reproductive output in imidacloprid-exposed colonies (Gill, Ramos-Rodriguez & Raine 2012; Whitehorn *et al.* 2012; Bryden *et al.* 2013).

A concern for bees about the use of neonicotinoids is the systemic nature of their application, which means that pesticide residues are taken up by all tissues in treated plants including the nectar and pollen (Cresswell 2011; Blacquière *et al.* 2012). These residues can persist in the nectar and pollen for the entirety of the blooming period, meaning that bees are potentially exposed for long periods (likely >28 days of this study) during the year (Rortais *et al.* 2005; Halm *et al.* 2006). Moreover, neonicotinoid residues are known to be found in nearby non-agricultural plants (for example in field borders, Krupke *et al.* 2012) and have been found to persist in soils at high concentrations (see Goulson 2013). Therefore, to achieve a more complete understanding of the risk posed by specific pesticides, such as neonicotinoids, to bees (and other insect pollinators), it is imperative that we assess the exposure profile in the field. This does not simply mean measuring the concentration of pesticide to which bees are exposed at

a single time point (e.g. Mullin *et al*. 2010; Thompson *et al*. 2013), but understanding the likely frequency and duration of exposure in the field to single and multiple pesticides. Currently the honeybee is the only insect pollinator for which validated ecotoxicological testing protocols exist. Even for this species, higher tier semi-field and field studies are not designed to specifically assess potential sublethal chronic effects of plant protection products on individual bees (which could perhaps be revealed by monitoring activity patterns of individuals using RFID technology) and are unlikely to detect colony-level effects as monitoring periods during these studies are often relatively short. Pesticide regulatory bodies must consider the chronic effect of specific pesticides on foraging performance of bees (and other pollinators) not only as this is important for bee colony success, but also because it is likely have fundamental consequences for the essential pollination services they provide.

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## Authors' contribution

RJG and NER designed and carried out the experiment and wrote the paper; RJG conducted data analysis; and NER conceived the project.

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## Supporting Information

Additional Supporting information may be found in the online version of this article:

**Data S1.** Supporting methods and results.
**Fig. S1.** Forager number as a function of effective colony size.
**Fig. S2.** Daily measures of foraging activity per colony by treatment.
**Fig. S3.** Daily mean duration of successful pollen foraging bouts conducted by a single forager.
**Fig. 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.
**Fig. S5.** Relationship between pollen load size brought back by foragers and previous forager experience per treatment.
**Fig. 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).
**Table S1.** Weekly analyses: statistical outputs from a Linear Mixed Effects model (LMER) are comparisons of treatment- with control colonies ('intercept').
**Table S2.** Weekly analyses: statistical outputs from pairwise chi-square tests ($\chi^2$) 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.
**Table S3.** Diversity and frequency of pollen types collected by foragers from each treatment group.
**Table S4.** Statistical outputs from chi-square tests ($\chi^2$) showing comparisons between control and treatment (*I*, LC and *M*) colonies.