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Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency

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Abstract Bumblebees and other pollinators provide a vital ecosystem service for the agricultural sector. Recent studies however have suggested that exposure to systemic neonicotinoid insecticides in flowering crops has sub-lethal effects on the bumblebee workforce, and hence in reducing queen production. The mechanism behind reduced nest performance, however, remains unclear. Here we use Radio Frequency Identification (RFID) technology to test whether exposure to a low, field realistic dose (0.7 ppb in sugar water and 6 ppb in pollen) of the neonicotinoid imidacloprid, reduces worker foraging efficiency. Whilst the nectar foraging efficiency of bees treated with imidacloprid was not significantly different than that of control bees, treated bees brought back pollen less often than control bees (40 % of trips vs 63 % trips, respectively) and, where pollen was collected, treated bees brought back 31 % less pollen per hour than controls. This study demonstrates that field-realistic doses of these pesticides substantially impacts on foraging ability of bumblebee workers when collecting pollen, and we suggest that this provides a causal mechanism behind reduced queen production in imidacloprid exposed colonies.

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Introduction

Around a third of all human food is thought to depend on insect pollination (McGregor 1976) and many crops benefit from this service, with adequate pollination increasing yields and improving crop quality (Klein et al. 2007). Recently, however, there has been growing concern over the use of neonicotinoid pesticides in agriculture and the sub-lethal effects they can have on pollinators (Bortolotti and Montanari 2003; Decourtye et al. 2004; Desneux et al. 2007; Yang et al. 2008; Aliouane et al. 2009; Henry et al. 2012; Whitehorn et al. 2012; Gill et al. 2012; Williamson and Wright 2013; Di Prisco et al. 2013; Matsumoto 2013), which has culminated in an EU-wide restriction on the use of three neonicotinoid pesticides. The ban comes into place in December 2013 and is a temporary, 2 year measure preventing the use of imidacloprid, clothianidin and thiamethoxam until further research can clarify the impact these pesticides are having on bees (European Commission 2013). Sub-lethal effects do not bring about direct mortality but impair an organism's ability to function in some other way, for example by impacting on activity, fecundity, neurophysiology, learning performance or other aspects of behaviour (Desneux et al. 2007; Laycock et al. 2012). There is mounting evidence that neonicotinoid pesticides, formulated to target neurotransmitter receptors in insects, are negatively impacting the foraging behaviour of bees by inducing memory and learning dysfunctions, and impairing navigational skills (Henry et al. 2012; Aliouane et al. 2009; Yang et al. 2008; Decourtye et al. 2004). The continuing decline of pollinators such as bumblebees and honeybees,

coupled with an increased focus on sustainable food production means that a greater understanding of the wider impacts of pesticides on pollinators is required.

Imidacloprid is the second most widely used agrochemical in the world (Pollak 2011), and is commonly used as a seed dressing to protect crops from insect pests. The pesticides used in these seed dressings are transported throughout the plant via the sap, ending up in both pollen and nectar at concentrations typically ranging from <1 to 10 ppb (parts per billion) (Cresswell 2011; EFSA 2012). Oil seed rape is the second most abundant arable crop grown in the UK in terms of area (Garthwaite et al. 2010) and its flowers are known to attract bumblebees, honeybees and other pollinating insects (Hayter and Cresswell 2006). The majority of growers that produce oilseed rape do so using dressed seeds, with recent figures suggesting that only around 4 % of seed sown in the UK remain untreated (Garthwaite et al. 2010). Fields of rape offer a mass flowering crop that blooms for several weeks and bees that forage on these plants are thus exposed over large scales to trace dietary insecticides such as imidacloprid.

A recent study by Whitehorn et al. (2012) found that *Bombus terrestris* (Linnaeus 1758) colonies exposed to field realistic doses of imidacloprid (0.7 ppb in sugar water and 6 ppb in pollen), produced 85 % fewer queens than control colonies. Bumblebees have an annual cycle, with new queens single-handedly founding the next generation of nests (Goulson 2010). There is evidence that only the most successful nests produce new queens (Muller and Schmid-Hempel 1992) and reductions in the number of queens produced 1 year will likely have knock on effects for the number of colonies founded the next. However, Whitehorn et al. (2012) did not attempt to elucidate the mechanisms underlying reduced performance of treated colonies, which might include reduced fecundity of the queen, or reduced foraging efficiency of the workers. Gill et al. (2012) provide a possible explanation; they found that exposure to imidacloprid at 10 ppb in sugar water reduced the foraging success of worker bumblebees. However, the highest concentration of imidacloprid found in the nectar of seed-treated oilseed rape to date is 0.8 ppb (EFSA 2012). In this study we examined the effect of a lower, more field realistic dose of imidacloprid to determine what effects it may have on the foraging ability of *B. terrestris* workers.

Methods

Six commercial *B. terrestris* colonies (Biobest N.V., standard hives) were used to examine the effect of field realistic doses of the pesticide imidacloprid on foraging activity over a 4 week period. Upon arrival, nests were small, evenly aged and consisted of the queen and up to 65 workers. They contained two internal tanks which supplied the bees with sugar solution during transportation, and these tanks were sealed prior to controlled feeding to ensure that bees only had access to the 'nectar' provided as part of the experiment. Colonies of approximately equal weights were randomly allocated to either a treatment or control group (three in each). Control colonies were fed ad libitum (ad lib) with pollen and a sugar water solution for a period of 14 days in the laboratory. Over the same period colonies assigned to the treated group were fed pollen and sugar water containing 6 and 0.7 μg kg^{-1} imidacloprid respectively, thus mimicking levels of imidacloprid found in oil seed rape (Bonmatin et al. 2003). During the 14 day period bees were provided with no alternative forage.

After 2 weeks of controlled feeding in the lab, all colonies were placed out in the field and the foraging behaviour of bees was monitored over a 4 week period (07.08.2012–04.09.12). The study was carried out in domestic gardens in an urban area of Stirling in the Central-belt region of Scotland. The nearest farmed area was over 1 km away. Bees were allowed to acclimatise to their surrounding for 24 h. After this time the first 12 bees exiting each nest that had undamaged wings were collected. In treated nests this first batch of bees would have been individuals that consumed contaminated pollen and nectar during their adult life, however given the 9 day pupation phase they are not likely to have been larvae reared on food contaminated with imidacloprid (Van Der Steen 2008). We used Radio Frequency Identification (RFID) technology to monitor the foraging duration of individual bumblebees, and an automated system to record the weight of bees entering and exiting the nest. RFID technology is increasingly being used to study the behaviour of insects (Robinson et al. 2009: ants; Streit et al. 2003; Molet et al. 2008: bumblebees; Sumner et al. 2007: paper wasps), and allows an accurate and automated way of monitoring their activity (Ohashi et al. 2010). A small RFID tag (mic3®-AG64 bit RO, iID2000, 13.56 MHz system, 1.0 × 1.6 × 0.5 mm; Microsensys GmbH, Erfurt, Germany) weighing 3 mg (<3 % of the weight of the smallest bee tagged) was glued to the dorsal surface of each bee's thorax. The weight of these tags was small relative to the average weight of nectar and pollen carried by bees; bumblebees are known to carry up to 90 % of their own body weight (Goulson et al. 2002). The tags were carefully positioned so that they would not hamper wing movement and bees were then released and left to forage independently for a period of 4 weeks. Treated and control colonies were randomly paired and each pair were monitored for a 24 h period every 3 day (approximately). A fully automated system was set up to record the time and weight

of bees departing from and returning to the nest: in a set-up similar to that used by Stelzer et al. (2010) a system of 2 cm tubes were used to connect the entrance of each colony to a clear plastic box mounted on top of a balance (weighing to 3 decimal places). A small clearance gap was left between the tube system and the weighing box to ensure that only the weight of bees in the box was recorded. In most cases the time it took for bees to traverse the box was sufficient to get a stable reading. However on about a third of occasions there were multiple bees in the box at one time which meant it was not possible to obtain an accurate weight of any one individual bee. If this occurred when a bee was returning to the nest, the trip was excluded from the analysis of weight data. However if it occurred when a bee was leaving the nest then an average weight of that bee was obtained from other departures made during the monitoring period, and this was used as proxy in the analysis. After traversing the box, bees then entered another length of tube leading them to the outside. RFID readers were mounted between the nest entrance and the first length of tube, which recorded the exact time bees entered and exited the nest. A motion detecting camera was set up to record the weight of bees as they passed over the balance, and to determine if bees returned carrying pollen. As in Stelzer et al. (2010), any trips that lasted for 5 min or less as well as those flights where bees lost mass were excluded from analysis as the majority of these were likely to have been orientation or defecation flights. These trips only accounted for a small number of the total trips recorded and numbers were similar in treated and control colonies (only 15 trips in total, nine for treated and 8 for control bees). Additionally any trips over 4 h in duration (seven in total, three for treated and four for control bees) were excluded from the analysis as these often occurred on rainy days where bees may have been prevented from returning to the nest due to adverse weather.

Fourteen days into the 4 week data capture period a further 12 bees from each nest were tagged. The development of *B. terrestris* workers in laboratory conditions includes $\sim$14 day of larval development during which larvae are frequency fed, followed by $\sim$9 days as a pupa. Thus in treated colonies, bees tagged at this point were likely to have been those reared on the pollen and nectar dosed with imidacloprid. At the mid-way stage of the experiment one control nest and one treated nest had to be removed from the experiment due to wax moth infestations; therefore no further bees were tagged in each of these colonies.

All statistical analyses were conducted using R version 2.15 (R Development Team 2012). Generalised Linear Mixed Effects models (GLMM; fitted by maximum likelihood using the lme4 package) with Gaussian errors were used to test the effect of imidacloprid treatment on, trip duration, weight of forage collected and the foraging efficiency (mg of forage collected per hour) of individual bees. The time of day for each trip and the number of days since each bee was tagged were included as covariates along with treatment as a fixed factor. Individual and colony I.D. were included as random factors to account for pseudo-replication between and within colonies. 'Batch' (whether the bees were tagged at the start of week one, or the start of week three) was also included as a fixed factor. All two way interactions were included in the starting model. Factors that did not contribute significantly to the model were removed in a stepwise manner, using $p = 0.05$ as a threshold for factor retention or removal. After each simplification step models were assessed using the Akaike's information criterion (AIC; Akaike 1974). When modelling both pollen and nectar foraging efficiency the most parsimonious model determined using the stepwise approach matched the model with the lowest AIC value.

A GLM, with quasibinomial errors to allow for over dispersion, was used to determine if there was a significant difference in the proportion of trips in which treated and control bees returned with pollen. The number of trips in which bees returned with pollen over the number of returns without pollen was modelled as the response variable and treatment and batch were included as fixed effects. Nest was included as a fixed effect in this analysis as, due to the relatively small sample size, models including nest as a random effect were unable to correctly separate out nest effects that were not due to treatment.

A Pearson's correlation was used to examine the relationship between time spent foraging and the weight of the load collected during each bout. Unless otherwise stated all averages are mean $\pm$ SD.

Results

Between 07 August 2012 and 04 September 2012 data were gathered from 256 foraging bouts; 21 foragers from control colonies were recorded making 113 foraging trips (5.4 $\pm$ 1.4 trips per bee), and 24 foragers from treated colonies made a total of 142 trips (5.96 $\pm$ 1.9 trips per bee). During the course of the study two bees from the treated group and one bee from the control group failed to return to the nest. There was no difference in the lifespan of bees from treatment and control groups, with all tagged bees (with the exception of the three that failed to return to the nest) surviving until the end of the study.

Control bees spent on average 25.44 $\pm$ 6.1 min foraging for nectar, with a mean weight of 42.6 $\pm$ 9.86 mg collected per bout, resulting in a nectar foraging rate of 101 $\pm$ 10.68 mg h^{-1}. This was not significantly different from the nectar foraging rate of treated bees (GLMM: $\chi^2 = 0.534$, df = 1, $p = 0.464$; Fig. 1a) who spent on

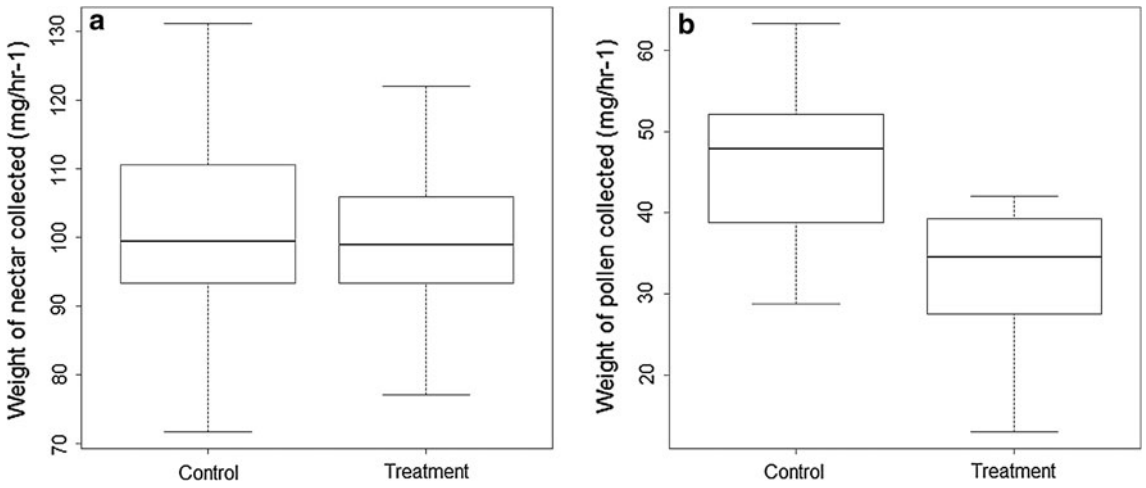

Fig. 1 The efficiency of foraging on pollen gathering trips was significantly lower for treated bees than untreated bees, whilst no significant difference was found in foraging efficiency on nectar gathering trips. The *box plots* depict median and interquartile range, with the *bars* representing the means of treated and untreated bees and their 95 % confidence interval

average 27.26 ± 8.4 min foraging for nectar, bringing back 44.7 ± 12.49 mg of nectar per bout resulting in a foraging rate of 99.24 ± 9.67 mg h^{-1}. Neither treatment nor any of the other proposed explanatory variables; time of day, batch and number of days since the bee was tagged, were significant in explaining trip duration, weight of nectar collected or nectar foraging efficiency.

The average length of time spent on pollen foraging trips (trips in which the returning bee had visible pollen loads) was 73.8 ± 14.38 min for control bees with a mean weight of 57.32 ± 11.22 mg being collected per bout, resulting in a pollen foraging rate of 47.71 ± 7.62 mg h^{-1}. The mean length of time spent on pollen foraging trips in treated bees was 77.85 ± 24.96 min, with the minimal model for trip duration including treatment, the number of days since the bee was tagged and the interaction between the two (GLMM: $\chi^2 = 9.99$, df = 1, $p < 0.01$). Trip duration in control bees remained approximately constant throughout the experiment, however in treated bees the duration of trips increased with time from tagging. The mean weight of pollen collected per bout by treated bees was 41.07 ± 12.72 mg, with treated bees bringing back significantly less pollen than control bees (GLMM: $\chi^2 = 4.76$, df = 1, $p < 0.01$), with no other factors remaining in the minimal model. This resulted in a mean foraging rate for pollen of 32.97 ± 9.43 mg h^{-1}, a 31 % reduction compared to control bees (GLMM: $\chi^2 = 18.06$, d.f = 1, $p < 0.001$; Fig. 1b). There was no significant effect of time of day, batch and number of days since individual bees were tagged in explaining pollen foraging efficiency. Treated bees were also significantly less likely than control bees to return to the nest carrying pollen (41 vs 65 % of foraging bouts respectively; t = −2.135, $n = 42$, $p < 0.05$).

The positive correlation between time spent foraging for pollen and the amount of forage collect was significant in both treated bees ($r = 0.576$, $n = 57$, $p < 0.001$) and control bees ($r = 0.729$, $n = 71$, $p < 0.001$).

In accordance with Whitehorn et al. (2012), treated nests gained less weight than control nests and also produced fewer workers and queens, but the number of nests used in our experiment was too few to permit meaningful statistical analysis of these differences.

Discussion

This study strongly corroborates the findings of previous studies, and shows that the neonicotinoid imidacloprid can have sub-lethal effects on free-flying worker bumblebees, and thus is likely to reduce colony success. Here we were able to quantify for the first time the change in bumblebee foraging efficiency as a result of field-realistic measures of imidacloprid exposure showing that, on pollen gathering trips, treated bees brought back 31 % less forage per hour than controls, representing a significant reduction in efficiency. This is in accordance with the findings of Gill et al. (2012) who ranked the pollen loads of bumblebees returning to the nest as small, medium or large and found that imidacloprid exposed bees brought back proportionally more small loads than unexposed bees. Gill et al. (2012) also found that imidacloprid exposed bees collected pollen on 59 % of their foraging bouts, versus control bees that collected pollen on 82 % of occasions (a 28 % decrease). Using a lower, field realistic dose we found that bees exposed to imidacloprid showed a 23 % reduction in the frequency of pollen-collecting trips, compared to controls.

Pollen is the main protein source for bumblebees and is particularly important for the rearing of young to replace older workers (Harder 1990). It has been suggested that foraging for pollen is more challenging than foraging for nectar (Raine and Chittka 2007), and it is usually restricted to dry, sunny weather, whereas nectar can be collected in most conditions except heavy rain (Peat and Goulson 2005), so that pollen rather than nectar shortages are more likely to limit colony success (Goulson 2010). This is reflected in the lower foraging efficiency of bees when gathering pollen versus nectar (Peat and Goulson 2005). Using the same concentrations of imidacloprid as the current study (6 ppb pollen and 0.7 ppb nectar), and the same two-week exposure period, Whitehorn et al. (2012) found an 85 % reduction in queen production in colonies exposed to imidacloprid. Developing queens are known to require more food during their developmental period and thus queen production is likely to suffer as a result of lower provisions of pollen. Whitehorn et al. (2012) also found that colonies exposed to imidacloprid gained significantly less weight over time than control colonies. In previous work studying *B. lucorum*, a species closely related to *B. terrestris,* a positive correlation was found between nest size and queen production (Müller and Schmid Hempel 1992). Hence our data provide a simple mechanism for the dramatic declines in queen production described by Whitehorn et al. (2012); a substantially reduced pollen supply to the colony.

In this experiment we made the assumption that if a bee returned with a visible pollen load then any increase in weight recorded was due to the pollen it had collected whilst foraging. In some cases however it is likely that bees foraged for a mixture of pollen and nectar. It is thus possible that the lower weight of forage brought back by bees exposed to pesticide was due to reduced nectar collection, or a combination of reduced pollen collection and reduced nectar collection. The former seems less likely since bees which returned only with nectar showed no significant impact of pesticide treatment.

It is worth noting that in the present study bumblebees were kept in the lab for 2 weeks and treated colonies were given no alternative but to feed upon pollen and nectar dosed with imidacloprid. This is perhaps unrealistic of field conditions as bees would normally be free to forage on a range of contaminated and uncontaminated resources. However, oil seed rape is the third most abundant arable crop grown in the UK (after wheat and barley) with the production area for this crop having increased by 17.75 % between 2010 and 2012, representing an increase of 114,000 ha (Garthwaite et al. 2010; DEFRA 2012). Rape is known to flower for around 3–4 weeks providing an abundant, if short-lived floral resource (Goulson et al. 2010). Both bumblebees and honeybees feed on oil seed rape (Hayter and Cresswell 2006) and given the general decline in floral resources in the countryside (Carvell et al. 2006) it is likely that, whilst in flower, oil seed rape constitutes a large component of many bees' diets. A recent study by Thompson et al. (2013) that examined the effects of three neonicotinoids on bumblebee colonies in field conditions failed to establish a negative control, demonstrating that these substances are widespread in agricultural environments. Furthermore, substantial concentrations of neonicotinoids (up to 9 ppb) have been found in wildflowers growing near to treated crops (Krupke et al. 2012), suggesting that exposure to these types of pesticide might not be restricted to bees foraging on the crops themselves. Hence we suggest that the level of exposure used here is likely to approximate that experienced by some wild bumblebee nests under field conditions.

Interestingly, we found no significant difference in foraging efficiency between the first batch of foragers tagged and the second. This suggests that impaired foraging continued to be seen in bees from treated colonies for at least 4 weeks after exposure. However, it is not clear whether this occurred because bees continued to be exposed to imidacloprid in honey stored within the nests, or whether the reduced performance of the second batch of foragers was due to exposure as larvae. Yang et al. (2012) describe impaired learning in honeybees following exposure to imidacloprid as larvae. Further studies could clarify the persistence of imidacloprid within the nest and the effects it may have on subsequent generations of workers. Whatever the mechanism, our data suggest that exposure to imidacloprid may reduce worker performance for at least 4 weeks after the source of exposure is removed.

Whilst this study has put forward a mechanism for reduced queen production in imidacloprid exposed colonies (Whitehorn et al. 2012) it is not able to fully explain the mechanism behind the reduced pollen foraging efficiency found in treated bees. In both treated and control bees a positive correlation was found between time spent foraging and the weight of pollen collected and no significant difference was found in the number of bees failing to return to the nest between treatments, which, coupled with the fact that there were no differences found in nectar foraging efficiency, suggests that navigation is not likely to have been the issue. Further studies are needed to clarify how imidacloprid impairs bumblebee's ability to forage for pollen, with the evidence gathered in this study suggesting that the pesticide may either reduce motivation to collect pollen, or impair the bees' ability to collect pollen from flowers, rather than affecting their trips to and from their foraging sites.

Agricultural intensification has been proposed as a primary driver behind the decline of bumblebees, with habitat loss as well as increased pesticide use believed to be two

important causal factors (Goulson et al. 2008). Whilst this study has focused on the effects of imidacloprid, the uses of other neonicotinoids have also been called into question, since the three most commonly used compounds, imidacloprid, thiamethoxam and clothianidin all have similar modes of action (Nauen et al. 2003). A recent study has reported harmful effects of thiamethoxam on honeybee homing abilities (Henry et al. 2012), whilst another found no significant impact of thiamethoxam on colony initiation in bumblebees (Elston et al. 2013). If ecologically and economically important pollinator populations are to be maintained then the advisability of any future use of neonicotinoids on flowering crops must be questioned and further work is needed to clarify their impacts.

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Conflict of interest The authors declare that they have no conflict of interest.

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