



Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid)

James E. Cresswell^{a,*}, Christopher J. Page^a, Mehmet B. Uygün^a, Marie Holmbergh^b, Yueru Li^a, Jonathan G. Wheeler^a, Ian Laycock^a, Christopher J. Pook^a, Natalie Hempel de Ibarra^c, Nick Smirnoff^a, Charles R. Tyler^a

^a Biosciences, College of Life & Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, United Kingdom

^b IFM Biology, Linköping University, SE-581 83 Linköping, Sweden

^c Psychology, College of Life and Environmental Sciences, University of Exeter, Washington Singer Building, Perry Road, Exeter EX4 4QG, United Kingdom

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ABSTRACT

Currently, there is concern about declining bee populations and the sustainability of pollination services. One potential threat to bees is the unintended impact of systemic insecticides, which are ingested by bees in the nectar and pollen from flowers of treated crops. To establish whether imidacloprid, a systemic neonicotinoid and insect neurotoxin, harms individual bees when ingested at environmentally realistic levels, we exposed adult worker bumble bees, *Bombus terrestris* L. (Hymenoptera: Apidae), and honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), to dietary imidacloprid in feeder syrup at dosages between 0.08 and 125 $\mu\text{g l}^{-1}$. Honey bees showed no response to dietary imidacloprid on any variable that we measured (feeding, locomotion and longevity). In contrast, bumble bees progressively developed over time a dose-dependent reduction in feeding rate with declines of 10–30% in the environmentally relevant range of up to 10 $\mu\text{g l}^{-1}$, but neither their locomotory activity nor longevity varied with diet. To explain their differential sensitivity, we speculate that honey bees are better pre-adapted than bumble bees to feed on nectars containing synthetic alkaloids, such as imidacloprid, by virtue of their ancestral adaptation to tropical nectars in which natural alkaloids are prevalent. We emphasise that our study does not suggest that honey bee colonies are invulnerable to dietary imidacloprid under field conditions, but our findings do raise new concern about the impact of agricultural neonicotinoids on wild bumble bee populations.

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1. Introduction

Currently, there is widespread concern about declining bee populations (Biesmeijer et al., 2006; Potts et al., 2010; Cameron et al., 2011) and the sustainability of the pollination services that they provide to agriculture and wild plants (Kremen et al., 2002; POST, 2010). One potential threat to bee health is the unintended impact of agricultural insecticides (Desneux et al., 2007), which some implicate as a contributory cause of bee declines (Hansard, 2011), although other detrimental factors also may be responsible, such as impoverished forage bases or diseases (Potts et al., 2010).

Over 100 pesticides are known to be variously toxic to honey bees depending on their chemical structure (Devillers et al., 2003). Systemic neonicotinoids, such as imidacloprid, are among the most widely used insecticides against pest herbivores (Elbert et al., 2008). These broad-spectrum neurotoxins disrupt the insect

nervous system by acting agonistically on nicotinic acetylcholine receptors (insect nAChRs), which are membrane proteins that induce membrane depolarization at nerve synapses (Matsuda et al., 2001; Thany, 2010). They are ingested by bees as trace residues (here defined as up to 10 μg active ingredient kg^{-1}) in nectar and pollen from flowers of treated crops, such as sunflower (*Helianthus annuus*) and oilseed rape (canola, *Brassica napus* L.) (Bonmatin et al., 2005; Rortais et al., 2005). In Europe and North America, honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) are important generalist pollinators that forage from mass-flowering crops (Free, 1993; Hoyle et al., 2007) and both have exhibited population declines (Pettis and Delaplane, 2010; Cameron et al., 2011). To establish whether residues of imidacloprid in pollen and nectar of treated crops could be implicated in these declines (e.g., Cresswell et al., 2012), it is important to determine whether individual bees are harmed by trace residues.

Previous studies demonstrate that trace dietary imidacloprid harms individual honey bees (Decourtye and Devillers, 2010) and that it is capable of doing so at environmentally realistic levels (Cresswell, 2011; Blacquière et al., 2012), where it reduces

* Corresponding author. Tel.: +44 1392263779.

E-mail address: j.e.cresswell@ex.ac.uk (J.E. Cresswell).

performance in learning tasks by worker bees under laboratory conditions by between 6% and 20% (Cresswell, 2011). However, substantially higher doses of imidacloprid are required to cause elevated rates of mortality in honey bees (Cresswell, 2011). In contrast, trace dietary imidacloprid at dosages of approximately $10 \mu\text{g kg}^{-1}$ in sugar syrup has caused elevated rates of mortality in bumble bees (Tasei et al., 2000; Mommaerts et al., 2010). These data suggest a differential sensitivity between honey bees and bumble bees and for theoretical reasons this is not unexpected. The evolutionary theory of plant defensive chemistry (Ehrlich and Raven, 1964) predicts differential sensitivity among insect herbivores through adaptive divergence (Bernays and Graham, 1988; Zangerl and Berenbaum, 2003). An extant species will be relatively less sensitive to a dietary chemical if its ancestors had the opportunity to adapt to it while feeding on the then available host plants. This theory has been extended to insect nectarivores (e.g., Adler, 2000) and, among bees, honey bees therefore may be better pre-adapted than bumble bees to feed on nectars containing synthetic alkaloids, such as neonicotinoid pesticides, by virtue of their ancestral adaptation to tropical nectars (Ruttner, 1987; Dyer, 2002; Dornhaus and Chittka, 2004) in which natural alkaloids are prevalent (Baker, 1977). Bumble bees may be more sensitive because of their ancestral evolution with montane and tundra nectars (Williams, 1985; Hines, 2008) in which alkaloids are rare (Baker, 1977). We therefore investigated the effects of dietary imidacloprid in nectar on honey bees and bumble bees in parallel in order to compare their sensitivities.

2. Materials and methods

2.1. Bees and imidacloprid diets

In the laboratory, we fed caged honey bees and bumble bees *ad libitum* on syrup (Attracker; Koppert B.V., Berkel en Rodenrijs, Netherlands) containing imidacloprid at a range of 10 dosages that both spanned and exceeded environmentally realistic levels. We measured three health indicators: feeding rate, locomotory activity and longevity. Bumble bees were caged individually (cage dimensions: $0.065 \text{ m} \times 0.05 \text{ m} \times 0.035 \text{ m}$) and honey bees were caged in groups of 10 (cage dimensions: $0.01 \text{ m} \times 0.08 \text{ m} \times 0.018 \text{ m}$) because they are more dependent on a social context for survival. All cages were wooden with the two largest faces made of fine plastic mesh. Bumble bees (*Bombus terrestris* L.) were obtained as domesticated colonies from a commercial supplier (Natupol Beehive; Koppert B.V., Berkel en Rodenrijs, Netherlands) and honey bees (*A. mellifera*, Buckfast) were captured immediately before use at the nest entrance of four hives that were maintained at the University of Exeter. Each cage of honey bees contained individuals from a single hive. Bees were maintained in a controlled environment room (temperature 25°C , 40% relative humidity, 12:12 h of light:darkness). In order to quantify their intrinsic variation in feeding rate due to variation in size, bumble bees were maintained on a control diet of syrup for three days before dosing began. In honey bees, we found little intrinsic variation among cages in feeding rate and so they were dosed immediately after caging. Once dosing began, each cage was provided with a syrup feeder containing either control syrup or a syrup with one of the following nine doses of imidacloprid in units of 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; $0.08 \mu\text{g l}^{-1}$. Imidacloprid was obtained as a solution in acetonitrile ($10 \text{ ng } \mu\text{l}^{-1}$, product code L 14283700AL; Dr. Ehrenstorfer GmbH, Augsburg, Germany) and we conducted trials in which the acetonitrile was either removed by evaporation with a vacuum dryer (ScanVac MaxiVac Beta; Labogene, Lyngby, Denmark) and the imidacloprid was suspended in water before being mixed into feeder syrup or the imidacloprid–acetonitrile mixture was mixed

into the syrup directly. Where the acetonitrile was not removed, it was present in syrups at a concentration of $100 \mu\text{l}$ per μg imidacloprid.

Bees were monitored daily for syrup consumption and longevity. Locomotory activity was quantified by subjecting a video of each cage to image analysis (ImageJ v. 1.44; National Institutes of Health, Bethesda, MD, USA). Bees were visible through the cage mesh and were filmed *in situ*. For honey bees, we analysed 30 s of video that was recorded four days after dosing began and calculated the mean speed of bees (distance walked per individual per hour) for each cage. For bumble bees, we similarly analysed at least 1 min of video recorded four days after dosing began. As a control for the effects of acetonitrile, we also conducted experiments on honey bees and bumble bees as described above but with an appropriate concentration gradient of dietary acetonitrile. Trials were conducted between June 2010 and November 2011.

To verify the concentration of imidacloprid in our doses, we prepared the usual range of experimental dosages, but in water rather than syrup to facilitate analysis. Samples were analysed in an Agilent 1200 series liquid chromatograph interfaced via an electrospray ionisation source to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Samples and standards ($10 \mu\text{l}$) were injected onto an Eclipse Plus (Agilent Technologies, Santa Clara, CA, USA) C_{18} reverse phase column ($150 \text{ mm} \times 2.1 \text{ mm}$, $3.5 \mu\text{m}$). Mobile phase A was 2% acetonitrile + 0.1% formic acid. Mobile phase B was 95% acetonitrile + 0.1% formic acid. The elution conditions were 0 min – 0% B, 1 min – 70% B, 10 min – 80% B, 10.2 min – 100% B, 12 min – 100% B, with a flow rate of 0.3 ml min^{-1} increasing to 0.45 ml min^{-1} at 10 min. The source N_2 gas temperature was held at 350°C with a flow of 11 l min^{-1} and a nebulizer pressure of 35 psi. The capillary voltage was 4 kV. Fragmentor and collision energy voltages were 40 V and 20 V, respectively.

Imidacloprid was identified and quantified by selected reaction monitoring (SRM) using the product ion m/z 209 derived from the precursor ion of m/z 256. Samples of each dosage were spiked with a reference standard of 100 mg l^{-1} [^2H]imidacloprid (Sigma–Aldrich, Gillingham, UK). The deuterated imidacloprid was detected using a precursor ion m/z of 260 and a product ion m/z of 213. Imidacloprid concentrations in the dilution series were quantified by comparing peak areas from imidacloprid-d4 to peak areas of non-labelled imidacloprid in SRM chromatograms. The instrument response was linear over the range 0.0619 – $125 \mu\text{g l}^{-1}$ imidacloprid and we found that all dosages contained appropriate levels of imidacloprid (measured imidacloprid = $1.14 \times \text{nominal dosage} + 0.86$; $r^2 = 0.999$).

2.2. Statistical analyses

We tested whether bees responded to variation in imidacloprid dosage and whether dose–response relationships differed between bee species by analysis of covariance (ANCOVA) with ‘dose’, or $\log_{10}(\text{dosage of imidacloprid in } \mu\text{g l}^{-1} + 1)$, as the covariate and with ‘species’ and ‘solvent’ as fixed factors, where ‘solvent’ denotes a binary variable indicating the presence or absence of acetonitrile. Where necessary, the response variable was log-transformed to meet test assumptions and we conducted model simplification as described in Crawley (2007). Where the dose–response relationship was manifestly non-linear, we used analysis of variance (ANOVA) instead of ANCOVA and treated ‘dose’ as a categorical variable. All statistical analyses were conducted in R version 2.7.1 (Ihaka and Gentleman, 1996).

For bumble bees, rates of feeding on dosed syrups were corrected for intrinsic variation among individuals, which was likely due to age and body size. For each experiment, we regressed the post-dose mean daily feeding rate (mg d^{-1}) on the pre-dose feeding rate, which explained approximately 10% of variation

(e.g., $r^2 = 0.12$, $F_{1,374} = 49.3$, $P < 0.001$). The adjusted post-dose feeding rate for each individual was expressed relative to the performance of an average bee by adding the individual's residual from this least-squares regression to the mean rate of post-dose feeding among all bees.

In our control experiments that tested the effects of dietary acetonitrile, only locomotory activity (m h^{-1}) of bumble bees was affected (see Fig. S1 in Appendix A). To factor out this effect

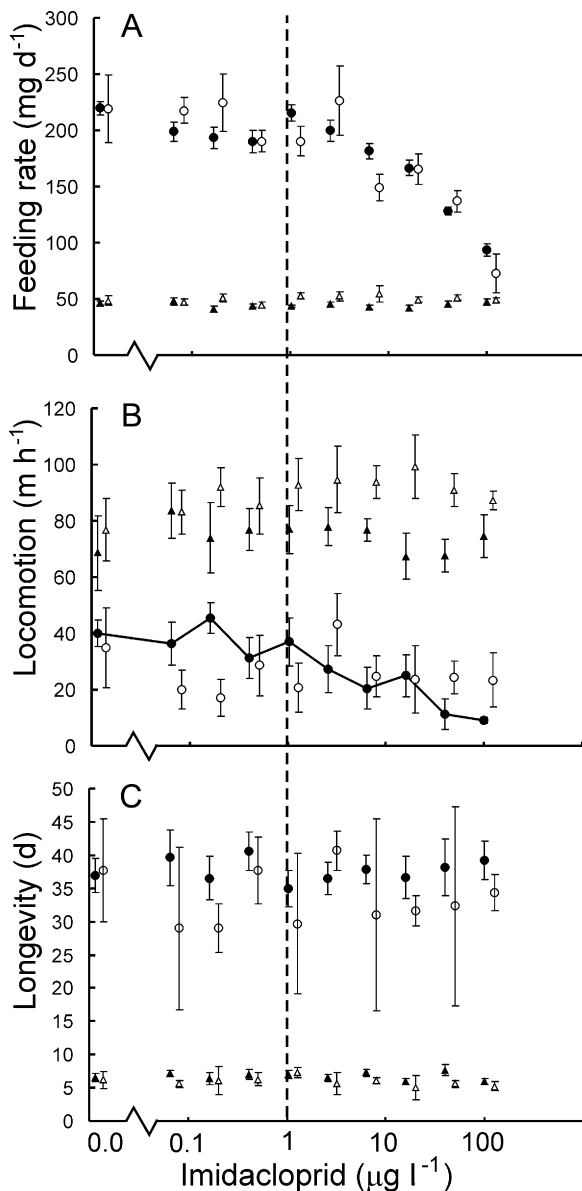


Fig. 1. Dose–response relationships for bees feeding on syrups containing imidacloprid at concentrations between 0.0 and 125 $\mu\text{g l}^{-1}$. (A) Mean daily feeding rate ($\text{mg syrup bee}^{-1} \text{d}^{-1}$); (B) mean rate of locomotion (m h^{-1}); (C) mean longevity in days under experimental conditions. Round symbols indicate mean measurements (± 1 s.e.m.) made on bumble bees, triangles indicate honey bees. Filled symbols indicate measurements made when diets contained acetonitrile (these are displaced slightly on the x-axis to reveal their error bars). Points in (B) are interpolated for ease of inspection. Dashed vertical line indicates 1 $\mu\text{g l}^{-1}$ for reference. Within treatments, individuals were distributed approximately evenly among levels of dosage and measurements were made on the following numbers of subjects: bumble bees, imidacloprid syrups $n = 107$ bees (except longevity, where $n = 30$), imidacloprid–acetonitrile $n = 292$; honey bees, imidacloprid syrups $n = 40$ cages, imidacloprid–acetonitrile $n = 100$. The solid line in (B) is for inspection purposes only and connects the mean locomotion rates of bumble bees fed on the acetonitrile–imidacloprid syrups.

from those experiments using acetonitrile–imidacloprid syrup, we regressed rate of locomotion while feeding on mixed syrups on the mean locomotion rate of individuals at the corresponding doses of acetonitrile. We then analysed the residual variation in locomotory activity for dependence on the concentration of dietary imidacloprid.

To determine whether the effect of dietary imidacloprid on feeding rate intensified over successive days, we regressed the mean daily feeding rate on days 2–6 of the exposure on the rate recorded during the first 24 h of feeding on dosed syrup. If the effect intensified, then the residuals from this least-squares relationship will vary systematically with dose. Specifically, the feeding rates of individuals at the higher doses of imidacloprid will be less than expected given their feeding on the first day and, therefore, the residuals associated with these individuals will be relatively more negative. We tested for systematic variation among the residuals by ANCOVA with ‘dose’ as the covariate and ‘solvent’ as a fixed factor to investigate the impact of acetonitrile. For bumble bees, the data on progressive changes in daily feeding rates for the imidacloprid-only diet were obtained from a separate experiment in which cages contained four individuals (cage dimensions: 0.12 m \times 0.12 m \times 0.04 m), but their use is appropriate because we ascertained that the highly characteristic twice-inflected shape of the dose–response curve for average daily feeding rate per individual across the 6-day exposure was qualitatively identical to that found among our individually caged bees.

3. Results

Individual bumble bees consumed more syrup per day than honey bees (ANOVA, $F_{1,484} = 3092.4$, $P < 0.001$; Fig. 1A) and the rate of feeding responded to the dosage of imidacloprid only in bumble bees (ANOVA: dose, $F_{1,484} = 50.0$, $P < 0.001$; dose \times species interaction, $F_{1,484} = 14.5$, $P < 0.001$). The form of the dose–response relationship in bumble bees was affected by the presence of dietary acetonitrile (ANOVA: species \times solvent, $F_{1,484} = 5.5$, $P = 0.02$), but the mean feeding rate declined significantly with increasing dosage of imidacloprid whether acetonitrile was present (Spearman’s correlation, $\rho = -0.82$, $P < 0.01$) or not ($\rho = -0.76$, $P < 0.02$). Each additional nanogram of imidacloprid in an individual bumble bee’s daily diet reduced syrup consumption by approximately 6% relative to that of undosed bees (Fig. 2).

Individual honey bees walked further than bumble bees (ANOVA, $F_{1,361} = 248.8$, $P < 0.001$; Fig. 1B), but locomotory activity responded to the dosage of imidacloprid only in bumble bees whose diet contained acetonitrile (ANOVA: dose, $F_{1,361} = 10.8$, $P = 0.001$; dose \times species interaction, $F_{1,361} = 5.3$, $P = 0.02$; dose \times solvent,

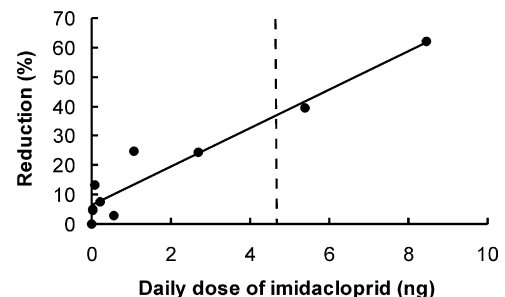


Fig. 2. Percentage reduction in feeding rate in dosed bumble bees relative to undosed bees as a function of their mean daily intake of imidacloprid in nanograms. The fitted line is a least-squares linear regression: percent reduction = $6.95 + 6.29$ (ng imidacloprid), $R^2 = 0.92$. The vertical dashed line indicates the maximum daily intake of imidacloprid in the dosed honey bees (i.e., 4.9 ng), whose feeding rate was unaffected relative to the controls.

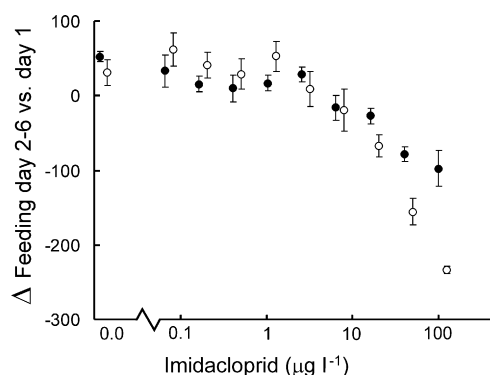


Fig. 3. Deviation of mean daily feeding rate of bumble bees (mg syrup bee⁻¹ d⁻¹) on days 2–6 after exposure to imidacloprid from the mean rate during the first 24 h (Δ feeding day 2–6 vs. day 1) in relation to the concentration of imidacloprid in the feeder syrup ($\mu\text{g l}^{-1}$). Filled symbols indicate measurements (± 1 s.e.m.) made when diets contained acetonitrile. Individuals were distributed approximately evenly among treatments and measurements were made on the following numbers of subjects: imidacloprid syrups $n = 52$, imidacloprid–acetonitrile $n = 149$.

$F_{1,361} = 10.9$, $P = 0.001$). In the presence of acetonitrile, mean locomotory rate declined significantly with increasing dosage of imidacloprid (Spearman's correlation, $\rho = -0.90$, $P < 0.001$).

Bumble bees lived longer than honey bees (ANCOVA, $F_{1,306} = 942.5$, $P < 0.001$; Fig. 1C), but the longevity of bees did not vary with the dosage of imidacloprid ($F_{1,306} = 1.0$, $P = 0.32$).

In bumble bees, the effect of dietary imidacloprid on feeding rate intensified over time, because feeding rates dropped progressively after the first day of exposure to the higher dosages of imidacloprid (ANCOVA, dose, $F_{1,194} = 74.4$, $P < 0.001$; Fig. 3). The magnitude of this effect depended on the presence of dietary acetonitrile (ANCOVA, dose \times solvent, $F_{1,194} = 144.9$, $P < 0.001$; Fig. 3); in its absence, individuals exposed to the highest dosage of imidacloprid eventually fed at approximately half the rate of undosed bees after >24 h of exposure, but dietary acetonitrile accelerated this effect so that it occurred within the first day of exposure with little subsequent intensification (Fig. 3).

4. Discussion

4.1. The dose–response relationship in bumble bees

We found that bumble bees were affected by dietary imidacloprid on two measures (daily feeding rate and locomotory activity, although the second effect was evident only in the presence of dietary acetonitrile). We argue that the detrimental influence of dietary imidacloprid on feeding rate in bumble bees was not principally the result of antifeedant properties, but instead has its basis in toxicity. Imidacloprid is an antifeedant at concentrations above $40 \mu\text{g l}^{-1}$ (DEFRA, 2007) but we have demonstrated that the effect of imidacloprid on feeding rate showed dose-dependent intensification over time. To account for this, we must postulate either that bees showed 'avoidance learning' (Bolles, 1970) after experiencing an aversive stimulus from feeding on dietary imidacloprid or that dietary imidacloprid reduced the bees' ability or need to feed. Either way, we must postulate that imidacloprid had a toxic effect on bumble bees.

We found that the feeding rate of bumble bees declined rapidly as the dosage of imidacloprid in syrup increased above $1.28 \mu\text{g l}^{-1}$ ($1.0 \mu\text{g kg}^{-1}$), which coincided with daily ingestion rates for individual bees of >1 ng. These results are consistent with previous laboratory studies which found that bumble bees are affected by dietary imidacloprid in the same range of dosages (Tasei et al., 2000; Mommaerts et al., 2010). However, whereas other studies

have found that dietary imidacloprid in syrups dosed at approximately $10 \mu\text{g kg}^{-1}$ was capable of reducing the longevity of *B. terrestris* individuals (Tasei et al., 2000; Mommaerts et al., 2010), we found no such effect. The disparity probably arises because the severity of imidacloprid's toxic effects depends on the test conditions. Like us, Mommaerts et al. (2010) found that bumble bees could survive very well in the laboratory while feeding in an enclosed nest box on syrup dosed with imidacloprid at $10 \mu\text{g kg}^{-1}$, but they also observed almost complete mortality within two weeks among individual bees that were forced to fly 6 m trips to forage on an equivalent syrup under glasshouse conditions. The physiological basis of this locomotion-dependent toxicity remains unknown.

For bumble bees, the dose–response relationship between feeding rate and the dosage of imidacloprid had a double inflection. Determining the physiological basis for the inflections is beyond the scope of our current study, but it deserves comment because we found that this distinctive pattern recurred in separate experiments and we therefore speculate as follows. The twice-inflected dose–response relationship is consistent with the existence of an inducible detoxification system. The experimental doses of imidacloprid below the induction threshold are toxic or impair feeding, but when the threshold is reached, detoxification is induced and feeding returns to normal. When the level of imidacloprid increases further, the system is eventually overwhelmed and toxic effects again become evident in proportion to the concentration of the dose. A twice-inflected dose–response relationship also links the concentration of certain antibiotic drugs (e.g., penicillin and caspofungin) to the percentage of microbes killed, where it is called the 'quadruphasic' dose–response effect (Wiederhold, 2009), the 'paradoxical effect' (Stevens et al., 2004), or the 'Eagle effect', after the microbiologist Harry Eagle, who first characterised it (Eagle and Musselman, 1948). The mechanism that underlies this phenomenon in microbes is not fully understood, but it is also thought to depend on the dose reaching a threshold level that is sufficient to induce derepression or activation of a resistance mechanism (Stevens et al., 2004), as we have similarly speculated.

The detrimental effect of dietary imidacloprid on locomotory activity was evident only when acetonitrile was present. Acetonitrile causes nervous disruption in mammals (Ahmed et al., 1992) and may have affected the bees either directly or through its metabolites, which include hydrogen cyanide (Ohkawa et al., 1972). In our study, dietary acetonitrile also accelerated the impact of imidacloprid on feeding rate. We therefore speculate that this organic solvent affected membrane permeability and thereby facilitated the entry of imidacloprid into the bodies of bees. Acetonitrile is not an ingredient in commercial pesticide formulations, so this physiological phenomenon is not environmentally relevant.

4.2. The dose–response relationship in honey bees

In our study, individual bees ingested approximately 50 mg d^{-1} of syrup at the highest dosage of $125 \mu\text{g l}^{-1}$ (a daily intake of about 4.9 ng) without exhibiting a detectable response on our measures. If taken in a single meal, this amount of imidacloprid is likely to be lethal to a honey bee, because the LD_{50} (level that kills 50% of subjects) for an acute dose is 4.5 ng (Cresswell, 2011). We suggest that the honey bees in our study survived because they ingested imidacloprid gradually throughout the day and metabolised it continuously and thereby avoided accumulating it to a lethal level.

Consistent with previous work (Suchail et al., 2004), our results suggest that honey bees have a substantial capacity to detoxify dietary imidacloprid. The apparent insensitivity of honey bees to dietary imidacloprid in our investigation contrasts with the results

of previous studies (reviewed in Cresswell, 2011), which found detrimental effects at dosages that were much smaller than our highest dose. We propose three possible explanations for this discrepancy.

First, our measurements of performance may have been insensitive as symptoms of toxicity. In contrast to our methodology, previous laboratory tests on individual honey bees (Decourtye et al., 2003, 2004) used the PER (proboscis extension response) paradigm (Bitterman et al., 1983), which reveals impacts on insect learning and memory (Gauthier, 2010). PER is a very sensitive assay and insects may respond to low doses that do not produce other obvious symptoms (Soliman and Cutkomp, 1963; Sharma, 1973). Consequently, it is possible that the measurements on honey bees that we made here simply were unable to reveal subtle neurotoxic disruption. Even so, it is surprising that we failed to detect an effect at our highest dosage ($125 \mu\text{g l}^{-1}$), which is expected to produce a performance deficit of approximately 60% in PER tests (Cresswell, 2011).

Second, it is possible that the discrepancy between our results and those of PER-based studies arises from differences in the way the bees were treated during the experiments. PER trials require bees to be temporarily refrigerated and subsequently immobilised in a jacket throughout the testing procedure (Bitterman et al., 1983). If a bee's metabolic rate falls during this enforced immobility, its detoxification processes may be impaired. For example, if neonicotinoids are metabolised by cytochrome P450 mono-oxygenases (Suchail et al., 2004), these are thermally rate-limited (Puntarulo and Cederbaum, 1989) and therefore refrigeration and immobilisation may impair detoxification and thereby increase neurotoxic impacts. In contrast, we tested freely roaming bees in our experiments and, in our laboratory, thermal camera measurements indicated that honey bees maintained their body temperature approximately 2°C above the environmental ambient of 25°C , which may have enabled them to metabolise and detoxify imidacloprid better than bees under a PER regime.

Third, we may have studied an unusually resistant stock of honey bees. However, bees drawn from the same hives exhibited the expected performance deficits in standard PER tests (Holmbergh and Hempel de Ibarra, pers. obs.).

In summary, the discrepancy between the results of our work on honey bees and those of previous studies could have arisen either because of differences in sensitivity to dose among the responses studied or because of dissimilar experimental procedures. To resolve this uncertainty, new studies must be conducted on the effect of temperature on imidacloprid metabolism and on the effect of imidacloprid on learning in freely roaming honey bees.

4.3. Relative sensitivity of bumble bees and honey bees

Individual honey bees were capable of ingesting up to 4.9 ng of imidacloprid per day without evident effect, whereas an equivalent intake is expected to cause a 38% reduction in feeding by bumble bees (Fig. 2). Our results therefore indicate that bumble bees were more sensitive to dietary imidacloprid than honey bees. This finding appears to contradict the conclusion of a survey of the toxicological literature (Hardstone and Scott, 2010), which was that bumble bees are less sensitive than honey bees to pesticides generally. However, Hardstone and Scott's review was based only on experiments where the xenobiotic was topically applied, which means that the disparity in sensitivity between honey bees and bumble bees could have originated in differences in absorption due to cuticle properties. Consequently, the greater sensitivity of bumble bees to oral dosing that we observed is not necessarily anomalous.

The differential sensitivity of the two bee species to dietary imidacloprid could originate in differences in either target-site sensitivity (Liu et al., 2005) or the capacity for detoxification by

enhanced metabolism (Puinean et al., 2010). We do not expect that honey bees are particularly likely to exhibit relatively high levels of target-site insensitivity, because the honey bee genome codes for a nicotinic acetylcholine receptor (nAChR) ligand binding domain that interacts with neonicotinoids (Matsuda et al., 2009). We therefore infer that enhanced metabolism of imidacloprid by honey bees (Suchail et al., 2004) is principally responsible for their low sensitivity relative to bumble bees. If generalized P450 mono-oxygenase activity is indeed responsible for rapid metabolism of imidacloprid in honey bees (Suchail et al., 2004; but see Iwasa et al., 2004), they may show some cross-resistance (Mota-Sanchez et al., 2006; Rauch and Nauen, 2003) to other neonicotinoids, but this is currently unstudied.

Our findings are consistent with the hypothesis that honey bees are better pre-adapted than bumble bees to feed on nectars containing neonicotinoid pesticides by virtue of ancestral adaptation to tropical nectars. Proving this hypothesis depends in part on accumulating instances where tropical nectars that contain natural alkaloids are toxic to bumble bees, but not to honey bees. Currently, there is insufficient information to provide this comparative toxicology (Adler, 2000). To our knowledge, there is only one other well-studied example, namely the alkaloid gelsemine, which occurs naturally in the nectar of *Gelsemium sempervirens* L. This instance neither supports the hypothesis, because it is not particularly toxic to bumble bees (Manson and Thomson, 2009), nor does it count strongly against it, because comparative toxicological tests on honey bees have not been conducted (Detzel and Wink, 1993). While our results begin to show that differential pre-adaptation between honey bees and bumble bees is plausible, more investigation is required to properly test the hypothesis.

4.4. Implications for the sustainability of bee populations

Our findings cannot be used to infer that honey bee colonies are invulnerable to dietary imidacloprid under field conditions and they do not in themselves eliminate imidacloprid as a potential contributor to declines in honey bee populations. Rather, we have revealed a relationship, namely that bumble bees are relatively more sensitive than honey bees to toxic effects caused by dietary imidacloprid. Our results therefore raise concern about the impact of widely used agricultural neonicotinoids on bumble bee populations, which have also been identified elsewhere (Whitehorn et al., 2012). In the environmentally relevant range for imidacloprid residues in nectars of treated crops, which is approximately $1\text{--}10 \mu\text{g l}^{-1}$, we have demonstrated that dietary imidacloprid reduced the feeding rate of individual bumble bees by approximately 10–30%. The demographic consequences of these effects and their ecological impact on pollination services are currently uncertain. In the United Kingdom, there is arguably a greater potential for impact from systemic pesticides on bumble bees than on honey bees because of their life cycle. In particular, fields of oilseed rape typically bloom in late spring (Hoyle et al., 2007), which coincides with the foraging activity of many bumble bee queens (Goulson, 2003), newly emerged from hibernation, whose individual performance determines the fate of an entire colony. If the flowers in these fields contain imidacloprid residues, bumble bees are exposed to potential toxicity at a critical stage in their colony development. For a factor to cause population declines among social bees, its detrimental effects must impact on the colony, which is the unit of reproduction, but some previous investigations of dosed bumble bee colonies have found no such detrimental effect (Franklin et al., 2004; Morandin and Winston, 2003). However, the power of the statistical testing in these colony-level studies was perhaps limited by high levels of within-treatment variation among colonies, as in previous studies of honey bee colonies (Cresswell, 2011). In contrast, imidacloprid has been shown to have a

detrimental effect on the production of queens by bumble bee colonies under field conditions (Whitehorn et al., 2012). Whether the detrimental effects on individuals that we have detected constitute the basis of a threat to the sustainability of bumble bee populations remains a target for further research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2012.05.003>.

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