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Effects of the neonicotinoid pesticide thiamethoxam at field-realistic levels on microcolonies of *Bombus terrestris* worker bumble bees

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ABSTRACT

Neonicotinoid pesticides are currently implicated in the decline of wild bee populations. Bumble bees, *Bombus* spp., are important wild pollinators that are detrimentally affected by ingestion of neonicotinoid residues. To date, imidacloprid has been the major focus of study into the effects of neonicotinoids on bumble bee health, but wild populations are increasingly exposed to alternative neonicotinoids such as thiamethoxam. To investigate whether environmentally realistic levels of thiamethoxam affect bumble bee performance over a realistic exposure period, we exposed queenless microcolonies of *Bombus terrestris* L. workers to a wide range of dosages up to 98 $\mu g\,kg^{-1}$ in dietary syrup for 17 days. Results showed that bumble bee workers survived fewer days when presented with syrup dosed at 98 μg thiamethoxam kg^{-1}, while production of brood (eggs and larvae) and consumption of syrup and pollen in microcolonies were significantly reduced by thiamethoxam only at the two highest concentrations (39, 98 $\mu g\,kg^{-1}$). In contrast, we found no detectable effect of thiamethoxam at levels typically found in the nectars of treated crops (between 1 and 11 $\mu g\,kg^{-1}$). By comparison with published data, we demonstrate that during an exposure to field-realistic concentrations lasting approximately two weeks, brood production in worker bumble bees is more sensitive to imidacloprid than thiamethoxam. We speculate that differential sensitivity arises because imidacloprid produces a stronger repression of feeding in bumble bees than thiamethoxam, which imposes a greater nutrient limitation on production of brood.

1. Introduction

The pollination services of wild bees help to maintain plant species in natural ecosystems and are worth billions of dollars annually to agriculture (Williams and Osborne, 2009; Winfree, 2010). Evidence of declining wild bee populations (Biesmeijer et al., 2006) and the extirpation of certain species (Burkle et al., 2013) are therefore issues of increasing concern (Vanbergen, 2013). It is widely acknowledged that several factors are driving declines in wild bees (Williams and Osborne, 2009; Potts et al., 2010). However, a group of neurotoxic pesticides, the neonicotinoids, have specifically been singled out for blame (Shardlow, 2013), which has led to calls for restrictions on their use in agricultural (EFSA, 2013a; Maxim and van der Sluijs, 2013) that have recently been implemented across the European Union (European Commission, 2013). The neonicotinoids, which include imidacloprid, thiamethoxam and clothianidin, are systemic and so the pesticide is distributed throughout plant tissues to control sucking insect pests (Elbert et al., 2008). Consequently, trace residues can appear in nectar and pollen (Blacquière et al., 2012) and bees are exposed to dietary neonicotinoids by foraging from the flowers of treated agricultural crops (Elbert et al., 2008).

Bumble bees are important wild pollinators that are detrimentally affected by neonicotinoids in laboratory studies, where dietary residues reduce food consumption and brood production of *Bombus terrestris* L. workers (Tasei et al., 2000; Mommaerts et al., 2010; Cresswell et al., 2012; Laycock et al., 2012), and in semi-field studies, where *B. terrestris* colonies under exposure exhibit reduced production of brood, workers and queens (Gill et al., 2012; Whitehorn et al., 2012). The majority of these studies focus solely on imidacloprid, which has historical relevance because it was the first neonicotinoid in widespread use (Elbert et al., 2008) and was identified publicly as a potential threat to bee health in 1999 (Maxim and van der Sluijs, 2013). However, newer neonicotinoid varieties, such as thiamethoxam and its toxic metabolite clothianidin, are increasingly preferred to imidacloprid in crop protection. For example, in 2011 imidacloprid made up just 10 percent of the total 80,000 kg of neonicotinoid applied to UK crops (FERA, 2013). Consequently wild bumble bees are at increased risk of exposure to these alternative neonicotinoids. We therefore chose to further investigate the effects of dietary thiamethoxam on bumble bees.

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Residues of thiamethoxam ranging from 1 to 11 μg kg^{-1} (=parts per billion or ppb) have been detected in nectar from treated crops including alfalfa, oilseed rape, pumpkin, sunflower, squash and *Phacelia tanacetifolia* (Dively and Kamel, 2012; EFSA, 2012; Stoner and Eitzer, 2012). In pollen, residues are typically higher, ranging from 1 to 12 μg kg^{-1} in sunflower, oilseed rape and squash, but reaching 39, 51 and 95 μg kg^{-1} in *Phacelia*, alfalfa, and pumpkin, respectively (Dively and Kamel, 2012; EFSA, 2012; Stoner and Eitzer, 2012). For bees, exposure to residues such as these probably occurs in transient pulses; for example, during the mass-flowering of treated oilseed rape that lasts for approximately one month and peaks over a period of around two weeks (Hoyle et al., 2007; Westphal et al., 2009). Detrimental effects on honey bees of dietary thiamethoxam at 67 μg L^{-1} have already been demonstrated (Henry et al., 2012), but the effects on bumble bees in a similar dosage range are unclear. For example, in one *B. terrestris* microcolony study 100 μg kg^{-1} thiamethoxam presented to workers in sugar solution increased mortality and reduced drone production while residues at 10 μg kg^{-1} had no detectable effect (Mommaerts et al., 2010). However, in another study 10 μg kg^{-1} thiamethoxam reduced workers' production of drone brood (the workers' eggs and larvae), while microcolony feeding rates were reduced at both 1 and 10 μg kg^{-1} (Elston et al., 2013). With evidence of thiamethoxam's effects currently inconsistent, it remains uncertain whether environmentally realistic residues are capable of having a detrimental impact on bumble bee populations. We therefore present an experiment designed to test the performance of bumble bees presented with dietary thiamethoxam at a wide range of concentrations, including dosages within the field-realistic range for nectar.

In this study, we made use of the reproductive capacity of *B. terrestris* workers in queenless microcolonies to investigate the effects of thiamethoxam on bumble bee performance. In microcolonies, small groups of bumble bee workers are maintained in the absence of a queen and, over a period of days, a dominant worker lays eggs that will develop into drones while the others forage and care for brood (Tasei et al., 2000). In a recent guidance document for risk assessment of plant protection products on bees (EFSA, 2013b), the use of microcolonies was recommended as part of 'higher tier' risk assessment studies in bumble bees. Using *B. terrestris* microcolonies, we characterised dose–response relationships that described thiamethoxam's effects on brood (eggs and larvae) production, food consumption and days survived by workers (Laycock et al., 2012) over an exposure lasting 17 days. Following laboratory exposure periods of similar length, imidacloprid produced substantive sublethal effects on feeding and brood production in *B. terrestris* microcolonies (Laycock et al., 2012) and reduced colony growth and production of new queens in queenright colonies allowed to develop for a further six weeks in pesticide-free conditions (Whitehorn et al., 2012). Here we applied dosages and some endpoints that were adopted in the imidacloprid microcolony study (*i.e.* Laycock et al., 2012) to enable us to compare the relative sensitivity of bumble bees to the two neonicotinoids.

2. Materials and methods

2.1. Microcolonies

We obtained four colonies of *B. terrestris* (subspecies *audax*) (Biobest, Westerlo, Belgium) each consisting of a queen and approximately 150 workers. One hundred queenless microcolonies were established by placing 400 individual workers (100 from each queenright colony) into softwood boxes (120 × 120 × 45 mm) in groups of four. The allocation of workers to boxes was randomized, but each microcolony contained workers from the same queenright colony. Each box was fitted with two 2 mL microcentrifuge tubes (Simport, Beloeil, Canada) that were punctured so as to function as syrup (artificial nectar) feeders. We maintained microcolonies for 18 days under semi-controlled conditions (23–29 °C, 20–40 percent relative humidity) and in darkness except during data collection. Specifically, all microcolonies were acclimatised to experimental conditions by feeding *ad libitum* on undosed control syrup (Attracker: 1.27 kg L^{-1} fructose/glucose/saccharose solution; Koppert B.V., Berkel en Rodenrijs, Netherlands) for 24 h prior to 17 days of exposure to thiamethoxam. A single bee that died during acclimatisation was replaced with a worker from its queenright source colony.

2.2. Thiamethoxam dosages

To produce a primary thiamethoxam stock solution (10^5 μg thiamethoxam L^{-1}), we dissolved 5 mg thiamethoxam powder (Pestanal®; Sigma-Aldrich, Gillingham, UK) in 50 mL purified water. Primary stock solution was further diluted (to 10^4 μg L^{-1}) in purified water and an aliquot of diluted stock was mixed into feeder syrup to produce our most concentrated dietary solution of 125 μg thiamethoxam L^{-1} (or 98.43 μg kg^{-1}=ppb). By serial dilution from the highest concentration we produced nine experimental dosages at the following concentrations: 98.43, 39.37, 15.75, 6.30, 2.52, 1.01, 0.40, 0.16, 0.06 μg thiamethoxam kg^{-1}. Following acclimatisation, microcolonies were fed *ad libitum* for 17 days with undosed pollen balls (ground pollen pellets, obtained from Biobest, mixed with water; mean mass=5.3 g, SE=0.1 g) and either undosed control syrup (19 control microcolonies) or syrup dosed with thiamethoxam (9 dosed microcolonies per thiamethoxam concentration, listed above). This level of replication (*i.e.* a minimum of nine replicates per concentration) is consistent with similar microcolony studies (Mommaerts et al., 2010; Laycock et al., 2012; Elston et al., 2013). Pollen balls were weighed before and after placement into microcolonies to quantify pollen consumption and syrup feeders were weighed each day to measure syrup consumption. We corrected for evaporation of water from syrup and pollen based on the mass change of syrup feeders and pollen balls maintained under experimental conditions, but not placed into microcolonies. Additionally, where syrup or pollen was collected by bees but not consumed, for example where syrup was stored in wax honey pots, its mass was determined and subtracted from consumption accordingly. We monitored microcolonies daily for individual worker mortality and the appearance of wax covered egg cells that indicate the occurrence of oviposition. To assess brood production, at the end of the experiment we freeze-killed workers in their microcolony boxes and collected all laid eggs and larvae from the nests. In our previous microcolony study (Laycock et al., 2012), we also investigated the effect of imidacloprid on ovary development because imidacloprid produced a dose-dependent decline in workers' brood production. Except at the highest dosages, thiamethoxam had no effect on brood production (*i.e.* microcolonies laid eggs at a statistically equivalent rate, see Section 3) and we therefore chose not to measure ovary development here. The experiment was conducted in two replicate trials between October and December 2012. Each trial comprised 50 microcolonies and dosage groups were approximately equally represented in both. The results of the two trials were qualitatively similar and so data were pooled for further analysis.

We verified the concentration of thiamethoxam in our doses using solid phase extraction (SPE) and liquid chromatography–mass spectrometry (LCMS) as follows. First, we dissolved our dosed syrups in LCMS-grade water (Fisher Scientific, Loughborough, UK). To extract thiamethoxam from syrup, the diluted samples were processed through 1 mL Discovery® DSC-18 SPE tubes (Sigma-Aldrich, Gillingham, UK) under positive pressure. Specifically, we conditioned the SPE tube with 1 mL LCMS-grade methanol (Fisher Scientific, Loughborough, UK) followed by 1 mL LCMS-grade water, prior to passing through a 1 mL diluted sample. The tube was washed with 1 mL LCMS-grade water and the thiamethoxam was eluted from the column with three separate, but equivalent, aliquots of LCMS-grade methanol, totalling 450 μL. Methanol was removed by evaporation in a ScanSpeed MaxiVac Beta vacuum concentrator (LaboGene ApS, Lynge, Denmark) and the remaining thiamethoxam was dissolved in 500 μL of LCMS-grade water. Extracted thiamethoxam 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), along with a calibration curve consisting of nine known thiamethoxam concentrations that ranged from 0.1 to 125 μg L^{-1}, using methods described in Laycock et al. (2012). The instrument response was linear over the range 0.1–125 μg L^{-1}, with the relationship of the calibration curve given by $instrument\ response = 228.42 \times thiamethoxam\ concentration + 265.87$, $R^2 > 0.99$). We used the calibration equation to determine the concentration values of our extracted samples and found that all dosages contained appropriate levels of thiamethoxam ($measured\ thiamethoxam = 1.16 \times nominal\ dosage + 1.57$, $R^2 > 0.99$).

2.3. Statistical analyses

In our experiments, endpoints responded only to the two highest dosages of thiamethoxam (see Section 3). We therefore analysed the variation in food consumption and days survived by workers in microcolonies that was due to thiamethoxam using one-way ANOVA, with *dosage* (dosage of thiamethoxam in

μg kg^{-1}) treated as a categorical variable, and compared the highest dosage groups to those below using orthogonal contrasts.

We tested whether the two highest thiamethoxam dosages were associated with an increased frequency of oviposition failure (zero brood produced) using a 2×2 contingency table and Pearson's Chi-squared test with Yates' continuity correction.

To determine whether brood production was dose-dependent below the two highest dosages, we used zero-inflated Poisson regression (ZIP) because of an excess of zero counts in our data (Lambert, 1992). We tested the appropriateness of the ZIP model by comparing it to a standard Poisson model using a Vuong non-nested test and confirmed that the ZIP model was the superior choice (*Vuong test statistic* $= -5.17$, $P < 0.001$).

In our analysis, the total number of eggs and larvae produced in microcolonies during the 17-day exposure period represents *brood* (brood were not produced during pre-dose acclimatisation). Where necessary, we log-transformed *dosage* to log (*dosage* + 1) to meet test assumptions. All statistical analyses were conducted in *R* v3.0.0 (Ihaka and Gentleman, 1996).

3. Results

Per capita consumption of syrup and pollen in microcolonies was significantly affected by thiamethoxam (ANOVA: *syrup consumption*, $F_{9,90} = 9.29$, $P < 0.001$; *pollen consumption*, $F_{9,90} = 15.14$, $P < 0.001$; Fig. 1). Specifically, a significant reduction in food consumption was evident only in microcolonies exposed at the two highest dosages, 39 μg kg^{-1} and 98 μg kg^{-1} (orthogonal contrast: *syrup consumption*, $F_{9,90} = 9.29$, $t = -8.87$, $P < 0.001$; *pollen consumption*, $F_{9,90} = 15.14$, $t = -11.22$, $P < 0.001$). No dose-dependent variation was detectable among microcolonies exposed to dosages ≤ 16 μg kg^{-1} (ANOVA: *syrup consumption*, $F_{7,74} = 0.39$, $P = 0.91$; *pollen consumption*, $F_{7,74} = 0.90$, $P = 0.51$). Despite consuming less syrup, microcolonies exposed to higher dosages nevertheless ingested larger amounts of thiamethoxam (Table 1).

In microcolonies, the frequency of oviposition failure at the two highest thiamethoxam dosages (94 percent failure) was greater than at lower dosages (48 percent) and these frequencies differed significantly (Chi-squared contingency table analysis: $X^2 = 11.33$, $df = 1$, $P < 0.001$; Fig. 2). Excluding the two highest dosages, thiamethoxam did not significantly affect the number of brood

Table 1
Frequency of successful oviposition in *Bombus terrestris* bumble bee microcolonies, with the number of brood (eggs and larvae) produced by successful ovipositors and the time at which first oviposition occurred. Microcolonies ($N = 100$) were presented with thiamethoxam (TMX) in feeder syrup at given dosages for 17 days (replicates per dosage group: control, $N = 19$; dosage treatments, $N = 9$ per concentration). *Per capita* consumption of TMX in microcolonies is provided for each dosage treatment. Only data from the 44 percent (44/100) of microcolonies that produced brood is provided in successful oviposition, brood given oviposition and day of first oviposition columns. Except for successful oviposition, data represent the mean ± SE. We found no detectable effect of dosage on brood production or timing of oviposition in successfully ovipositing microcolonies (Spearman's correlation, $P > 0.05$).

TMX dosage (μg kg^{-1} ppb^{-1})	TMX consumed (ng bee^{-1} day^{-1})	Successful oviposition (percent)	Brood, given oviposition	Day of first oviposition
Control	0.000 ± 0.000	63	5.4 ± 1.1	10.7 ± 0.7
0.1	0.021 ± 0.002	67	4.3 ± 1.8	11.0 ± 0.5
0.2	0.051 ± 0.003	78	5.7 ± 1.6	13.1 ± 1.5
0.4	0.131 ± 0.004	22	11.0 ± 3.2	9.8 ± 3.5
1.0	0.324 ± 0.027	22	3.0 ± 0.0	9.5 ± 1.5
2.5	0.777 ± 0.068	33	5.3 ± 1.8	11.3 ± 3.6
6.3	1.809 ± 0.085	44	3.3 ± 1.0	12.8 ± 2.6
15.7	5.101 ± 0.509	67	5.0 ± 1.6	11.3 ± 0.6
39.4	7.379 ± 0.602	11	5.0 ± 0.0	12.0 ± 0.0
98.4	14.785 ± 2.076	0	–	–
	All ovipositing microcolonies		5.3 ± 0.6	11.4 ± 0.5

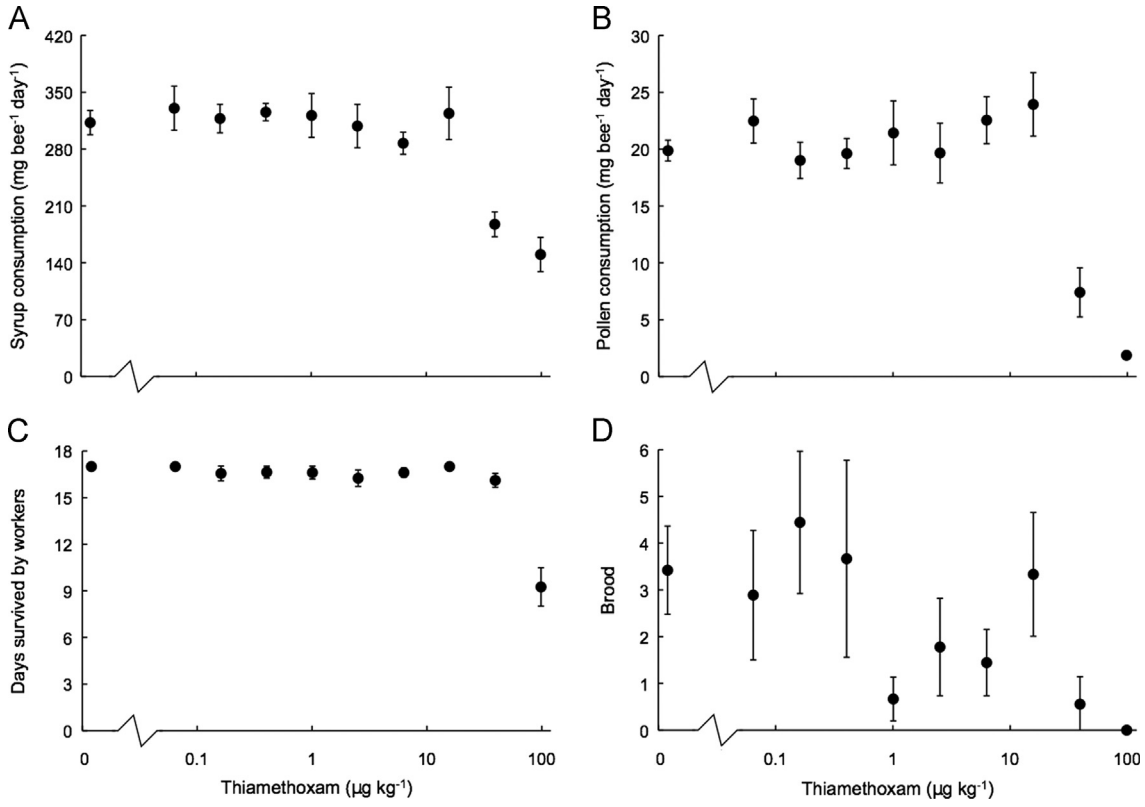

Fig. 1. Daily *per capita* feeding rates, days survived by workers and brood production in *Bombus terrestris* bumble bee microcolonies following 17 days of exposure to thiamethoxam in dosed syrup (μg kg^{-1} = parts per billion). (A) Daily *per capita* consumption of dosed syrup; (B) daily *per capita* consumption of undosed pollen; (C) number of days workers survived while under exposure (maximum = 17 days); and (D) brood production (eggs and larvae produced; data includes microcolonies that failed to oviposit). Data represent the means and error bars indicate ± SE (replicates per dosage group: control, $N = 19$ microcolonies; dosage treatments, $N = 9$ microcolonies per concentration). Control data (0 μg kg^{-1}) are displayed slightly displaced on the *x*-axis for ease of inspection.

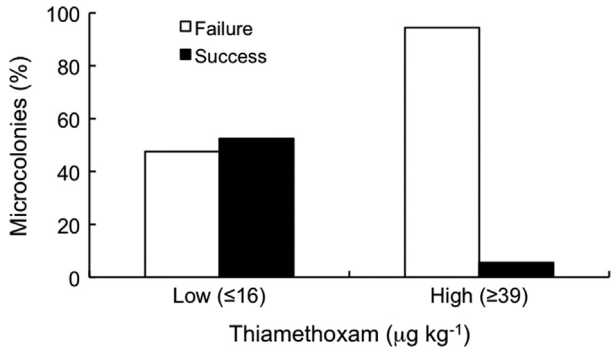

Fig. 2. Frequency of oviposition failure and success in *B. terrestris* bumble bee microcolonies presented for 17 days with thiamethoxam in dosed syrup ($\mu g\ kg^{-1}$ = parts per billion). Low dosage group ($N=82$) and high dosage group ($N=18$) consist of microcolonies exposed to dietary thiamethoxam at concentrations of ≤ 16 and $\geq 39\ \mu g\ kg^{-1}$, respectively. Open bars represent failure to produce brood (zero brood produced) and filled bars represent success ($\geq$ one brood individual produced). Frequency of oviposition failure in the high dosage group (94 percent) differed significantly from that in low dosage group (48 percent; Chi-squared contingency table analysis, $P<0.001$).

produced (ZIP regression: *brood count*, $z=-1.26$, $P=0.21$; *zero brood production*, $z=0.45$, $P=0.65$; Fig. 1).

Among microcolonies that produced brood, there was no effect of dosage on the number of brood produced or on the timing of first oviposition (Spearman's correlation: *brood vs. dosage*, $\rho=-0.03$, $N=44$, $P=0.85$; *days until oviposition vs. dosage*, $\rho=0.08$, $N=44$, $P=0.63$; Table 1).

The number of days survived by workers in microcolonies varied significantly with thiamethoxam dosage (ANOVA: $F_{9,90}=27.43$, $P<0.001$; Fig. 1), but it was reduced only at 98 $\mu g\ kg^{-1}$ (orthogonal contrast: $F_{9,90}=27.43$, $t=-15.44$, $P<0.001$) and did not differ at lower dosages (ANOVA: $F_{8,82}=1.25$, $P=0.28$).

4. Discussion

4.1. Thiamethoxam effects

We found that thiamethoxam reduced feeding and brood production in *B. terrestris* microcolonies that fed on syrup with a dietary concentration of 39 $\mu g\ kg^{-1}$ or above for 17 days. At lower dosages, microcolonies consumed syrup and pollen at normal control rates and brood production was not detectably dose-dependent. These results are consistent with those of a previous *B. terrestris* microcolony study in which dietary thiamethoxam produced an EC_{50} for drone production of 35 $\mu g\ kg^{-1}$ and had no observable effect on workers at 10 $\mu g\ kg^{-1}$ (Mommaerts et al., 2010). However, another recent study reported that 10 $\mu g\ kg^{-1}$ thiamethoxam was capable of reducing syrup feeding and brood production in microcolonies (Elston et al., 2013). These contrasting results may have arisen because bumble bees consumed different amounts of thiamethoxam in nominally equivalent treatment groups, with Elston et al. (2013) having dosed both syrup and pollen at 10 $\mu g\ kg^{-1}$, whereas Mommaerts et al. (2010), like us, dosed only syrup. Additionally, our results correspond with studies of clothianidin, which is thiamethoxam's primary toxic metabolite and becomes active during thiamethoxam exposure (Nauen et al., 2003). Specifically, dietary clothianidin at 38 $\mu g\ kg^{-1}$ negatively influenced honey bee foraging behaviour (Schneider et al., 2012), but lower dosages had no adverse effects on colonies of *Bombus impatiens* Cresson bumble bees (Franklin et al., 2004).

Where thiamethoxam was presented to microcolonies at 39 $\mu g\ kg^{-1}$ or above, we observed an association between impaired feeding on syrup and pollen and failure to produce brood. A similar association was observed in *B. terrestris* microcolonies fed imidacloprid across a range of dosages (Laycock et al., 2012). The hypothesis proposed by Laycock et al. (2012), that nutrient limitation imposed by an imidacloprid-induced reduction of feeding may be responsible for repression of brood production in bumble bees, can also be applied in our current study to explain thiamethoxam's detrimental effect on brood production at higher dosages. We therefore postulate that the capacity to impair bumble bee feeding behaviour is common amongst neonicotinoids, particularly at high dosages, and this may provide a general mechanism for reduced brood production (Gill et al., 2012; Laycock et al., 2012; Elston et al., 2013).

Consistent with previous findings (Mommaerts et al., 2010), the number of days survived by workers was significantly reduced in microcolonies fed approximately 100 $\mu g\ kg^{-1}$ thiamethoxam. For honey bees, relatively large dosages of thiamethoxam (67 $\mu g\ L^{-1}$) also impact on worker survival (Henry et al., 2012). Apparently, these relatively high concentrations of dietary thiamethoxam are highly toxic to bees in general.

4.2. Differential sensitivity of bumble bees to thiamethoxam and imidacloprid

In other toxicology studies the biological efficacy of thiamethoxam is said to be comparable to other neonicotinoids (Nauen et al., 2003), but relative toxicity is somewhat inconsistent among studies and species. For example, the LD_{50} for bees was lower for imidacloprid than thiamethoxam in topical and oral toxicity studies (Iwasa et al., 2004; Mommaerts et al., 2010), but higher when other beneficial arthropods and pest species were tested (Magalhaes et al., 2008; Prabhaker et al., 2011). Our study indicates that bumble bees may be less sensitive to thiamethoxam than imidacloprid at dosages in the realistic range typically found in nectars of treated crops (approximately 1–11 $\mu g\ kg^{-1}$; Dively and Kamel, 2012; EFSA, 2012; Stoner and Eitzer, 2012). Whereas we found no detectable effect on *B. terrestris* microcolonies of thiamethoxam in this range, a previous study conducted under approximately identical conditions found that dietary imidacloprid was capable of substantively reducing brood production and food consumption in microcolonies at concentrations as low as 1.0 and 2.5 $\mu g\ kg^{-1}$, respectively (Laycock et al., 2012). Similar differences in sensitivity have been demonstrated in aphids, *Myzus spp.*, with imidacloprid repressing feeding at concentrations as low as 6 $\mu g\ L^{-1}$ (Nauen, 1995; Devine et al., 1999) and thiamethoxam failing to repress feeding even at higher dosages (Cho et al., 2011). However, we note that these two *B. terrestris* microcolony studies offer only an approximate comparison. For example, in the present study brood production was lower overall than that observed by Laycock et al. (2012), perhaps because of the intrinsic variation in reproductive success that exists between bumble bee colonies (Müller and Schmid-Hempel, 1992). In future work it will be important to compare the sensitivity of bumble bees from the same colony.

Differential sensitivity may be due to imidacloprid producing a stronger repression of feeding in bumble bees than thiamethoxam at field-realistic dosages (Cresswell et al., 2012; Laycock et al., 2012). Such differences perhaps arise because of thiamethoxam binding to target sites that are distinct from those of imidacloprid (Kayser et al., 2004; Wellmann et al., 2004; Thany, 2011) or because imidacloprid has a greater affinity for insect nicotinic acetylcholine receptors (nAChRs) (Wiesner and Kayser, 2000). However, while imidacloprid is only a partial agonist of native nAChRs in several insects including honey bees (Déglise et al., 2002; Brown et al., 2006; Ihara et al., 2006), clothianidin is a 'super' agonist of *Drosophila* nAChRs (Brown et al., 2006) and has a

higher agonist efficacy than imidacloprid in cockroach nAChRs (Ihara et al., 2006). We assume that thiamethoxam is metabolised to clothianidin in bumble bees as it is in other organisms (Nauen et al., 2003), but whether the metabolite is a superior agonist of bumble bee nAChRs is currently unknown. If clothianidin has the higher agonist efficacy in bumble bees, the differential sensitivity we observe may be attributable to the superior hydrophobicity of imidacloprid (Ihara et al., 2006), which could determine the neonicotinoids' accessibility to the receptor and therefore its insecticidal potency (Ihara et al., 2006). While our results show that differential sensitivity of bumble bees to neonicotinoids is possible, further research is required to understand the mechanistic basis of this phenomenon.

4.3. Environmental relevance

In our study, realistic dietary residues of thiamethoxam between 1 and 11 $\mu g\ kg^{-1}$ had no detectable effect on the performance of bumble bee workers in microcolonies. We extrapolate our results to wild bumble bee populations with caution because additional work is clearly necessary to determine the impact of thiamethoxam on bumble bee queens and their colonies. We also note that our study considers only the effects of dietary thiamethoxam in nectar and not pollen. Furthermore, we test an exposure period of 17 days, whereas environmental exposure could extend across a month or more as bumble bees forage on mass-flowering crops throughout their bloom (Westphal et al., 2009). Consequently, we may underestimate the effects of field-realistic exposures. However, our failure to detect an effect in this range is consistent with a recent field study in which *B. terrestris* colonies produced new queens successfully despite being found to contain stored forage comprising thiamethoxam at an average of 2.4 $\mu g\ kg^{-1}$ in nectar and 0.7 $\mu g\ kg^{-1}$ in pollen (Thompson et al., 2013).

Our findings suggest that environmentally realistic residues of imidacloprid have the potential to make a greater impact on bumble bees than residues of thiamethoxam, which could have important implications for future neonicotinoid usage in agriculture. However, further research is required to establish thiamethoxam's impact on queenright colonies in wild populations.

Disclosure statement

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