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Original article

Sub-lethal effects of thiamethoxam, a neonicotinoid pesticide, and propiconazole, a DMI fungicide, on colony initiation in bumblebee (*Bombus terrestris*) micro-colonies

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Abstract – This study investigated whether field-realistic exposure to a neonicotinoid insecticide and a fungicide affected nest building or brood production in queenless *Bombus terrestris* micro-colonies in the laboratory. Bees were exposed to honey water and pollen paste containing field-realistic mean or field-maximum exposure rates of thiamethoxam (1, 10 μg/kg) or propiconazole (23, 230 mg/kg) for 28 days. Thiamethoxam: Both doses reduced consumption of honey water solution and resulted in fewer wax cells. At 10 μg/kg, nest building initiation was delayed, fewer eggs were laid and no larvae produced. Propiconazole: Both doses reduced consumption of honey water solution. At 23 mg/kg, fewer wax cells were produced. Thus, at realistic (mean) exposure rates of these pesticides, no adverse impacts on brood production were found. Pesticide-free alternative forage will reduce field exposure by dilution and thus the impact of maximum rates.

Bombus terrestris **/ thiamethoxam / propiconazole / nectar consumption / nest building / brood**

1. INTRODUCTION

During recent years documented declines in several insect pollinator species and their relation to the central role of such invertebrates in agricultural and horticultural production systems have been the subject of considerable debate and concern (Kearns et al. 1998; Velthuis and van Doorn 2006; Gallai et al. 2009; Potts et al. 2010; Klein et al. 2007). A primary focus of work investigating the causes of these declines has been on honey bees, with fewer studies on bumblebees and solitary bees (Goulson et al. 2008; De la Rúa et al. 2009; Laycock et al. 2012).

Although bees represent only a proportion of the invertebrate species that contribute to the pollination of crops, the potential impact of the observed decline in their numbers on crop yields has resulted in a significant research focus in this area (vanEngelsdorp et al. 2010; Blacquiere et al. 2012). No single factor has been found to explain the decline, and it is considered to be the result of a range of interacting effects including climate change, xenobiotic substances, habitat fragmentation and natural enemies such as bee pests and diseases (Williams and Osbourne 2009; Decourtye et al. 2010; Neumann and Carreck 2010; Kluser et al. 2011). Amongst xenobiotic influences, the non-target effects of pesticide use are often cited as a contributing factor, although again bumblebees and solitary bees have received limited attention.

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Conventional pesticides are widely used in agriculture in intensive production systems, and the use of neonicotinoids has increased significantly since the introduction of imidacloprid during the 1990s. Applied to a wide range of crops for the control of major pests (Laycock et al. 2012), they now represent one of the most broadly used classes of insecticides and are formulated as foliar sprays, seed dressings and soil additions. The products act as agonists of nicotinic acetylcholine receptors (Elbert et al. 2008), and following application are known to be translocated systemically within plants to the pollen and nectar, representing a potentially important exposure pathway for bees (Blacquiere et al. 2012). Laboratory and field studies investigating the non-target effects of such pesticides and their ecological importance rely on the establishment and use of realistic exposure levels (Laycock et al. 2012), and there is an increasing literature describing neonicotinoid residues in bee collected pollen and honey that support the design and interpretation of laboratory experiments investigating their consequences in the field (Chauzat et al. 2006, 2009, 2011; Cutler and Scott-Dupree 2007; Pirard et al. 2007; Nguyen et al. 2009; Bernal et al. 2010; Garcia-Chao et al. 2010; Genersch et al. 2010; Higes et al. 2010; Mullin et al. 2010).

Studies have shown a range of lethal and sub-lethal effects resulting from exposure to different dose rates of various neonicotinoid insecticides (Blacquiere et al. 2012). Topical and oral application of nitro-containing neonicotinoids (including thiamethoxam) was found to be more acutely toxic to honey bees than those containing a cyano-group such as thiacloprid (Isawa et al. 2004; Laurino et al. 2011). A similar relative toxicity of the nitro- and cyano-neonicotinoids has been observed in the bumble bee *Bombus terrestris* following chronic exposure in micro-colonies (Mommaerts et al. 2010). Metabolites of some neonicotinoids, including clothianidin which is a product of thiamethoxam, also contribute to toxicity (Nauen et al. 2003).

Modified behaviour that adversely affects foraging efficiency can significantly reduce colony growth (Thompson and Maus 2007); neonicotinoid exposure has been shown to result in symptoms such as knockdown (paralysis), trembling, uncoordinated movement and hyperactivity (Lambin et al. 2001; Colin et al. 2004). In addition, Cresswell (2011) argues that sub-lethal effects of neonicotinoids on learning and memory, and gustatory responses can result in a reduction in general fitness of bee colonies. However, other responses such as avoidance behaviour following contact with contaminated plants may reduce exposure (Decourtye and Devillers 2010; Maus et al. 2003). Where foragers return to the nest with contaminated nectar and pollen, potential for adverse effects on egg and larval development or brood number of honey bees has been highlighted for imidacloprid (e.g. Lu et al. 2012), solitary bees (Abbot et al. 2008) and bumble bees (Tasei et al. 2000; Mommaerts et al. 2010; Laycock et al. 2012; Gill et al. 2012). The full consequences of exposure in the field will result from a combination of these effects; for example, colonies of *Bombus impatiens* foraging on plants treated with imidacloprid have displayed a reduction in the rate of nest development (brood chambers, honey pots and worker biomass) and foraging activity (Gels et al. 2002). Thus, to draw conclusions on the potential of pesticide exposure in the field on colony development, information should be generated on a range of individual responses linked to the apical end point of successful brood rearing. There are few data available in the literature on the impact of thiamethoxam (but see Mommaerts et al. 2010) on bumble colonies, and this study investigates a range of sub-lethal responses leading to successful brood rearing, at realistic field exposure rates.

Fungicides are routinely applied to flowering crops in which bees forage and high residues of some fungicides have been detected in bee products including pollen and wax (Mullin et al. 2010). Propiconazole, a demethylation inhibiting (DMI) fungicide, has been detected in bee wax and pollen at concentrations of 227 and 361 μg/kg, respectively (Johnson et al. 2010), and although such fungicides are not generally considered to be acutely toxic to bees, exposure in the field and uncertainties relating

to toxicity of pesticide mixtures results in the need for further research (Thompson 1996; Krupke et al. 2012). Most of the current literature addresses the impact of only a subset of the neonicotinoids (frequently imidacloprid) and fungicide products, resulting in there being an urgent requirement for further investigation of the impact of other products, particularly in relation to colony-level effects. In addition, Gill et al. (2012) highlight simultaneous chronic exposure of bees to multiple pesticides in the agricultural environment and possible combinatorial effects that may result. Investigation of such effects relies on an initial understanding of the impact of the individual products at realistic exposure levels, which can then be compared to their impact under conditions of simultaneous exposure. This study used queenless micro-colonies of *B. terrestris* (similar to those described by Mommaerts et al. (2010)) to generate data on the impact of exposure to honey solution and pollen contaminated with environmentally realistic dose rates of thiamethoxam and propiconazole on bee mortality, honey solution consumption, nest building, the number of honey pots built and brood (eggs and larvae) number.

2. MATERIALS AND METHODS

Stock colonies of *B. terrestris audax* obtained from Agralan Ltd, Swindon, UK (originating from Biobest, Belgium), each consisting of a queen and approximately 60 worker bees, were maintained in the laboratory at 21 °C. Each colony was offered 1–2 g of mixed source pollen (Agralan Ltd.; supplemented at 2-day intervals) and ad libitum proprietary liquid sugar solution feed (Biobest). Worker bees used in experiments were collected from the colony and anaesthetised using a 1-min exposure to carbon dioxide before being weighed and placed in experimental cages. Bees that had not recovered after 20 min were replaced.

Queenless micro-colonies consisting of three worker bees were established in colony cages consisting of 500 ml plastic containers closed with muslin mesh (11 cm diameter×7 cm deep). A lidded plastic tube (length 10 cm; diameter 1 cm) with a 2-mm-diameter feeding hole pierced at one end was inserted through a hole in the side of the colony cages and secured at a slight downwards angle with the hole facing upwards as a feeding tube. Artificial nectar solution (Rowse Pure Honey and water at 60 %, *w*/*v*) was offered ad libitum using the feeding tube. Pollen dough (1 g dried mixed pollen (Agralan) soaked in the honey-water solution) was placed on a 2×2-cm^2 piece of metal foil in each colony cage and supplemented weekly. To reduce condensation, the base of the cage was lined with absorbent tissue, and a cotton wool ball and a small plastic bottle top smeared with pollen paste were placed in the cage to encourage nest-building. Micro-colonies were maintained in a controlled temperature room at 27 °C and 70 % relative humidity, with a 8:16-h L/D photoperiod during experiments (Tasei et al. 2000; Tasei and Aupinel 2008). Thiamethoxam and propiconazole were obtained as analytical standards (Pestanal >99 % purity) from Sigma Aldrich, UK.

Four treatments were presented in the artificial nectar solution and pollen paste with ten replicates (micro-colonies) per treatment. A field-realistic maximum dose ("high-dose") thiamethoxam treatment, consisting of 10 μg/kg in honey water solution and 10 μg/kg in pollen paste and a field-realistic mean dose ("low-dose") thiamethoxam treatment, consisting of 1 μg/kg in artificial nectar and 1 μg/kg in pollen paste was used based on the exposure data reviewed in EFSA (2012c). A field-realistic maximum dose ("high-dose") treatment of propiconazole, consisting of 230 mg/kg in nectar and 230 mg/kg in pollen, and a field-realistic mean dose ("low-dose") treatment of propiconazole, consisting of 23 mg/kg in nectar and 23 mg/kg in pollen, were applied, based on an application rate of 0.23 kg ai/ha and a residue per unit dose of 100 to calculate the residue in milligrams per kilogram pollen (which is between the 95th percentile suggested in EFSA 2012a and the maximum suggested in EFSA 2012b).

Solvent control micro-colonies were fed honey water solution with 2,000 μg/kg of acetone and pollen dosed with 2,000 μg/kg acetone (the same level of acetone as in the treatments). Untreated micro-colonies were fed honey-water solution and untreated pollen dough throughout the experiment.

All pollen and honey water solutions were stored at 4 °C, and new solutions were prepared weekly.

2.1. Assessments

In each treatment, the quantity of honey water solution consumed was assessed by weighing and replacing the feeders at 2-day intervals. Food consumption was calculated from the difference between feeder weight at the start and end of each 2-day period, and these were combined to calculate the total consumption over the 28-day experimental period. It was not possible to assess pollen consumption due to the incorporation of the pollen supplied into the nest by the bees. Daily assessments were made of worker mortality, nest-building activity and egg laying, and the behaviour of the bees was noted (including lack of co-ordination, aggression, stumbling and grooming behaviour); if no adverse effects were observed, the observation was terminated after 5 min per colony resulting in a mean observation period of 3 min per colony.

The micro-colonies were maintained for 28 days, after which the colonies were frozen, the remaining honey solution weighed and the workers weighed. Adult bee mass was expressed as the sum of the weights of the three bees in each micro-colony. The nest was dissected and the number of eggs and larvae recorded. Bees that died during the experiment were removed but not replaced.

2.2. Statistical analysis

Statistical analyses used the statistical program R 2.14.2 (Crawley 2007). The relationship between different treatments and micro-colony properties was investigated using linear modelling or generalised linear modelling dependent on the structure of residuals (Crawley 2007).

2.2.1. Dataset 1: honey water solution consumption data

The effects of treatment type and individual micro-colony bee mass (in grams) on honey water consumption (in grams) were analysed by analysis of covariance using linear models, as there was at least one continuous explanatory variable (bee mass) and one categorical explanatory variable (treatment type). The response variable was honey water consumption, and there is one experimental factor (treatment type) with six levels (thiamethoxam "high dose" and "low dose", propiconazole "high dose" and "low dose", control with acetone and control) and one covariate bee mass. The full models were simplified by removing non-significant terms to achieve the minimal adequate model with the lowest Akaike information criterion (Crawley 2007).

2.2.2. Dataset 2: adult mortality data

The effects of treatment type and bee mass (in grams) over the 28-day period on adult bee mortality within the micro-colonies were analysed using generalised linear models with binomial error distribution and log link function. A binomial error structure was used as the data describe proportions of live and dead bees per replicate. The models were simplified by removing non-significant terms, and models were compared using ANOVA. The independence of treatment, time (in days), bee mass (in grams) and mortality data was tested using chi-squared test.

2.2.3. Dataset 3: onset of nest building data

The time from when the experiment started to the day on which nest building (production of wax cells) was first observed in micro-colonies was recorded and analysed as a function of treatment type and bee mass (in grams) by using linear models and analysis of variance (AOV function). The categorical explanatory variable was treatment (with six levels as above); the response variable was the day on which nest building was first observed.

The number of wax cells (honey pots) produced in each treatment was analysed as a function of treatment type and individual micro-colony bee mass (in grams) using generalised linear models with Poisson error distribution and log link function. The variance of treatment and micro-colony properties was compared using Fischer's F test.

2.2.4. Dataset 4: egg and larvae production data

The number of eggs and larvae produced in each treatment was analysed as a function of treatment

type and individual micro-colony bee mass (in grams) using generalised linear models with Poisson error distribution and log link function. Poisson error was used as the data were count data (eggs and larvae). Fischer's F test was used to compare the variance of treatment and micro-colony properties. Again, models were simplified by removing non-significant terms.

3. RESULTS

Bees exposed to both propiconazole treatments (230 and 23 mg/kg) and both controls remained active throughout the experiment and displayed no uncoordinated movement or excessive grooming. In the thiamethoxam 1 μg/kg treatment, a total of three records of uncoordinated movement and one of extensive grooming/rubbing together of the hind legs were noted from the 70 cage observations made in the first week. There were a total of 210 observations made in weeks 2–4, during which three events of excessive grooming and two of uncoordinated behaviour were recorded. In the first week of exposure to the 10 μg/kg thiamethoxam treatment, uncoordinated movement and extensive grooming of the abdomen/rubbing together of the hind legs were noted in 17 of the 70 observations, and thereafter in the following 3 weeks (210 observations), no uncoordinated movement was noted and excessive grooming recorded during only seven observations.

3.1. Honey water consumption

There was a significant effect of treatment on consumption of honey water solution (F=74.26, P<0.01, $d.f.$=713). Micro-colonies exposed to the 230 mg/kg propiconazole treatment consumed a mean of 2.2 g less honey water solution per 2-day period over the 28-day period than control micro-colonies (P<0.001) (Figure 1). Those exposed to 23 mg/kg propiconazole treatment consumed a mean of 0.75 g less honey water solution per 2-day period than control micro-colonies (P<0.001). Micro-colonies exposed to treatments of thiamethoxam (10 μg/kg thiamethoxam and 1 μg/kg thiamethoxam) consumed significantly less nectar than control colonies, with micro-colonies exposed to 10 μg/kg thiamethoxam consuming a mean of 1.54 g less artificial nectar per 2-day period in comparison to controls (P< 0.001) and micro-colonies exposed to 1 μg/kg thiamethoxam consuming a mean 1.16 g less nectar per 2-day period than control micro-colonies (P<0.001). This was not the result of exposure to the acetone carrier as consumption by bees exposed to the control with acetone treatment was not significantly different from the control (P=0.20). Bee mass was not a

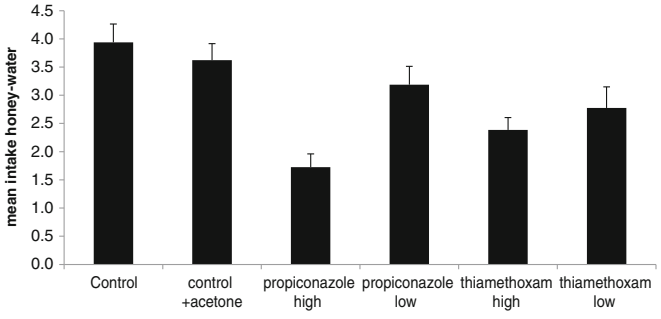

Figure 1. Consumption of honey water solution (in grams) per 2-day period over the 28-day experimental period by *B. terrestris* micro-colonies (n=10 per treatment). *propiconazole high* high-dose propiconazole treatment (P<0.001); *propiconazole low* low-dose propiconazole treatment (P<0.001); *thiamethoxam high* high-dose thiamethoxam treatment (P<0.001); *thiamethoxam low* low-dose thiamethoxam treatment (P< 0.001); *control+ acetone* solvent control (P=0.20); *control* untreated.

significant factor influencing honey water solution consumption (P=0.83).

3.2. Mortality

There was no significant effect of treatment on bee mortality during the 28-day period (F=0.253, *d.f.*=59); all treatments were not significantly different to the control: control acetone (P=0.999); propiconazole 230 mg/kg (P=0.399); propiconazole 23 mg/kg (P=0.146); thiamethoxam 10 μg/kg (P=0.088); thiamethoxam 1 μg/kg (P=0.149); bee mass (P=0.975).

3.3. Nest building and brood production

There was a significant difference between the time to initiation of nest building activity in the 10 μg/kg thiamethoxam micro-colonies and controls (F=1.384, P=0.017, *d.f.*=53), with only 20 % of micro-colonies in the treated cages building a nest within the 28-day experimental period. There were no significant differences in the time to nest building initiation between any other treatment type (control acetone, P=0.882; thiamethoxam 1 μg/kg, P=0.258; propiconazole 230 mg/kg, P=0.248 and propiconazole 23 mg/kg, P=0.154). Bee mass was not found to be significantly related to either the percentage of colonies building nests (P=0.819) or the number of days after the start of the experiment that nest building was first recorded (P=0.819).

Although the bees collected the honey water solution from the feeder, not all was consumed immediately as shown by the proportion of micro-colonies which contained honey pots at the end of the study (Figure 2). There was a significant relationship between the number of wax cells (honey pots) built and treatment (F=3.65, P=0.0063, *d.f.*=54). Significantly fewer honey pots were produced in comparison to the control in micro-colonies exposed to the 10 μg/kg thiamethoxam (P=0.008), the 1 μg/kg thiamethoxam (P=0.033) and the 23 mg/kg propiconazole treatments (P=0.033). There was no significant difference between the 230 mg/kg propiconazole treatment or the control with acetone and the untreated control (P=0.588).

There was a significant relationship between the number of brood (eggs and larvae) present after 28 days and treatment (F=10.61, P=0.002, *d.f.*=53, Figure 3). No significant difference was found between untreated controls and the 23 mg/kg propiconazole (P=0.120), the 230 mg/kg propiconazole (P=0.070) or the 1 μg/kg thiamethoxam treatment (P=0.051). There was no significant difference between untreated controls and the control with acetone (P=0.500).

Following exposure to the 10 μg/kg thiamethoxam treatment, significantly fewer eggs and larvae were produced over the

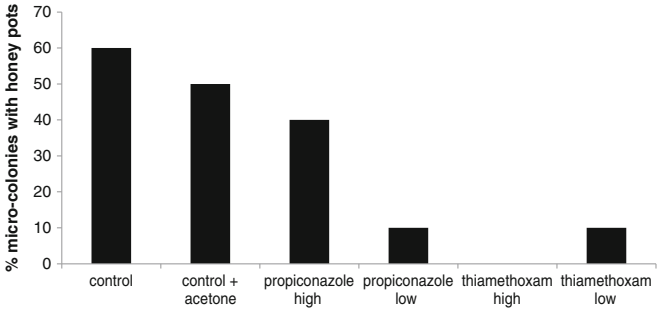

Figure 2. Proportion of *B. terrestris* micro-colonies producing honey pots during the 28-day experimental period (n=10 per treatment). *propiconazole high* high-dose propiconazole treatment; *propiconazole low* low-dose propiconazole treatment; *thiamethoxam high* high-dose thiamethoxam treatment; *thiamethoxam low* low-dose thiamethoxam treatment; *control+ acetone* solvent control; *control* untreated.

experimental period (P=0.020). In contrast to all other treatments, exposure to the 10 μg/kg thiamethoxam treatment also resulted in the production of no larvae during the experiment. The mean number of eggs and larvae produced by micro-colonies was not significantly related to bee mass (P=0.69).

4. DISCUSSION

Micro-colonies of queenless workers facilitate investigation of the sub-lethal effects of systemic pesticides on bumblebees, enabling the comparison of multiple replicates of contaminated food treatments under standardized conditions (Tasei et al. 2000; Mommaerts et al. 2010; Blacquiere et al. 2012; Laycock et al. 2012). In this study, the technique was used to expose *B. terrestris* to two-dose rates of both the neonicotinoid insecticide thiamethoxam and the DMI fungicide propiconazole to provide a baseline study of sub-lethal effects (there were no significant effects of the treatments on mortality of individuals) on colony initiation in bumblebee micro-colonies.

Laboratory studies of the risks posed by pesticides to non-target organisms rely on the use of environmentally relevant dose rates of pesticides (Blacquiere et al. 2012; Dechaume-Moncharmont et al. 2003). The exposure levels used in this study can be compared with residue data held by European Union Member States and collated by EFSA (2012c). The highest residue levels of thiamethoxam in nectar recorded in a range of European field crops did not exceed concentrations of 5.2 μg/kg, and the maximum residues in pollen were 51.0 μg/kg. Thus, although bumblebees in the field may be exposed to maximum concentrations as high as the high-dose thiamethoxam treatment used in this study (10 μg/kg), the 1 μg/kg thiamethoxam treatment represents a more realistic mean exposure rate. Although the higher-dose rates selected reflect the highest level of exposure that bees are likely to encounter in the field, diverse forage sources result in a discontinuous distribution of treated plants in the natural environment offering bees the opportunity to select untreated plants to feed on; thus, the experimental procedure simulates a worst-case exposure which may rarely be encountered in the field. Similar data are not directly available for propiconazole; however, residues in pollen and nectar for sprayed products (EFSA 2012b) suggest that maximum residues may lie around 5,250 μg/kg in nectar and 37,500 μg/kg in pollen, and the field-maximum residue in this study was greater than these values to ensure any effects at higher levels would be identified.

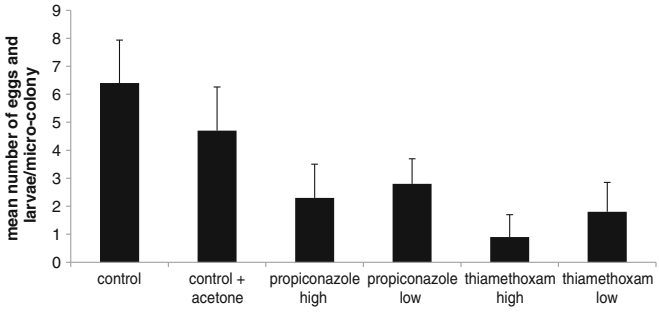

Figure 3. Mean (± SE) number of brood (eggs and larvae) produced per treatment during a 28-day exposure to propiconazole and thiamethoxam (n=10 per treatment). *propiconazole high* high-dose propiconazole treatment (P=0.070); *propiconazole low* low-dose propiconazole treatment (P=0.120); *thiamethoxam high* high-dose thiamethoxam treatment (P=0.020); *thiamethoxam low* low-dose thiamethoxam treatment (P=0.051); *control+ acetone* solvent control (P=0.500); *control* untreated.

In this study, significant reductions in the intake of artificial nectar by micro-colonies fed on all dose rates of thiamethoxam or propiconazole were recorded. Micro-colonies exposed to thiamethoxam also exhibited reduced storage with only 10 % of colonies in the low-dose treatment building wax cells (honey pots) and no wax cells being produced in high-dose treatments. Nectar stored in these cells is essential to feed adults and for brood production (Tasei and Aupinel 2008). If the behaviour of micro-colonies in the laboratory reflects those of queen-right colonies, there is potential for a significant reduction in food consumption of worker and queen bees to impact on colony initiation and reproduction in the field. Reduced storage can potentially result in lower egg laying rates contributing to a reduction in colony fitness (Laycock et al. 2012), particularly if uncontaminated sources are not available or when adverse weather conditions are not conducive to foraging.

Laycock et al. (2012) also observed reduced feeding of syrup and pollen dosed with the neonicotinoid insecticide imidacloprid in *B. terrestris* micro-colonies, consistent with observations by Bortolotti et al. (2003) in which concentrations of 500 and 1,000 μg/kg imidacloprid in a sucrose solution exerted a repellent effect on *Apis mellifera* adults. Reduced consumption rates when food is contaminated with pesticides have been recorded for several insecticide groups particularly in relation to honeybees (Solomon and Hooker 1989) and natural enemies inhabiting agricultural crops (e.g. Singh et al. 2004; Thornham et al. 2007) and have been explained by a reduction of the insects ability to feed through a lack of coordination resulting from neurotoxic insecticides, or an increased handling time.

In this study, 24 % of the observations made during the first week of exposure of micro-colonies subject to the 10 μg/kg thiamethoxam treatment recorded characteristic behavioural responses commonly associated with neurotoxic insecticides (e.g. uncoordinated movement and extensive grooming of the abdomen; Nauen et al. 2001; Colin et al. 2004). There was also a significant reduction in nest building activity in this treatment group with only two micro-colonies building a nest within the 28 day experimental period and with no larvae being produced. Mommaerts et al. (2010) reported that after 11 weeks of exposure, the no observed effect concentration for drone production was 10 μg/kg thiamethoxam, but they did not measure the food intake or numbers of eggs and larvae produced as in this study. Laycock et al. (2012) have reported that ingestion of another neonicotinoid, imidacloprid, by *B. terrestris* workers substantially reduced the fecundity of worker bumble bees in micro-colonies. Workers feeding on syrup containing 98 μg/kg imidacloprid did not fully develop ovaries whilst those exposed to lower levels developed ovaries but egg laying was reduced at levels as low as 1 μg/kg. Tasei et al. (2000) reported that exposure of *B. terrestris* workers to dietary imidacloprid at 10 μg/kg in a micro-colony study was followed by a 43 % reduction in subsequent larval production possibly because of sublethal effects on broodcare or direct toxic effects on larvae. Similar mechanisms to those elucidated by Laycock et al. (2012) may explain the current work and extension of the study period would determine whether egg laying and hatch were delayed rather than suppressed in the high-dose thiamethoxam treatment micro-colonies. It has been suggested (Decourtye and Devillers 2010) that reduced reproductive capacity can be more detrimental to a colony than mortality of foraging bees; thus, if *B. terrestris* encountered this very high level of exposure in the field, colony initiation and development may be impeded. However, it is currently unclear how representative the effects observed in bumble bee micro-colonies in the laboratory are of queen-right colonies in the field. The absence of significant effects at the lower (1 μg/kg) thiamethoxam treatment does, however, indicate that these responses would only be likely to occur if there is no alternative uncontaminated forage within the 1.5–2 km foraging range of bumble bee colonies (Goulson et al. 2008), and consequently, bees

are consistently exposed to concentrations similar to those encountered in the higher (10 μg thiamethoxam/kg) treatment.

Few significant responses to either propiconazole treatment were recorded in this study, and no overt behavioural symptoms such as knockdown, trembling, uncoordinated movement and hyperactivity (Lambin et al. 2001; Colin et al. 2004) were observed. At the higher-dose rate of propiconazole, although significantly less artificial nectar was consumed, there were no significant effects of the treatment on nest building, brood production, activity or coordination. If the exposure of bumblebees in the field is no greater than the highest propiconazole concentrations used in this study, then *B. terrestris* adults are unlikely to be significantly adversely affected by this pesticide. This reflects the conclusions of Ladurner et al. (2005) who found that propiconazole has little effect on the solitary bee *Osmia lignaria* and the honeybee *A. mellifera* at recommended application rates.

4.1. Implications for queen-right colonies

B. terrestris have an annual lifecycle, with young queens surviving the winter to found new colonies in the spring. Consequently, if queen-right colonies in the field have access only to nectar and pollen containing the field maximum for thiamethoxam (10 μg/kg) and their responses reflect those in micro-colonies in the laboratory, a combination of a delay in colony development, lowered nectar storage in wax cells and a reduction in the number of eggs and larvae may have significant negative implications for colony initiation and survival. Whitehorn et al. (2012) recorded reduced colony growth and queen production in queen-right *B. terrestris* colonies exposed to a realistic exposure level of dietary imidacloprid for 2 weeks in the laboratory before placing the colonies in the field for 6 weeks. They concluded that even a small reduction in colony size may have an adverse effect on *B. terrestris* queen production and hence colony survival. In this study, the experimental design resulted in micro-colonies receiving higher and longer exposure to pesticide residues than would occur in the field where diverse forage sources are available. The impact of diverse forage may be enhanced by the ability to modify feeding behaviour in response to contaminated food sources that has been reported for at least one species, *A. mellifera* (Decourtye and Devillers 2010). Further, the short-term pulsed exposure to dietary neonicotinoids resulting from the restricted flowering periods of treated crops discussed by Laycock et al. (2012) may also result in the frequency at which higher levels of exposure are encountered being low and fecundity recovering as levels of dietary neonicotinoids decline. Further research is required to evaluate the recovery capacity of bumblebees once the flowering period of treated crops in the vicinity of the colony is over. Two studies have reported no negative effect on *B. terrestris* foraging on plants treated with either thiamethoxam or imidacloprid (Colombo and Buonocore 1997; Tasei et al. 2001) suggesting that diverse foraging may limit exposure.

Further work is also required to address synergism as studies have shown increased acute toxicity of neonicotinoid pesticides in honey bees co-exposed to DMI fungicides (triflumizole, propiconazole, triadimefon, epoxiconazole; Isawa et al. 2004). The DMI fungicides act by disrupting ergosterol biosynthesis via cytochrome P450 inhibition, and these compounds can also inhibit insect P450s (Brattsen et al. 1994; Isawa et al. 2004). Studies have indicated that DMI fungicides synergize pyrethroid toxicity in the honeybee (Colin and Belzunces 1992, Pilling and Jepson 1993, Thompson and Wilkins 2003) and propiconazole at high doses increases the toxicity of neonicotinoids such as thiacloprid and acetamiprid by as much as 559-fold (thiacloprid) in the laboratory (Isawa et al. 2004). There are no studies reporting the effects of these mixtures in bumble bees.

This study has highlighted that constant exposure to high levels of thiamethoxam in pollen and nectar has the potential to affect the initiation and development of bumble bee micro-colonies under laboratory conditions. However, these effects

were not observed following constant exposure to more realistic residues of thiamethoxam or to propiconazole. The short flowering periods of treated crops, availability of alternative forage and any behavioural responses to contaminated pollen and nectar are likely to further reduce exposure of bumble bee colonies in the field

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Effets sublétaux du thiamethoxam, pesticide néonicotinoïde, et du propiconazole, fongicide inhibiteur de la déméthylation, sur la construction de nids dans des micro-colonies de bourdons (*Bombus terrestris)*

***Bombus terrestris* / pesticide / consommation de nectar / construction du nid / couvain**

Subletale Effekte von Thiametoxam, einem Neonikotinoid, und Propiconazol, ein DMI-Fungizid, auf die Koloniegründung bei Hummel-Minivölkern (Bombus terrestris)

***Bombus terrestris* / Thiamethoxam / Propiconazol / Nektarverbrauch / Nestbau**

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