The combined effects of a monotonous diet and exposure to thiamethoxam on the performance of bumblebee micro-colonies

Dance, C., Botías, C., & Goulson, D.

School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG

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Abstract

There is a pressing need to better understand the factors contributing to declines of wild pollinators such as bumblebees. Many different contributors have been postulated including: loss of flower-rich habitats and nesting sites; monotonous diets; impacts of invasive pathogens; exposure to pesticides such as neonicotinoids. Past research has tended to investigate the impacts of these stressors in isolation, despite the increasing recognition that bees are simultaneously exposed to a combination of stressors, with potentially additive or synergistic effects. No studies to date have investigated the combined effects of a monotonous diet and exposure to pesticides. Using queenless micro-colonies of *Bombus terrestris audax*, we examined this interaction by providing bees with monofloral or polyfloral pollen that was either contaminated with field-realistic levels of thiamethoxam, a commonly used neonicotinoid, or not contaminated. Both treatments were found to have a significant effect on various parameters relating to micro-colony performance. Specifically, both pesticide-treated micro-colonies and those fed monofloral pollen grew more slowly than those given polyfloral pollen or pollen without pesticides. The two factors appeared to act additively. Micro-colonies given monofloral pollens also exhibited lower reproductive efforts and produced smaller drones. Although further research is needed to examine whether similar effects are found in whole colonies, these findings increase our understanding of the likely effects of multiple stressors associated with agricultural intensification on bee declines.

Introduction

Considering the invaluable ecosystem services provided by bees, particularly through their pollination of wildflowers and crops (Gallai et al., 2009), emerging evidence for declines of some species are a great cause for concern. For wild bees, evidence of decline is most clear in bumblebees (Rasmont et al., 2005; Biesmeijer et al., 2006; Kosior et al., 2007; Goulson et al., 2008; Xie et al., 2008; Grixti et al., 2009; Williams & Osborne, 2009; Cameron et al., 2011; Goulson et al. 2015)

Many factors have been implicated in contributing to worldwide losses in pollinator stocks, the most prominent of which are habitat loss and degradation, exposure to harmful agrochemicals such as pesticides, competition from invasive species, pathogens and parasites and diet stress, and climate change is only likely to further exacerbate these existing pressures (Brown & Paxton, 2009; Potts et al., 2010; Goulson et al., 2015). Generally recognised as the most significant driver of declines in biodiversity at a global scale is land-use change and its concomitant habitat loss (Foley et al., 2005), and the same is true for losses of bees (Goulson et al., 2008; Brown & Paxton, 2009; Potts et al., 2010; Winfree, 2010; Goulson et al., 2015). As increasing amounts of natural, flower-rich habitat is converted to agricultural land, the availability of suitable, undisturbed nesting sites and consistent and varied floral resources, on which many species of wild bee depend, is reduced (Carvell, 2002; Williams & Osborne, 2009; Goulson et al. 2015). For example, the range and abundance of many plants on which bumblebees tend to forage have declined in the United Kingdom (Carvell et al., 2006; Kleijn & Raemakers, 2008), with 97% of flower-rich grasslands having been lost in Britain in the 20th century (Howard et al., 2003; Goulson et al., 2015). Often what is left is a more homogenous landscape, characterised by short, temporally and spatially isolated blooming periods of mass-flowering crops such as oilseed rape and canola (Westpal et al., 2006; Osborne et al., 2008). These landscapes are generally less suited to pollinators; in a meta-analysis of 54 studies, Winfree et al. (2009) found habitat loss to be the most significant contributor to losses in wild bee richness and abundance. Similarly, Ricketts et al. (2008), in a review of 23 studies detected a negative correlation between wild bee diversity and distance from areas of natural habitat.

Due to these losses in the extent of wildflowers, it has been proposed that mass-flowering crops could provide valuable resources for pollinators (Westphal et al., 2003). However, as they are only available for such short period of time, they might not be sufficient to sustain viable pollinator populations (Kremen et al., 2007). Furthermore, bees inhabiting areas of intensive farmland will almost certainly have more monotonous diets than they would have done in their evolutionary past (Goulson et al., 2015) and this has caused concern that pollinators may be adversely affected by inadequate nutrition, although the effects of diet stress have been little investigated. It is well known that the nutritive quality of both pollen and nectar of different plants is highly variable (Hanley et al., 2008). For example, pollen protein content can range from 2.5 to 61% (Roulston et al.,

2000). Therefore, it is not surprising that pollen diet can have important implications for the development of bee colonies. One study examining the effects of pollen quality and diversity on honey bees found that bee physiology and immune system function were both increased when pollen diet was of higher quality (i.e. higher protein content) and more diverse (i.e. polyfloral; pollen originating from multiple plant species) (Di Pasquale et al., 2013). Furthermore, studies on bumblebees have also indicated the importance of pollen diet in colony development and brood production, the general trends being that colonies perform better when pollen source is varied or of higher quality (Génissel et al., 2002; Tasei & Aupinel, 2008a; Vanderplanck et al., 2014; Baloglu & Gurel, 2015; Moerman et al., 2015). Whilst these studies have primarily been intended for maximising the efficiency of commercial bumblebee rearing for crop pollination, they nevertheless may help in the understanding of the influence of agricultural intensification on bee health and nutrition (Di Pasquale et al., 2013).

Not only does agricultural intensification lower the availability of suitable habitats and food sources, remaining habitats may be further degraded due to the use of agrochemicals, such as herbicides, fungicides and insecticides, many of which are toxic to pollinators (Williams & Osborne, 2009; Goulson et al., 2015). Of the pesticides to which bees are likely to be exposed, neonicotinoids have attracted most attention and debate. Since their development in the 1980s and their commercial availability in the 1990s (Kollmeyer et al., 1999), they have rapidly become the most widely used class of insecticides in the world (Goulson, 2013). As nicotinic acetylcholine receptor (nAChR) agonists, they bind to receptors in the central nervous system (Elbert et al., 2008). In low concentrations, this causes nervous stimulation but higher doses can lead to paralysis and death. Their water solubility and systemic nature means that they are readily absorbed by roots and leaves and transported around the whole plant protecting all the plant tissues. This however has important implications for pollinators as varying concentrations of these chemicals are often found in the pollen and nectar of both treated crops and nearby wildflowers (Botías et al., 2015). Whilst the concentrations of neonicotinoids are generally not sufficient to cause rapid mortality in pollinators (Goulson, 2013), a wide range of sub-lethal effects have been documented including reductions in foraging and homing abilities, (Yang et al., 2008; Schneider et al., 2012), weakened immune function (Di Prisco et al., 2013), reduced food consumption (Tasei et al., 2000) reduced nest growth, and lower reproductive capacity (Gill et al., 2012; Laycock et al., 2012; Whitehorn et al., 2012). The majority of the controversy over the effects of neonicotinoids has been concerned with whether bees actually encounter large enough amounts in the wild to cause them significant harm (Godfray et al., 2014), and this may in part be down to the huge variability in concentrations of these chemicals found in the field (Blacquière et al., 2012). However, recent studies have shown that

persistence of neonicotinoids in untreated wildflowers means that exposure is likely to be more extensive than previously thought (Botías et al., 2015).

The majority of studies to date have focussed on the impacts of imidacloprid on bees, but other neonicotinoids such as thiamethoxam and clothianidin are now used more frequently (Laycock et al., 2014). Whilst detrimental effects of thiamethoxam to honey bees and bumblebees have been documented at fairly high doses, ranging from 67 ng/g to higher than 100 ng/g (Mommaerts et al., 2010; Henry et al., 2012), residues in crops and wildflowers do not tend to reach these levels and are more often in the range of 1 to 12 ng/g (Arnold et al., 2012; Dively & Kamel, 2012; Stoner & Eitzer, 2012; Botías et al., 2015). Evidence of effects at field-realistic levels on bumblebees is conflicting; Elston et al. (2013) detected a significant reduction in nest building and brood production at levels as low as 1 ng/g and 10 ng/g respectively , whilst others found no effects with doses of 10 ng/g (Mommaerts et al., 2010; Laycock et al., 2014). This discrepancy may be in part explained by differences in the methodologies of the studies. The two latter studies only exposed bees to thiamethoxam in dietary syrup and not pollen (Mommaerts et al., 2010; Laycock et al., 2014), despite the fact that neonicotinoids are present in both pollen and nectar. Most recently, Goulson (2014) found that concentrations of thiamethoxam in pollen stores of free-flying bumblebee nests in the range 0 to 1.6 ppb strongly and negatively correlated with colony performance, but these nests were also exposed to a cocktail of other neonicotinoids so disentangling effects of particular compounds is difficult.

The majority of scientific literature and public debate on the topic of bee health has tended to focus on the impacts of the individual drivers of pollinator declines in isolation, with the emphasis often on attempting to identify the sole or primary cause of bee declines (Potts et al., 2010; Goulson et al., 2015). However, it has been increasingly recognised that these drivers rarely act in isolation, and that in the wild, bees will commonly be faced by combinations of numerous different stressors that may interact additively or synergistically (Potts et al., 2010; Goulson et al., 2015). These kinds of interactions have been documented between different agrochemicals, whereby chemicals such EBI fungicides greatly increase the toxicity of insecticides (Pilling & Jepson, 1993; Schmuck et al., 2003; Sgolastra et al., 2016). Furthermore, there is increasing evidence that exposure to pesticides can lower immune system function, making bees more susceptible to damage from pathogens, such as *Nosema ceranae* (Alaux et al., 2010; James & Xu, 2012; Pettis et al., 2012; Di Prisco et al., 2013). Diet stress has also been implicated in affecting the ability of bumblebees to fight off infection from a trypanosome parasite, with starved bees experiencing much higher mortality rates (Brown et al., 2000). Moreover, a recent study found that the combined exposure to poor quality pollen and the neonicotinoid thiamethoxam had detrimental effects on hypopharyngeal gland development of honeybees (Renzi et al., 2016). It has thus been hypothesised that nutritional stress may have the

potential to lower bees' capacity to withstand the effects of pesticides (Goulson et al., 2015) although this has yet to be tested in bumblebees.

Here we investigate the combined effects of a monotonous diet and exposure to thiamethoxam on bumblebee micro-colonies. Queenless micro-colonies are considered to be reliable indicators of trends in larger queenright colonies (Tasei & Aupinel, 2008b), and are recommended for risk assessments of agrochemicals by the European Food Safety Authority (EFSA, 2013). Monotonous and varied diets were simulated by feeding micro-colonies either monofloral or polyfloral diets. Simultaneously, half the micro-colonies in each diet treatment were also exposed to environmentally-realistic levels of thiamethoxam in both pollen and syrup over a period of 17 days, after which point uncontaminated pollen and syrup were provided. Colonies were observed and performance parameters were recorded both during and after exposure to determine the effects of the two treatments and their interaction on measures of colony performance.

Methods

Honeybee-collected *Cistus spp.* pollen was purchased from Pollenergie® (France) and a honeybee-collected polyfloral pollen blend was purchased from Biobest (Belgium) via Agralan Ltd (Swindon, UK). As honeybee pollen loads can potentially contain viable *Nosema ceranae* spores (Higes et al., 2008), deformed wing virus (Singh et al., 2010) and other bee pathogens (Graystock et al., 2016), all the pollen provided to our micro-colonies was sterilized to exclude honeybee pathogen spill-over effects. Polyfloral pollen was sterilised by Biobest using gamma irradiation with a cobalt-60 source at dose rates between 25-45 kGy. We were unable to use this approach so monofloral pollen was sterilised by a 30 minutes cycle exposure to ultraviolet germicidal light (254 nm). Whilst a single study has indicated that gamma irradiation has no effect on pollen protein content (Junjie et al., 1998), it is possible that the different sterilisation methods may have affected some other nutritive quality of pollen. However, the effect of sterilisation techniques on pollen quality has been largely unexplored.

Protein content of both the monofloral and polyfloral pollens was calculated to be 10.15 and 12.60%, respectively, using the Bradford method (Bradford, 1976), adapted for use with the NanoDrop 2000/2000c (Thermo Scientific, 2010). The polyfloral pollen was examined using a microscope and four dominant types of approximately equal representation were identified (Asteraceae *Taraxacum* type, 23.4%; Rosaceae *Rubus* type, 20.3%; Rosaceae *Crataegus/Malus* type, 18.6%; Papaveraceae *Papaver* type, 14.9%). The remaining 22.8% was made up of 7 more pollen types, each representing less than 5% of the total volume. *Cistus* pollen was added to the polyfloral blend so that it was at a similar proportion to the 5 main pollen groups. This was in order to

minimise any detrimental or favourable effects of any toxin and/or additional nutrient present in *Cistus* pollen acting only on monofloral treatment colonies.

Four colonies of *Bombus terrestris audax*, each with approximately 100 workers, were purchased from Biobest (Belgium) via Agralan Ltd (Swindon, UK). Forty queenless micro-colonies were established by placing 5 workers from one of the four queenright colonies into circular plastic boxes (diameter 11cm, height 9 cm) with an aluminium mesh cover to allow air ventilation. Micro-colonies were kept in a dark room with controlled conditions throughout the entire study period (50 ± 5% humidity and 24 ±1°C). Workers were left for 2 days to acclimatise to their new environment, during which time uncontaminated polyfloral pollen and syrup were supplied *ad libitum*. After the 2 days, micro-colonies were assigned to one of four treatment groups with 10 micro-colonies per group. Micro-colonies with workers originating from each queenright colony were assigned evenly to the four different treatment groups in order to control for effects of the workers' colony of origin on performance. Micro-colonies were weighed and a small amount of wax from the corresponding queenright colony was then added to stimulate oviposition. Half of the micro-colonies received monofloral pollen and half were given polyfloral pollen. All groups received the same inverted sugar syrup solution (50% Ambrosia syrup, EH Thorne Ltd), and groups were supplied with their particular pollen diet throughout the 5 week study period. Within each diet treatment, half of the colonies were exposed to thiamethoxam and the others were provided with uncontaminated food. Pollen and nectar were dosed with thiamethoxam at field realistic levels of 3.5 ppb (Botías et al. 2015). The period of exposure to thiamethoxam lasted 17 days, after which time, all groups were supplied with uncontaminated pollen and nectar.

Colonies were observed and performance parameters including worker mortality, micro-colony growth, reproductive effort and food collection were recorded. Daily observations consisted of counting and removing any dead workers or newly emerged males. Males were weighed and their thoraxes were measured using callipers. Their lipid content was measured using a protocol slightly modified from Brown et al. (2000). Briefly, the whole body of each bumblebee was dried at 70 °C for 5 days and weighed on a precision balance. Every dried bee was then placed in an Eppendorf tube containing 1 ml diethyl ether for 24 h to dissolve lipids, vortexing the tubes for 30 seconds every 3-4 hours (except for the overnight period). The bees were then rinsed in fresh diethyl ether, and subsequently dried at 70 °C for a further 5 days and finally reweighed. The amount of fat in each bumblebee was taken from the difference between the first and second weight measurements.

Every three days, syrup and pollen feeders were weighed to measure collection and fresh pollen and syrup were provided. Data on food collection were also used to calculate the average amount of active compound collected by each bee. We consider this pollen and syrup collection rather than consumption as some syrup was stored in nectar pots and pollen was used to provision

brood. Five identical plastic boxes to those used for the bee micro-colonies were kept with full syrup feeders and weighed every 3 days in order to control for any effects of evaporation in syrup collection analyses. The micro-colonies were also weighed and the number of brood cells and nectar pots was noted.

At the end of the fifth week, all the colonies were frozen and dissected. The numbers of larvae and pupae were counted and the workers were weighed.

All statistical analyses were carried out using SPSS 21.0. Data were first tested for normality using a Shapiro-Wilk test. Where data were normally distributed, generalized linear models (GLM) were used to test for effects of pollen diet and exposure to pesticides, and any interactions between the two, on the colony performance parameters. Where distributions were not normal (e.g. numbers of males produced), non-parametric tests were used. Analyses were also carried out to determine whether there were any significant differences between colony growth and the food collection of pesticide treated groups during the period of exposure and after the period of exposure. Two linear regressions were calculated for each micro-colony, one for each time period, to determine the relationship between time and each of the three variables: syrup collection; pollen collection; weight gain. The slopes of the regressions for each time period were analysed for effects of pesticide exposure using a GLM.

Results

Worker mortality & weight change

Over the 5 week study period, a total of 6 worker bees died with at least one death per treatment group. No one micro-colony had more than 1 death and the total number of deaths was too few for further analysis. All workers lost weight during the study (fig. 1). However, workers in colonies that were exposed to pesticides lost significantly more weight than those that received uncontaminated food (GLM: χ^2=5.10, df=1, p=0.02). There was no significant effect of pollen diet on worker weight change (GLM: χ^2=0.69, df=1, p=0.41), nor was there any significant interaction between pesticide exposure and pollen diet (GLM: χ^2=0.01, df=1, p=0.93).

Micro-colony growth, reproductive success & male quality

The amount that micro-colonies grew was significantly affected by both pollen diet and exposure to pesticides, with colonies receiving polyfloral pollen without pesticides performing best, and those receiving monofloral pollen contaminated with pesticides performing worst (Pollen, GLM: χ^2=9.37, df=1, p=0.002; pesticide, GLM: χ^2=6.32, df=1, p=0.012). There was no significant interaction between

the two factors (GLM: χ^2=1.57, df=1, p=0.210). Micro-colonies that received a monofloral diet grew on average 15.5% less than those that received a polyfloral pollen. Furthermore, micro-colonies that received uncontaminated syrup and pollen grew on average 15.6% more than micro-colonies that were exposed to pesticides (fig. 2 & fig. 3). Comparing the rate of weight gain during and after exposure, micro-colonies that received pesticides grew faster once they were no longer being exposed to pesticides than micro-colonies that received uncontaminated food throughout the experiment (GLM: χ^2=6.44, df=1, p=0.011) (fig. 2).

The number of males produced was significantly affected by the treatment applied (Kruskal-Wallis: χ^2=21.27, df=3, p<0.001) (fig. 4A). Micro-colonies that received monofloral pollen produced significantly fewer males than those fed polyfloral pollen (Mann-Whitney U: U=44, df=38, p<0.001). Although pesticide treated micro-colonies produced fewer males than those that received uncontaminated food, this difference was not significant (Mann-Whitney U: U=142, df=38, p=0.108). There was also a significant effect of pollen diet on the average number of brood per treatment group (GLM: χ^2=18.78, df=1, p=<0.001). Micro-colonies that received monofloral pollen produced on average 32 fewer larvae and pupae and this represented a 40% reduction compared to polyfloral groups (fig. 4B).

Furthermore males from micro-colonies that were supplied with monofloral pollen were on average 0.05 g lighter than those fed polyfloral pollen (GLM: χ^2=29.5, df=1, p<0.001). The weight of males was not significantly affected by pesticide exposure (GLM: χ^2=0.50, df=1, p=0.481) (fig. 5A.). The male thorax width was also significantly lower in micro-colonies given monofloral pollen compared to polyfloral pollen (GLM: χ^2=20.1, df=1, p<0.001). There was also a significant effect of pesticide exposure on male thorax width, with thoraxes being narrower in micro-colonies that were exposed to pesticides (GLM: χ^2=5.57, df=1, p=0.018). There was a marginally non-significant interaction between pesticide exposure and pollen diet on male thorax width (GLM: χ^2=3.74, df=1, p=0.053) (fig. 5B). The fat content of males, measures as a proportion of body weight, was significantly higher in micro-colonies fed on a polyfloral diet ($F_{1,24}$ = 24.2, p<0.001) but there was no significant effect of pesticide contamination ($F_{1,24}$ = 1.31, p=0.264), and no significant interaction between the two (fig. 6).

Food collection

Micro-colonies that received monofloral pollen collected significantly less syrup than micro-colonies that received polyfloral pollen (GLM: χ^2=7.42, df=1, p=0.006) (fig. 7A). However, when controlling for variation in the amount of weight gained by micro-colonies (weight gain was added as a covariate to the GLM), this difference was non-significant (GLM: χ^2=2.07, df=1, p=0.150). There was no significant effect of pesticide exposure on the amount of syrup collected by micro-colonies, both

before and after controlling for micro-colony weight gain (before, GLM: χ^2=1.06, df=1, p=0.304; after, GLM: χ^2=0.12, df=1, p=0.726).

There was no significant effect of pollen diet on the amount of pollen collected by micro-colonies, both before and after controlling for the amount of weight gained (before, GLM: χ^2<0.001, df=1, p=0.985; after, GLM: χ^2=2.88, df=1, p=0.090). There was also no significant effect of pesticide exposure on the amount of pollen collected by micro-colonies, both before and after controlling for weight gain (before, GLM: χ^2=3.27, df=1, p=0.070; after, GLM: χ^2=0.39, df=1, p=0.534).

Micro-colonies that received pesticide contaminated food collected both pollen and syrup in significantly greater quantity in the period after exposure than micro-colonies that received non-contaminated food throughout (syrup, GLM: χ^2=7.897, df=1, p=0.005; pollen, GLM: χ^2=6.441, df=1, p=0.011).

The average amount of thiamethoxam removed from the feeders per worker over the experimental period was 27.22 ng (comprising 2.26 ng from pollen and 24.97 ng from syrup) in the monofloral micro-colonies and 29.25 ng (comprising 2.07 ng from pollen and 27.18 ng from syrup) in the polyfloral micro-colonies.

Discussion

Both managed and wild pollinators are increasingly exposed to a wide range of threats, with many different pressures implicated in driving losses in their stocks at a global scale (Brown & Paxton, 2009; Potts et al., 2010; Goulson et al., 2015). Intensification in agricultural practices results not only in large-scale habitat loss, fragmentation and degradation, but also homogenises landscapes, reducing the diversity of floral resources, and often exposing bees to cocktails of harmful agrochemicals (Potts et al., 2010; Goulson et al., 2015). Here, we investigate for the first time the combined effects of varying diet quality and exposure to a neonicotinoid pesticide, thiamethoxam, on bee colony performance.

Consistent with the findings of previous studies, neither a monotonous diet nor exposure to thiamethoxam at field-realistic levels (3.5 ppb) were sufficient to cause any significant worker mortality during the period studied. Reductions in worker survivorship relating to pollen diet have only been documented when workers are fed solely on syrup and deprived of pollen altogether (Duchateau & Velthuis, 1989; Génissel et al., 2002; Smeets & Duchateau, 2003). Furthermore, studies relating to the effects of thiamethoxam in bumblebees have only detected significant reductions in worker life expectancy at concentrations above 100 ppb (Mommaerts et al., 2010; Laycock et al., 2014), nearly 30 times higher than the dosage applied to pollen and syrup in this study.

Although there were no lethal effects, we detected a variety of sub-lethal effects in this study. For example, micro-colonies that received monofloral pollen gained less weight (figs. 2 & 3), and exhibited lower reproductive effort; monofloral micro-colonies produced fewer males (fig. 4A) and had fewer larvae and pupae than micro-colonies that received a polyfloral diet (fig. 4B). Not only was the total reproductive output of monofloral micro-colonies lower, the quality of drones produced was also significantly reduced, with males being lighter (fig. 5A) and smaller (fig. 5B) and with lower lipid content (fig. 6). Our findings are broadly in agreement with those of previous studies which generally find that colonies perform comparatively poorly when fed a monofloral diet (Génissel et al., 2002; Tasei & Aupinel, 2008; Baloglu & Gurel, 2015). However, caution is needed in interpreting our results. The protein content of the monofloral pollen was 24% lower than the polyfloral pollen blend; studies by Greenberg (1982) and Regali & Rasmont (1995) have shown that higher pollen protein consumption can increase the size of bees. Alternatively, differences between treatments may be a consequence of differences in other nutritive properties, such as the composition of amino acids or sterols. A recent study by Vanderplanck et al. (2014) investigating how pollen chemistry of five different monofloral pollens affected the development of bumblebee colonies found that the most important factors determining pollen performance were the polypeptides/total amino acids concentration and sterol composition; the two pollens that performed the best contained high concentrations of polypeptides/total amino acids and the sterol 24-methylenecholesterol.

Some studies have indicated that monofloral pollens of better quality can produce levels of colony performance comparable to polyfloral blends (Génissel et al., 2002; Baloglu & Gurel, 2015) and *Cistus* pollen specifically has been shown to perform badly compared to other monofloral pollens (Tasei & Aupinel, 2008a; Baloglu & Gurel, 2015; Moerman et al., 2015); Moerman et al. (2015) demonstrated that *Cistus* fed colonies grew slower than colonies fed either *Salix* or *Actinidia deliciosa* pollen and that this was down to *Cistus* pollen's lower amino acid concentration. Overall, it is clear that pollen diet has profound implications for bee colonies, but further works is required before we can draw general conclusions as to what constitutes a healthy diet for bees.

Pesticide exposure had fewer detectable effects than diet. Micro-colonies that received contaminated food gained less weight than micro-colonies that received uncontaminated food (figs. 2 & 3), but their total reproductive output was comparable to that of uncontaminated micro-colonies (fig. 4). Our findings seem to be largely in accordance with those of Elston et al. (2013), who detected reductions in micro-colony performance at thiamethoxam doses within a field-realistic range. Conversely, both Mommaerts et al. (2010) and Laycock et al. (2014) did not detect any effects, even when doses were 10 ppb which is nearer the likely upper limit for average field exposure (Botias et al. 2015). As previously mentioned however, both of these studies only dosed

dietary syrup and not pollen, and so undoubtedly underestimate extent of exposure that would be experienced by bees in the wild. Moreover, these studies only investigated quantitative measures of reproduction (i.e. numbers of brood) and not qualitative measures such as offspring size and lipid content. We found that pesticide exposure decreased the size of males (as measured by thorax width). As previous work has found that micro-colonies are representative analogues of whole colonies (Tasei & Aupinel 2008b), it is plausible that similar patterns would also be seen in the production of new workers and queens. As smaller queens and males generally experience lower reproductive success, with smaller queens being less likely to survive winter hibernation and smaller males less likely to mate (Beekman et al., 1998a; Beekman 1998b; Amin et al., 2012; Vanderplanck et al., 2014), this could have important consequences for reproductive success and fitness at the population level.

It should also be noted that, as food was provided directly within micro-colony boxes, there was no need for bees to forage. One of the main influences of neonicotinoids on bees is through their impairment of foraging behaviour and homing ability (Yang et al., 2008; Schneider et al., 2012; Feltham et al. 2014), and Mommaerts et al (2010) demonstrated that bumblebees are up to 10 times more sensitive to imidacloprid when they have to forage compared to when food is provided directly. Additionally, wild bees may be exposed to a wide range of additional stressors, such as other chemicals including EBI fungicides and infection from pathogens, both of which have been shown to amplify the adverse effects of neonicotinoids (Pilling & Jepson, 1993; Schmuck et al., 2003; Di Prisco et al., 2013; Sgolastra et al., 2016). Thus we might expect greater effects of pesticides on bee colonies under more natural settings.

When micro-colony weight gain was added as a covariate, there was no significant difference in how much syrup was collected by micro-colonies, suggesting that the size of the micro-colonies was the most significant factor in explaining the variation in syrup collection (fig. 7A.). In contrast to syrup collection, pollen quality did not significantly affect pollen collection. This is in accordance with Mommaerts et al. (2010) and Vanderplanck et al. (2014) suggesting that workers do not adjust their consumption or larval provisions when pollen is nutritionally poor. There is evidence that bumblebees can assess the chemical quality of pollen, enabling them to select pollen of superior quality (Robertson et al., 1999; Hanley et al., 2008; Kitaoka & Nieh, 2009; Leonhardt & Blüthgen, 2012), yet it does not seem that they compensate when collecting low quality pollen by collecting more.

We detected that in both groups that were exposed to pesticides, micro-colonies collected pollen and nectar more quickly when they had been provided with uncontaminated food, suggesting that there may have been an anti-feedant effect imposed by thiamethoxam. Whilst it has been shown that anti-feedant properties of neonicotinoids can lead to reduced reproduction (Gill et al.,

2012; Laycock et al., 2012; Elston et al., 2013; Laycock et al., 2014), these anti-feedant effects have only been noticed when doses were in excess of 10 ng/g. As the reproductive effort of colonies that were exposed to pesticides was comparable to those received uncontaminated food (fig. 4), it seems that micro-colonies were able to compensate for anti-feedant effects by eating more and growing more quickly once no longer exposed to thiamethoxam (fig. 2).

Overall, our findings suggest that both dietary pollen quality and exposure to the neonicotinoid thiamethoxam have multiple, measurable adverse effects on bumblebee micro-colonies, though there were no strong interactions between the two stressors. Although micro-colonies are regarded as being good proxies for whole colonies, it would be informative to investigate the effects of these factors using whole colonies in more realistic field settings where other stressors are present and where bees have to forage to collect food.

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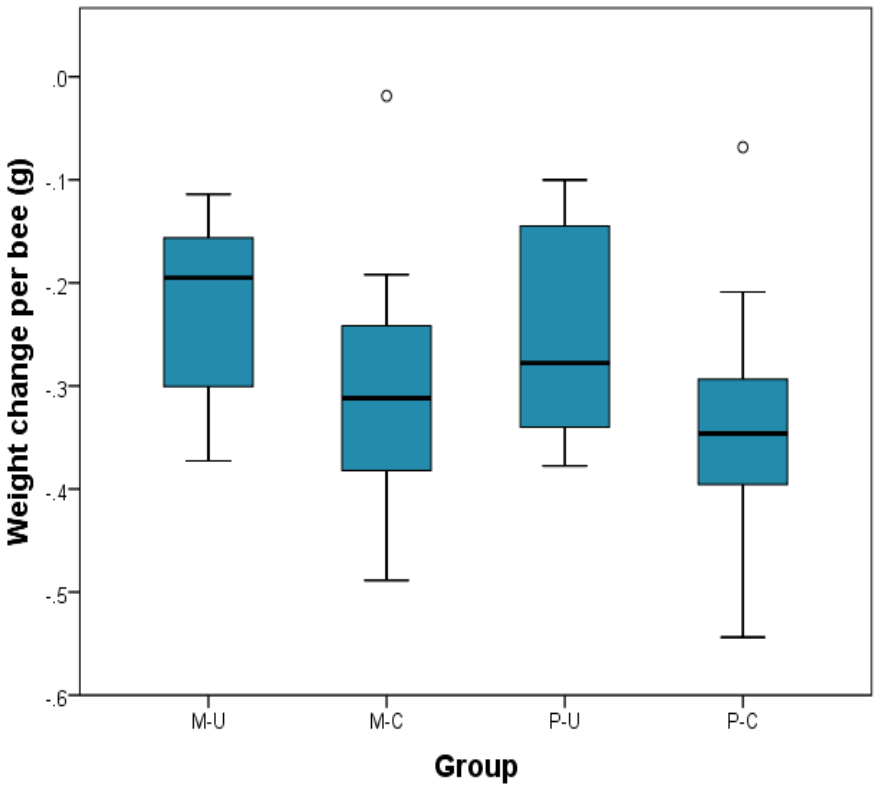

Figure 1. The average worker weight change per individual bee for each treatment group across the 5 week study period (median and interquartile range). All workers lost weight. M = monofloral, P = polyfloral, U = uncontaminated, C = contaminated with pesticide.

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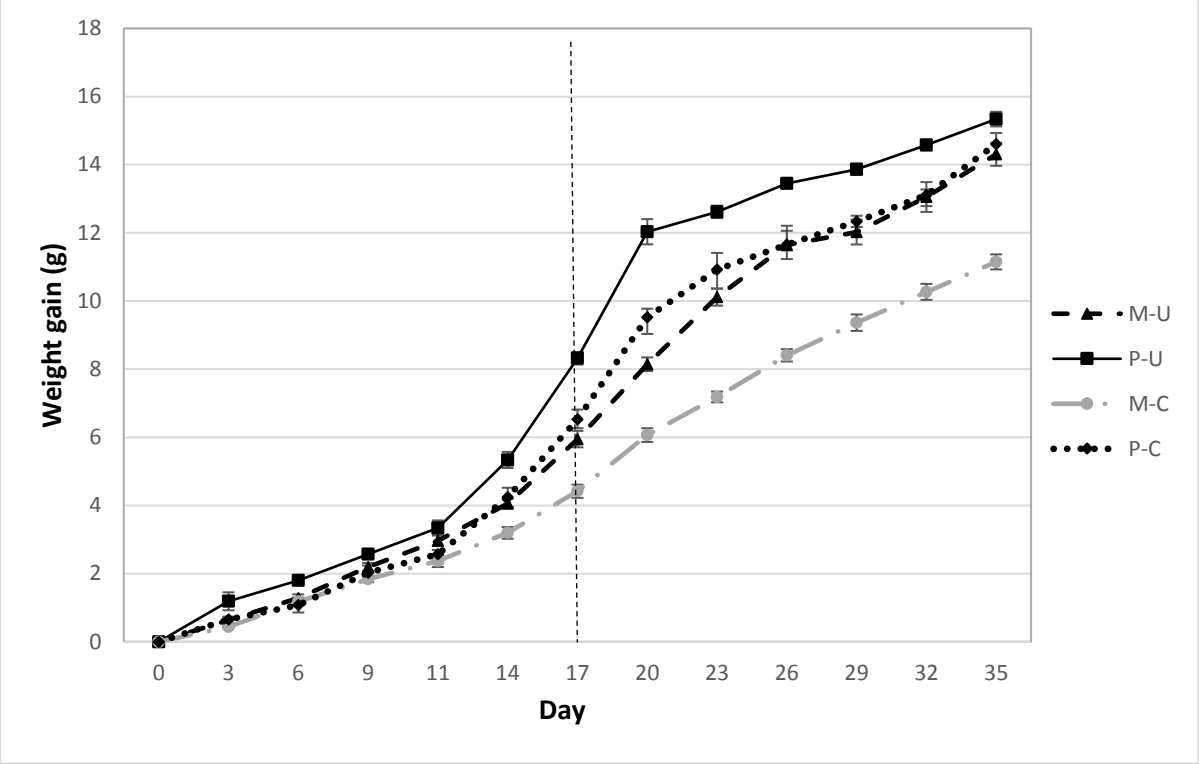

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Figure 2. The average cumulative weight gain per micro-colony of treatment groups through time. The error bars show the standard error of all the microcolonies in each group. The vertical dashed line indicates the periods during and after pesticide exposure. Uncontaminated pollen and syrup was provided to all groups from day 17 onwards.

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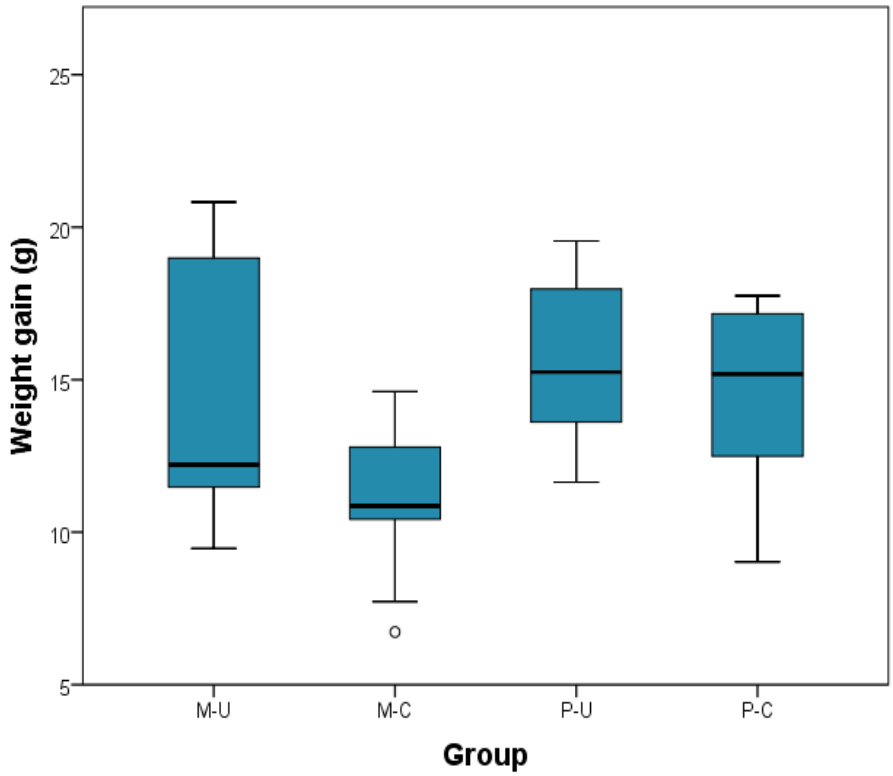

Figure 3. Total weight gain. The average weight gain of microcolonies from each treatment group at the end of the 5 weeks. M = monofloral, P = polyfloral, U = uncontaminated, C = contaminated with pesticide.

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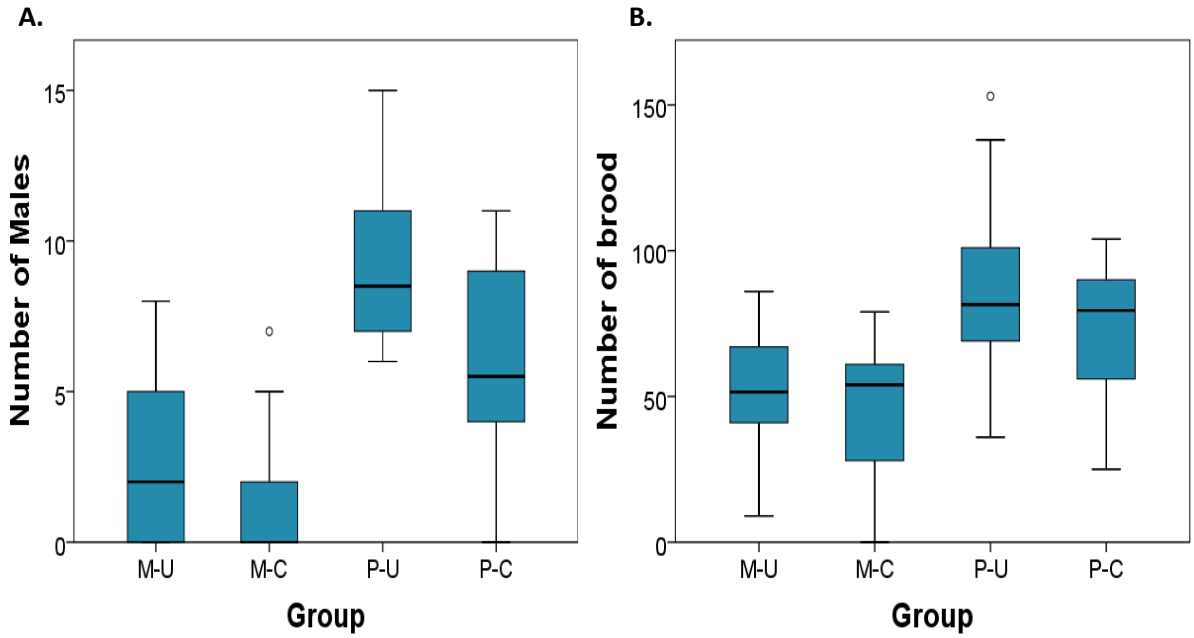

Figure 4. A. The average number of males produced by microcolonies from each group throughout the 5 week study. **B.** The average number of brood per micro-colony from each treatment group. M = monofloral, P = polyfloral, U = uncontaminated, C = contaminated with pesticide.

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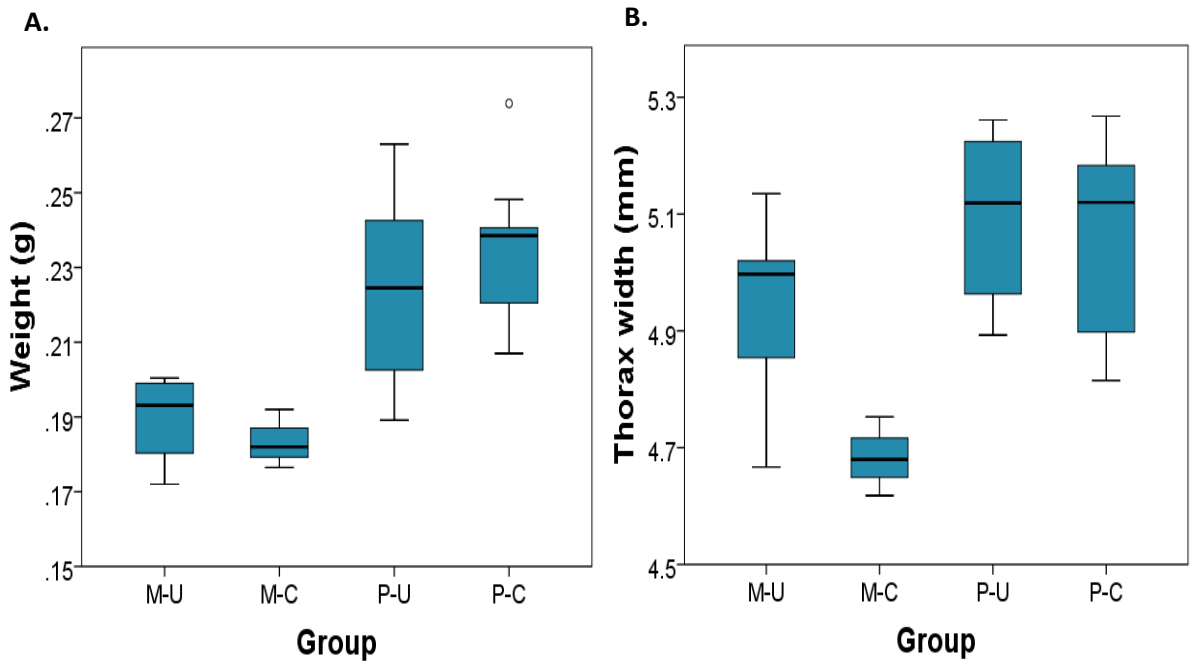
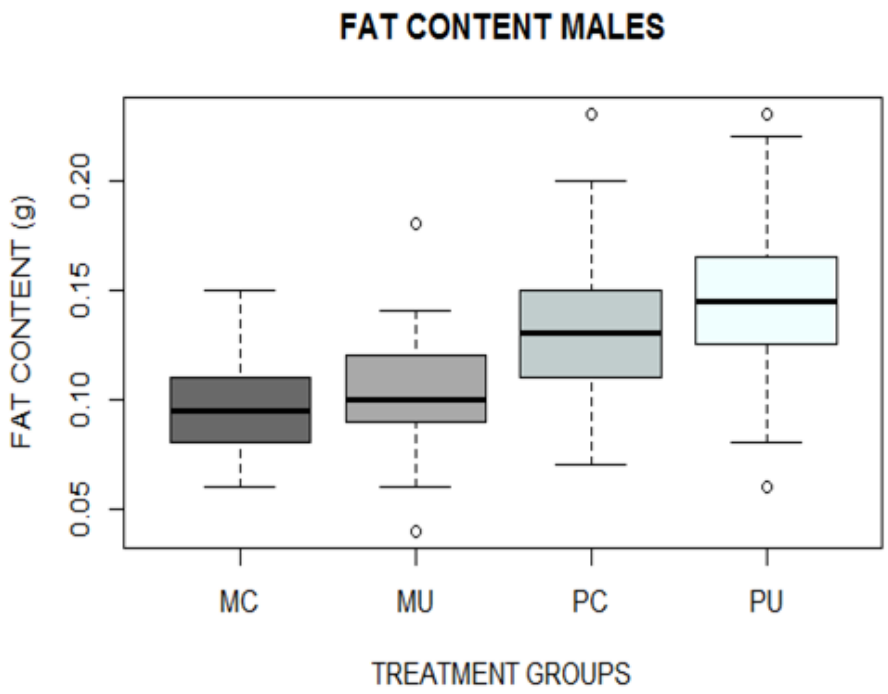


Figure 5. A. The average weight of individual males produced by each treatment group. **B.** The average thorax width of males per group. M = monofloral, P = polyfloral, U = uncontaminated, C = contaminated with pesticide.



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605 **Figure 6. A.** The average fat content of male offspring, expressed as a proportion of body weight, from
606 each treatment group. M = monofloral, P = polyfloral, U = uncontaminated, C = contaminated with
607 pesticide.

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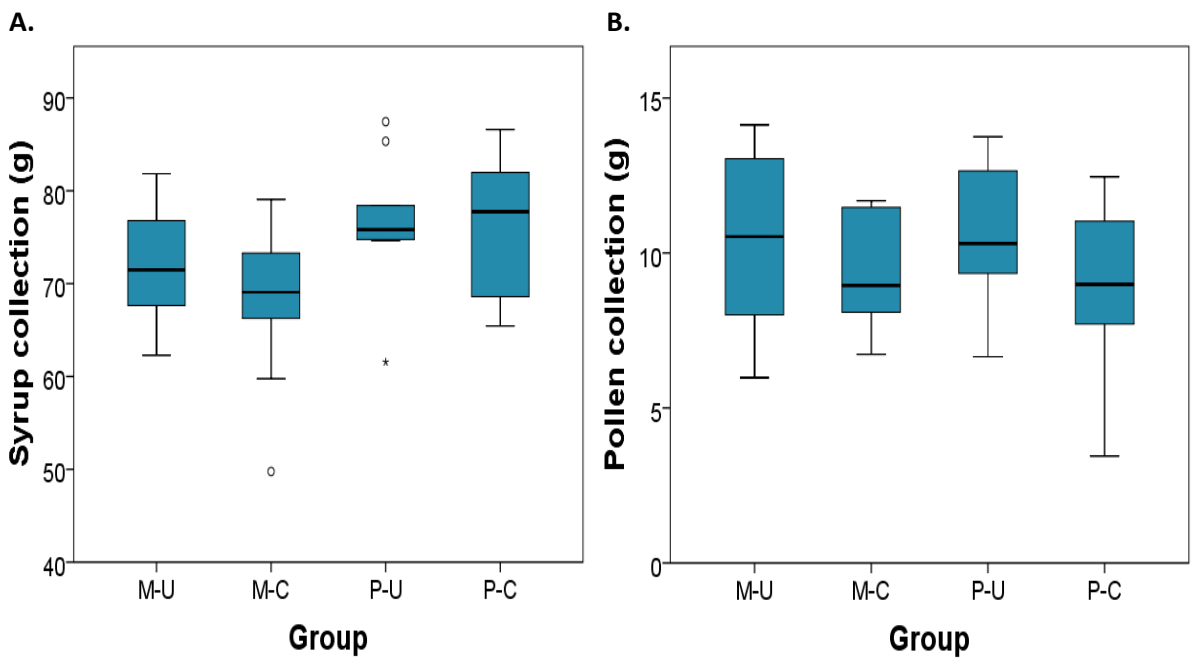

Figure 7. A. The average amount of syrup collected by microcolonies of each treatment group across the 5 week study period. **B.** The average amount of pollen collected by microcolonies of different treatment groups across the 5 weeks.