



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



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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.

1. 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, 2015; Xie et al., 2008; Grixti et al., 2009; Williams and Osborne, 2009; Cameron et al., 2011).

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 and 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, 2015; Brown and Paxton, 2009; Potts et al., 2010; Winfree, 2010). 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 and 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 and 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 (Westphal et al., 2003; 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 polli-

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nators (Westphal et al., 2003). However, as they are only available for such a 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 and Aupinel, 2008a; Vanderplanck et al., 2014; Baloglu and 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 and 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 (Jeschke et al., 2011). 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; 2015), 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 1–12 ng/g (Arnold et al., 2012; Dively and Kamel, 2012; Stoner and 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 (2015) found that concentrations of thiamethoxam in pollen stores of free-flying bumblebee nests in the range 0–1.6 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 as ergosterol biosynthesis inhibiting (EBI) fungicides greatly increase the toxicity of insecticides (Pilling and Jepson, 1993; Schmuck et al., 2003; Sgolastra et al., in press). 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 and Xu, 2012; Pettis et al., 2012; Di Prisco et al., 2013; Sanchez-Bayo et al., 2016). 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 and 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.

2. 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*

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