Helping Agricultural Pollination & Bees in Farmland

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Declaration

I declare that the work carried out in this thesis is entirely done by me, and that any help provided by other individuals with data collection and analysis is fully acknowledged.

I certify that this thesis has not and will not be submitted - in whole or part, to another university for the award of any other degree.

Signature:

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Summary

Previous research has shown that bees are vital to crop pollination. However, modern agricultural practices are occupying an increasing share of the world's land area and have been heavily linked to declining bee populations. This thesis explores: i) the foraging behaviour of the honey bee (*Apis mellifera*) and its influence on crop pollination, and ii) the impact of current farmland management on bees and other flower visiting insects.

Chapter 3 demonstrates, via waggle dance decoding, that the majority of honey bee foraging was outside the orchards in which our hives were located, and that oilseed rape (*Brassica napus*) is a significant competitor to orchard flowers for honey bee visits.

Chapter 4 indicates that competitive interactions between honey bees and wild pollinators can influence honey bee flower choice, foraging behaviour and, potentially, their cross-pollination services.

Chapter 5 presents a survey of an area of agri-environmental farmland previously identified as a foraging 'hotspot' via waggle dance decoding. The data show that the five plant species with the most flower visitors were agricultural weeds, and that the abundance of flowers was a key determinant of flower visitor abundance.

Chapter 6 suggests that the proximity of honey bees to neonicotinoid (thiamethoxam) seed-treated oilseed rape has little impact on their long-term colony performance.

Chapter 7 shows that larger mature seed-treated plants have higher neonicotinoid residues in two widely grown crops: oilseed rape and maize (*Zea mays*).

Chapter 8 implies that the performance and reproduction of bumble bee colonies in an agricultural landscape is similar whether located adjacent to or distant from fields of thiamethoxam seed-treated OSR.

Publications arising from this thesis

Balfour, N. J. & Ratnieks, F .L. W. (In Submission) Honey bee foraging during a commercial pollination event determined via waggle dance decoding (**Chapter 3**).

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Chapter One: General Introduction

1.1 The European Honey Bee

The European honey bee (*Apis mellifera*) is the principal organism studied in this thesis and the focus of four of the following experimental chapters. *A. mellifera* is a eusocial species which lives in perennial colonies with a reproductive queen, up to 60,000 workers and during spring and summer several hundred males (drones). The survival and reproduction of this species, like most bees, relies mainly on flowers (Kevan and Baker, 1983). The nectar and pollen their flowering partners produce are a colony's primary sources of carbohydrate and protein, respectively. However, there are some other natural sources of energy, such as the sugary excretions of some Hemiptera, known as 'honey dew' (Winston, 1987).

Fig. 1.1 Illustration of Tree Beekeeping, practiced in Northern European forests from 2000 or 1000 BC to circa AD 1700 (image taken from *Oekonomische Encyklopädie*, 1774).

A. mellifera has been partly domesticated by humans for as long as 4,500 years (Crane, 2013), although early beekeeping techniques were quite different from those used today, see Fig. 1.1. Further understanding of honey bee biology during the 18th Century (e.g. Huber, 1792) and the invention of hives with moveable frames (Langstroth, 1857) facilitated the development of modern commercial beekeeping. Honey bees have since been exported from their native continents of Europe and Africa to almost every country. Today, there are an estimated 81 million managed honey bee colonies worldwide (FAO, 2015).

Although the honey bee is only one of over 270 species of bees (Falk and Lewington, 2015) in the United Kingdom (UK), they are disproportionally important to humans. In 2013 commercial British hives produced c. 6.4×10^6 kg of honey and 6.5×10^4 kg of beeswax, generating approximately £20m (FAO, 2015). However, the main economic

importance of honey bees is their crop pollination services, which are thought to be worth £400m per year to the UK agricultural sector (POST, 2010).

1.2 Agricultural Pollination

The majority (84%) of European (Williams, 1994) and 70% of the world's (Klein et al., 2007) crop species benefit from pollination by insect to varying degrees. Overall, it is estimated that c. 9% of the total global value of agricultural output relies on insect pollination (Gallai et al. 2009). Although the contribution of groups such as hoverflies (Syrphidae) and beetles (Colepotera) should be recognised (Rader et al., 2015), bees (Apoidea) are generally considered to provide the majority of these pollination services (Williams, 1994; Klein et al., 2007).

Fig. 1.2 Honey bee (*Apis mellifera*) visiting a flower of a an apple tree (*Malus domestica*). Image by Nick Balfour.

Situating honey bee colonies among fruit, vegetable and seed crops has been recommended for over a century (Benton, 1896) and this practice is known to improve both crop yield (e.g. Free, 1962; Bommarco et al., 2012) and quality (e.g. Changon et al., 1993; Garrett et al. 2014a). Accordingly, growers regularly rent hives from beekeepers to pollinate crops such as apples (*Malus domestica*; Fig. 1.2), oilseed rape (*Brassica napus*), blueberries (*Vaccinium* sp.) and almonds (*Prunus dulcis*; Morse & Calderone, 2000).

Because honey bee colonies are versatile, cheap, convenient and available in large numbers during most of the year they are well suited to modern commercial pollination (Klein et al., 2007). Consequently, it has recently been estimated that honey bees perform as much crop pollination as all other bee species combined (Kliejn et al, 2015). Although the number of honey bee colonies are increasing worldwide, the demand for their pollination services is rising at a greater rate (Aizen and Harder, 2009; Breeze et al., 2014). In Chapter Three honey bee waggle dance decoding (Von Frisch, 1967) is used to investigate the foraging ecology of colonies located within commercial apple and pear orchards.

Wild bees, such as bumble bees (*Bombus*) are also known be important to agricultural pollination (Garibaldi et al. 2013) and, of course, to the life cycle of many wildflower species (Corbet et al., 1991). In recent years bumble bee colonies, commonly *B. terrestris*, have been available commercially and are largely used in specialist agricultural settings such as glass and plastic houses to pollinate fruits like tomato (Morandin et al. 2001) and strawberries (Dimou, 2008). In particular their 'buzzing' behaviour, high frequency vibration produced by their wings to liberate pollen grains, favours the pollination of certain crop flowers (Heinrich, 1979).

Solitary bee species are also thought to be extremely effective pollinators (Corbet et al., 1991; Klein et al., 2003) and often more efficient than social species. This is because solitary bees switch more between plants, increasing cross-pollination (Wilmer and Stone, 1989) and in the process of collecting more pollen and less nectar than social bees, more frequently brush the reproductive parts of flowers (Corbet, 1987; Freitas and Paxton, 1998). However, solitary bee species are often not abundant (e.g. Klein et al., 2003) and visit only a narrow range of plant species (Falk and Lewington, 2015).

Interestingly, the presence of wild bees has been shown to increase the pollinating efficacy of honey bees foraging on almond flowers (Brittain et al. 2013) and sunflowers (Greenleaf et al. 2006) by causing more movements between patches. In Chapter Four I investigate the mechanics underlying the competitive interactions of wild bees and honey bees foraging on lavender (*Lavandula x intermedia*) flowers. In particular, this study examines the effect of competition on bee flower choice, foraging behaviour and their cross-pollination services.

1.3 Agricultural Change & British Biodiversity

At present 71% of the British (DEFRA, 2013) and 38.5% of the world's (FAO, 2015) landscape is occupied by agricultural activity. Given that the global human population is projected to grow substantially over the course of this century, food production will also need to be increased (Tilman et al., 2011). Indeed, agricultural production and consumption is projected to be 60% higher in 2050 than it was in 2005/2007 (Alexandratos and Bruinsma, 2012).

The expansion and increasingly intense management of this land during the last century has often been linked to a general decline in British biodiversity: birds (reviewed in

Newton, 2004), amphibians and reptiles (Arnold, 1995), plants (Barr et al., 1992) and mammals (reviewed in Battersby, 2005). Parallel population declines and range contractions of the UK's pollinating insects are also considered to be a consequence of modern farming practices: hoverflies (Biesmeijer et al., 2006) , butterflies (Asher et al., 2001) and bees and wasps (Ollerton, 2014).

There are multiple factors that could be responsible for these declines, including habitat loss (Green, 1990) and fragmentation (Goulson, et al, 2008), increased use of herbicides (Ollerton, 2014) and mechanisation (Robinson and Sutherland, 2002). However, the increased application of insecticides has probably been the most high profile of these factors (e.g. Carson, 1962). A relatively modern class of insecticides, neonicotinoids (in use since 1991; Elbert et al., 2008), has been under considerable scrutiny in recent years. At present there is a little agreement between the findings of field research, which has generally found no effect (Cutler and Dupree, 2007; Pilling et al. 2013), and the results of laboratory studies which have shown sub-lethal affects to bumble bee colonies (Whitehorn et al., 2012) and to individual honey bees (Henry et al., 2012). In 2013 The European Commission imposed a two-year moratorium on the use of neonicotinoid seed dressings on bee attractive crops to allow time for further research. In this thesis I investigate the effects of proximity to neonicotinoid (thiamethoxam) seed-treated crop on honey bee (Chapter Six) and bumble bee (Chapter Eight) colony performance. Furthermore, I explore the relationship between plant size and the neonicotinoid residues of mature seed-treated crops in Chapter Seven.

1.4 Helping Bees in Farmland

Changes to farming practices and land-use during the last century have also negatively impacted floral resources available to bees in the British agricultural environment. Firstly, there has been a long-term decline in heathland (Ericaceae; Webb, 1996), haymeadows (reviewed in Jefferson, 2005) and hedgerows (reviewed in Robinson and Sutherland, 2002). Second, there has been a shift away from the use of legumes, such as *Trifolium pratense* and *Lotus corniculatus*, to maintain soil fertility (Carvell et al. 2006; Kleijn and Raemakers, 2008). Third, increased use of herbicides (Whitehead & Wright 1989), artificial fertilizers (Ollerton et al., 2014) and other farming changes (reviewed in Robinson and Sutherland, 2002) have severely impacted wild flower populations (Stroh et al. 2014).

Fig 1.3 Flower rich pasture under high level agri-environmental management, Castle Hill National Nature Reserve, East Sussex. Image by Nick Balfour.

However, since the mid-1990's The European Union has sought to halt the general decline of farmland biodiversity by subsidising (2007-13: € 22.2 billion; EUROPA, 2011) less intensive crop management and by taking some agricultural land completely out of production (reviewed in Bignall, 1998). These agri-environmental schemes are now widespread and cover the majority (59%) of the UK's agricultural land (DEFRA, 2013). Encouragingly, farmland operating under these schemes have been shown to have richer communities of plants (Taylor and Morecroft, 2009), moths (Fuentes-Montemayor et al., 2011) and bumble bees (Pywell et al., 2006; Carvell et al., 2007). Furthermore, recent research suggests that honey bees are attracted to land under high level agri-environmental management during the late summer (July-August, Couvillon et al. 2014b). In Chapter 5 I present a survey of the habitats, flower-visiting insects and flowering plants in this area of farmland, see Fig. 1.3.

1.5 Aims and Objectives

The research above demonstrates that bees and agriculture are inextricably co-dependent. Flower visiting insects are vital to crop pollination; while modern agriculture is occupying an increasing land area, and has been stongly linked with recent bee declines. As such, the aims of this thesis are to: i) further the understanding of the foraging ecology of honey bees in agricultural crops and ii) identify farmland practices that are to the benefit of bees. I hope this research can make a contribution to realising their future harmony.

Chapter Two: General Methodologies

This chapter presents an overview of the main experimental methods used in the following research. Further information is availble in each of the experimental chapters (Three to Eight).

2.1 Beekeeping

Modern beekeepers generally house honey bee colonies (up to 60,000 workers) in hives with moveable frames. The hive can have one or more boxes of frames, depending on the size of the population and honey stores. In spring and summer the queen is often confined to the lower one or two boxes with a wire mesh (queen excluder) with gaps large enough for only the smaller worker caste to pass through.

Fig. 2.1 Luciano Scandian, Hasan Al Toufailia and myself monitoring honey bee colonies adjacent to blooming oilseed rape (*Brassica napus*) fields. Image by Heloise Blanchard.

Extensive beekeeping was needed to manage and monitor the 72 colonies used in the experiment in which we measured colony performance in relation to foraging on oilseed rape seed-treated with neonicotinoid insecticide (Chapter Six). Close management of these colonies was a required and included: i) ensuring a marked (with acrylic paint on the thorax), wing clipped and egg-laying queen is present; ii) monitoring and treating pests (e.g. varroa mites; *Varroa destructor*) and brood diseases (e.g. chalk brood; *Ascosphaera apis*); iii) providing sufficient space for brood production and food

storage; iv) destroying developing queen cells to prevent swarming; and v) checking that the colony has ready access to adequate quantities of stored honey. Failure to quickly identify and rectify problems that arose, may have led to these colonies swarming, failing or even absconding. Any of these outcomes would have introduced huge additional variation and/or reduced our sample size.

To research and record behaviour within the colony, such as the waggle dance (Von Frisch, 1967), honey bees are often housed in glass-fronted hives (observation hives). These hives are generally smaller in volume and in worker bee population (c. 5,000 workers) than those used in normal beekeeping practice. For ease of observation these hives are ordinarily kept indoors and plastic piping used to connect the colony to the outside world. In Chapter Three, which investigated spatial foraging patterns of honey bees during the apple and pear bloom, via waggle dance decoding, observations hives were kept in sheds within our study orchards. Maintaining these colonies in these unnaturally confined conditions required relatively intensive management, such as: i) weakening the hive by removing frames of developing brood or excess workers to prevent swarming ii) offering supplemental sucrose syrup (via feeders) if there is little stored honey, and iii) providing extra insulation (300mm thick polystyrene sheets attached to the glass front) because the frames in an observation hive are arranged vertically, rather than horizontally, and colonies often struggle to maintain normal brood temperature (33-36 ° C; Winston, 1987), especially at night.

2.2 Honey bee Waggle Dance Recording and Decoding

The waggle dance is the mechanism by which honey bee colonies communicate the location of profitable foraging resources to nestmates (Seeley, 1995). When a honey bee forager working a profitable flower patch returns to the hive she will recruit nestmates to this location via the waggle dance. This figure of eight movement consists of 'waggle runs' and 'return returns'. The waggle run, see Fig. 2.2, encodes vector information, distance and direction from the hive to the foraging location. The number of times a waggle run is repeated is correlated with the profitability of the resource indicate relative to the local alternatives (Seeley, 1995).

Fig. 2.2 A honey bee worker performing a waggle dance. Image by Christoph Grueter.

To investigate honey bee foraging by decoding waggle dances, we first videoed the area of observation where the dances were taking place (the dance floor). Dances were later decoded using frame-by-frame playback on a computer, using a protractor and the media player timer (e.g. StreamClip, 2015). The methodology used in Chapter Three followed that of Couvillon et al. (2012), where we recorded the angle (relative to plumb lines suspended in front of the glass front) and duration of six consecutive waggle runs, excluding the first and last run (which are both known to be inaccurate). These durations and angles were averaged to give a mean direction (relative to the sun's azimuth; Von Frisch, 1967) and distance (from a dance calibration curve, where one second is approximately equal to 750m; Couvillon et al., 2012) per dance.

Because both the distance and direction encoded in the waggle dance are known to be inherently inaccurate (Towne and Gould, 1988), dance vectors were plotted as probability distributions using the methodology developed by Schurch et al. (2013). This technique involved simulating multiple vectors for each dance. The locations generated are then binned into 25 m^2 quadrats . A raster image (a dot matrix data structure) of multiple dances was then used to create a heat map of a colony's overall foraging pattern. This methodology also enabled us to determine the median and confidence interval estimates of the proportion of waggle dances indicating oilseed rape fields and apple and pear orchards in Chapter Three.

2.3 Pollen Trapping and Analysis

Chapters Three and Six used the long established methodology (e.g. Todd and Bishop, 1940) of identifying pollen pellets collected from returning honey bees to determine a colony's foraging patterns. Pollen pellets were gathered using pollen traps with a 5.0 mm plastic mesh fitted to the hive entrance. As returning pollen foragers entered their hives they were forced to squeeze through this narrow mesh which dislodged pellets held in their pollen baskets (corbiculae) into the collection tray below. Because honey bees generally only visit one flower species per foraging trip, the majority (93%; Betts, 1920) of these pollen pellets will have been collected from a single species. Pollen collection periods were alternated between morning and afternoon to allow for the different diurnal patterns of pollen presentation by individual plants species (e.g. Pervical, 1950).

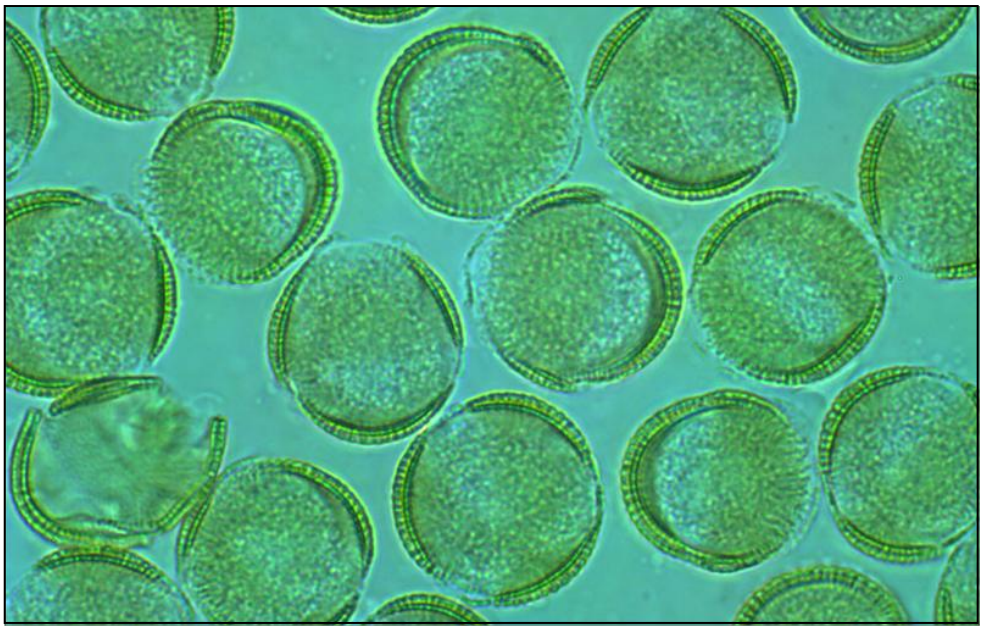

Fig. 2.3 Oilseed rape pollen grains (*Brassica napus*) at ×600 magnification. Image by Nick Balfour.

Subsequently, a random sample of the pollen grains collected (per day, per hive) were sorted into colour groups. Those pellets that were determined to be within the colour range of the flower species of interest using pollen colour identification guides (e.g. Hodges, 1952), were then prepared for ×600 microscopic examination. First, a shaving of an individual pellet was mixed with distilled water and mounted on a microscope slide. Then the size, shape and texture of the pollen grains, see Fig. 2.3, were compared

to specimens collected directly from the anthers of the study flower species or to pollen grain identification guides (e.g. Kirk, 2006).

2.4 Identifying and Quantifying Flower Visiting Insects

Fig. 2.4 Recording the flower visiting insects in a transect along a field margin. Image by Katie Fensome.

To sample the abundance of flower visiting insects in the survey of agricultural land presented in Chapter Five a stratified random approach was taken. Firstly, we conducted an initial survey of the flowers and habitats present in the c. 5 km^2 area previously identified as a late summer hotspot for honey bee foraging in 2009-11 via waggle dance decoding (Couvillon et al., 2014b). As it was not feasible to survey such a large area in detail, we conducted an intensive survey of 12 fields, three fields from each of four habitat types: (i) pasture fields, (ii) field margins/hedgerows of arable fields, (iii) set-asides: uncultivated field corners (ESS options: EF1/HF1; NE, 2012a; NE, 2012b), (iv) nature reserve: pasture fields in the Castle Hill NNR (National Parks and Access to the Countryside Act 1949) and an adjacent Site of Special Scientific Interest (Wildlife and Countryside Act 1991). Fields were further stratified into three sub-habitat types (scrub, short grass or long grass) and sampling effort was apportioned according to their relative areas. Transect sampling was used to quantify the abundance and richness of the flower visiting insects and flower plant species in these areas, Fig. 2.4. This methodology approximately followed that of the United Kingdom Butterfly Monitoring

Scheme (UKBMS, 2016), where a recorder walked the centre of a 2 × 100m transect recording 1m to the left and right side in open habitats, noting the flower visitors and the species of flower they were visiting. In field margins recording was done on only one side in 1 × 200m transects. These two transects gave equal survey areas ($200m^2$).

Although it is reasonably straightforward to identify some bee species on the wing (e.g. *Apis mellifera*), the majority require detailed examination to distinguish between closely related species. For example *Bombus lapidarius* can be easily confused with the much rarer *B. ruderarius* as the basic colour pattern is similar. However, the females of these species may be separated by inspecting the colour of their corbicular (pollen basket) hairs: which are black in the case of *B. lapidarius* and orange/red in *B. ruderarius*. As such many individual bees were often captured with a butterfly net, examined with a ×20 hand lens and identified with the aid of field-guides (e.g. Edwards and Jenner, 2005; Baldock and Collins 2008). The majority of butterflies, on the other hand, are easily identified by the colour markings on their wings. Hoverflies (Syrphidae) and wasps present more of a challenge and individuals were only identified to genus in this study.

2.5 Identifying and Quantifying Flowering Plants

The stationary nature of flowering plants makes them somewhat easier to identify than insects. Often detailed examination of the basal leaves is necessary to separate between closely related species, in particular those with composite flower (Asteraceae). During the survey presented in Chapter Five flowering plant species were identified with the aid of field guides (Streeter and Hart-Davies 2009, Sterry, 2010).

In this study flower abundance was quantified by counting the number of 'flower units' for all flowering species inside 1m × 1m quadrats (Southwood, 1966). Flower units were categorised as a single flower (e.g. *Rubus fruticosa*), stem (e.g. *Galium verum*) or inflorescence (e.g. *Centaurea nigra)* as appropriate. We recorded the number of flower units per species in five quadrats along the length of our flower visiting insect transects (described above), one every 20 metres. Quadrats locations were alternated between the left and right of the midline of each transect.

Because flowers and flower units are of different sizes, comparing the quantity present per species is not straightforward. Therefore, we employed a measure of flower

abundance that allowed different flowering plant species to be assessed in a relative manner. This was achieved by calculating the mean 'petal area' represented by a flower unit for each of the flower species recorded. The petal area per flower unit was determined by collecting ten flower units per species. 30 flowers (or florets) from each were then cut open, placed flat on graph paper and photographed. Petal images were later categorised to the closest approximate geometric shape (circle, semi-circle, quarter circle, rectangle etc.) and relevant measurements (e.g. diameter, height) determined using ImageJ software (ImageJ, 2014).

2.6 Quantifying Nectar Volume and Concentration

In the study of the influence of competition on honey bee foraging behaviour (Chapter Four) nectar volumes were quantified by probing the base of the lavender corolla tube with a 1µl capillary micro-pipette (Drummond Microcaps), Fig. 2.6. The length of liquid held in these 64 mm long pipettes was then measured to ±0.5mm which quantified nectar with an accuracy of ±0.008µl.

Fig 2.6 Measuring the nectar volumes held by lavender (*Lavandula x intermedia*) flowers using a micro-pipette tube. Image by Francis Ratnieks.

To measure the sugar content of lavender flowers in this study thirty flowers were emptied of nectar with a micro-pipette. Flowers were then covered with a fine mesh (0.2 x 0.2mm) to exclude all insects. After two hours the accumulated nectar was collected

with a micro-pipette and the sugar concentration (degrees Brix) determined using a hand-held refractometer (Kruss HR 25/800). This methodology avoids the potential effects on sugar concentrations that may be caused by nectar dilution via precipitation or nectar evaporation caused by exposure to warm temperatures.

Chapter Three: Honey Bee Foraging During Commercial Pollination Determined via Waggle Dance Decoding

3.1 Abstract

Managed honey bees play an important role in global crop pollination. Farmers often pay beekeepers to temporarily relocate hives among crops bloom. Here, and for the first time, we use a unique behaviour, the waggle dance, to investigate honey bee foraging in a crop under commercial pollination.

Over two springs we videoed and then decoded 834 waggle dances made by forager bees from six observation hives located in two apple and pear farms in Kent, UK. We also obtained pollen samples from returning foragers and made counts of insects visiting apple and pear flowers. From these data we determined the foraging patterns of our six honey bee colonies during and after bloom. We also quantified visitation to nearby fields of another spring-flowering crop, oilseed rape (OSR).

Honey bees were the most common insect recorded visiting apple and pear flowers (honey bees: 64%, solitary bees: 19%, bumble bees: 10%, flies: 7%). Almost half of the pollen loads collected from returning foragers were apple or pear (47%). Dances from the bloom period indicated lower mean foraging distances than dances recorded two weeks post bloom (0.98 v 1.57 km, $p = 0.005$). Almost a quarter (24%) of dances indicated foraging in orchards, a significantly higher proportion ($p < 0.001$) than for OSR fields (13%). Most dances (84%) indicated locations outside of the orchards in which the hives were located.

Taking into account the distance of orchards and OSR fields from our study hives, the amount of foraging per hectare was greater in OSR fields than in orchards ($p<0.001$). Furthermore, in 2013 the proportion of waggle dances for OSR, per colony, was negatively correlated with the proportion for orchards ($p = 0.029$). Orchards >0.5 km from our hives were little visited.

Our results indicate that OSR, a widely grown and predominately spring flowering crop, is a significant competitor to apple and pear flowers for honey bee visits. Most (95th percentile) honey bee foraging occurred within 2.1 km of the hives. This potential foraging area (13.9 km^2) is approximately 35 times larger than the orchards on the two study farms. Therefore, maximising the pollination services of managed honey bee

colonies requires an ecosystem level approach that takes into account farm and foraging scale, and competing floral sources.

3.2 Introduction

Apples (*Malus domestica*) are the fourth most widely grown fruit crop in the world (Pommer and Murakami, 2009; FAO, 2011; FAO, 2015). Of the top three fruit crops, grape flowers are principally wind pollinated (Morse and Calderone, 2000) and commercial plantain and banana varieties produce fruit without fertilization (Heslop-Harrison and Schwarzacher, 2007), leaving apple as the most widely grown insect-pollinated fruit crop globally. Apples are often grown in concert with pears (*Pyrus communis*; Jackson, 2003). Together, these two crops are grown in 93 countries and generate approximately US$80bn per year (FAO, 2015).

Insects, particularly bees, play a major role in the pollination of much of the world's fruit, seed and vegetable crops (Kleijn et al., 2007). These ecosystem services are known to benefit both crop yield (e.g. Free, 1962; Bommarco et al., 2012) and quality (e.g. Changon et al., 1993; Garrett et al., 2014a). Although, wild insects can be effective crop pollinators (Garibaldi et al., 2013), the economic value added by managed honey bees (*Apis mellifera*) is perhaps equal to that of all other bee species combined (Kliejn et al, 2015).

It has been estimated that 85% of apple and 65% of pear production is dependent on insect pollination (UKNEA, 2011). This is because commercial apple and pear varieties, to varying degrees, require cross-pollination (MacDaniels and Heinicke, 1929). Cross pollination deficit is challenging because both apple and pear flowers are receptive for only a few days (Williams, 1966). Furthermore, because commercial apple and pear production is confined to temperate regions, the weather during their spring bloom is often not conducive to insect activity (e.g. Boyle-Makowski and Philogene 1985; Vicens and Bosch, 2000).

Early reports suggested that wild pollinators alone provide sufficient orchard pollination in the UK (e.g. Wilson 1929) and the US (Howlett, 1934). However, due to the decline in wild bee populations through the second half of the last century (Batra, 1995; Biesmeijer et al. 2006) this is no longer thought to be the the case (Free, 1993; Volz et al., 1996). Thus, to ensure sufficient pollination, apple and pear producers often rent

honey bee hives during bloom (Benton, 1896; Morse and Calderone, 2000; Park et al., 2010).

The waggle dance (Von Frisch, 1967) is the mechanism by which honey bee colonies share information on profitable foraging resources (Seeley, 1995). This unique behaviour has been used to explore many aspects of honey bee foraging ecology (e.g. Visscher and Seeley 1982; Beekman and Ratnieks, 2000; Couvillon et al., 2014a). Although crop pollination is a well-researched topic (see Free, 1993), to our knowledge the waggle dance has not previously been used to determine where honey bees from colonies located in a crop under commercial pollination are actually foraging. Maximising the potential of honey bees is likely to be of increasing importance to the global food supply, given that the demand for their pollination services is rising at a greater rate than are hive numbers (Aizen and Harder, 2009; Breeze et al., 2014).

Here we present a two-year on-farm study of the foraging ecology of honey bee colonies located in two commercial apple farms in Kent, the main apple-producing region of the UK (DEFRA, 2015). We videoed and decoded honey bee waggle dances to determine their foraging distances and locations during and after the spring bloom of apple and pear trees. Dance information was combined with analysis of pollen trapped from returning foragers and insect counts on pear and apple flowers. We also used dance decoding and trapped pollen analysis to assess foraging on nearby fields of oilseed rape (*Brassica napus*) a widely grown (DEFRA 2014a) and predominately spring blooming (DEFRA, 2014b) crop, which may compete with orchard flowers for bee visits.

3.3 Materials and methods

3.3.1 Experimental Design

This study was carried out on two farms 0.5 km apart in West Kent. Both farms were owned by commercial growers, Adrian Scripps Ltd. (Moat Farm) and Darbyshire Ltd. (Capel Grange Farm). The farms lay, respectively, to the north and south of the village of Five Oak Green (lat. 51.183563, long. 0.356381). The combined areas of their apple and pear orchards were approximately equal, 0.40 km^2 at Moat Farm and 0.36 km^2 at Capel Grange Farm. To augment pollination, Moat Farm rented 12 and 10 honey bee hives in 2012 and 2013, respectively, and situated them in their main pear orchard, as is

generally recommended (Free, 1993). By contrast, Capel Grange did not rent hives. Both growers had pollenizer apple (crab apple, *Malus sylvestris*) and pear (var. Doyenné du Comice) trees at intervals along the rows of crop trees, and were growing several crop varieties in most of their apple orchards.

Three honey bee observation hives were housed in a single shed at each farm in 2012 and 2013. These two sheds were 1.2 km apart. Hives were moved into the sheds approximately one week prior to the start of the pear bloom, which precedes that of apple by 1-2 weeks. The observation hives held three deep Langstroth frames and were insulated with polystyrene when not being videoed. Each colony had: a marked laying queen and approximately 5000 workers, were free of visible signs of disease, and had space for honey and pollen storage throughout the experiment. Colonies were given access to supplemental sucrose syrup via feeders.

The locations of flowering oilseed rape (OSR) fields and orchards within foraging range, <5 km during April, May and June (Couvillon et al., 2014a; Garbuzov et al., 2015), of our honey bee colonies, were determined via one aerial survey in May 2012 and another in May 2013. This information was used in conjunction with waggle dance data to construct distribution maps of honey bee foraging over the bloom period of the apple and pear varieties at the study orchards (April and May, occasionally extending into June). Hourly weather data were collected via a weather station (TFA 35.1095 Sinus, Ammerzwil, Switzerland) at Capel Grange Farm.

3.3.2 Videoing, Decoding, Mapping and Analysing Waggle Dances

Honey bee waggle dances were videoed from all six observation hives using camcorders (Sony HDR-CX115) during weather conditions suitable for honey bee foraging and waggle dance activity (≥13°C, light or no wind). Videos were recorded on nine days throughout the whole apple/pear bloom period in 2012 (10 April to 13 May) and on six days in 2013 (1 to 31 May). In order to compare foraging locations during the bloom versus those post-bloom, further videos were made during two days approximately two weeks after the end of apple bloom in 2012 (23 and 25 May) and 2013 (12 and 14 June).

Waggle dances were later decoded by observing videos played at a speed of 25fps using Streamclip (2015) software using established methods (Couvillon et al., 2012). Four waggle runs per dance, excluding the first and last waggle run, were decoded to obtain

the mean duration (which encodes distance) and angle relative to vertical (which encodes direction relative to the solar azimuth) of each dance. Probability distributions of the errors in the dance vector were combined and plotted using established methods (Schurch et al., 2013), to give heat maps of overall foraging patterns. This methodology also enabled us to determine the median and confidence interval estimates of the proportion of waggle dances for orchards and OSR fields.

3.3.3 Pollen Analysis

Pollen pellets were collected from returning foragers during apple/pear bloom in 2012 and 2013 using pollen traps (5.0mm plastic mesh, E.H. Thorne, UK) fitted to the entrance of each observation hive. Pollen samples were collected for 2-3 hours from each colony during five days in 2012 and five days in 2013. We analysed a total of 1500 pellets, 50 from each of the 30 samples deemed large enough to provide an accurate picture of a colony's foraging (≥50 pollen pellets).

50 randomly selected pellets were examined at 600× magnification in order to determine the proportion of apple/pear and OSR pollen present in each of these 30 samples. Pollen grains were confirmed as OSR or apple/pear by comparison to voucher specimens collected directly from flower anthers in April 2012. Other pollen loads were identified, where possible, with the aid of pollen pellet identification guides (Hodges, 1974; Kirk, 2006).

3.3.4 Quantifying Apple & Pear Tree Flower Visitors

Insect counts were made on apple and pear flowers of different varieties to assess the abundance of flower visiting insects in the study orchards. Five trees per row were carefully examined for flower visitors during good foraging conditions (≥13°C and light or zero wind). Rows were selected using a random number generator and counts were taken at every seventh tree. Observations from 50 trees were recorded on four separate days during the full bloom period of four apple varieties (Kanzi, Gala, Cox and Bramley) and one pear variety (Conference) in 2012 and 2013 (n = 400 trees per variety). The period of full bloom was defined as the date when 50% of the flowers were open. Bloom dates were provided by Scripps Ltd, who carefully monitor the phenology of their trees. The insect counts per tree were adjusted to allow for the

relative number of flowers counted on each variety (on ten trees per variety at full bloom).

3.3.5 Statistical Analysis

Statistical analyses used 'R' software (R-Project, 2015). Maps and geospatial measurements were generated in ArcMap 10.2 (ESRI, 2015). We used Generalized Linear Mixed-effect Models (GLMM, 'R' package lme4, version 1.1-7), proportion tests (PropTest, 'R' command: prop.test) or regression analysis (LM, 'R' command: lm). 'Year' and 'Apiary' were held as a random effect in GLMM analysis ('R' command: glmer (response variable~explanatory variable + (1|Year) + (1|Apiary)). Waggle dance analysis used mean values per hive, with the exception of foraging per hectare in which we grouped the hives at each farm per year. The analysis of insect counts used means per variety, per day. Proportion data were arcsin transformed prior to GLMM analysis. All R^2 values presented are adjusted. Data are presented as mean ± 1 standard deviation per hive, unless stated otherwise. Dates are presented as day of the year.

3.4 Results

Table 3.1 Area (km^2) of orchards and flowering oilseed rape (OSR, *Brassica napus*) within 1 and 2 km of the two observation hive locations (Moat Farm [MF], and Capel Grange Farm [CG]) over the apple (*Malus domestica*) and pear (*Pyrus communis*) bloom period in 2012 and 2013. Also shown is the median percentage (and in brackets: 2.5% and 97.5% confidence intervals) of waggle dances to all orchards, 'target' orchard (orchard area within the farms in which the hives were located) and OSR fields.

Year	Apiary /Farm	Orchard area (km^2) within 1, 2 km	All Orchard foraging (%)	Target farm orchard foraging (%)	OSR area (km^2) within 1, 2 km	OSR foraging (%)
2012	MF	0.43, 1.20	20.3 (15.0, 25.6)	13.5 (9.8, 16.5)	0.00, 0.42	8.3 (3.8, 12.0)
2012	CG	0.82, 1.87	40.2 (35.3, 45.1)	27.7 (23.9, 32.6)	0.28, 0.52	17.4 (13.0, 21.7)
2013	MF	0.43, 1.20	10.7 (7.7, 13.6)	8.9 (6.5, 11.6)	0.34, 1.02	18.5 (14.2, 23.1)
2013	CG	0.82, 1.87	22.8 (17.9, 27.7)	13.8 (10.6, 17.9)	0.04, 0.47	6.5 (3.3, 10.6)

Peak bloom was earlier in 2012 than 2013 for the pear (Conference: day 108 vs 127) and apple (mean of Kanzi, Bramley and Gala: 119 vs 132) varieties studied. We recorded only 94 (2012) and 132 hours (2013) of temperatures conducive to honey bee foraging (≥13 °C) during the bloom. In both years our honey bee colonies had average areas of 0.61 km^2 of flowering OSR and 1.20 km^2 (Scripps apiary) and 1.87 km^2 (Darbyshire apiary) of orchards within a 2 km radius, see Table 3.1. The nearest OSR fields to the hives at Moat Farm and Capel Grange Farm were, 1 km and 0.3 km in 2012 and 0.3 km and 0.7 km in 2013, respectively.

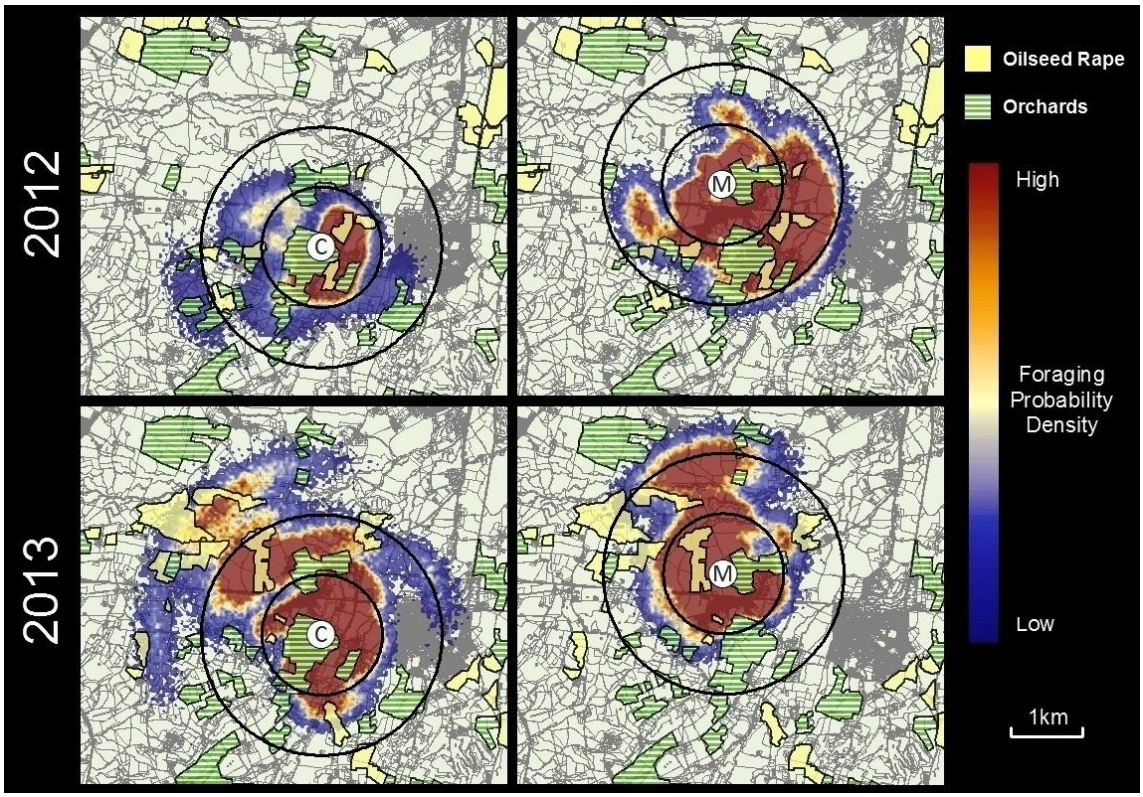

Fig. 3.1 Probability distributions of honey bee foraging from the observation hives at Capel Grange (C; 51.178698, 0.36155149) and Moat (M; 51.188063, 0.35361215) Farms, Kent, UK, in relation to flowering oilseed rape fields (yellow) and orchards (green & white stripes) in spring 2012 and 2013. The apiaries were 1.2 km apart. Circles around the apiaries represent distances of 1 and 2 km. Colour spectra show the range of foraging probabilities, as determined by waggle dance simulations, binned into 25 m^2 quadrats, from blue to red (548 - 4032, depending on the dataset). The following landmarks can be used to locate the study area on maps. The urban area (grey) in the centre-right of each map is Paddock Wood. The approximately horizontal line in the centre of each map is the Tonbridge-Ashford railway line, and the approximately vertical line near the top right corner is the A228 road.

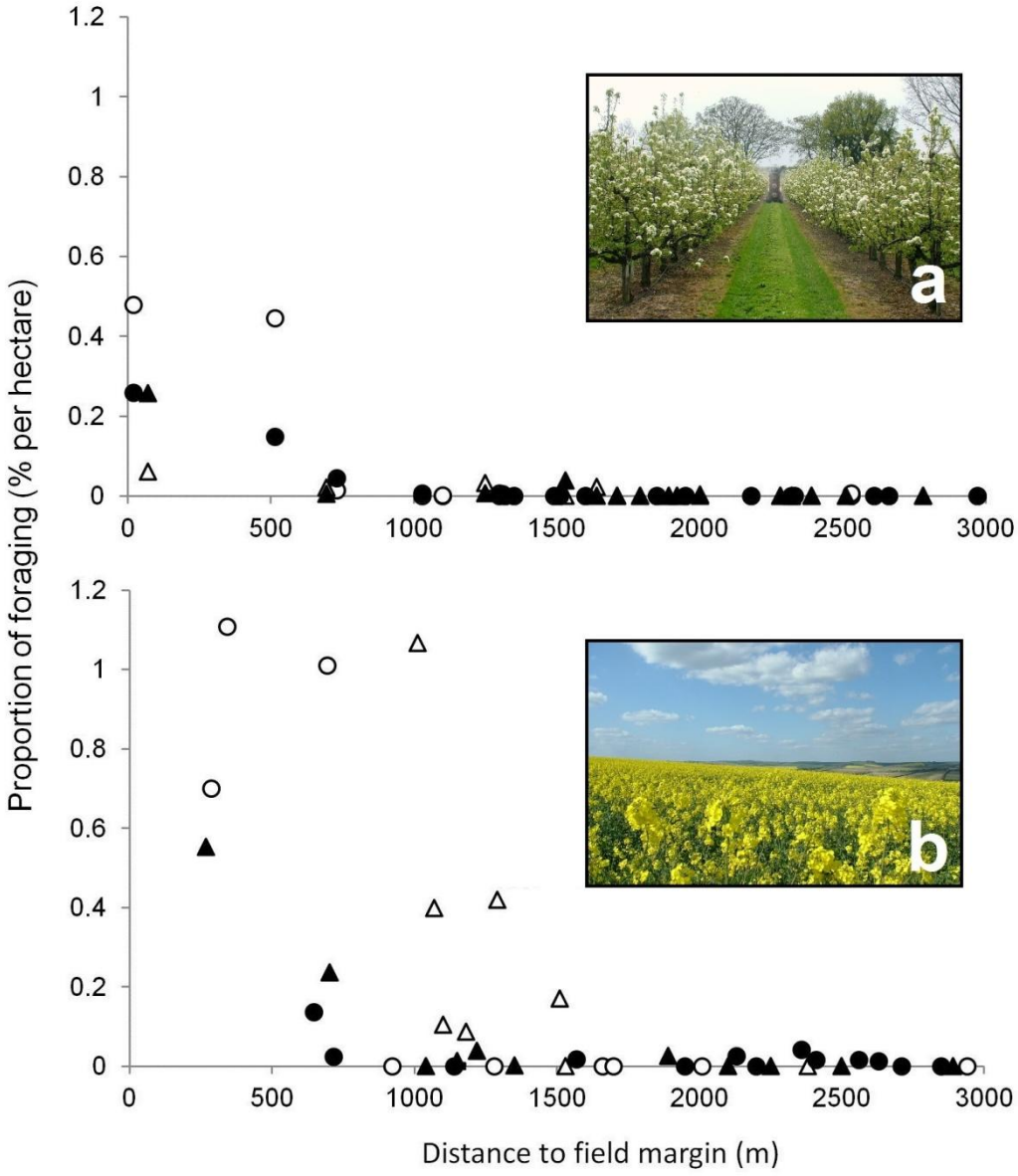

Fig. 3.2 Proportion of foraging per hectare in a) orchards and b) oilseed rape fields as a function of apiary distance from the closest field or orchard margin. Contiguous orchards and fields were grouped into one area. There were no waggle dances for fields >3 km from the two apiaries (Moat Farm, circles; Capel Grange Farm, triangles) in 2012 (open symbols) or 2013 (black symbols).

834 waggle dances were decoded, 428 from 2012 and 406 from 2013 (Fig. 3.1). Approximately one-third (30.6%) of dancing bees were carrying visible pollen loads in their corbiculae. The mean waggle dance distances indicated by pollen-carrying bees were significantly shorter (0.95 ± 0.39 km) than those without pollen (1.22 ± 0.45 km, GLMM, $\chi^2 = 4.02$, df = 1, $p = 0.045$). However, this situation was reversed after the apple bloom (pollen: 1.67 ± 0.84 vs nectar: 1.34 ± 0.43 km), although the differences

were not significant (GLMM, $\chi^2 = 1.22$, df = 1, $p = 0.269$). During the apple/pear bloom the 95th percentile foraging distance was 2.13 km. On average, mean distances from waggle dances during the bloom (0.98 ± 0.30 km) were significantly shorter than those after the bloom had ended (1.57 ± 0.69 km, GLMM, $\chi^2 = 7.79$, df = 1, $p = 0.005$).

Almost one-quarter (mean: 23.5%) of waggle dances signalled orchards, a significantly higher proportion (GLMM, df = 1, $\chi^2 = 24.76$, $p < 0.001$) than for OSR fields (12.7%), see Table 3.1. However, only 16.0% of dances indicated the 'target' orchards within the two farms in which the hives were sited. The proportion of dances indicating OSR fields and target orchards were not significantly different (GLMM, df = 1, $\chi^2 = 0.18$, $p = 0.670$).

Honey bees preferentially forage at closer locations from their hives, presumably because this reduces energy costs (Seeley, 1995). When the distance from our study apiaries to OSR fields or orchards is taken into account, the proportion of foraging per hectare in OSR fields was actually greater than in orchards (GLMM, df = 1, $\chi^2 = 23.2$, $p<0.001$), see Fig. 3.2. In addition, orchards located >0.5 km and OSR fields >1.5 km from our apiaries were little visited. OSR fields and orchards closer to our apiaries elicited a greater proportion of foraging per hectare (GLMM, df = 1, $\chi^2 = 56.4$, $p<0.001$).

The proportion of waggle dances indicating OSR fields was negatively correlated with that for fruit orchards in 2013 (LM, $R^2 = 0.67$, df = 5, F = 11.07, $p = 0.029$). However, this pattern was not observed in 2012 (LM, df = 5, F= 3.41, $p = 0.139$) or when the data from both years were combined (LM, df = 11, F= 0.53, $p = 0.485$).

Almost half (47.2%) of the pollen pellets we identified were from apple/pear flowers. Given the difficulty of distinguishing between the pollen grains of Roseaceae species, a small proportion may have been from *Crataegus monogyna*, a common hedgerow shrub, and other closely related species. However, *C. monogyna* flowering overlapped with that of apple and pears during only one week in 2013. The remaining pollen pellets were from OSR (19.2%), *Vicia faba* (7.8%), *Allium* spp. (4.9%), *Prunus spinosa* (3.5%), *Taraxacum officinale* (2.8%), *Salix* spp. (1.8%), *Pinus sylvestris* (0.7%), or were undetermined (12.1%).

Insects were observed at a density of approximately one per five apple trees (mean of Kanzi, Gala, Cox and Bramley: 0.21/tree) and one per ten pear trees (Conference: 0.12/tree). However, the mean counts per day did not differ significantly between these five apple/pear varieties (GLMM, df = 1, $\chi^2 = 4.20$, $p = 0.380$). Honey bees were the most common insect observed visiting apple and pear flowers combined (63.7%; PropTest, df = 1, $\chi^2 = 75.95$, $p<0.001$). There was no significant difference in the number of insects (GLMM, df = 1, $\chi^2 = 0.06$, $p = 0.812$) or honey bees (df = 1, $\chi^2 = 0.68$, $p = 0.409$) recorded per tree at the two farms. Nearly one fifth (18.6%) of the flower visitors were solitary bee species, predominately *Andrena* (*A. haemorrhoa*, *A. fulva*, *A. wilkella*), which were especially common on pear flowers (45.4% of all visitors). One tenth (10.7%) were bumble bees (*Bombus terrestris/lucorum*, *B. pascuorum*, *B. pratorum* and *B. hypnorum*), over a third (38.3%) of which were queens. The remainder of the visitors recorded were flies (7.0%), mostly (68.9%) Bibionidae.

3.5 Discussion

Our data give a unique picture of honey bee foraging in a crop during pollination and highlight the limitations of determining their ecology via pollen trapping alone (Free, 1968). The mean foraging distance of worker bees from hives located within the apple farms determined from waggle dances recorded during bloom was considerably shorter (c. 1 km) than the maximum range of honey bee colonies (>10 km; Beekman and Ratnieks, 2000). This finding accords with previous research, which found that most foraging occurs within 1 km (mean 0.98km) of the hive during spring in two other locations in England: Sussex, some 30km from our study site (Couvillon et al., 2014a) and the Sheffield area (Beekman and Ratnieks, 2000).

Because honey bees are rational foragers (e.g. Seeley, 1995), these results, and those of others (Von Frisch, 1967; Schepe et al., 2014), indicate that there is an abundance of floral resources available to honey bees during spring which leads to foraging close to the hives. We found that waggle dances recorded approximately two weeks after the orchard bloom had ended indicated significantly greater foraging distances (1.57 km).

The proportion of apple and pear pollen pellets combined (47.2%) was higher than our waggle dance data suggested (23.5% of dances were for orchards). This is likely because the pollen-collecting bees were foraging closer to the hive, on average, during apple and pear bloom. This should favour pollination, as pollen-foraging honey bees are

more effective pollinators of apple flowers (Free and Williams, 1974). This also indicates that apples and pears are a better source of pollen than of nectar.

Overall, almost a quarter (23.5%) of waggle dances indicated foraging in orchards, a significantly higher proportion than for OSR fields (12.7%). However, when distance to the hive is factored in, the picture was reversed (Couvillon et al., 2014b), see Fig. 3.2. OSR fields at up to 1.5 km were relatively frequently visited, in agreement with previous research on honey bee foraging on OSR (Garbuzov et al., 2015). By contrast, orchards at >500 m were very rarely visited (Fig. 3.2a) and the proportion of foraging per hectare in OSR fields was greater than in orchards. Moreover, the proportions of waggle dances per colony for OSR fields were negatively correlated with those for orchards in 2013 ($R^2 = 0.67$, $p = 0.029$).

Our results strongly suggest that OSR, which occupies 3% of the UK land area (DEFRA, 2014a) and predominately blooms in spring (95%; DEFRA, 2014b), is a major competitor to fruit trees for honey bee visitation. Indeed, it has been shown previously, via pollen trapping, that mustard (*Sinapis alba*; Brassicaceae), a close relative of OSR, also attracts honey bee foragers from hives located in orchards (Stephen, 1958). OSR is attractive to honey bees (Cook et al., 2003; Rundolf et al., 2015; Garbuzov et al., 2015) and proximity to OSR fields is known to increase the abundance of several other bee species (Westpal et al. 2003; Holzschuh et al., 2013). OSR pollen is high in essential amino acids relative to many other flower species (Weiner et al., 2010). Furthermore, OSR nectar is rich in sugar (45%, Mesquida et al., 1988), comparable to commercially grown apple nectar (42%, Butler, 1945) and more rewarding than pear nectar (15%, Butler, 1945). However, sugar concentrations reported in the literature vary widely, and for comparisons to be ecologically relevant they should be taken from nearby plants on the same days.

Prior research has found that honey bees commonly visit competing flower species, such as dandelions (*Taraxacum officinale*), within apple orchards (Free, 1968). However, our findings, and those of others (Stephen, 1958), indicate that competing floral resources outside orchard boundaries also play an important role in determining the foraging patterns of honey bees from hives located within orchards. Indeed, it is often the case that the crops that honey bees are rented to pollinate are less attractive or rewarding than alternative flowering plants within their foraging range (Jay, 2000). To

maximise pollination, honey bee colonies are usually placed within or at close proximity to their target orchard, and spread out in small groups, which increases visitation, pollen collection (Braun et al., 1953) and crop yield (Free, 1962).

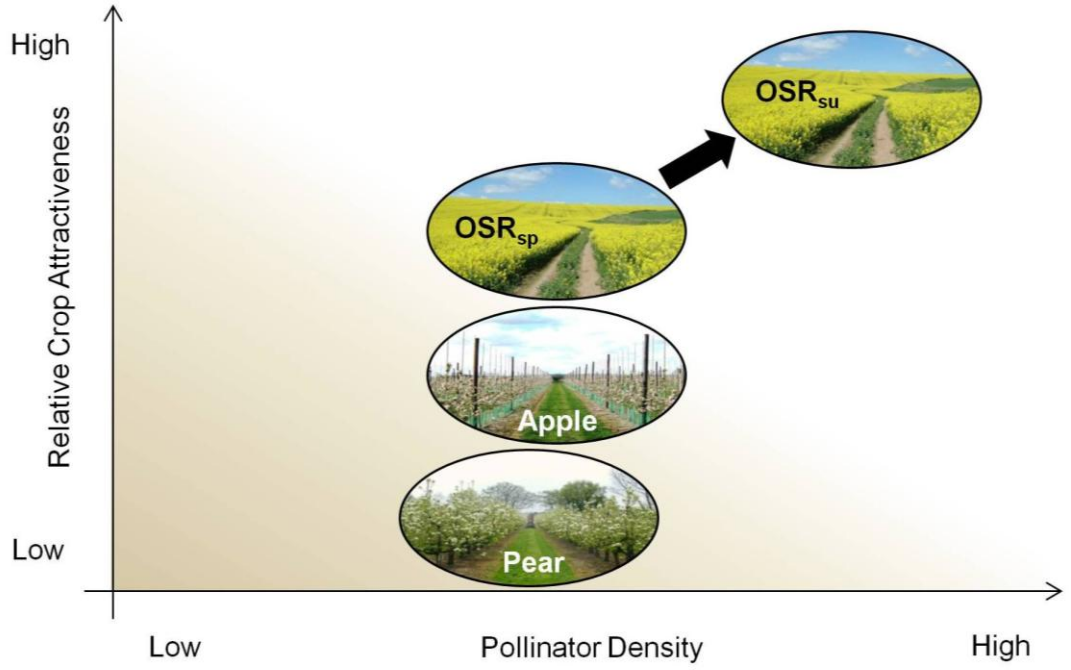

Fig. 3.3 Visualisation of the pollination requirements of pear (*Pyrus communis*), apple (*Malus domestica*) and oilseed rape (OSR; *Brassica napus*) in two key dimensions: crop attractiveness relative to local competing alternatives and the local density of pollinating insects. As the axis scales are qualitative, the locations given are only general. Crops closer to the origin (i.e. relatively unattractive and/or in areas with low pollinator densities) will likely suffer from pollination deficits (darker shading). Pear is slightly to the left of apples and OSR because it blooms a week or two earlier, which will mean fewer pollinators as *Bombus* and *A. mellifera* colonies will have fewer workers. Pear is also below apple as its nectar contains less sugar. Apples and pears bloom only in spring, but OSR can be grown to bloom in spring (late summer planted) or summer (spring planted). Summer flowering OSR (OSR_{su}) will likely be relatively more attractive than spring flowering OSR (OSR_{sp}) to pollinators due to the relative dearth of alternative floral resources during these months and, furthermore, more pollinating insects should be on the wing (e.g. *Bombus* and *A. mellifera* colonies will be at peak population), hence it is positioned further from the origin in both the horizontal and vertical dimensions.

Honey bees were the most common insects recorded visiting apple and pear flowers (64%), which echoes the findings of prior research (see Free, 1993). However, solitary bees were also quite abundant (19%). The majority of these were *Andrena* species, many of which are commonly on the wing during the spring in Britain (Faulk and

Levington, 2015) and have previously been observed to frequent fruit tree flowers (Chambers, 1946). Bumble bees represented only one in ten (11%) of the insects recorded. This is to be expected, given that most bumble bee colonies are founded in the spring or early summer and reach a maximum number of foragers during summer (Edwards and Williams, 2004). The phenology and colony cycle of *Bombus* was reflected in the high proportion of queens observed (38%).

Because half of all crop pollination worldwide, in financial terms, is provided by honey bees (Kliejn et al., 2015), effective managment of their pollination services is important for crop production; especially given the ongoing challenges of environmental change (e.g. Robinson and Sutherland, 2002; Biesmeijer et al., 2006), pests and diseases (e.g. Ratnieks and Carreck, 2010) and the fact that crops requiring insect pollination constitutes an increasing part of the human diet (Aizen and Harder, 2009; Breeze et al., 2014).

Our data from dance decoding indicate that crop pollination decisions should not just be made at the individual farm level, but at the landscape level (Jay, 1986; Steffan-Dewenter et al., 2002). Considerations beyond the farm level are particularly relevant to the management of honey bees due to their long range foraging (>10 km; Beekman and Ratnieks, 2000) relative to other bee species (Greenleaf et al., 2007). We found that the majority of honey bee foraging during bloom was within 20% (95th percentile, 2.1 km) of their maximum range, similar to that found previously during spring (Couvillon et al., 2014a). However, this represents a large potential foraging area (13.9 km^2), approximately 350 times that of the average British orchard (0.04 km^2; FOE, 2002) and 35 times larger than the total orchard areas at the two study farms.

Landscape consideration of foraging ecology is particularly relevant in locations where there is a low density of pollinators or if the target crop requiring pollination is relatively unattractive foraging resource (e.g. pear, soybean, tomatoes; McGregor, 1976; Free, 1993) compared to available alternatives. Fig 3.3 presents these two factors graphically and highlights the circumstances in which pollination deficits may occur (e.g. Vaissière et al., 2009; Garratt et al., 2014b). Also highlighted is the effect of blooming season, which potentially has consequences for both local pollinator density and crop attractiveness relative to local competing alternatives. Another factor not shown is the effect of pollination on crop yield and value. The yield of crops is almost

totally dependent on insect pollination, whereas in others it provides just a small increase.

It is obvious that the decision whether or not to procure additional pollinating insects, such as honey bee colonies, depends on multiple factors. Understanding these factors and their interactions is not so simple, especially when this requires knowledge of the distances that pollinators commonly fly. It is fortunate that the most important pollinating bee, *Apis mellifera*, allows researchers to eavesdrop on its communication system by decoding waggle dances, allowing us to investigate this important pollination parameter. Our study contributes important insights into the potential use of waggle dance decoding as an aid in commercial crop pollination, and could be followed by studies of other crops. An obvious candidate is the California almond crop, for which approximately one million bee hives are rented each year during spring bloom (Morse and Calderone, 2000) and which has an annual value of $5.7 billion (FAO, 2015).

Chapter Four: Exploitative Competition Alters Bee Flower Choice and Foraging Behaviour

4.1 Abstract

In this field experiment we test and support the hypothesis that exploitative competition between bees can influence several aspects of their foraging behaviour. Three treatments of lavender patches were set out: bumble bees excluded, honey bees excluded, control.

Bumble bees are known to handle lavender flowers more rapidly than honey bees, partly due to their longer tongues. As predicted, excluding these superior competitors consistently (n = 4 trials) and greatly increased honey bee numbers per patch (14-fold increase; $P<0.001$). The exclusion of bumble bee also caused multiple changes to honey bee foraging behaviour: time spent on a patch (+857%; $P<0.001$), flower handling time (+16%, $P=0.040$), interval between probed flowers (-27%, $P=0.012$), proportion of inter-flower flights (-26%, $P<0.001$) and flowers rejected (-12%, $P<0.001$).

Conversely, and also as predicted, excluding honey bees had no effect on bumble bee numbers or foraging behaviour. A key consequence of bumble bee exclusion was to increase the mean flower nectar content from 0.007 to 0.019 µl (+171%). By constructing an energy budget, we show that this leads to honey bees making a substantial, rather than a marginal, energetic profit per flower visited.

Our results show the foraging behaviour of individual bees is extremely flexible and greatly influenced by the effects of interspecific competition on nectar rewards. Collectively, these individual decisions can have rapid and important consequences at the community level, including competitive exclusion.

4.2 Introduction

Exploitative competition, where multiple species share a limited resource, is considered the most common form of competition amongst terrestrial animals (Schoener, 1983). However, it is challenging to determine its influence on foraging behaviour and ecology as it often co-occurs with interference competition, in which there is direct inhibition,

such as aggression, among individuals (e.g. Persson, 1985; Hart, 1987; Eccard and Yolnen, 2002; Ward et al., 2007; Segers and Taborsky, 2012).

Bee foraging is a excellent system to investigate the consequences of exploitative competition. Bees are diverse, with many species typically foraging in the same area and visiting the same flower species. Foraging bees compete for two largely generic resources, nectar and pollen, which are often scarce. Indeed, the majority (>90%, Heinrich 1976) of the "standing crop" of nectar produced by plants attractive to bees is consumed each day, leaving many flowers either empty of nectar or containing only small volumes (c. 0.1 µl, Wetherwax, 1986; Williams, 1998; Herrerra, 1989; Balfour et al., 2013). Consequently, both honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) workers need to visit hundreds of flowers per foraging trip (e.g. Ribbands, 1949; Heinrich, 1979). Although some species defend patches of flowers from potential competitors (see Severinghaus et al., 1981; Biesmeijer and Slaa, 2006), bees generally forage without physically interacting (e.g. Inouye, 1978; Schaffer et al., 1979). In addition, bees are highly mobile and very sensitive to changes in floral rewards (e.g. Free, 1965; Heinrich, 1979; Seeley, 1995). As such, they have the potential to respond quickly to resource depletion resulting from competition.

The fundamentals of interspecific competition and resource partitioning between bee species are thought to be reasonably well understood (e.g. Kevan and Baker, 1983). Virtually all of this understanding, however, is from studies of different bumble bee species (e.g. Brian, 1957; Inouye, 1978; Harder, 1983). As such, the frequency and scope of competition between honey bees and bumble bees remains largely unknown, even though they frequently co-occur (e.g. Herrera, 1989; Goulson and Sparrow, 2009; Balfour et al., 2013) and in spite of indirect evidence for competitive effects (Thomson, 2004; Goulson and Sparrow, 2009). Further understanding these interactions is relevant to recent concerns regarding their conservation (Goulson et al., 2008; Potts et al., 2009), the colonisation of honey bees and bumble bees to areas where they are not native (e.g. Paini, 2004; Ishii, 2007), and their pollination services (e.g. Greenleaf et al., 2006; Brittain et al., 2013).

There is evidence that competition between *A. mellifera* and *Bombus* negatively impacts the latter (Thomson, 2004; Goulson and Sparrow, 2009). Presumably, the opposite can also occur. Bumble bees are known to handle flowers faster than honey bees (Free,

1968; Heinrich, 1979; Kevan and Baker, 1983) and also have longer tongues, both of which may provide a competitive advantage. Recent research has shown that *Bombus* visit lavender (*Lavandula x intermedia* 'Grosso') flowers over three times faster than honey bees (Balfour et al., 2013). Experimental shortening of lavender corolla tubes (from c. 7 to c. 3mm) showed that this was partly due to the longer tongues of bumble bees (7.8-8.9mm vs 6.6mm in honey bees). Superior tongue-length allows *Bombus* easy access to Grosso's concealed nectar, the main reward being sought by lavender visitors (Herrera, 1989; Balfour et al., 2013). Bumble bees were c. 10 times as common as honey bees on lavender flowers (Balfour et al., 2013; Garbuzov and Ratnieks, 2013) despite an abundance of honey bees on nearby patches of borage (*Borago officinalis*) suggesting that bumble bees were outcompeting honey bees on lavender.

In this study we test the hypothesis that bumble bees are deterring honey bees from foraging on lavender via exploitative competition. We predicted that excluding bumble bees would increase standing nectar rewards and the number of honey bee foragers. Conversely, we predicted that honey bee exclusion would not affect bumble bee numbers, because the few *A. mellifera* normally present on the lavender flowers would little affect nectar availability.

4.3 Materials & Methods

4.3.1 Study Site, Species and Experimental Setup

In August 2012 and July-August 2014 we studied honey bees and bumble bees foraging on three experimental 2.0 x 1.1m patches of lavender, variety Grosso (*Lavandula x intermedia*). Each patch comprised 66 plants, each in a three litre pot. Plants were in full flower, each with 8-23 inflorescences of 3-16 open flowers (sometime called florets).

The patches were 100 metres apart on the University of Sussex campus, southern England, in a sheltered, grassy area with full sun. Grosso is the most widely-grown lavender variety for oil production (Upson and Matthew, 2004), and has blue flowers with petals fused basally to form a long (average depth 7.2mm) narrow corolla tube with basal nectaries.

We studied bee foraging on Grosso as our previous research (Balfour et al., 2013) on this variety had directly led to the hypothesis that exploitative competition between bumble bees and honey bees was deterring honey bees from foraging on this plant.

We excluded bumble bees from one lavender patch (BBE) and honey bees from another (HBE). No insects were excluded from a third, control (CON), patch. All insects were excluded overnight, 1800-0900h, by covering all patches with a 4mm mesh. Between 0900-1800h when foraging data were collected, we excluded bees foraging in the 'wrong' patch (e.g. bumble bees on BBE patch) by gently tapping them with a bamboo. Care was taken not to disturb bees foraging in the 'correct' patches. Results (Fig. 2) show that this method was highly effective. For example, the mean numbers of bumble bees in the BBE patches, was <0.1 per count. The few bees recorded forging in the 'wrong patch' were due to the difficulty of differentiating, without close inspection, smaller *Bombus pascuorum* from honey bees, both of which were approximately the same size and colour. In 2012 a single five-day trial was made. In 2014, three trials were made by switching treatments at the start of Days 4 and 7 (3 and 6 August 2014) so that each patch was studied under all three treatments (BBE, HBE and CON).

4.3.2 Quantifying Flower Visitor Numbers & Bee Behaviour

To determine the overall effect of bumble bee exclusion on the number of honey bee foragers per patch, and *vice versa*, we quantified the number of all insects foraging on the lavender flowers at each patch once every 30 minutes from 1000-1800h by making a near instantaneous count (see Garbuzov and Ratnieks, 2013). Foraging insects were identified to species, except for *Bombus terrestris* and *B. lucorum* (as these species cannot be distinguished in the field) and non-Syrphidae Diptera. For analysis insects were placed into four categories: (i) honey bees, (ii) bumble bees, (iii) butterflies and (iv) hoverflies. Although several other species were observed in the vicinity the only bees recorded visiting Grosso during the course of the experiment were honey bees, bumble bees and the wool carder bee *Anthidium manicatum*. Lavender flowers begin producing nectar before 6.00 and foraging can continue until after 19.00 (*L. latifolia*, Spain, Herrera, 1990). Our observations were made during between peak foraging hours (10.00 - 18.00; Herrera, 1990).

Several aspects of the foraging behaviour of individual honey bees and bumble bees (see Table 4.1 and Balfour et al., 2013), on all three patch types, were quantified by watching videos frame by frame on Streamclip software (Streamclip, 2014). Videos were made using a Sony HDR-CX115 camcorder during ideal foraging weather (>20C, wind <5kph) on days 3 and 4 (2012) when bee numbers per patch had stabilised. Individual bees were located opportunistically and videoed while they foraged on 20 consecutive flowers, then captured, placed in a honey bee queen marking cage and paint marked. Data were not used from bees that were already marked, evaded capture, or followed for <20 flowers.

4.3.3 Quantifying Nectar Volume & Sugar Concentration

To assess the impact of bee exclusions on nectar volumes we collect nectar samples on Days 3 and 4 (2012) and 3, 6 and 10 (2014) on each of our experimental patches. Thirty nectar samples, per day, were taken 1200-1400h, the period of peak nectar production (Herrera, 1989) from 30 flowers (10 each from upper, middle and lower locations) of randomly-selected inflorescences of randomly-chosen plants in each patch. Nectar volumes were quantified to ±0.008µl by measuring the length of liquid (±0.5mm) in a 64 mm long, 1µl micro-pipette (Drummond Microcaps).

In order to calculate the potential energy profits to bees foraging on lavender, we evaluated the sugar content of Grosso. To gather sufficient volume of nectar to make a reliable measure of sugar content (c. 1ul) from uncovered Grosso plants would involve probing c. 100 flowers (the average volume per flower being c. 0.01 µl) during which time the evaporation of the nectar within the capillary tube would increased the sugar concentrations and in turn compromise our results. Therefore, thirty flowers were emptied of nectar with a micro-pipette on 29 August 2012 at 1200h. Flowers were then covered with a fine mesh (0.2 x 0.2mm) to exclude all insects. After two hours the accumulated nectar was collected with a micro-pipette and the sugar concentration (degrees Brix) determined using a hand-held refractometer (Kruss HR 25/800). By restricting the data collection to 12.00 and 14.00 we reduced any potential biases caused by daily fluctuations in nectar production or concentration.

4.3.4 Determining the Energetic Returns Per Flower Visited

We predicted that the exclusion of bumble bees would greatly affect the energetic profitability of lavender flowers to honey bees, but not *vice versa*. To test this we constructed an energy budget (see Appendix A), per lavender flower visited. These calculations use the foraging data collected in this study (Table 4.1), the sugar content of Grosso (described above), data from Balfour et al. (2013) and data on bee respiration rates (O_2 consumption or CO_2 production of resting, walking and flying bumble bees and honey bees) taken from 17 previous studies. We made our energy budget per flower as this is the key measure of overall energetic profitability, which would also include the energetics flights to and from the nest etc. In particular, if foraging is not energetically profitable per flower, then it will not be profitable when additional energy expenditures are included.

To calculate the energy gain (in joules, J) of a foraging bee per Grosso flower we first calculated the mean energy available (N) in the nectar of one flower. This is given by the product of the mean volume (v in ml) of nectar per lavender flower, the sugar concentration (s) of nectar lavender (39%; see Results), the specific gravity (g) of a solution which is 39% sugar (1.148 Brix) and the energy content of sucrose (c; 16480 J/g):

$$N = vsgc \quad (1)$$

From this we subtracted the energy expended in all activities (flying, walking, handling) by a nectar collecting bee, per flower. The energy expended flying per flower (f), for example, is given by the product of the metabolic rate (r) of activity in J/g/s, the mean bee weight (b, in grams, g) and the mean duration (t) used in this activity per lavender flower:

$$f = rbt \quad (2)$$

4.3.5 Statistical Analysis

All analyses uses data collected during Days 3 and 4 (2012) and 3, 6 and 10 (2014), during which bee numbers per patch had stabilized, unless stated otherwise. When quantifying the number of 'Grosso' flower visitors we undoubtedly counted the same insect on more than one occasion. Therefore to average away the pseudoreplication in

the data (Crawley, 2014), 'Patch' (n = 4) was considered as the experimental unit in our count data analysis, i.e. prior to analysis, counts were averaged across four days: Day 3 (2012) and 3, 6, 10 (2014). Statistical analyses were conducted using 'R' software (R-Project, 2013). We used Generalized Linear Mixed-effect Models (GLMM 'R' package lme4, version 1.1-7) or one-way ANOVA. 'Day' or 'Patch' was held as a random effect in GLMM analysis ('R' command: glmer(response variable~treatment+(1|Day/Patch), family=binomial). Delta Akaike information criterion (ΔAIC) is given by the AIC of the null model minus the AIC of the alternative model. Tukey's Honestly Significant Differences tests (HSD, 'R' package multcomp, version 1.3-6) followed significant (P<0.05) ANOVA. All values are presented as mean ± 1 standard error.

4.4 Results

Honey bees (*Apis mellifera*: 34%) and bumble bees (60%: *Bombus terrestris* and *B. lucorum*: 42% [these species cannot be distinguished in the field], *B. pascuorum*: 17%, *B. lapidarius*: <1%, *B. hortorum*: <1%, *B. hypnorum*: <1%, *B. vestalis*: <1%) comprised 94% of all insects counted on all lavender flowers in the three patch types in 2012 and 2014 combined. The other 7% were butterflies (4%), hoverflies (2%), other Diptera (<1%) and wool carder bees (*Anthidium manicatum*, <1%). No aggressive or otherwise antagonistic interactions between foraging insects were observed with the exception of male wool carder bees, which are known to aggressively defend flower patches (Severinghaus et al. 1981). When seen on our experimental patches they were removed. We repeated the experiment in 2014 to confirm the 2012 result and to show that consistent effects resulted from bumble bee and honey bee exclusions.

As predicted, bumble bee exclusion greatly increased honey bee numbers. Across the four trials the same trend was always observed, with the mean number of honey bees on the bumble bee-excluded patches (BBE) being 14 times greater than on the control patches (Fig. 4.1 and 4.2; 8.4 ± 0.67 vs 0.6 ± 0.23; GLMM; ΔAIC = 73.41, df = 1, χ^2 = 75.41, P<0.001). However, the number of bumble bees on the honey bee-excluded patches (HBE), was not different from the control patches (7.1 ± 0.4 vs 7.3 ± 0.4; GLMM; ΔAIC = 0.51, df = 1, χ^2 = 2.26, P = 0.107).

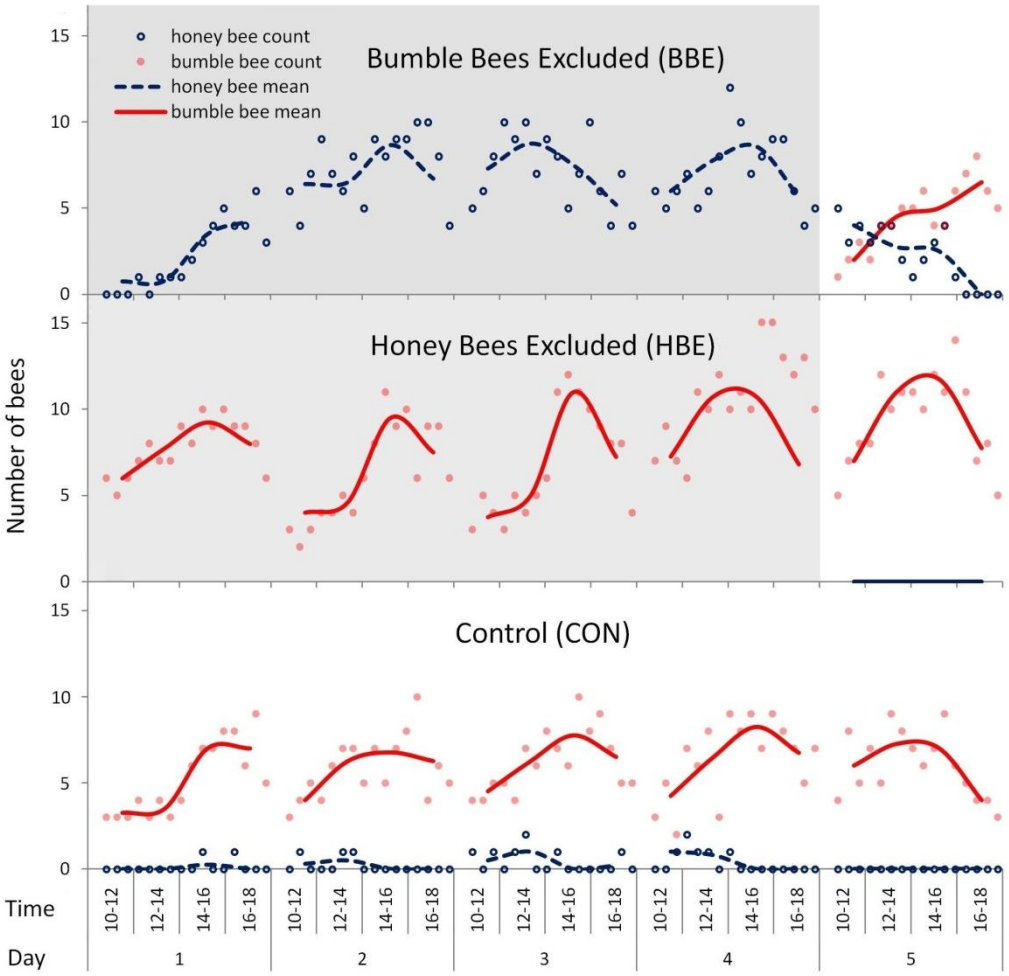

Fig. 4.1 Numbers of honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) present on three patches of lavender (*Lavandula intermedia* 'Grosso') with and without bee exclusion from counts made every 30 minutes (data points) and the moving average of four consecutive counts (lines). The shaded area indicates the period of bee exclusion on the two treatment patches. Bees were not excluded from any patches on Day 5. Bees were excluded from all patches overnight (1800-0900h) using 4mm mesh. Day 1 = 16 August 2012. Bumble bee counts are for all species combined.

Five measures of honey bee foraging behaviour were affected by bumble bee exclusion (BBE vs control), Table 4.1: (i) time between consecutively probed flowers (search time) was significantly less (-27%; 1.07s vs 1.48s; ANOVA; df = 40, F = 6.924, *P* = 0.012); (ii) extraction time, the time spent imbibing nectar from a single flower, was significantly greater (+16%; 1.44s vs 1.24s; ANOVA; df = 40, F = 4.502, *P* = 0.040); (iii) significantly fewer flowers were rejected (43% vs 55%; GLMM; ΔAIC = 10.51., df = 1, χ^2 = 12.51, *P*<0.001); (iv) the proportion of flights between flowers, versus the alternative of walking, was reduced (flying 38% vs 64%; GLMM; ΔAIC = 154.80, df =

1, χ^2 = 156.77, *P*<0.001), and (v) individuals spent ten times as long foraging in a patch after arriving (1111s vs 117s; ANOVA; df = 18, F = 35.54, *P*<0.001).

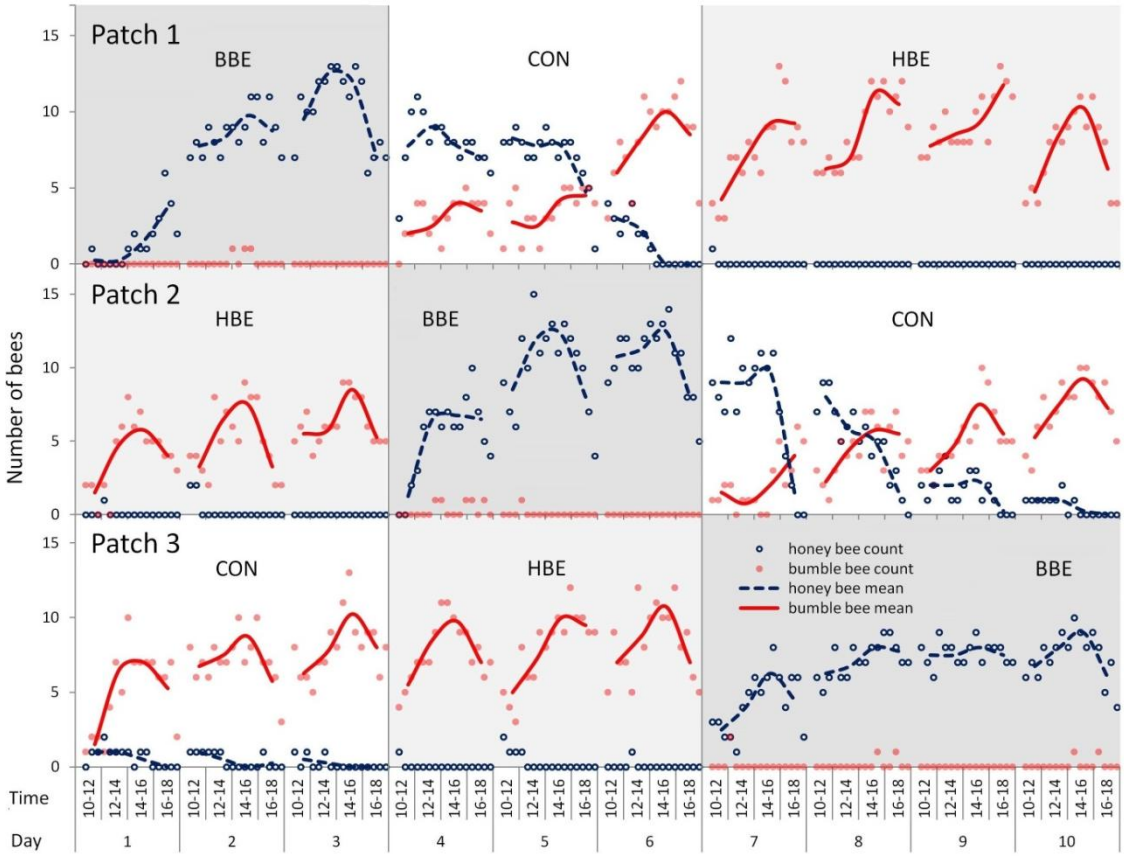

Fig. 4.2 Numbers of honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) present on three patches of lavender (*Lavandula intermedia* 'Grosso') with and without bee exclusion from counts made every 30 minutes (data points) and the moving average of four consecutive counts (lines). The shaded area indicates the period of bee exclusion (BBE: bumble bees excluded, HBE: honey bees excluded, CON: control). The vertical black lines separating Days 3 from 4 and 6 from 7 indicate the switching of Treatments between the three patches. Bees were excluded from all patches overnight (1800-0900h) using 4mm mesh. Day 1 = 30 July 2014. Due to rain data were not collected on Day 4 and patches were left covered with nets. Bumble bee counts are for all species combined.

Conversely, the same measures of bumble bee foraging behaviour were unaffected by honey bee exclusion (HBE vs control): (i) search time (0.34s vs 0.35s; ANOVA; df = 22, F = 0.221, *P* = 0.643); (ii) extraction time (0.43s vs 0.45s; ANOVA; df = 22, F = 0.134, *P* = 0.718); (iii) proportion of flowers rejected (14% vs 13%; GLMM; ΔAIC = -1.34, df = 1, χ^2 = 0.05, *P* = 0.834) and (iv) mode of locomotion between flowers (flying: 16% vs 19 %; GLMM; ΔAIC = 1.24, df = 1, χ^2 = 0.76, *P* = 0.382), (v) time spent foraging in a patch (649s vs 684s; ANOVA; df = 22, F = 0.038, *P* = 0.848).

Hoverfly and butterfly counts did not differ significantly between treatments (hoverflies: BBE: 0.16, HBE: 0.24, control: 0.08; ANOVA; df = 11, F = 1, *P* = 0.405; butterflies: BBE: 0.33, HBE: 0.35, control: 0.44; ANOVA; df = 11, F = 0.083, *P* = 0.921).

Table 4.1 Foraging behaviour of honey bees (*A. mellifera*) and bumble bees (*B. terrestris/lucorum*).

Bee and patch type	N, bees	Extraction time (s)	Search time (s)	Flower rejection (%)	Inter-flower flights (%)	Time on patch (s)
honey bees, BBE	21	1.44 ± 0.37	1.07 ± 0.46	43	38	1111± 547
		***P = 0.040**	***P = 0.012**	***P <0.001**	***P <0.001**	***P <0.001**
honey bees, CON	21	1.24 ± 0.38	1.48 ± 0.55	55	64	117 ± 61
bumble bees, HBE	12	0.43 ± 0.08	0.34 ± 0.06	14	16	649 ± 469
		P = 0.718	*P = 0.643*	*P = 0.834*	*P = 0.382*	*P = 0.848*
bumble bees, CON	12	0.45 ± 0.13	0.35 ± 0.08	13	19	684 ± 389

Bees foraged on Grosso lavender in patches from which bumble bees (BBE) or honey bees (HBE) had been excluded, and a control patch (CON) without exclusion. Based on video footage, a bee's foraging sequence across 20 flowers was divided into identifiable components: a) Extraction time: imbibing nectar at the flower; b) Search time: travel between probed flowers, including any time spent on rejected flowers, c) percentage of flowers rejected, d) Inter-flower flights are the proportion of movements between flowers made by flying (versus walking) and e) Time on patch: seconds between a bee entering and departing the experimental lavender patches. P-values show comparison of exclusion patch with control (ANOVA or GLMM).
Data are presented as mean ± 1 standard deviation. * Significant difference (*P* <0.05)

Mean nectar volumes per flower were greater in the absence of bumble bees (GLMM; BBE: 0.019 ± 0.036µl vs control: 0.007 ± 0.013µl; HSD; *P* <0.001). In contrast, the exclusion of honey bees did not affect nectar volume (GLMM; HBE: 0.007 ± 0.013µl vs control; HSD; *P* = 0.719). The proportion of flowers without detectable nectar was also significantly lower with bumble bee exclusion (BBE: 44% vs control: 60%; two-sample proportion test, $\chi^2 = 7.065$, df = 1, *P* = 0.008), but was not reduced significantly by honey bee exclusion (HBE: 55% vs control; two-sample proportion test; $\chi^2 = 0.492$, df = 1, *P* = 0.483). Grosso nectar sugar concentration was 39 ± 3.3% (n = 30).

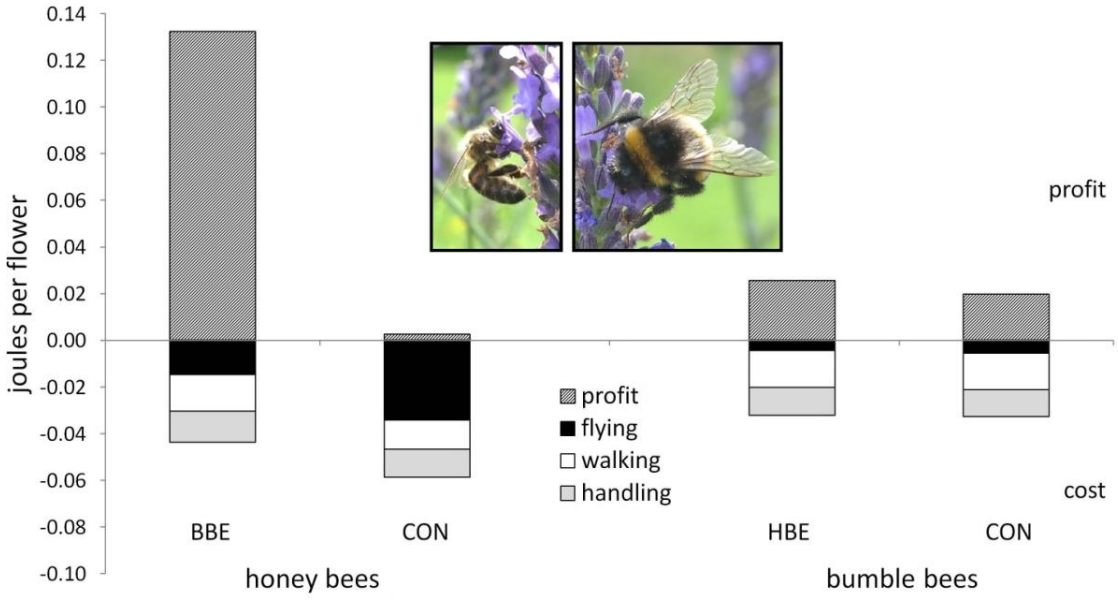

Fig. 4.3 Energy budgets of honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris/lucorum*) per lavender (*Lavandula intermedia* 'Grosso') flower in our three experimental patch types (BBE: bumble bees excluded, HBE: honey bees excluded, CON: control). Each bar represents the total the energy value of nectar in an average lavender flower on each patch type. The hatched area at the top of each bar represents the mean energy gained (profit) per lavender flower. The black, white and grey areas below the line represent the mean energy expended (cost) per flower. See Appendix A for calculations. The energy used flying is greater for honey bees than bumble bees because honey bees usually fly (64%) between flowers, while bumble bees usually walk (81%). In addition, bumble bees visit lavender flowers at three times the rate of honey bees. Therefore, bumble bee profit per flower would need to be multiplied by three to compare profits per unit time.

At first glance, it is perhaps surprising that a nectar volume increase of only 0.01 µl per flower would be sufficient to cause such a large increase in honey bee visitation. However, this corresponds to a 171% increase and our energy budget calculations (see Appendix A) shows that, for honey bees, this turns a marginal net energy gain per flower in the control patches into a substantial "profit" in the bumble bee excluded patches (CON vs BBE), Fig 4.3. Parallel calculations show that the energetic gains of bumble bees (*B. terrestris/lucorum*) are approximately the same per lavender flower whether honey bees are present or not (HBE vs CON, Fig. 4.3). Although we did observe a few honey bees foraging on the control patches, these stayed only a short time compared to those foraging on the bumble bee-excluded patch (1111s vs 117s). This suggests that these honey bees were scouts sampling and then rejecting the patch as unprofitable.

4.5 Discussion

Our results clearly show that excluding bumble bees has a major effect on lavender flower visitation by honey bees. Indeed, honey bees effectively experienced competitive displacement by bumble bees as they were virtually absent from the control patches but were consistently much more numerous, on average 14 times more, on patches from which bumble bees were excluded (Fig. 4.1 and 4.2). Although predicted by ecological theory, competitive displacement has rarely been documented in the field (Simberloff et al., 1997; Reitz and Trumble, 2002).

Our results, like those of others (e.g. Inouye, 1978; Schaffer, 1979), suggest that flower selection by generalist bees is often determined through ongoing competition rather than by the 'ghost of competition past'. This is not surprising as there is very strong selective pressure on bees to respond quickly to changes in floral rewards whether from competition or other factors. Indeed, our data show considerable plasticity in the structure of flower-pollinator communities in response to exploitative competition.

Ecological release from interspecific competition has long been thought to allow niche expansion (e.g. Van Valen, 1965) and this phenomenon has been observed in a variety of animal groups. Ocelots, for example, consume larger prey in areas where jaguars, which are much larger, are absent (Moreno et al., 2006). Comparable responses to reduced competition have also been documented in bird (Diamond, 1970), fish (Persson and Hansson, 1999) and lizard (Lister, 1976) communities. Competition for resources can be considered a reduction in habitat quality and at the individual level has been shown to reduce survival and reproductive success (e.g. Eccard and Ylonen, 2002).

At the start of each of the two field trials (Day 1, 2012 and 2014) honey bee numbers took longer to plateau than did bumble bee numbers: approximately 1.5 days for honey bees in the bumble bee-excluded patch versus <1 day for bumble bees in the honey bee-excluded and control patches (Fig. 4.1 and 4.2). This may be a result of their contrasting scouting strategies. Whereas each bumble bee forager acts as its own scout (Heinrich, 1979), only c. 10% of honey bee foragers scout, with most new foragers being recruited to flower patches via waggle dances (Seeley, 1995).

Competition for resources between honey bees and bumble bees may be beneficial to pollination services. Our results show that exploitative competition with bumble bees

reduces the amount of time spent in a patch by honey bees from 2 to 20 minutes. The increased movement of foragers between patches would give greater cross pollination per honey bee, although there would be fewer bees in total. Our results hint at the mechanisms underlying recent studies showing that wild bees increase the pollination efficiency of honey bees on almond flowers (Brittain et al., 2013) and sunflowers (Greenleaf et al., 2006) through causing more movements among patches.

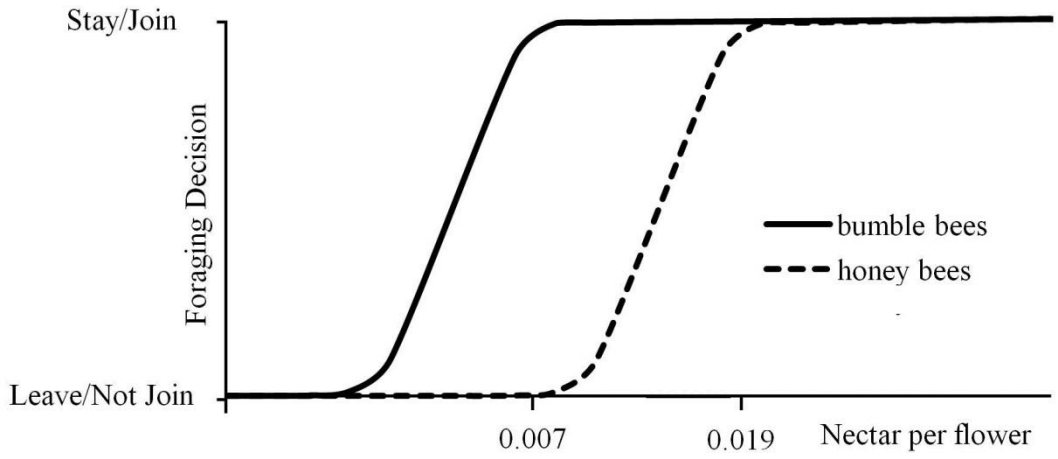

Fig. 4.4. Predicted response (stay or leave) of bumble bees (*Bombus* spp.) and honey bees (*A. mellifera*) to nectar amounts per flower. The numbers on the horizontal axis are taken from the mean nectar volume per flower in our control (0.007µl) and bumble bee excluded patches (0.019µl), see Results. The bumble bee curve is to the left of the honey bees curve because Bombus can make a profit at 0.007µl and visit flowers 3 times more quickly than honey bees (Balfour et al. 2013). Figure based on Fig. B2.3 in Stephens and Krebs (1986).

Hoverfly and butterfly numbers were not significantly affected by bumble bee or honey bee exclusion. This may have been due to the lack of statistical power because of their relatively low numbers on our lavender patches (6% of all insects, respectively 0.2 and 0.4 per count). However, we predict that nectarivores, such as hoverflies and butterflies, may not be as readily affected by exploitative competition as bees. Unlike female bees which collect resources not only to provision themselves, but also nestmates and their colony, butterflies and most other insects feed only to fuel their own activities. As a result they are not under the same constraints to forage efficiently.

When faced with two alternative forage resources with differing rewards, optimal foraging theory predicts that an animal should focus on the more rewarding alternative (Stephen and Krebs, 1986). In theory, this can lead to a 'step' function in which the less

rewarding resource is abandoned. Fig. 4.4 shows how this step function may be operating for bees foraging for lavender nectar in this study. Nectar being the primary reward sought by bees from lavender (Herrera, 1989; Balfour et al., 2013). Because bumble bees forage more efficiently than honey bees, they can profitably forage at a nectar content of 0.007μl per flower in the control patch (Fig. 4.3). However, honey bees cannot make a significant net energy profit at this level, we would expect them to reject the patch. Indeed, our data show that honey bees stayed, on average, less than two minutes foraging in these patches. By contrast, in the bumble bee excluded patches, nectar levels were higher (0.019μl) and honey bees could make a considerable energy profit (Fig. 4.3). Many more honey bees were present in these patches and stayed ten times longer. Other nectarivores, such as butterflies, are predicted to continue foraging at lower reward rates than bees. As such, the butterfly curve would be to the left of the bumble bee curve in Fig. 4.4.

Our results show that exploitative competition can play a hidden but powerful role in shaping flower visitor communities. Without experimental manipulation, an obvious inference from observational data alone (Garbuzov and Ratnieks, 2013) would be that lavender is inherently much less attractive to honey bees than to bumble bees. But this would be an erroneous conclusion. In the absence of bumble bees, numerous honey bees forage on lavender. As illustrated in Box A our data show that a foraging advantage in one common competitor can have an important effect at the community level, leading to the complete, voluntary, exclusion of another species via exploitative competition.

Chapter Five: Following the dance: Ground Survey of Flowers and Flower-visiting Insects In a Summer Foraging Hotspot Identified via Honey Bee Waggle Dance Decoding

5.1 Abstract

Decoding of honey bee waggle dances has previously shown that average foraging distances are longest during July and August in Sussex, United Kingdom, indicating a scarcity of summer floral resources. However, it also identified a summer foraging 'hotspot' in agricultural land at 2-3km distance. Dance decoding does not yield precise foraging locations or information on the flower species visited. Therefore, we surveyed this hotspot during July and August 2012 and 2013 in order to identify the habitats and flower species used by honey bees and other flower-visiting insects (FVI).

The hotspot area consisted predominantly of four habitat types: pasture fields, field margin/hedgerow of arable fields, set-aside and a National Nature Reserve. We surveyed three fields within each habitat type. The abundance of flowers was found to be a key determent of FVI abundance per field ($p = 0.002$). Field margins/hedgerows were the most flower abundant habitat type ($p = 0.002$) and had more than twice (235%) the FVI abundance ($p = 0.001$) and species richness ($p = 0.035$) per unit area than did pasture fields. Areas with long grass had greater flower abundance ($p<0.001$) and FVI species richness ($p = 0.009$) than those with short grass (≤30cm). The five plants on which we recorded the greatest number of FVI were species considered to be agricultural weeds.

Honey bees represented 19% of all FVI, showing that dance decoding had located a hotspot that was an important foraging location not just for honey bees but also for other types of FVI. Honey bee abundance, per transect, was strongly correlated with that of other FVI ($p = 0.001$), particularly bumble bees ($p<0.001$). However, FVI groups were not found uniformly across our study site and honey bee abundance was only weakly linked to overall species richness ($p = 0.069$).

5.2. Introduction

Agriculture is occupying a growing share of the Earth's land area (Tilman et al., 2011). This, together with increasingly intense management of farmland during the last century has often been linked to declining population of flower-visiting insects: hoverflies

(Biesmeijer et al., 2006), butterflies (Asher et al., 2001), bees and wasps (Ollerton et al., 2014). However, since the mid-1990's the European Union's (EU) Common Agricultural Policy (CAP) has sought to halt the general decline of farmland biodiversity (reviewed in Robinson & Sutherland, 2002) by subsiding (2007-13: € 22.2 billion; Europa, 2011) less intensive crop management and by taking some land entirely out of production (Reviewed in Bignall, 1998). These agri-environmental schemes are now widespread and cover 59% of the UK's agricultural land (DEFRA, 2014a).

Research that identified honey bee (*Apis mellifera*) foraging locations by decoding their waggle dances (Von Frisch, 1967) has indicated that late summer (July-August) is the period of year with the greatest average foraging distances in two UK localities (Beekman and Ratnieks, 2000; Couvillon et al., 2014a). As honey bees are well known to be economically rational foragers, this implies a relative shortage of floral resources during these months. These may not be just a British phenomena as a relative dearth in late-season flowers has also been reported in the Dutch agricultural landscape (Schepe et al., 2014).

In one of these studies, Sussex, UK, dance decoding identified an area located 2-3 km from the study hives as a foraging 'hotspot' during the challenging months of July and August (2009-11; Couvillon et al., 2014b). This hotspot is agricultural land in the South Downs, and encompasses Environmental Stewardship Scheme (ESS) farmland, including the Castle Hill National Nature Reserve (NNR) and an adjacent Site of Special Scientific Interest (SSSI). Although honey bee dance decoding is a useful and unique tool for studying honey bee foraging locations (reviewed in Couvillon et al., 2014b) it cannot pinpoint exact foraging locations (Schürch and Couvillon, 2013). As such dance decoding cannot differentiate between adjacent habitats (Schürch et al., 2013), nor can it indicate the species of flower visited by the dancing bee.

In order to ascertain the flower species and habitats which honey bees are visiting, we surveyed the hotspot during July and August in both 2012 and 2013. We also recorded the habitats types which occurred there, the species of flowers in bloom, the other insects present and the flower species they were visiting.

With these data we first aim to identify which features of this agricultural landscape are attracting honey bees over long distances. Second, we explore whether the hotspot

identified via honey bee dance decoding is also important to other flower visitors. Thirdly, we identify which sub-habitats and flower species are most commonly utilised.

5.3 Materials &Methods

5.3.1 Study Site and Initial Survey

During 2-15 July 2012 we conducted an initial survey of the flowers and habitats present in c. 5 km^2 in the Castle Hill area (United Kingdom, latitude: 50.84425916, longitude: -0.05170996). This centred on the area identified as a late summer hotspot for honey bee foraging in 2009-11 via waggle dance decoding (Couvillon et al., 2014b). The majority (>95%) of this area comprised 38 agricultural fields: pasture fields (25), arable fields (12) and set-aside (1). The remainder was a small wood (2.5 ha), farm outbuildings and a road (A270).

The initial survey showed that this land comprised four main habitat types potentially attractive to honey bees and other flower-visiting insects (FVI): (i) *Pasture* fields, (ii) *Field Margin/Hedgerow*: <5m strip of relatively untended land on the boundary of arable fields, (iii) *Set-Aside*: fenced, uncultivated, arable field corners of approximately 0.5ha (ESS options: EF1/HF1; NE, 2012a; NE, 2012b), (iv) *Nature Reserve*: pasture fields in the Castle Hill NNR (1949 National Parks and Access to the Countryside Act) and the adjacent SSSI (1991 Wildlife and Countryside Act). The woodland and arable fields were found to be almost bereft of blooming plants and hence were not surveyed further.

As it was not feasible to survey such a large area in detail, we conducted an intensive survey of three fields considered the most FVI-attractive within each of the four habitat types. Therefore, we selected the most FVI-friendly fields in our study area by combining the abundance and diversity of flowers estimated in our initial survey with waggle dance 'hotspot' approximations from July and August 2009-2011 (Couvillon et al., 2014b).

5.3.2 Quantifying Flower-visiting Insect Abundance

Transect sampling was used to quantify the abundance and richness of the FVI on flowers and the flower species they were visiting. Transects were conducted in the 12 study fields between 10.00-15.00, July and August 2012 and 2013, during weather conditions suitable for FVI activity (≥16°C and light wind). Insects actively visiting

flowers, and the plant species, were recorded in 1 x 200m in Field Margin/Hedgerow and 2 x 100m transects in the three other habitat types. In order to give equal survey areas, we walked the centre of each 2 x 100m transect recording 1m to the left and right side, but recorded on only one side in the 1 x 200m transects.

In each study field we made multiple transects (mean 26; range 16-42). The number per field was determined by its relative area, from Ordinance Survey maps. Fields were further stratified into three sub-habitat types (scrub, short grass or long grass) and sampling effort was again apportioned according to their relative areas. The first transect began approximately in the centre of the sub-habitat area and followed the direction of a randomly-generated compass angle. Field Margin/Hedgerow transects followed the edge of the field boundary, always in the same direction to distribute sampling effort equally. The next transect began where the previous one ended. If a field boundary or the edge of the sub-habitat was reached, the transect was redirected by 90 degrees back into the study area.

FVI were identified using field-guides (Chinery, 1989; Chinery, 1993; Baldock and Collins 2008; Ball et al.; 2013). All FVI were recorded except Coleoptera and Neuroptera which were not numerous, and non-Syrphidae Diptera, which could not be adequately identified. Flower visitors were identified to species or genus with the exception of some parasitoid wasps and sawflies (Symphyta), which accounted for less than 1% of all FVI. As such, all biodiversity indexes are calculated at the genus level, except from the number of FVI species per transect. This was achieved by further identifying FVI to morphospecies during each transect. Due to the difficulty of differentiating between cryptic species in the field (e.g. *B. terrestris* and *B. lucorum*; Wolf et al., 2010) the number of species per transect may be slightly underestimated. However, many individuals were caught and/or photographed for detailed examination. We also recorded whether visible pollen loads were present on foraging bees.

5.3.3 Quantifying Flower Abundance

Flower abundance was quantified by counting the number of 'flower units' for all blooming insect attractive species inside five 1m x 1m quadrats per transect, one every 20m (Southwood, 1966). We alternated quadrats locations between the left and right of the midline of each transect, except in Field Margin/Hedgerow where this was not possible (i.e. 1 x 200m transects). Flower species were identified using field guides

(Streeter and Hart-Davies 2009, Sterry, 2010). Flower units were categorised as a single flower (e.g. *Rubus fruticosus*), stem (e.g. *Galium verum*) or inflorescence (e.g. *Centaurea nigra*) as appropriate.

Because flowers and flower units are of different sizes, it is not straightforward to compare the quantity present per species. Therefore, we employed a measure of flower abundance that allows different plant species to be assessed on a more even footing. This was realised by calculating the mean 'petal area' represented by a flower unit for each species recorded. The petal area per flower unit was determined by collecting 10 flower units per species. 30 flowers (or florets) from each were then cut open, placed flat on graph paper and photographed. Petal images were categorised to the closest approximate geometric shape (circle, semi-circle, quarter circle, rectangle etc.) and relevant measurements (e.g. diameter, height) determined using ImageJ software (ImageJ, 2014, version 1.48).

5.3.4 Statistical Analysis

Statistical analyses used 'R' software (R-Project 2014, version 3.1-1). Generalized Linear Models (GLM) were simplified using backwards elimination of non-significant variables and model comparison using ANOVA. Because our count data was overdispered a quasipoisson error structure was in used all GLM analysis (O'Hara and Kotze, 2010). In the correlation analysis between honey bee abundance and other FVI abundance and species diversity (GLM), honey bee data were removed from the response variables and the analyses performed separately. Petal area calculations were used in all flower abundance analyses. Each habitat was stratified into scrub (areas dominated by *Rubus fruticosus* and *Ulex europaeus*), long grass (>30 cm) or short grass (≤30cm) in GLM analysis. Tukey's Honestly Significant Differences tests (HSD, 'R' package multcomp, version 1.3-6) followed significant ($p < 0.05$) ANOVA when appropriate. All values are presented as mean, or mean ± 1 standard deviation. Shannon-Wiener genus diversity (H') was calculated using the standard formula, where p_i is the proportion of the sample represented by species i:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

5.4 Results

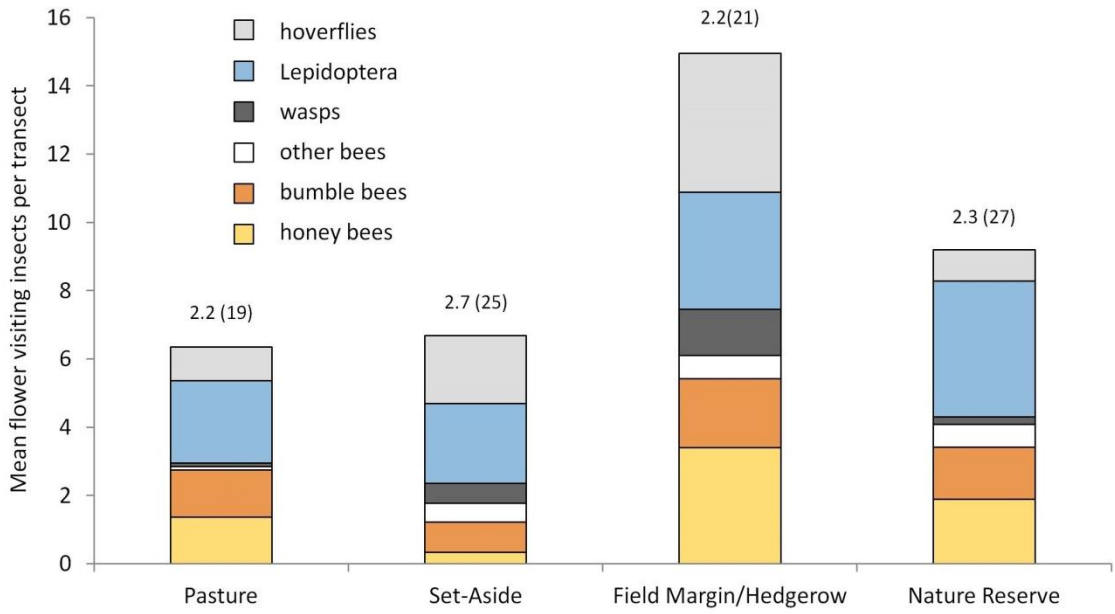

Fig. 5.1 Mean numbers of flower-visiting insects (FVI) per transect (200 m^2) in each habitat type by insect group for 2012 and 2013 combined. The numbers above each bar are Shannon-Wiener FVI genus diversity values, followed in brackets by the mean FVI genus richness recorded per field for each habitat type.

A total of 311 transects were conducted, 152 in 2012 and 159 in 2013, on which we recorded 2,807 FVI. The most abundant were Lepidoptera (35%) followed by honey bees (19%), hoverflies (19%), bumble bees (16%), wasps (6%) and other bees (5%), see Fig. 5.1. Of all the bees recorded, 16% of were observed carrying pollen loads. The mean number of FVI per transect was 16% less in 2013 than in 2012. In particular, in 2013 there were fewer honey bees (-78%), hoverflies (-70%), solitary bees (-38%) and wasps (38%) and more Lepidoptera (+157%) and bumble bees (+17%).

Nature Reserve fields had the greatest number of Lepidoptera (2.8/transect). Field Margins/Hedgerows had the highest numbers of honey bees (5.0/transect), hoverflies (6.0/transect) and wasps (2.3/transect), predominately in fields located far (>1.5km) from the NNR. Bumble bees were ubiquitous and fairly evenly distributed across all four habitat types (1.1-1.7/transect). Solitary bees were mostly observed in Field Margins/Hedgerows, Set-Aside and Reserve fields (0.6-0.7/transect).

Flowering plants were surveyed in a total of 1555 $1m^2$ quadrats. Across all four habitat types an average of 2.1 ± 1.0 flowering species were recorded per quadrat and 2.6 ± 1.1

m^2 of petal area per 200m^2 transect. We recorded approximately three times less petal area per quadrat in 2013 relative to 2012 (-65%). This same pattern was observed in the mean number of flowering species per quadrat (-53%).

Field Margin/Hedgerow was the most flower-abundant habitat type, see Table 5.1. However, it was relatively species-poor and dominated by *Heracleum sphondylium* (67% of total petal area). Nature Reserve fields were the most species rich (3.3 per quadrat), but had only marginally more petal area than other habitat types. Pasture fields had below average petal area, but were relatively species rich (2.4 per quadrat). Set-Aside fields had the lowest petal area of the habitats studied and also had relatively low flower species richness.

Over half of all FVI (55%) were recorded on only five plant species: *Heraculeum sphondylium* (18%), *Centurea nigra* (12%), *Cirsium averense* (9%), *Jacobaea vulgaris* (9%) and *Rubus fruticosus* (7%). However, the plant species with the highest number of FVI per unit petal area were: *Succisa pratensis*, *Chamerion angustifolia*, *Carlina acaulis*, *Dipsacus sylvestris* and *Reseda lutea* (see Fig. 5.2). The only species recorded visiting *C. acaulis* was the chalkhill blue butterfly (*Polyommatus coridon*). The flower species with the highest Shannon-Weiner FVI genus diversity values were *Jacobaea vulgaris* (3.2), *Heraculeum sphondylium* (3.1) and *Daucus carota* (2.7).

There was considerable overlap in the proportions of honey bees and bumble bees seen on different flower species: *Centaurea nigra* (Apis: 27%; Bombus: 11%), *Rubus fruticosus* (23%; 12%), *Heracleum sphondylium* (17%; 8%) and *Trifolium repens* (7%; 12%). However, other bees were observed predominately on composite flowers: *Heracleum sphondylium* (17%), *Leontodon hispidus* (10%), *Crepis capillaris* (10%) and *Cirsium vulgare* (9%).

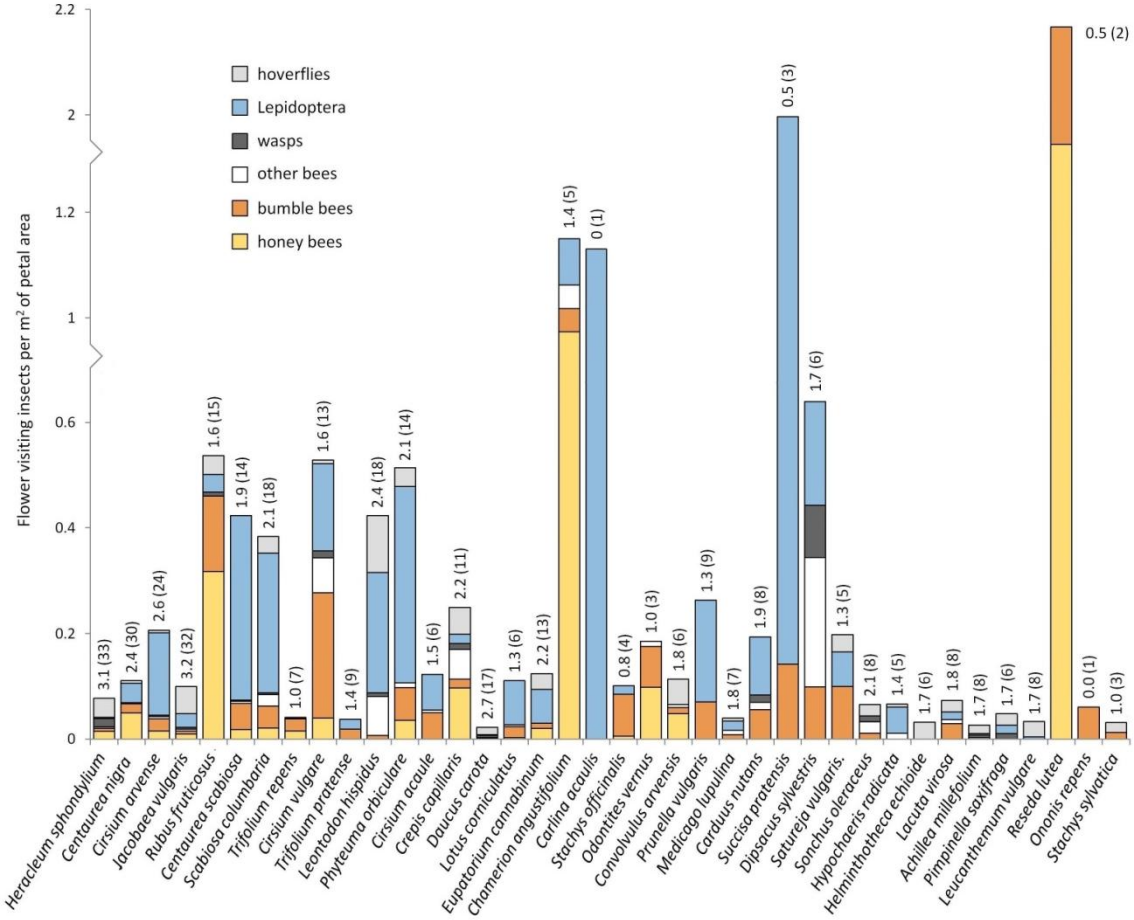

Fig. 5.2 Flower-visiting insects (FVI) per square metre of petal area for all flower species on which ten or more individuals were recorded. Flower species are ordered, from left to right, by the total number of FVI recorded per species. The numbers above each bar are Shannon-Wiener FVI genus diversity index values per flower species, followed in brackets by FVI genus richness.

Hoverflies and wasps were mainly recorded on species with open flowers and accessible nectaries: *Heracleum sphondylium* (Syrphidae: 46%; wasps: 72%), *Jacobaea vulgaris* (25%; 6%) and *Daucus carota* (both 5%). Lepidoptera were generally found nectaring on tall species such as *Cirsium arvense* (19%), *Centaurea scabiosa* (18%), *C. nigra* (11%, Asteraceae) and *Knautia arvensis* (8%, Caprifoliaceae).

5.5 Discussion

5.5.1 The Waggle Dance as an Indicator of Flower Visitor Attractive Habitats

Our results clearly show that honey bee waggle dance can also be informative in regard to other types of FVI. The study area was previously identified as a hotspot for summer

foraging by honey bees, located 2-3km from their hives, via dance decoding (Couvillon et al., 2014b). In our survey of this area we recorded honey bee foragers at a density of 1.7 per 200m^2 transect. However, other types of FVI were also abundant and honey bees constituted only 19% of all FVI. Lepidoptera (35%) were the most commonly observed FVI group followed by hoverflies (19%), bumble bees (16%), wasps (6%) and other bees (5%). In addition the survey data show that the abundance of honey bees per transect was positively correlated with those of other FVI (GLM, $p<0.001$, see Appendix B), in particular bumble bees ($p<0.001$). However, the different FVI groups were not found uniformly across the study area and honey bee abundance per transect was only weakly correlated to overall species richness (GLM, $p = 0.069$). Furthermore, there was no correlation between honey bee abundance and that of Lepidoptera ($p = 0.153$), wasps ($p = 0.169$), hoverflies ($p = 0.419$) and solitary bees ($p = 0.595$).

We also observed little overlap between the flower species commonly visited by honey bees (e.g. *Centaurea nigra* and *Rubus fruticosus*) and those frequented by other FVI groups, with the exception of bumble bees. Solitary bees, hoverflies and wasps were mainly recorded on species with open accessible nectaries (e.g. *Heracleum sphondylium, Jacobaea vulgaris*), consistent with their short tongue-lengths (Southwood and Juniper, 1986). Conversely, Lepidoptera were found predominantly in the four study fields in or adjacent to the Castle Hill NNR (63%), visiting flowers with relatively long corolla tubes and upright dense corymbose inflorescence or capitulum (e.g. *Cirsium arvense*, *Centaurea scabiosa*) which are well suited to their long tongues (Corbett, 2000).

5.5.2 Foraging Patterns in the Study Area

In accordance with previous studies we found a correlation (GLM, $p = 0.001$) between the diversity of flower species and FVI abundance (e.g. Lagerlof et al., 1992; Kleijn et al. 2001). However, flower abundance (i.e. petal area) better explained both FVI abundance (GLM, $p<0.001$) and richness ($p<0.001$). In particular petal area was positively correlated with the abundance of other bees (GLM, $p = 0.014$), wasps ($p<0.001$) and hoverflies ($p<0.001$). Furthermore, regression analysis indicated that a field's flower abundance was a very important factor (LM, $R^2 = 0.597$, $p = 0.002$) in FVI abundance (Fig. 5.3). By contrast, using the same analysis, flower species richness per field was unrelated to FVI abundance ($R^2<0.001$, $p = 0.958$). This was exemplified

by one Field Margin/Hedgerow, which was ranked fourth of 12 in terms of FVI abundance (Fig. 5.3), despite being almost a floral monoculture of *Heracleum sphondylium* (97% of total petal area). However, *H. sphondylium* was the plant species on which we recorded the greatest number of FVI and it attracted a notably diverse assemblage (FVI genus: 33, Shannon-Weiner: 3.1).

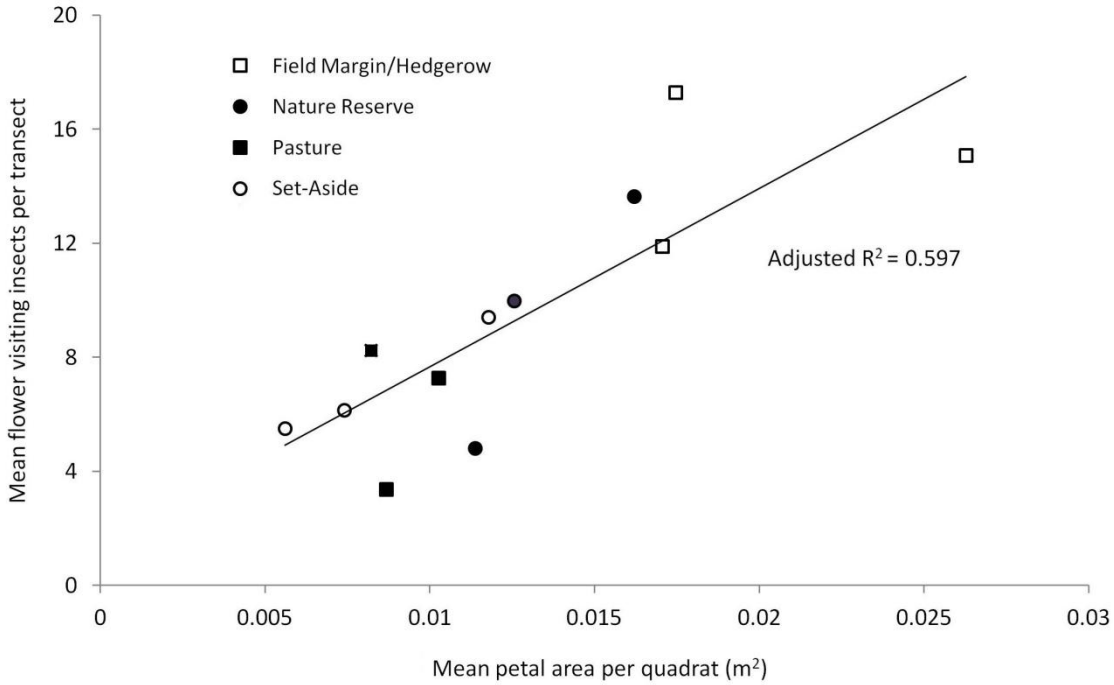

Fig 5.3 Relationship between the number of flower-visiting insects recorded per transect (200 m^2) with mean petal area per quadrat (1m^2) per study field. There is a significant positive relationship, shown by the regression line (t = 4.16, df = 10, p = 0.002).

Over half of all FVI (55%) were recorded visiting only five common, vigorous, flower species (*Heracleum sphondylium*, *Centaurea nigra*, *Cirsium arvense*, *Jacobaea vulgaris*, *Rubis fruticosus*). However, this situation may not be due to the inherent attractiveness of these species to FVI, but simply a reflection of the flower species frequently encountered in the study area. The four flower species with the greatest number of FVI in fact had relatively few FVI per unit petal area (Fig. 5.2). The flower species that were less abundant but had more FVI per unit petal area e.g. *Succisa pratensis*, *Chamerion angustifolia*, *Carlina acaulis*, may be of greater potential value to FVI.

Table 5.1 Mean (± SD) petal area per transect, mean flower species richness per quadrat and the most common flower species (by petal area) in each of the four habitat types studied. All values presented as mean ± 1 standard deviation.

Habitat type	Mean petal area/200m^2 transect (m^2)	Mean number species in bloom/ 1m^2 quadrat	Flower species with greatest petal area
Field Margin/Hedgerow	4.1 ± 1.0	1.2 ± 0.6	*Heracleum sphondylium* (67%) *Cirsium arvense* (8%) *Daucus carota* (7%)
Nature Reserve	2.7 ± 1.0	3.3 ± 1.0	*Centaurea nigra* (17%) *Galium verum* (15%) *Trifolium pratense* (12%)
Pasture	1.8 ± 0.2	2.4 ± 0.5	*Trifolium pratense* (28%) *Trifolium repens* (13%) *Jacobaea vulgaris* (11%)
Set-Aside	1.7 ± 0.6	1.6 ± 0.1	*Jacobaea vulgaris* (34%) *Heracleum sphondylium* (10%) *Cirsium arvense* (6%)

Nature Reserve fields were, perhaps surprisingly, not significantly more diverse or abundant in FVI than pasture fields (Table 5.1). However, both FVI (GLM, $p = 0.030$, Table B, see Appendix B) and lepidopteran ($p<0.001$) abundance fell with increasing distance from the Castle Hill NNR. This area is locally famous for its biodiversity and the majority of FVI we recorded in the NNR were Lepidoptera (43%) which are often considered good indicators of high quality habitat (moths: Merckx et al., 2009; butterflies: Thomas, 2005). In an effort to expand the flower-rich chalk grassland of Castle Hill NNR, Natural England (personal comm. NE, 2014) have recently promoted the conversion of the nearby arable fields to pasture via the terms of new leases offered to tenant farmers. Our data suggests that FVI, in particular butterflies, are concentrated in the NNR but are dispersing into nearby fields. However, the local 'high priority' (*Polyommatus bellargus*) and two of the local 'medium priority' (Butterfly Conservation, 2000) butterfly species (*Polyommatus coridon*, *Argynnis aglaja*) were only recorded in the NNR or adjacent habitats. This may be due to these butterflies being restricted to their favoured habitats by the distribution of their larval food plants (e.g. Brakefield, 1982).

The majority (89%) of FVI we recorded in this study were foraging primarily for nectar, rather than pollen: Lepidoptera (35%); male, and half of the female (Gilbert, 1981), hoverflies (14%); wasps (7%) and bees without visible pollen loads (33%). This

indicates that nectar is likely a more important, and more limiting, summer floral resource than pollen. Indeed, during July and August researchers have observed nectar depressions (Von Frisch, 1967; Lack, 1982), intense nectar competition (e.g. Inouye, 1978; Weatherwax, 1986; Balfour et al., 2013) and the beginning of marked nectar robbing between honey bee colonies (e.g. Sakofski et al., 1990; Downs and Ratnieks, 2000). Indeed, intense nectar competition may have had a strong, but unseen, effect on the flower-FVI relationships recorded in this study (Balfour et al., 2015).

5.5.3 Augmenting Late Summer Agricultural Floral Resources

Ironically, our data show much overlap between native, nitrogen-tolerant agronomic weed species and FVI attractive plant species. Four of the five plant species on which we recorded the greatest number of FVI are described in the Higher Level Stewardship Manual (NE, 2010) as undesirable weeds: *Heracleum sphondylium*, *Cirsium arvense*, *Jacobaea vulgaris* and *Rubus fruticosus*. The fifth, *Centaurea nigra*, is also considered an agricultural weed of special importance (Percival, 1949). Moreover, three of the nine plant species on which we recorded the greatest number of FVI (*Cirsium arvense*, *Jacobaea vulgaris* and *Cirsium vulgare*) are classified as 'injurious weeds' under a 1959 Parliamentary Act which aims to arrest their spread (UKGOV, 1959). On the other hand, weeds are also thought to be the fastest declining category of plant species in the UK agricultural landscape, primarily due to the use of herbicides (Whitehead and Wright, 1989; Barr, 1993) and probably also artificial fertilisers (Ollerton et al., 2014).

Environmental Stewardship Scheme (ESS) options which could potentially benefit flower visitors (buffer strips, field corners, nectar flower mixes) come with recommendations to suppress weed species by planting non-aggressive grasses, mowing, ploughing or spot herbicide application (NE, 2005). However, we note a softening of this approach in the latest ESS Handbooks (NE, 2012a; NE, 2012b). Tolerating the presence of native weeds in these areas represents a less expensive and a more sustainable option than the sowing of 'wildflower mixes' which are generally short-lived (Pywell et al., 2006) and of non-native seed stock (Akeroyd, 1994).

The sowing of wildflower mixes is generally encouraged in non-cropped arable areas (ESS options: EF4/HF4, EF10/HE10, EF11/HE11; NE, 2012a; NE, 2012b). Whilst the flower species recommended for these mixes may prove attractive to bumble bee foragers (Pywell et al., 2006; Carvell et al., 2007) they may not cater for other FVI

groups. The management of these habitats for the benefit of FVI is important not only for crop and wildflower pollination (e.g. Kearns et al., 1998) but also for pest-control services (Kruess and Tscharntke, 1994). As such, it would seem logical to recommend more open-flowered species with short corolla tubes and accessible nectaries (e.g. composite flowers) suitable for beneficial insects such as wasps and hoverflies (Southwood & Juniper 1986).

There was a conspicuous absence of flowering trees in our study area. This is primarily due to the phenology of the UK's native tree species, which almost all bloom in spring and early summer (31 of 32, Mitchell, 1982). *Tillia cordata* (small-leaved lime) is the only native UK tree species that blooms in July. Lime tree flowers are well known to be attractive to bees, flies and moths (Anderson, 1976). During early July 2013 we estimated that a solitary *T. cordata* on the University of Sussex campus was visited by c. 400 FVI, mostly bees. That level of FVI activity is roughly equivalent to what we recorded on 6000 m^2 of the Castle Hill NNR. Increasing the number of lime trees on UK farmland represents an inexpensive, long-term, low maintenance option for increasing nectar availability during the late summer.

Field margins/Hedgerows had the greatest petal area (GLM, $p = 0.002$) and FVI, per unit area (see Fig. 5.1). In our pair-wise analysis, Field Margin/Hedgerow had statistically higher abundances of all FVI (HSD, $p = 0.001$), wasps ($p<0.001$), other bees ($p<0.001$) and hoverflies ($p = 0.050$), and greater species richness ($p = 0.035$) than did pasture fields. Field Margin/Hedgerow are effectively oases of wild habitat and are often noted for their conservation value. Previous studies have highlighted the importance of field margins (Lagerlof et al., 1992, Kells et al., 2001; Marshall and Moonen, 2002) and hedgerows (Hannon and Sisk, 2009, Merckx et al., 2009) to agricultural biodiversity. However, we found the flower species richness (GLM, $p<0.001$) and diversity of FVI (Shannon-Wiener, Fig. 5.1) were generally lower in Field Margin/Hedgerow than in other habitat types.

Long grass areas had greater petal area (HSD, $p<0.001$), were richer in FVI ($p = 0.009$) and more abundant in wasps ($p = 0.044$) and hoverflies ($p = 0.013$) than those with short grass (≤30cm). This is because both summer grazing (Stewart and Pullin, 2008) and mowing (Morris, 2000) change vegetation composition and structure by reducing the ability of plants to flower and seed, which is known to have negative consequences for

associated insects (e.g. Morris, 2000). Occasional mowing is of course needed to prevent the encroachment of scrub, which in our study area is an issue in the Castle Hill NNR (*Rubus fruticosus* and *Ulex europaeus*). However, our findings suggest that mowing should probably kept to a minimum during the late summer.

Encouragingly, farms operating under the EES have been shown to have a greater abundance and diversity of plants (Taylor and Morecroft, 2009), moths (Fuentes-Montemayor et al., 2011) and bumble bees (Pywell et al., 2006; Carvell et al., 2007). Our results show that honey bees are attracted to land under higher level EES (Couvillon et al. 2014b) principally due to the presence of weeds or wildflowers. There is growing evidence that uncultivated areas and the weeds they harbour play an important role in the agricultural environment, not only for bees (Hald, 1999; Hyvönen and Huusela-Veistola, 2008; Requier et al., 2014), but also for other FVI (Nicholls and Altieri, 2013), Orthoptera, (Kleijn et al., 2006), predatory insects (Dennis et al., 1994; Lee et al., 2001), mammals (Shore et al., 2005) and birds (Marshall et al., 2003). Moreover, tolerating untended agricultural areas could also help to reverse the declining populations of our native wildflowers (Stroh et al., 2014).

Chapter Six: Effects of Foraging on Neonicotinoid-Treated Oilseed Rape on Honey Bee Colony Performance and Survival

6.1 Abstract

Mass flowering crops may benefit bees by providing nectar and pollen but may cause harm if treated with insecticides. In this year-long UK field study we investigated these potential benefits and costs to honey bee colonies. During spring bloom, 36 hives were placed in three apiaries adjacent to large fields of oilseed rape (OSR) grown from neonicotinoid (thiamethoxam) treated seeds. Another 36 hives were in three apiaries sufficiently distant (>1.25km) from OSR to result in low to near-zero OSR foraging. After OSR bloom, hives were relocated to common apiaries.

We found no effect of neonicotinoid exposure on colony survival or queen replacement. Adjacent colonies gained more weight during the first bloom month, but less during the following two months and 24% less over the year. Adjacent colonies also had less brood during winter. Our results indicate that proximity to thiamethoxam-treated OSR is neither beneficial nor deterimental to honey bee colonies.

6.2 Introduction

Honey bees (*Apis mellifera*) are important, producing c. 1.6 x 10^6 kg of honey worldwide annually (FAO, 2015) and performing as much crop pollination, in financial terms, as all other bee species combined (Kleijn et al. 2015). As such it is important to understand the effects of neonicotinoid insecticides on honey bee colonies. Presently, there is considerable controversy in this area. Several field studies have found no adverse effects on colonies foraging on seed-treated crops (Cutler and Scott-Dupree, 2007; Pilling et al., 2013; Rundlöf et al., 2015) and there is indirect evidence that honey bee populations have not been reduced (Cresswell et al., 2012). By contrast, laboratory studies have shown that individual workers can suffer sub-lethal effects if exposed to neonicotinoids, such as impaired navigation (Henry et al., 2012; Fischer et al., 2014). However, the concentrations studied may have exceeded the residues that bees would encounter when foraging on seed-treated crops under normal field conditions (Carreck and Ratnieks, 2014). These studies were influential in the European Commission's (EC) decision to impose a two-year moratorium on the use of three neonicotinoid seed

dressings on bee attractive crops (Europa, 2013a) and highlight the need for further field research.

Oilseed rape, or canola, is a globally important crop, with 36 million ha grown in 2013 (FAO, 2015). It is the principal UK non-cereal arable crop, occupying 3% of the land area during 2010-2013 (DEFRA, 2014a). Most (95%) is planted in late summer to bloom the following spring (DEFRA, 2014b). OSR flowers are attractive to bees (Rundlöf et al., 2015) and proximity to this mass flowering crop has been shown to enhance bee abundance (Westphal et al., 2003; Holzschuh et al., 2013). Beekeepers often move hives to OSR to enhance honey production (Carreck et al., 1997) and OSR yields are in turn increased by the additional pollination (Bommarco et al., 2012).

One reason for uncertainty surrounding the effects of neonicotinoids on honey bees is the difficulty of conducting controlled experiments with known exposure under agricultural field conditions. Given the considerable foraging range (>100 km^2) of honey bee colonies (Seeley, 1995), one challenge is to achieve control conditions with zero exposure (Carreck and Ratnieks, 2014). Another is to study treated areas large enough to be relevant to agricultural practice (Cutler and Scott-Dupree, 2007).

Our experiment investigated the net effect on honey bee colony performance of proximity to OSR grown from seeds treated with the neonicotinoid thiamethoxam, which may include both benefits (enhanced floral resources) and costs (insecticide exposure). Thiamethoxam was one of the two most widely-used neonicotinoids used in the UK to treat OSR seeds in the three years preceding the EC moratorium (Carreck and Ratnieks, 2014). We achieved this by siting apiaries at different distances from fields of flowering OSR. This resulted in a wide range of foraging on OSR within an existing agricultural environment, ranging from near zero to an ecologically-relevant maximum. (i.e., a very large field of flowering OSR <5m from the apiary).

6.3 Materials & Methods

6.3.1 Study Location & Experimental Design

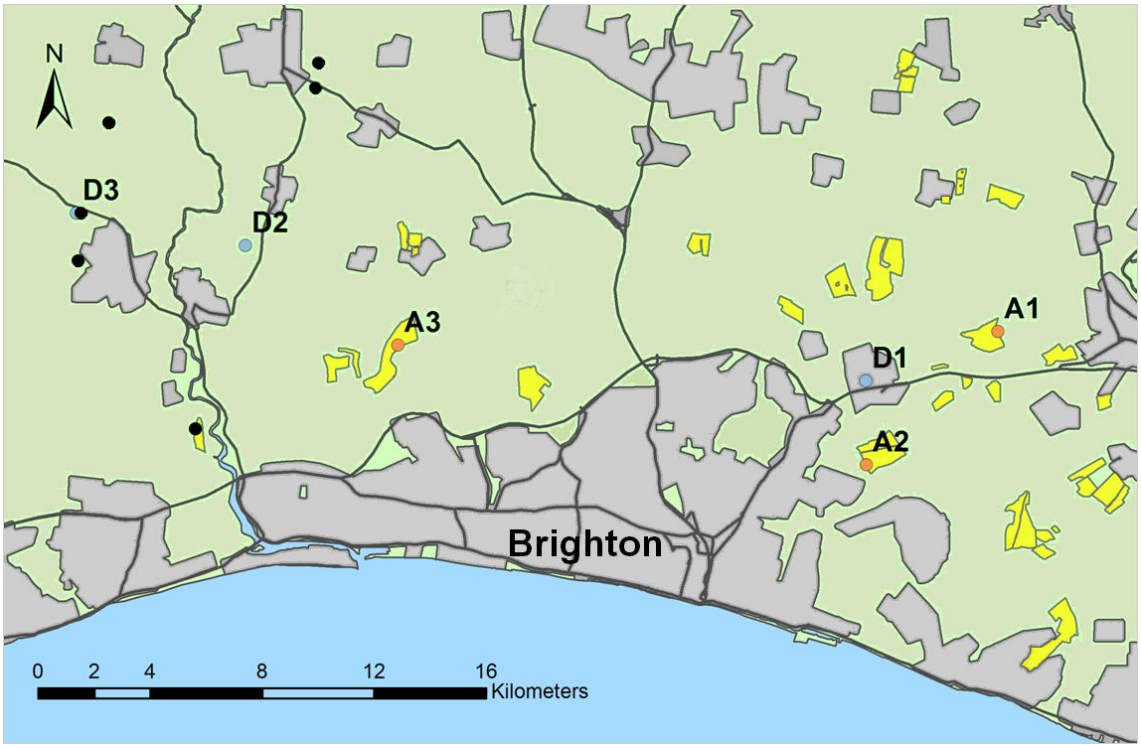

Fig. 6.1 Locations of spring apiaries, Adjacent (orange circles, A1- A3) to oilseed rape fields (shaded in yellow) and Distant (cyan circles, D1- D3). Also shown are the common apiaries (black circles) where colonies were relocated after the oilseed rape blooming period. Urban areas are shaded grey and rural areas green. Spring blooming oilseed rape fields (yellow) were located via an aerial survey in May 2014.

We assessed the impact of thiamethoxam (and its metabolite clothianidin) exposure on honey bee colonies foraging on a seed-treated bee-attractive agricultural crop in the field. During February and March 2014 we selected six rural apiary locations in a 6 x 20 km zone of predominately agricultural land in the South Downs, Sussex, UK. Three apiaries were adjacent (<5m) to large (0.38, 0.55, 0.64 km^2) oilseed rape (OSR) fields and three were at distances of 1.25km, 3.05km and 4.55km from the nearest OSR field. As such the 12 colonies in each apiary had variable areas of spring blooming OSR within their foraging range and would experience a range of neonicotinoid contamination levels (see Table 6.1). Crops were grown by commercial farmers from seeds planted in late summer 2013, before the EC moratorium, and treated with thiamethoxam (Cruiser OSR®, Syngenta Ltd., Basel, Switzerland). In April 2014 we set out six apiaries, each with 12 hives. We determined the effects of neonicotinoid

exposure by monitoring multiple indicators of honey bee colony performance during the subsequent 12 months: monthly weight change, number of frames of brood per colony, queen replacement and colony survival.

Apiary locations (Fig. 6.1) were selected with the aid of knowledge gained from previous studies of honey bee foraging in the study area. Although honey bees can forage at distances of up to 12km (Couvillon et al. 2014), average foraging distances are short, <1.1 km (Couvillon et al. 2014), during the OSR spring blooming period (April-May), with OSR fields located >2km from hives being little visited (Garbuzov et al., 2015). We also made an aerial survey (see Methods), which showed that the proportion of OSR in our study area (2.6%) was close to the UK average (3.0%).

During early OSR bloom stage (10% of flowers on the main raceme open), 2-4 April 2014, 12 colonies were moved into each of these six 'spring' apiaries. The OSR crops were being grown commercially and had been planted in late summer 2013, before the moratorium, using seeds treated with the neonicotinoid insecticide thiamethoxam (Cruiser OSR, Syngenta Ltd., Basel). The bloom stages of the three study fields were temporally synchronized. Near the end of the OSR bloom (10% of flower buds remaining), 20-22 May, all 72 colonies were moved from their spring apiaries to six 'common' apiaries. Each common apiary housed two hives from each spring apiary. All colonies were moved at night. To map all OSR fields in the area (Fig. 6.1) an aerial survey was undertaken on 12 May 2014.

6.3.2 Honey Bee Colony Management

Colonies were managed according to standard UK beekeeping methods and housed in hives consisting of a single 'commercial' brood chamber (11 frames of 43.8 x 25.4 cm, volume 56.4 litres). Each hive was given a queen excluder and additional boxes ('supers') of wax combs for honey storage as required. We removed and extracted the honey from one to two full supers per colony during June and July. Colonies had access to both honey stores and empty frames throughout the experimental period. Colonies were equalized on 31 March or 1 April 2014 during unfavourable foraging conditions to ensure that the vast majority of foragers were within the hive and worker population could be assessed. Each had a marked laying queen, 4 frames of brood, 6 frames of

adult worker bees, 2-3 frames of honey, 0.5-1 frames of pollen and two frames of empty wax foundation comb. All colonies were apparently disease free.

Any colonies with failed queens were made queen-right with mated queens at the earliest opportunity. To control varroa mite (*Varroa destructor*) each colony was given two Apistan (Vita Europe, Basingstoke, UK) strips in August 2014 and twice treated with oxalic acid, 2.25g via sublimation, in December 2014 and January 2015 (Al Toufailia et al., 2015). During the swarming period (May-June) additional inspections were made every nine days to destroy queen cells and prevent swarming. Additionally, we employed a modified version of the Brother Adam swarm prevention technique (Adam, 1987) between 15 May and 2 June 2014. This involved removing the queens from all 72 colonies for 10-14 days. During this period queens were housed in mating nuclei with several hundred workers and all queen cells were destroyed in the original colonies.

6.3.3 Measuring Colony Performance

At approximately monthly intervals from 2 April 2014 to 20 April 2015 we quantified four measures of colony performance: (i) hive weight (from which we determined weight change, after allowing for the weight of any additional hive equipment added or removed from each hive), (ii) frames of brood (iii) colony survival and (iv) queen survival/replacement.

To determine colony weight, hives were suspended and weighed using a digital hanging scale (PCE Instruments, model: PCE-HS 150N, Accuracy: ± 0.20 kg) immediately prior to being moved into spring apiaries and thereafter at approximately monthly intervals. Weighing was undertaken during poor weather conditions so that the majority of foragers were within the colony. At monthly intervals we also inspected all hives and estimated the number of sealed brood frames per colony (to the nearest quarter of a frame).

6.3.4 Determining Neonicotinoid Contamination

To determine neonicotinoid concentrations in the honey stored during the OSR bloom period we collected honey samples from all 72 colonies near the end of the OSR bloom (15 May 2014). Recently sealed honey was collected from multiple frames and locations within each colony to provide a representative sample from the OSR bloom period. To assess whether neonicotinoids were still present during the following winter we collected additional honey samples from sealed cells from all surviving colonies on 10 April 2015.

To monitor foraging on OSR during the bloom period pollen samples were collected for two 24h periods from all colonies during early (12 April 2014) and full bloom (23 April 2014) stages using pollen traps (Fairweather pollen trap; E.H. Thorne, Market Ransen, UK). To monitor the neonicotinoids present in the wider environment we also collected pollen samples from all colonies after the OSR bloom had finished (12 June 2014). All honey and pollen samples were stored at -20 °C prior to analysis.

During August 2014 we prepared 30 pooled samples, four per spring apiary, for chemical analysis. Each sample consisted of a homogenised composite of 5g of honey or 2g pollen from each colony for each of the six spring apiaries. The 12 honey samples were: (i) six from material collected 15 May 2014 and (ii) six from 10 April 2015. The 18 pollen samples were: (i) six from pollen from pollen traps collected 12 April 2014, (ii) six from 23 April 2014 and (iii) six from 12 June 2014.

Samples were analysed for neonicotinoid concentrations (thiamethoxam and its metabolite clothianidin) by SAL (Scientific Analysis Laboratory Ltd., Cambridge), an accredited (UK Accreditation Service) contract analytical laboratory that routinely analyses plant and food materials for the farming and food industries. SAL's extraction method is based on the Quechers extraction technique (Kamel, 2010) which uses water and acidified acetonitrile as an extraction solvent. Magnesium sulphate and ammonium acetate (as a buffer) were added to induce solvent partitioning. Quantitation was assessed against a series of known calibration standards dissolved in a methanol:water solution. Deuterated clothianidin (Clothianidin-d3) was used as an internal standard pre-extraction, to correct for losses during extraction and to compensate for matrix effects

(suppression or enhancement) during analysis. The limit of quantification (LOQ) and detection (LOD) were 0.1 μg per kg.

6.3.5 Pollen Analysis

We determined the proportion of OSR pollen present, by fresh weight, in two random samples of pollen pellets collected during the early (12 April 2014) and mid bloom periods (23 April 2014). 100 pollen pellets from each colony from each date were initially sorted by colour and then weighed. Pellets that were within the OSR pollen colour spectrum (bright yellow to light green; Kirk 2006) were subsequently examined at x 600 magnification and pollen grains were confirmed as OSR by comparison to voucher specimens collected directly from OSR flower anthers in April 2014.

6.3.6 Statistical Analysis

Statistical analyses used 'R' software (R-Project, 2015). We used Generalized Linear Mixed-effect Models (GLMM 'R' package lme4, version 1.1-7), regression analysis (LM, 'R' function: lm) or one-way ANOVA ('R' function: aov). 'Apiary' was held as a random effect in GLMM analysis ('R' command: glmer(response variable~treatment+(1|Apiary), family=Gaussian). Data are presented as means, or means $\pm$ one standard error. All R^2 values present are 'adjusted'. Proportion data were arcsin transformed prior to analysis. To determine the overall level of contamination per spring apiary we calculated two weighted averages of the neonicotinoid residues present in the two pollen (p) samples collected during the OSR bloom (12 and 23 April 2014) and honey (h) samples (collected 15 May 2014). The first (Equation 1) was simply the average of the two: $(p + h)/2$. The second (Equation 2) allowed for the fact that honey bee colonies gather c. 6 times more nectar than pollen (Seeley, 1995): $(p + 6h)/7$. In our statistical analysis we used both equations and they yielded approximately the same results (Fig. 6.2). Choice of equation made little difference to our statistical analyses due to the high correlation between the neonicotinoid contamination found in the pollen and honey samples per apiary (R^2 = 0.79, df = 5, F = 20.38, P = 0.011). Samples with no detectable neonicotinoid contamination (<0.1 μg/kg) were given a zero value in analysis.

6.4 Results & Discussion

As predicted, OSR pollen collection during the bloom varied widely, ranging from 0.2% (Apiary D3, 4.55 km from the nearest OSR filed) to 54% (Apiary A2) across the six apiaries and was related to proximity to the nearest OSR field. Across the three adjacent apiaries the mean was 48.7%, SE ± 1.87 (apiary means: 39, 53 and 54%) with mean OSR pollen collection being greater during early versus late bloom (67.1%, 12 April vs 30.6%, 23 April). By contrast, colonies in the three distant apiaries collected much significantly less OSR pollen (mean: 9.4% ± 1.95) than adjacent colonies (ANOVA, df = 5, F = 14.07, P = 0.020), ranging from near zero (0.2%) in apiary D3 to 8% in D2 and 21% in D1. Our design, therefore, gave a comprehensive spread in terms of OSR foraging.

Average neonicotinoid concentration (thiamethoxam plus clothianidin) for honey and pollen combined (using Equation 1, see Methods) ranged from 0.04 to 1.18 μg/kg (Table 6.1) across the six apiaries. This is within the lower range reported by previous studies (Blacquiere et al., 2012). The mean proportion of OSR pollen per apiary was positively correlated with neonicotinoid contamination in both honey (LM, $R^2 = 0.68$, df = 5, F = 11.85, *P* = 0.026) and pollen samples (LM, $R^2 = 0.60$, df = 5, F = 8.48, *P* = 0.044). Overall, neonicotinoid contamination was significantly higher in honey (mean: 0.77, range: <0.10 - 1.51 μg/kg) than in pollen samples (mean: 0.34, range: <0.10 - 0.84 μg/kg) and for adjacent (mean: 0.86 μg/kg) versus distant apiaries (0.25 μg/kg; ANOVA, df = 5, F = 8.07, P = 0.048).

Mean residue levels of the honey and pollen collected in apiaries adjacent to OSR fields were relatively low (0.29 to 1.51 μg/kg) despite being located <5m from large fields (38, 55, 64 ha) of neonicotinoid treated OSR. The mean proportion of OSR pollen collected by these colonies, 49%, was lower than that used for calculations of 'field realistic doses' of neonicotinoids in some laboratory studies which assumed 100% foraging on a treated crop.

Contamination levels were low during June, post OSR bloom, with non-detectable neonicotinoid levels (<0.1 μg/kg) in pollen samples (12 June 2014) from five of the common apiaries. The sixth common apiary was adjacent to a field containing post-bloom OSR. Pollen analysis showed that three of the twelve colonies in this apiary were

foraging on late blooming OSR, resulting in a mean pollen neonicotinoid level of 0.32 µg/kg.

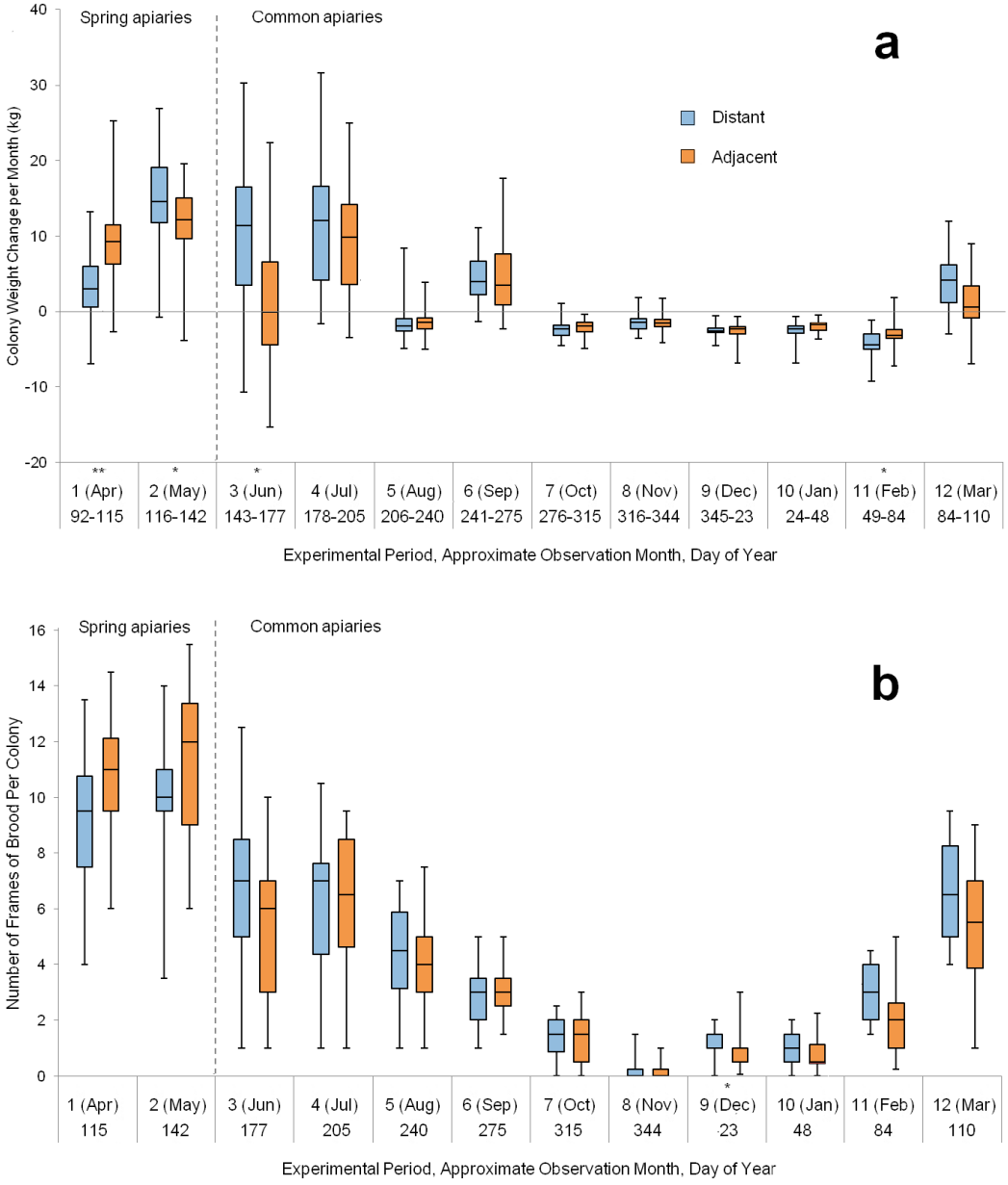

Fig. 6.2 Colony weight change (a) and frames of brood (b) per colony per approximate study month (day of the year displayed on horizontal axis) in three spring apiaries situated >1.25km from oilseed rape fields (Distant) and three apiaries located on the edge of oilseed rape fields (Adjacent). Figure shows the median (horizontal line within box), Q1 and Q3 (box) and range (bars). *significance levels (*P<0.05, **P<0.01, ***P<0.001) from Generalized Linear Mixed-effect Models analysis.

Table 6.1 Oilseed rape (OSR) forage availability, honey bee OSR pollen collection and neonicotinoid contamination of pollen and honey per spring 2014 apiary.

.					Neonicotinoid Contamination, thiamethoxam + clothianidin (µg/kg)			
Spring Apiary	**OSR within 0.5 & 2 km of apiary (km^2)**		**OSR pollen collection, early & mid bloom (%)**		**Pollen, early & mid OSR bloom**		**Honey, OSR bloom period**	**Honey, following winter**
Adjacent 1	0.30	0.60	51.0	29.9	1.05	0.63	1.51	0.1
Adjacent 2	0.28	0.55	70.6	36.5	0.47	0.29	0.79	<0.1
Adjacent 3	0.33	0.91	77.9	25.4	0.64	0.29	1.18	0.1
Distant 1	0.0	0.59	28.6	14.7	0.39	0.15	0.70	<0.1
Distant 2	0.0	0.0	0.5	15.8	<0.1	<0.1	0.46	<0.1
Distant 3	0.0	0.0	0.0	0.3	0.16	<0.1	<0.1	<0.1

During the first month of OSR bloom (April 2014) colonies adjacent to OSR fields gained significantly more weight than did the distant colonies (GLMM, df = 1, χ^2 = 13.68, P <0.001; Fig. 6.2a). This indicates that easy access to this mass flowering crop provided a short-term increase in honey production, detected as weight gain.

However, this benefit was not seen during the second month of OSR bloom (May) and was actually reversed (GLMM, df = 1, χ^2 = 4.72, P = 0.030). One contributing reason for this may have been a greater availability of alternative flowers in the wider environment. However, this alone would not have reversed the pattern and indicates that adjacent colonies were now suffering mid-term adverse effects of ongoing neonicotinoid exposure. Whilst the residue levels in the pollen and honey collected by adjacent colonies were unlikely to be acutely lethal to honey bees (Blacquire et al. 2012), they may have been enough to impair their orientation and foraging (Henry et al. 2012; Fischer et al. 2014). Indeed, this pattern was repeated during June 2014. Importantly, in June all colonies were now situated in six common apiaries under equal foraging conditions. During June the previously distant colonies gained more weight than did the previously adjacent colonies (GLMM, df = 1, χ^2 = 9.63, P = 0.002.

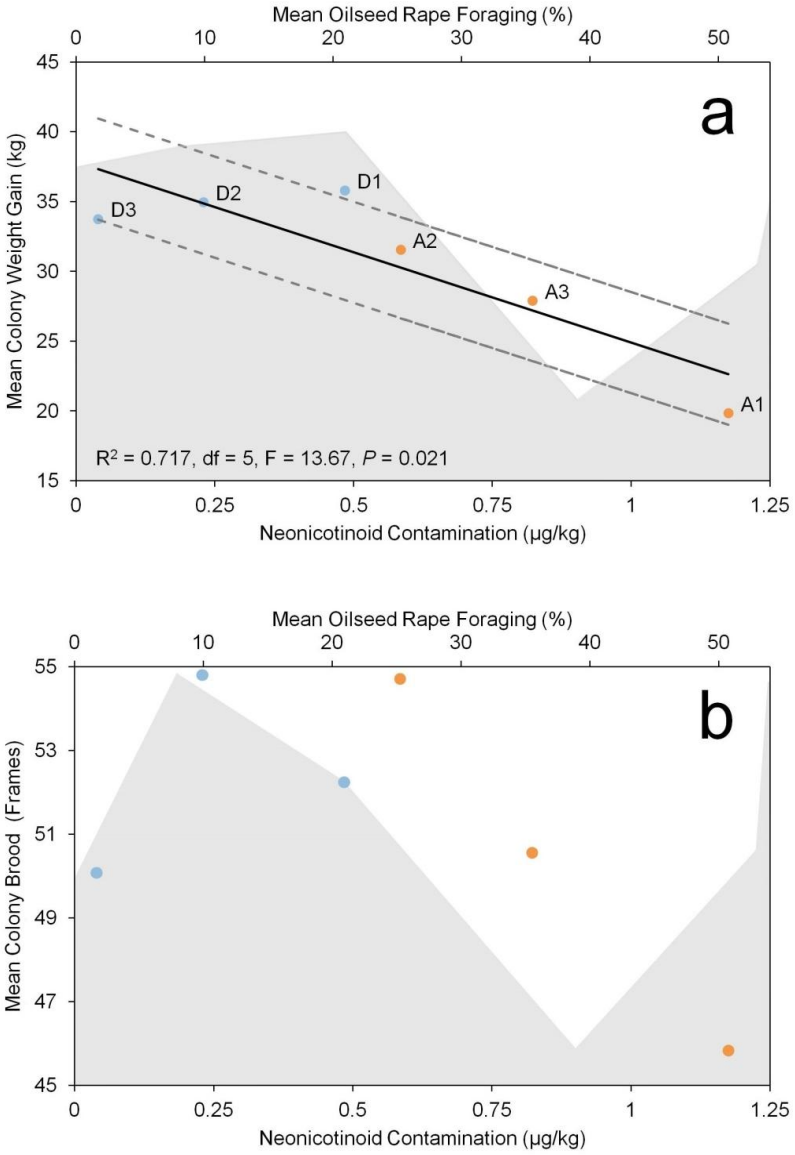

Fig 6.3 a) Colony weight gain and b) Brood production as a function of neonicotinoid contamination and oilseed rape foraging. Variation of neonicotinoid concentrations (mean in honey and pollen samples) collected during the oilseed rape (OSR) bloom period and mean colony OSR foraging during bloom period, per apiary, as a function of a) mean colony weight gain and b) mean colony brood production, per apiary, over one approximate calendar year. Spring apiaries locations are indicated as adjacent (A1-A3, orange) or distant (D1 -D3, blue) OSR fields. The grey shaded area shows the relationship between mean oilseed rape foraging and a) colony weight gain, and b) brood production, per apiary. The regression line (solid line) and the 95% confidence intervals (dashed lines) show the significant negative relationship between neonicotinoid concentrations and colony weight gain. All other relationship are non-significant (P>0.05). The analysis presented uses the Equation 1: $(p + h)/2$ to calculate neonicotinoid contamination per apiary (see Methods). Alternative analysis using Equation 2: $(p + 6h)/7$: a) R^2 = 0.64, df = 5, F = 9.74, P = 0.036.

From July-November 2014, monthly colony weight changes were similar between colonies relocated from adjacent versus distant apiaries. This indicates that the mid-term harmful effects of foraging on neonicotinoid-treated OSR seen in May and June had worn off. A likely reason for this is that honey bee workers are continuously replaced during spring and summer. Adult workers live for approximately one month and are reared from egg to adult in just three weeks (Seeley, 1995).

During the ensuing winter, however, colonies that had been adjacent to OSR fields during the previous spring had fewer frames of brood (GLMM, December 2014: df = 1, χ^2 =, P <0.001, February: df = 1, χ^2 = 3.85, P = 0.049 and March 2015: df = 1, χ^2 = 4.03, P = 0.045) than those previously distant (Fig. 6.2b). Analysis of winter honey stores (samples taken on 10 April 2015) detected neonicotinoid residues (0.1 µg/kg) only in colonies from two previously adjacent apiaries (A2 and A3, see Fig. 6.1). These two apiaries also had the highest contamination levels in spring 2014 (Table 6.1). One obvious interpretation of these data is that reduced winter brood rearing occurred because these colonies were consuming contaminated OSR honey stored during the previous spring.

The lower populations of colonies from the previously adjacent apiaries during February and March 2015 appear to be a consequence of reduced colony population at the turn of the year, rather than to ongoing negative effects of neonicotinoid contamination in early spring 2015. The evidence for this is that colony growth (the proportional increase in the number of frames of brood per colony) was similar for both groups of colonies between January-February (GLMM, df = 1, χ^2 = 0.09, P = 0.755) and February-March (GLMM, df = 1, χ^2 = 3.06, P = 0.080). In addition, the previously distant colonies lost more weight than previously adjacent colonies during February 2015 (df = 1, χ^2 = 6.37, P=0.012). This was presumably a consequence of the greater brood rearing of the distant apiary colonies used more of a colony's food stores. During the winter honey bee colonies maintain several thousand workers and continue to produce brood, consuming c. 20 kg of previously stored honey and pollen in the process (Seeley, 1995).

Overall, our data indicate that the net effect of proximity to seed-treated OSR to honey bee colonies in terms of honey production is negative. Over a full year (April 2014 to April 2015) colonies adjacent to OSR fields gained 8.4 kg on average, 24%, less than

distant colonies and neonicotinoid contamination of honey and pollen collected during spring 2014 was negatively correlated with cumulative hive weight gain per apiary (Fig. 6.3a, LM, R^2 = 0. 72, df = 5, F= 13.67, *P* = 0.021).

Importantly, weight gain across the year was not related to the proportion of pollen collected from OSR (LM, R^2 = 0.09, df = 5, F= 1.52, *P* = 0.285). These contrasting results indicate that ready access to this mass flowering crop is not in itself detrimental to colony performance. This possibility is, of course, unlikely given that proximity to OSR is known to augment the abundance of several bee species (Westphal et al., 2003; Holzschuh et al., 2013). The reason for these seemingly inconsistency data is that OSR pollen collection was not perfectly correlated with the neonicotinoid levels in our honey (R^2 = 0.68) and pollen samples (R^2 = 0.60). This is partly due to the mean neonicotinoid residues in the OSR plants in one of our study fields (that next to apiary A2) being 80% lower than those recorded at the other two fields (Balfour et al., 2016).

Our results provide the first evidence that neonicotinoid exposure negatively impacts honey bee colony performance under natural conditions. Importantly, however, colony survival (distant: 75%, adjacent: 86%; LM, df = 5, F = 1.33, *P* = 0.313), queen replacement (distant and adjacent: 28%; LM, df = 5, F = 0.26, *P* = 0.640) and brood production across the year (Fig. 6.3b; LM, df = 5, F = 2.30, *P* = 0.204) were not negatively correlated with neonicotinoid contamination. Our data, therefore, do not indicate that honey bee populations are significantly affected by the growing of OSR from treated seeds.

Our result highlight the difficulty faced by regulators and policy makers. Is any level of harm sufficient to justify banning a product? The European Food Safety Authority state '*it is not acceptable for colony size to fall by more than 7% as a result of exposure to pesticides at any time'* (Europa, 2013b). Our data from December 2014 show that the mean number of brood frames per colony from adjacent apiaries (0.87 $\pm$ 0.12) was 18.7% lower than from apiaries distant from OSR (1.23 $\pm$ 0.12) the previous spring. Perhaps because over-wintering honey bee workers normally have a protracted lifespan, and also because brood rearing is reduced in the winter, colonies are especially vulnerable during these months. However, this winter effect was small and could be avoided if beekeepers were to manage their hives to ensure that honey from treated crops was not used for winter stores, and this is one recommendation arising from the

present study. Our results also indicate relocating hives to thiamethoxam treated OSR fields is of no benefit to beekeepers. This may have consequences for OSR yields, which are known to benefit from increased pollination (Mesquida et al., 1988).

Our findings, and those of others (Cutler and Scott-Dupree, 2007; Pilling et al., 2013; Rundlöf et al., 2015), show that honey bees colonies are not severely harmed by chronic, low-level neonicotinoid exposure. However, honey bees are only one species, and a recent field study has found that Swedish solitary bee populations were adversely affected by proximity to neonicotinoid treated OSR (Rundlöf et al., 2015). This difference may reflect between the perennial life cycle of honey bees or perhaps their greater foraging range relative to temperate solitary species (Greenleaf et al., 2007). More prosaically, there is strong evidence that different insect species react differently to particular insecticides (Robertson et al., 2007; Cresswell, 2012) and that honey bee workers readily detoxify neonicotinoids (Cresswell et al., 2014). Indeed, there is probably no agricultural insecticide that is harmless to all non-target species or affects them all equally.

Chapter Seven: Size Matters: Significant Negative Relationship Between Mature Plant Mass and Residual Neonicotinoid Levels in Seed-treated Oilseed Rape and Maize Crops

7.1 Abstract

Neonicotinoid insecticides have been under scrutiny in recent years due to their potential to harm bees. The European Union recently imposed a two year moratorium (2014-15) on their application as a seed-treatment for certain bee-attractive crops. In this study we investigated the effect of mature plant size on residual neonicotinoid concentration in two widely grown, bee-attractive crops: oilseed rape (*Brassica napus*) and maize (*Zea mays*). Plants were collected from four commercial farms in Sussex, United Kingdom, three growing oilseed rape and one maize. All were grown from seeds treated with the neonicotinoid thiamethoxam. For both crops there was a significant negative relationship between mature plant mass and residual neonicotinoid (thiamethoxam and its metabolite clothianidin) concentrations ($p < 0.001$). Concentrations in plant tissues roughly halved with a four-fold increase in plant weight. These results indicate that agronomic practices that result in larger mature plants might have the potential to reduce the exposure of bees to neonicotinoid contamination of pollen and nectar.

7.2 Introduction

Neonicotinoids, a relatively new class of insecticides in use since 1991 (Elbert et al., 2008) have been under scrutiny in recent years due to research indicating negative impacts on non-target species, both directly (bees: e.g. Whitehorn et al., 2012; aquatic invertebrates: Beketov and Liess, 2008) and indirectly (insect-feeding birds: Hallman et al., 2014). Presently, there is a moratorium on neonicotinoid use as seed treatments, or as granules, on certain “bee attractive crops” such as maize, sunflower and oilseed rape in the European Union (EU) due to "high acute risks" to bees (Europa, 2013). In the EU, neonicotinoid insecticides were primarily applied as a broad-spectrum seed-treatment to protect crops from insect pests during their early growth phase (Elbert et al., 2008). However, residues in the nectar and pollen of mature flowering plants may be ingested by foraging bees.

Neonicotinoids concentrations present in pollen and nectar is the principal determinant of toxicity to individual bees or their colonies (Carreck and Ratnieks, 2014). Although the acute lethal dose for honey bees has been established under laboratory conditions (LD_{50}, thiamethoxam: 5ng/bee, clothianidin: 3.8 ng/bee; EFSA 2013), determining the effects of chronic exposure in the field is more challenging. Thus it is unclear what levels would be acceptable in the nectar and pollen of flowering crops (However, see Sanchez-Bayo and Goka, 2014). The recent change in the active ingredient commonly used in seed-treatments (from imidacloprid to clothianidin and thiamethoxam; Goulson, 2013) further compounds the uncertainties around this issue. As a guideline however, the chronic dose of the better studied compound, imidacloprid is 20 μg/kg (LC_{50}: Mommaerts, 2010) and the acute lethal dose is 3.7 ng/bee (LD_{50}, Schmuck et al. 2001). Nevertheless, it would seem likely that lowering residual neonicotinoid concentrations in the pollen and nectar of seed-treated crops would help to mitigate harm to bees (see meta-analysis in Creswell, 2011).

We hypothesised that this could be achieved via agronomic practices that increase mature plant size, thereby diluting neonicotinoid residues in the pollen and nectar. To test this hypothesis we measured residual concentrations of the neonicotinoid thiamethoxam and its metabolite clothianidin over a range of mature plant sizes in two widely-cultivated (FAO, 2014), bee-attractive crops: oilseed rape (*Brassica napus*) and maize (*Zea mays*). The flowers of these species are visited by honey bees and other bees and insects to collect pollen (oilseed rape and maize) and/or nectar (oilseed rape only).

7.3 Materials & Methods

We studied plants grown commercially on farms in Sussex and planted in the spring (maize) and late summer (oilseed rape) of 2013, before the EU moratorium took effect.

We analysed neonicotinoid residues in plant tissue samples of mature plants of different sizes, as gathering sufficient amounts of nectar and pollen per size class for chemical analysis would have been extremely challenging in the case of oilseed rape. Previous studies have found similar concentrations in plant tissue (leaves/panicles), nectar and pollen (Schmuck et al., 2001; Bonmatin et al., 2005). This is because neonicotinoids are water-soluble and thus readily translocated throughout the plant (Elbert et al., 2008). As such, this methodology was appropriate for determining the effect of plant size on residues in a way that is relevant to both nectar and pollen.

Flowering oilseed rape plants grown from seeds that had been treated with thiamethoxam (Cruiser OSR, Syngenta Ltd., Basel) were gathered from three farms in East Sussex, near Brighton, UK, between 8-11 June, 2014. To minimise within-field environmental variability, we collected all plants from a 15 m^2 section of each field. Plants were cut at ground level and weighed using a portable scale (sensitivity 1g). We then removed the upper-leaves and panicles of ten plants in each of five weight ranges (e.g. 0-100g, 100-200g, 200-300, 300-400, 400-500g). Samples were subsequently stored at -20°C. During August 2014 fifteen oilseed rape sub-samples were prepared from these, five from each weight category per farm. Each sub-sample consisted of an equal amount of material from ten plants homogenised in water (1g fresh weight of flower panicles plus 1g of leaves x 10 plants, plus 20g of water).

Nine whole seed-treated (Cruiser 5FS, Syngenta) flowering maize plants were collected from three fields on 29 August, 2013 at Sefter Farm, near Bognor Regis, West Sussex. One plant from each of three size categories (small, medium and large) was selected per field and cut at ground level. Samples were later weighed and stored at -20°C. Whole plant samples were analysed.

The 15 oilseed rape samples and nine whole maize plants were analysed by SAL (Scientific Analysis Laboratory Ltd., Cambridge) an accredited (UK Accreditation Service) contract analytical laboratory. For further details of the methodology used by SAL, see section 6.3.4 (Chapter 6).

Statistical analyses were conducted using 'R' software (version 3.1.1; R-Project, 2014). Linear Model (LM) analysis were simplified using backwards elimination of non-significant variables and model comparison using ANOVA. Oilseed rape LM analysis used the mean weights of the ten plants in each sample. All values are presented as mean ± 1 standard deviation.

7.4 Results

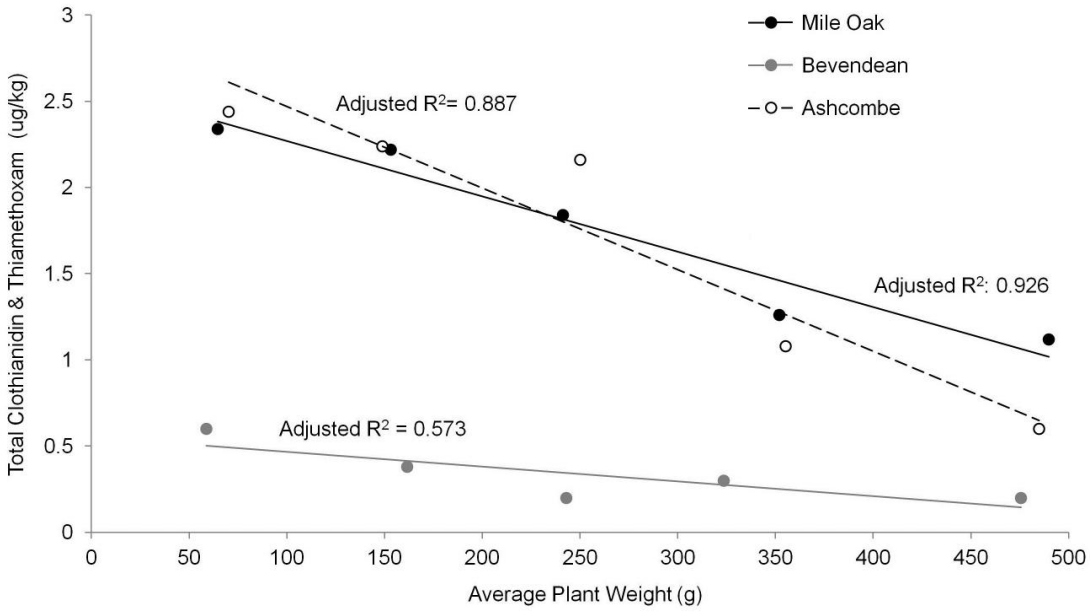

Fig 7.1 Variation in neonicotinoid concentrations in plant tissues with oilseed rape plant weight for three farms: Mile Oak, Bevendean and Ashcombe.

In the oilseed rape samples, neonicotinoid concentrations differed significantly among the three farms sampled (df = 2, F = 33.06, p <0.001). Therefore, the data were not pooled across farms and 'farm' was held as a co-variable in LM analysis. Neonicotinoid concentrations decreased significantly with increasing oilseed rape plant weight (df = 1, F = 28.073, p <0.001), Fig. 7.1.

In the maize samples neonicotinoid concentrations did not differ significantly between the three fields (df = 2, F = 0.074, p = 0.930). Therefore, the data were pooled. Neonicotinoid concentrations were $\log_{10}$ transformed prior to analysis (West et al. 2001) as this gave a substantially better statistical fit (R-squared: linear: 0.592, exponential: 0.826). Neonicotinoid concentrations were found to decrease significantly with increasing maize plant weight (df = 8, F = 33.42, p <0.001), Fig. 7.2.

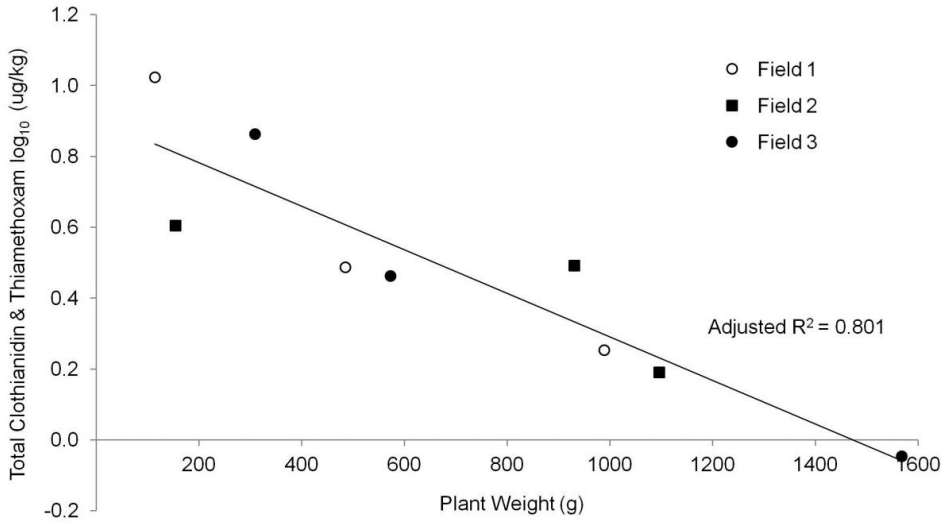

Fig. 7.2 Variation of $\log_{10}$ neonicotinoid (thiamethoxam and it metabolite clothianidin) concentrations in whole maize plant weight for three fields.

7.5 Discussion

Our results clearly show that residual neonicotinoid concentrations in the tissues of mature flowering plants are negatively correlated with plant mass in both oilseed rape and maize. For both crops, concentrations roughly halved with a four-fold increase in plant weight (Figs. 7.1 and 7.2). If the residual levels in pollen and nectar are also proportional to plant size, as the results of this study suggest, our data may also provide an insight into the wide range of residual pollen and nectar concentrations reported in the literature (0-36 ppb, reviewed in Blacquière et al., 2012), i.e. samples may have been collected from plants of widely differing sizes.

The results of our study strongly suggest that neonicotinoid residues in the nectar and pollen of seed-treated crop plants are likely inversely related to plant size.

The range of neonicotinoid concentrations that we observed (0.2-10.5 μg/kg) are similar to those found by previous researchers (reviewed in Bonmatin et al., 2015; Godfray et al., 2014). Previous work on the uptake of imidacloprid, a neonicotinoid widely used until it was recently replaced with clothianidin and thiamethoxam (Carreck and Ratnieks, 2014), by seed-treated maize (Bonmatin et al., 2005) and sunflower plants (Schmuck et al., 2001) found equal or slightly higher concentrations in leaves and panicles than nectar and pollen.

Neonicotinoid concentrations in the maize plants (3.91 ± 3.10 μg/kg) were found to be three times higher than in the oilseed rape samples (1.27 ± 0.86 μg/kg), on average. This is likely because maize is treated with approximately three to five times the amount of active ingredient, per seed, than is oilseed rape (reviewed in Goulson, 2013; Godfray et al., 2014).

Our results suggest that it should be possible to substantially reduce residual levels of neonicotinoid residues in seed-treated crop plants using agronomic practices that result in larger mature plants. Importantly, such measures would not affect the concentrations in seedling plants, which the seed-treatments are primarily designed to protect.

There are several feasible agronomic practices that could achieve this, the most obvious being to plant fewer seeds per unit area. Plants with more space and less competition for light and nutrients grow to a larger size, as shown with maize (Edmeades & Daynard, 1979) and oilseed rape (Morrison et al., 1989). Compensatory growth at lower seeding rates has been shown to result in similar yields per hectare for both oilseed rape (Scarisbrick et al., 1982) and maize (Marsalis et al., 2010). Moreover, planting fewer treated seeds per unit areas would have the added advantage of causing less total environmental contamination, given that only a small proportion (2-20%) of the chemical applied to seeds is absorbed by the plant (Shmuck et al., 2001, Sur and Stork, 2003). This is important as neonicotinoids have been shown to readily enter soils (Bonmatin et al., 2015) and to persist for a number of years (reviewed in Goulson, 2013).

Employing crop varieties which grow larger or develop over a longer period and flower later is another option. For example, late maize hybrids normally produce larger plants with more leaves than early varieties (Sangoi, 2001) and some oilseed rape varieties have an average flowering time some thirty days later than others (Wang et al., 2011), allowing more time for plant growth and, consequently, greater neonicotinoid dilution.

Similarly, planting oilseed rape in spring to bloom in the summer ("spring rape") will also result in smaller plants than autumn sowing for spring bloom ("winter rape"). Winter rape is more commonly grown than spring rape in the UK and EU (Kimber and McGregor, 1995) because it is has a longer growing season, resulting in bigger plants and higher yield potential (Scott et al., 1973). However, winter rape is not a viable

option in Canada and parts of Asia where harsh winters favour spring planting (Orvolius, 2003).

Given that the global human population is projected to grow substantially over the course of this century, food production will also need to be increased (Tilman et al., 2011). In addition, oilseed rape and maize have become important sources of biodiesel and bioethanol (FAO, 2013). An ongoing challenge of pesticide use is to minimise, or negate if possible, the harm to non-target species. Our results suggest that efforts to increase mature plant size in bee-attractive crops might have the potential to reduce neonicotinoid contamination originating from seed dressings, thereby reducing potential harm to bees and the wider environment.

Chapter Eight: Is Bumble Bee Colony Performance Affected by Proximity to Neonicotinoid Seed-treated Oilseed Rape?

8.1 Abstract

In 2013 the European Commission imposed a two-year moratorium on the use of neonicotinoid insecticides on certain bee-attractive crops due to concerns raised by several laboratory-led studies. Here, we assessed the impact of proximity to neonicotinoid (thiamethoxam) seed-treated oilseed rape (OSR; *Brassica napus*) on bumble bee colony (*B. terrestris*) performance and reproduction in the field.

In spring 2014, we set out 12 commercial hives at three sites adjacent to large fields of OSR (0.38, 0.55, 0.64 km^2) grown from treated seeds by local farmers. Another 36 colonies were at three sites sufficiently distant (1.25 km, 3.05 km, 4.55 km) from OSR to result in near-zero OSR foraging. Colonies were allowed to forage naturally for six or eight weeks, then collected for analysis.

OSR pollen foraging by the bumble bee colonies adjacent to seed-treated OSR fields was high (mean: 41.2%) and near-zero (mean: 1.6 %) at distant sites. The combined concentrations of thiamethoxam plus clothianidin found in the composite honey and pollen samples from adjacent colonies were within the lower range found previously (0.18- 0.72 ppb), and were below the detection level in the distant colonies (<0.1 ppb).

We found no significant differences in the number of male + worker ($p = 0.98$) or queen cocoons ($p = 0.79$) in the distant vs. adjacent colonies. A similar pattern was also seen in terms of colony weight gain ($p = 0.23$) and nest volume ($p = 0.37$). The only significant differences observed were that adjacent colonies had fewer males (-35.5%; $p = 0.032$) and more workers (+6.8 %; $p = 0.002$) than distant colonies.

Our results indicate that the performance and reproduction of *B. terrestris* colonies in an agricultural landscape are similar whether located adjacent to or distant from fields of thiamethoxam seed-treated OSR. This suggests that any cost or harm to colonies through residual amounts of insecticide in crop pollen and nectar are similar to any benefit from this additional and nearby foraging source, and that overall seed-treated OSR is neutral in its effects on colony performance.

8.2 Introduction

In recent years there have been concerns regarding the population declines and range contractions of many bumble bee species (e.g. Koisor et al., 2007; Goulson et al., 2008; Cameron et al., 2011). Multiple factors that could be responsible for these declines have been proposed, including habitat loss (Green, 1990) and fragmentation (Goulson, et al, 2008), increased use of artificial fertilizers (Ollerton, 2014) and other features associated with agricultural intensification (Robinson and Sutherland, 2002). However, the role of insecticides has for a long time (e.g. Carson, 1962) been the highest profile possible cause of bee declines. In particular, the effect of a relatively new class of insecticide, neonicotinoids (in use since 1991; Elbert et al., 2008) have gained particular prominence in recent years (see Carreck and Ratnieks, 2013; Eisenstein, 2015).

In response to the concerns about the possible negative effects of neonicotinoid insecticides on bees, the European Commission (EC) imposed a two-year moratorium on their use as seed treatments, or as granules, on "bee-attractive crops" such as oilseed rape (OSR), maize and sunflower in 2013 (Europa, 2013). In Europe, neonicotinoid insecticides have been primarily applied as a broad-spectrum seed-treatment to protect crops from insect pests during their early growth phase (Elbert et al., 2008). Because neonicotinoids are water-soluble they are readily translocated throughout the plant (Balfour et al., 2016) and act systemically and residues can be found in the nectar and pollen of mature flowering crop plants where they may be ingested or collected by foraging bees.

Neonicotinoid levels recorded in the nectar and pollen of seed-treated crops vary widely, but are generally in the range of 0-2 ppb (reviewed in Blacquière et al., 2012; Godfray et al., 2014). Although these levels are not acutely harmful or fatal to forager bumble bees, they may be high enough to cause sub-lethal effects to their colonies, such as reduced growth and reproduction (e.g. Whitehorn et al. 2012). Much of the previous research on this subject, including two papers in high-impact journals which were influential in the EC moratorium (Gill et al., 2012; Whitehorn et al., 2012), were laboratory-led and used methodologies in which colonies of *Bombus terrestris* were fed 'field-realistic doses', which may have been higher than those that bees actually encounter in the field (Ratnieks and Carreck, 2015). Nevertheless, a recent Swedish field study has shown that bumble bee colonies adjacent to fields of neonicotinoid (clothianidin) seed-treated OSR do not perform as well as those adjacent to untreated OSR fields (Rundlöf et al., 2015).

In this study we assessed the impact of proximity to neonicotinoid (thiamethoxam) seed-treated oilseed rape (*Brassica napus*) on bumble bee colony (*B. terrestris audax*) performance and reproduction. Proximity to flowering OSR can have both benefits (abundant floral resources; Westphal et al. 2003) and costs (insecticide exposure; Rundlöf et al., 2015) to bumble bee colonies. Here we assess the net effect of these costs and benefits by comparing the performance of colonies adjacent to seed-treated OSR fields (both benefits and costs) to colonies in the same area but with no OSR fields within their normal foraging range (neither benefits nor costs). By contrast, previous studies have compared the effect on bumble bee colonies of access to organically grown OSR (benefits) versus conventionally grown OSR (benefits and costs). However, less than 7% of UK arable land is currently under organic management (DEFRA, 2013; DEFRA, 2014c). Here, we address a question that is more relevant to the situation occurring in agricultural ecosystems: are bumble bee colonies affected by proximity to OSR, a bee-attractive mass flowering crop which has also been seed-treated with thiamethoxam, versus being situated out of range of OSR, but within the same agricultural landscape?

8.3 Materials & Methods

8.3.1 Study Location & Experimental Design

During February and March 2014 we selected six rural sites in a 6 x 20 km zone of predominately agricultural land in the South Downs, Sussex, UK to place our study bumble bee colonies. Three sites were adjacent (<5m) to large OSR fields (0.38, 0.55, 0.64 km^2) grown by commercial farmers from seeds planted in late summer 2013, before the EC moratorium, that had been treated with thiamethoxam (Cruiser OSR®, Syngenta Ltd., Basel, Switzerland). The three 'distant' sites were 1.25 km, 3.05 km and 4.55 km from the nearest OSR field boundary, see Fig. 8.1. The study species, *B. terrestris*, normally forage at <1 km of their nests (Wolf and Moritz, 2008). Therefore, no seed-treated OSR fields were within the normal foraging range of the distant colonies. However, *B. terrestris* have been recorded visiting attractive mass-flowering crops at distances of up to 1.75 km (Walther-Hellwig and Frank, 2000). Therefore, our design was expected to result in zero or little foraging on OSR from the distant colonies.

During early OSR bloom (10% of flowers on the main raceme open), 4 April 2014, we set out 72 commercially reared *B. terrestris audax* (tomato Audax type) colonies, 12 per

sites. Colonies were supplied by Syngenta Bioline (Clacton-on-Sea, England), were approximately 10 weeks old and contained a queen and approximately 60 workers on receipt (4 April). They were housed in the normal commercial hive boxes supplied by Syngenta, consisting of a 8.16 litre nest cavity inside a plastic membrane protected by a cardboard box. All colonies had access to an internal feeder containing sucrose solution and had a layer of cotton wool for insulation.

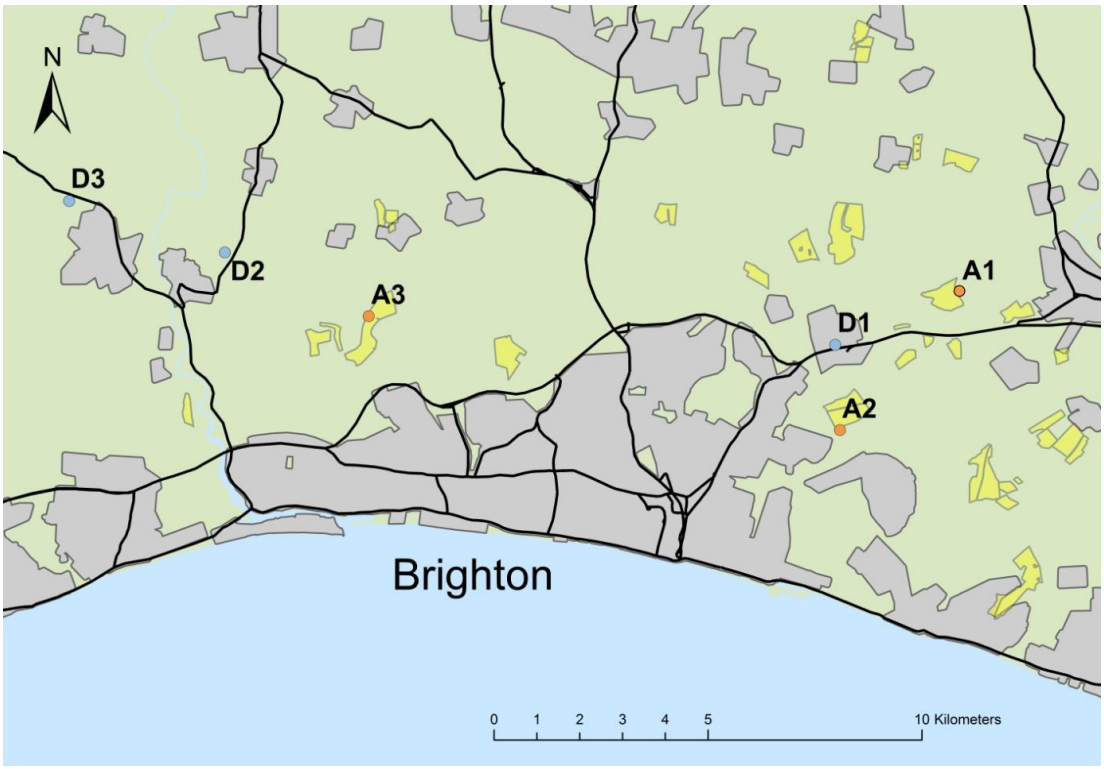

Fig. 8.1 Locations of 72 study bumble colonies, 12 per study site: adjacent (orange circles, A1- A3) to oilseed rape fields (shaded in yellow) and distant (cyan circles, D1- D3) in the South Downs in Sussex, UK. The proportion of oilseed rape in our study area (2.6%) was close to the UK average (3.0%). Urban areas are shaded grey and rural areas green. Spring blooming oilseed rape fields (yellow) were located via an aerial survey in May 2014.

Each of the study hives was given a polythene 'roof' to protect the cardboard box from rain and tied to a wooden stand staked one metre high above ground to prevent water ingress. The hive entrances were then opened and the colonies allowed to forage naturally. The suppliers advised us that these colonies would reach their reproductive stage approximately six weeks after receipt. At this point bumble bee colonies are at their maximum size and weight. Therefore, to assess colony performance and reproductive success, half the hives, six from each site, were collected after six weeks (16 May) and half after eight weeks (30 May). The blooming stages of the three study

fields were temporally synchronized and were near the end of the OSR bloom (c. 10% of flower buds remaining) when the hives were collected. Thus, colonies had 6 or 8 weeks opportunity to forage on OSR. Two days before colony collection, the hive entrances were switched from open to the inward pointing cone option provided by the hive manufacturer. This allows bees to enter the hive but not to exit, and ensured that on collection our hives contained all the bees living in the colony, including foragers. Hives were then stored at -20 °C until they were sorted to take samples and quantify nest contents.

8.3.2 Determining Colony Performance and Reproductive Success

Bumble bee colony performance was determined by quantifying several measures of productivity per colony: i) number and caste of fully-emerged bees present: workers, males or queens; ii) number of capped and uncapped cocoons, sorted as worker or male versus queen cocoons; iii) nest weight change; and iv) nest volume.

The number of fully-emerged worker, male and queen bees in each colony was determined by first separating the females from the males by examining the hind legs for corbiculae (pollen baskets), which are found in both queens and workers but not males. The females were then differentiated by size, with the queens being the conspicuously larger of the two castes (Heinrich, 1976).

The number of capped and uncapped cocoons per colony was determined by grouping together worker and male cocoons and separating them from the larger queen cocoons. This is possible because queens are on average twice the weight of males (Duchateau and Velthuis, 1988). Therefore, queen cocoons are larger than those of both males and workers. Separating male cocoons from those of workers was not possible due to their overlapping sizes.

To measure colony weight change the plastic membrane (containing the nest) and the internal feeder of each hive were weighed separately on portable balance (model 1066, Salter Ltd., Tonbridge, sensitivity 1g) on the day of receipt (4 April 2014) and again on the day they was collected from the field (16 or 30 May).

Nest volume was determined by filling the empty space in each of the 8.16 litre plastic hive membranes with polystyrene packing chips. The chips were then weighed to calculate their volume, and from this the volume of the nest.

8.3.3. Pollen Analysis

To determine the proportion of pollen foraging on OSR we examined 100 pollen shells (exines) in three faecal samples per colony (n = 300 exines per colony). This is possible because the hard exine layer of pollen grains pass through the digestive system of bumble bees without bursting (Roulston and Cane, 2000). Three samples from three sub-locations were taken from the faecal pile within the nest of each colony. Individual samples were placed on a microscope slide and distilled water was slowly added via a dropper. The sample was then squeezed and the faeces removed. The remaining liquid contained thousands of pollen exines. The samples were then mounted and examined at ×600 magnification using a compound transmission light microscope (model N-117N, Brunel Microscopes Ltd., Chippenham). We counted the proportion of OSR exines present in a transect of 100 visible exines. Transects began approximately in the centre of the microscope slide and the direction taken alternated between the four directions provided by the controls on the microscope's slide stage (i.e. up, down, left, right). Pollen grains were confirmed as OSR by size, shape and texture comparison to voucher specimens collected directly from OSR flower anthers.

8.3.4 Chemical Analysis

To determine the neonicotinoid residues present in the honey and pollen stored by our bumble bee colonies one composite sample of honey and another of pollen was prepared for chemical analysis per site. Initially, all the honey and pollen pots within each colony were collected. All colonies had at least 2 g of stored honey and all but six had 2 g of stored pollen (two from site D2, and one each from sites A1, A3, D1 and D3). All the available material from each colony was then homogenised into twelve composite samples (mean weight 42.3 g) to give one composite honey and one pollen sample per site.

Samples were analysed for neonicotinoid concentrations (thiamethoxam and its metabolite clothianidin) by SAL (Scientific Analysis Laboratory Ltd., Cambridge), an accredited (UK Accreditation Service) contract analytical laboratory. For further details of the methodology used by SAL see section 6.3.4 (Chapter 6).

8.3.5 Statistical Analysis

Statistical analyses were conducted using 'R' software (version 3.1.1; R Project, 2015). We used Generalized linear mixed-effect model (GLMER, 'R' package lme4, version 1.1-7). Samples with no detectable neonicotinoid contamination (<0.1 μg/kg) were given a zero value in analysis. Proportion data were arcsin transformed prior to analysis. All values are presented as mean ± 1 standard deviation. Study Site, Volume of Syrup Consumed and date of collection (six or eight weeks) were held as a random effects in GLMM analysis ('R' command: lmer (Response Variable~Treatment+(1|Site)+(1|Syrup)+(1|Month), family=Gaussian)).

8.4 Results & Discussion

Table 6.1 Oilseed rape (OSR) forage availability, mean percentage of faecal OSR pollen exines identified (n = 300 per colony) and neonicotinoid contamination thiamethoxam + clothianidin of pollen and honey per study site (n = 12 hives per site).

			Neonicotinoid contamination, thiamethoxam + clothianidin (μg/kg)	
Study location	**OSR within 1 km of (km^2)**	**OSR faecal pollen, mean ± s.e.m (%)**	**Pollen**	**Honey**
A1	0.37	40.5 ± 4.8	0.49	0.23
A2	0.55	41.3 ± 3.6	0.18	<0.1
A3	0.56	41.9 ± 4.9	0.42	0.25
D1	0	2.97 ± 1.02	<0.1	<0.1
D2	0	0.96 ± 0.28	<0.1	<0.1
D3	0	0.88 ± 0.23	<0.1	<0.1

The analysis of OSR pollen faecal exines indicated that OSR foraging by the bumble bee colonies at the adjacent sites was high (mean: 41.2%) and near-zero at sites distant (>1 km) from the nearest OSR field (mean: 1.6 %), see Table 8.1. Our design, therefore, resulted in the OSR foraging patterns we had predicted, high vs. near-zero.

The neonicotinoid concentrations (thiamethoxam plus clothianidin) found in the composite honey and pollen samples collected from the distant colonies were all below the level of detection (0.1 ppb), see Table 8.1. The residue levels of the honey (<0.1 - 0.23 ppb) and pollen (0.18 - 0.87 ppb) collected from colonies adjacent to OSR fields are within the range reported by the majority of prior studies (0-1 ppb, Blacquiere et al.,

2012; Godfray et al., 2014). This was despite these colonies being located <5m from large fields (38, 55, 64 ha) of neonicotinoid seed-treated OSR.

The mean proportion of OSR foraging by adjacent colonies (41%) and the mean neonicotinoid residues recorded in the honey (0.16 ppb) and pollen (0.53 ppb) samples were far lower than the values used in the calculations of the 'field realistic doses' in the high-profile lab-led studies of 2012 (Gill et al., 2012; Whitehorn et al., 2012).

Our colonies each had an internal syrup feeder (volume: 1.5 litres) provided by the supplier. On average 577 g sucrose solution was consumed since the colonies were set up, but only 117 g of this was during the experimental period. The fact that little syrup was consumed over the 6 or 8 weeks the colonies were in the field, probably reflects the fact that there was abundant nectar available. Although the syrup in the feeder may have reduced the amount of nectar collected and the neonicotinoid residues recorded, this effect would be small. Bumble bee colonies collect c. 50 g of nectar per day (Heinrich, 1979). Therefore, the amount of syrup consumed during the experimental period is equivalent to only 2-3 days of foraging. The syrup from the feeders would have constituted only c. 5% of a colony's energy use over the field period.

A significantly greater number of fully-emerged workers were present in the colonies adjacent to a seed-treated OSR field (80.3 ± 11.9) than in colonies distant from any OSR fields (75.2 ± 5.5; GLMER, df = 1, χ^2 = 9.5, p = 0.002). By contrast, the number of emerged males was significantly greater in distant (110.0 ± 12.4) than adjacent (81.2 ± 16.7) colonies (GLMER, df = 1, χ^2 = 4.6, p = 0.032). There was no difference in the number of queens at the distant vs. adjacent sites (24.3 ± 4.3 vs. 24.3 ± 4.6, GLMER, df = 1, χ^2 = 2.5, p = 0.620).

Although the number of male and worker cocoons was slightly higher in the distant colonies (712.9 ± 39.3) than in those adjacent to OSR fields (693.9 ± 58.4), this difference was not significant (GLMER, df = 1, χ^2 = <0.01, p = 0.98). This was also the case in the analysis of the number of total number of queen cocoons recorded in the distant (50.3 ± 11.5) vs. adjacent (39.1 ± 11.4) colonies (GLMER, df = 1, χ^2 = 0.07, p = 0.79). , see Fig. 8.2.

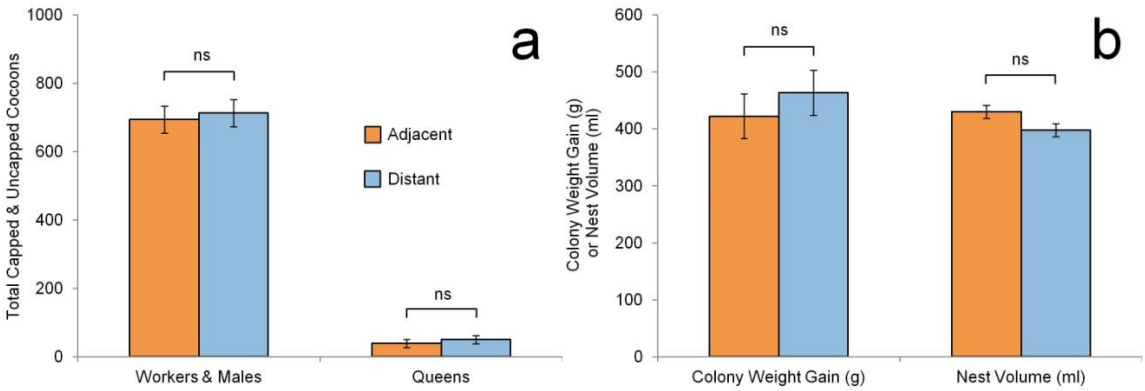

Fig 8.2 (a) Mean number of capped and uncapped cocoons (sorted as workers + males or queens) and (b) mean weight gain during the period in the field (six or eight weeks) and final nest volume of *B. terrestris* colonies in three sites situated >1.25km from any oilseed rape fields (Distant) and three located on the edge of large neonicotinoid seed-treated oilseed rape fields (Adjacent). Also shown is the standard error of mean (error bars) and statistical significance from Generalized Linear Mixed-Effect Models analysis (ns = not significant).

A similar pattern was seen in the weight gain of our colonies during the experimental period. Distant colonies (463.6 ± 49.42) gained more weight than those at adjacent sites (422.2 ± 48.2) but again this was not significant (GLMER, df = 1, $\chi^2 = 1.4$, $p = 0.23$). The mean nest volumes of adjacent colonies (4.30 ± 0.13) was greater than the colonies at distant sites (4.03 ± 0.15), but not significantly so (GLMER, df = 1, $\chi^2 = 0.8$, $p = 0.37$).

Overall, our results indicate that the performance and reproduction of *B. terrestris* colonies in an agricultural landscape was very similar, whether they were located adjacent to or distant from fields of thiamethoxam seed-treated OSR. The only significant differences were that colonies adjacent to OSR had significantly fewer males (-35.5%) and a greater number of workers (+6.8 %) than distant colonies.

These findings are in contrast to another field study that found that *B. terrestris* colonies adjacent to fields of neonicotinoid (clothianidin) seed-treated OSR did not perform as well as those adjacent to untreated OSR fields (Rundlöf et al., 2015). The likely explanation for this difference is experimental design. Our study determined the net effect of the benefits (abundant floral resources) and costs (insecticide exposure) of proximity to OSR vs. being elsewhere in the agricultural environment (neither OSR cost or benefit). On the other hand, Rundolf et al. (2015) measured the cost and benefit of seed-treated OSR vs. only the benefits of access to OSR (untreated with insecticides).

Taken together, the findings of Rundolf et al. (2015) suggest that neonicotinoid residues in the nectar and pollen negatively impact bumble bee colonies, while our study suggests that the negative impact is balanced by the benefit of the increased floral resources provided. Furthermore, our data indicate that bumble bee colonies perform approximately equally in other locations within the modern agricultural ecosystem. Therefore, it is tempting to suggest that factors other than the widespread use of neonicotinoids are responsible for ongoing bumble bee declines. Notably, the general decline in the UK's biodiversity (reviewed in Barr et al., 1993) predates the widespread use of neonicotinoids (in use since 1991; Elbert et al., 2008). Indeed, recent analysis of UK flower visitor extinctions suggest the main period of species loss was between the two World Wars and may be linked to the introduction of artificial fertilizers (Ollerton, 2015). Ultimately, agricultural changes over the last century have been profound and are multi-factorial (reviewed in Robinson and Sutherland, 2002).

Chapter Nine: Final Discussion & Future Directions

9.1 Bees and Insecticides

Chemical analysis of honey and pollen samples presented in Chapters Six and Eight, and the crop plants in Chapter Seven indicate that the neonicotinoid residues found in mature oilseed rape plants grown from treated seeds is in the range of <0.1 - 2.5 ppb. This is significantly lower than the 'field realistic' doses administered to bees in many laboratory studies (reviewed in Carreck and Ratnieks, 2014). Furthermore, many lab-based studies assumed 100% foraging on a treated crop to calculate these doses. However, the mean proportions of oilseed rape pollen collected by our study honey bee colonies located adjacent to very large fields was far lower (49%) than these researchers assumed. The findings of these lab experiments were influential in the European Commission's decision to impose a moratorium on the use of neonicotinoid seed dressings on bee attractive crops.

Contrary to these well publicized laboratory studies (e.g. Henry et al., 2012), the data presented in Chapter Six, and other finding (Cutler and Scott-Dupree, 2007; Pilling et al., 2013; Rundlöf et al., 2015), indicate that proximity to flowering seed-treated oilseed rape fields has little impact on long-term honey bee colony performance. Furthermore, our data suggest that performance and reproduction of *B. terrestris* colonies in an agricultural landscape were similar whether they are located adjacent to or distant from fields of thiamethoxam seed-treated OSR (Chapter Eight). The recent proliferation of papers linking insecticides with bee declines has perhaps diverted focus away from other factors affecting honey bee health. Importantly, pollinator declines predate the widespread use of all modern pesticides, not just neonicotinoids. This was shown, for example, by a recent analysis of UK bee and aculeate wasp extinctions which suggests that the main period of species loss was between the two World Wars (Ollerton, 2015). These findings are consistent with the impacts of agricultural intensification which are multi-factorial (reviewed in Robinson and Sutherland, 2002).

That said, the data in Chapter Six do suggest a small sub-lethal effect of neonicotinoid exposure on honey bee colony performance. However, the results of Chapter Seven indicate that neonicotinoid residues are roughly halved with a four-fold increase in crop plant weight. The concentration of neonicotinoids residues in pollen and nectar is the principal factor determining whether individual bees or their colonies are harmed

(Carreck and Ratnieks 2014). Although the acute insecticide exposure levels that kill individual honey bees in a laboratory are easily established (LD_{50}, thiamethoxam: 5ng/bee, clothianidin: 3.8 ng/bee; EFSA 2013), determining the effects of chronic exposure in the field is more challenging. As a result, it is not clear what concentrations would be acceptable in the nectar and pollen of flowering crops (however, see meta-analysis in Creswell, 2011). Because there is strong evidence that different insect species react differently to the same insecticide (Robertson et al., 2007; Cresswell, 2012; Rundlöf et al., 2015), further research is needed to determine a 'safe' threshold for all flower visiting species.

9.2 Floral Resources & Competition

If this thesis were written fifty years ago, it would likely have concluded that the results of Chapter Four are further proof that competition is the major organising force in ecological communities (e.g. Hutchinson, 1959). However, since the early 1980's the consensus has generally been that competition is too '*rare and sporadic to be of major significance*' (Sharrocks et. al, 1984). Nevertheless, the data shown in Chapter Four indicate that competition is alive and well among bees.

Recent research on the foraging choices of nectarivores has tended to ignore competition and instead focus on topics such as convergent evolution between flowers and pollinators. Implicit in this concept of 'pollinator syndromes' (e.g. Fenster et al. 2004) is an assumption of tight co-evolution leading to specialisation (e.g. morphological) between individual flower and visitor species. However, bee-flower relationships are often generalized (Waser et al., 1996), dynamic (Miller-Struttmann , 2015) and can be extremely competitive (Inouye, 1978). These features are clearly shown by the results of Chapter Four which indicate that there is great flexibility in bee-flower ecology. Indeed, we may expect competitive interactions between bees to be even more common in the future, given the challenges posed by current environmental changes, such as global warming, accidental or deliberate introduction of non-native bees and habitat loss and degradation. Consequently, the role of exploitative competition in bee-flower ecology probably deserves more attention.

Many previous studies have indicated that honey bee competition has negative impacts on other flower visiting species (see Paini, 2004; Thomson, 2004; Goulson and Sparrow, 2009). However, the data in Chapter Four shows that the reverse can also be

true. Further understanding is needed to determine the importance of exploitative competition in structuring insect-flower communities. Future research should also determine how these interactions influence other types of flower visitors. For example, i) are social bees more sensitive to nectar depletion than other flower visitors? and ii) are there flower species from which honey bee foraging deters bumble bee visitation?

9.3 Bee-friendly Farmland

The survey in Chapter Five clearly shows that agricultural weed species such as thistles (*Cirsium* spp.), knapweeds (*Centurea* spp.) and hedgerow plants such as bramble (*Rubus fruticosus*) are important summer forage resources for flower visitors. These findings add to the growing body of evidence that untended farmland and the weeds it supports are beneficial to bees (Hald, 1999; Hyvönen and Huusela-Veistola, 2008; Requier et al., 2014). Encouraging farmers to tolerate these plants in uncultivated areas instead of 'farming' for bees (planting nectar strips) may represent a more sustainable, cost-effective and bee-friendly farmland practice. Future research to explore this hypothesis would help inform agri-environmental policy. Furthermore, amending the 1959 Weeds Act, which classifies *Cirsium arvense*, *Jacobaea vulgaris* and *Cirsium vulgare* as 'injurious weeds', might greatly benefit summer flower visitors.

The results in Chapter Five also shows that there were few flowering trees during the late summer. Our data also indicate that a solitary lime tree (*Tillia cordata*) blooming in early July on the University of Sussex campus attracted as many nectarivores as 6000 m^2 of flower-rich National Nature Reserve chalk grassland. Surprisingly little is known about the relative attractiveness of Britain's tree species to flower visitor. Further research is needed to address this and to quantify the number of flower visitors trees attract relative to areas of wildflowers.

9.4 Agricultural Pollination

The study of honey bee foraging in apple and pear orchards (Chapter Three) clearly indicates that to maximise the potential of this important pollinator requires fruit growers to think on a scale greater than their own farm. Honey bee colonies are capable of foraging over a very large area (>100 km^2; Seeley, 1995) and this can be both an advantage and disadvantage in their use as agricultural pollinators. It may be useful to use dance decoding to determine whether the foraging of colonies sited in other mass-

flowering crops follow similar patterns. An obvious candidate is the California almond crop, for which approximately one million bee hives are rented each year during the spring bloom (Morse and Calderone, 2000).

The data presented in Chapter Three also shows the limitations of monitoring honey foraging via pollen trapping and analysis alone. This is because attractive pollen and nectar sources may not always overlap, as shown in Chapters Three and Four. Honey bee colonies also store approximately six times more honey than pollen (Seeley, 1995). Moreover, the demand for (Seeely, 1995) and the relative availability of these two resources are known to fluctuate through the seasons (Couvillon et al., 2015).

The results of Chapter Four also illustrate the economics underlying the findings of previous research showing that wild bees increase the pollination efficiency of honey bees on almond flowers (Brittain et al., 2013) and sunflowers (Greenleaf et al., 2006) by causing more movements between patches. However, our data indicate that another consequence of intense honey bee-wild bee competition is that honey bees may choose to forage on alternative flower species. Depending on the location, colonies sited in flowering crops may visit alternative floral resources within their wide foraging range if there is marked competition on crop flowers. Furthermore, as shown in Chapter Three, if the target crop flowers are relatively unrewarding, honey bees may forage elsewhere (i.e. oilseed rape fields).

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Appendix A: Energy Budget Calculations per Lavender Flower for Foraging Honey Bees and Bumble Bees

To calculate the energy budget of a bee foraging on a lavender (*Lavandula intermedia* 'Grosso') flower we need to know the mean nectar energy reward per flower (Equation 1, below) and from this subtract the energy expended in nectar collection per flower, which comprises three behavioural components: flying, walking and flower handling (Equation 2, 3). Energy expenditure is usually measured by the rate of either oxygen consumption or carbon dioxide production. One millilitre of oxygen used is equivalent to an energy expenditure of five calories, or 20.92 joules from carbohydrate metabolism (Heinrich, 1975a).

Whilst we have a high degree of confidence in our own data on calculations of the mean energy available per flower and the duration of foraging times collected, we expect errors to exist in our energy expenditure calculations due to the inconsistencies of the values available in the literature. To counter this we have averaged all the appropriate data identified. Moreover, our overall conclusions are not dependent on these figures being precisely correct.

The metabolic rate of honey bee (*Apis mellifera*) and bumble bee (*Bombus* spp.) flight can vary with temperature and the associated thermoregulation costs (e.g. Heinrich, 1975b; Rothe and Natchigall, 1989), study species (e.g. Ellington et al., 1990), caste (Cooper, 1993 in Wolf et al. 1999), nectar load (Wolf et al.,1989), reward rate (Moffatt, 2001; Nieh et al., 2006), wind speed (Wolf et al., 1999) and measurement technique (Rothe, 1983 in Rothe and Natchigall, 1989). Estimates of the metabolic rate of bumble bee free-flight at 25 C (mean temperature at study site during observations) range from 0.262 (Sivola, 1984) to 0.438 J/g/s (Heinrich, 1975b). Honey bee flight energetic expenditure estimates, at 20-30 C, are similar, ranging from 0.240 (Rothe and Natchigall, 1989) to 0.587 J/g/s (Wolf et al., 1989). Therefore, we used a mean of all available estimates; eight estimates from six studies for bumble bees (Table A) and nine estimates from eight studies for honey bees (Table A). Encouragingly, the mean values are very similar: bumble bees, 0.365 J/g/s, honey bees 0.372 J/g/s.

Data on the energy expended by walking honey bees is itself highly variable (Wolf et al.,1989). We calculated a mean of 0.244 J/g/s from four measurements taken from three studies (Table A). The only energetic estimates available for walking bumble bees

(both 0.046 J/g/s) are from indolent individuals alternating between walking and resting (at 25C), Kammer and Heinrich (1974) and Sivola (1984) and so are not relevant. Therefore, we also use the *A. mellifera* walking mean value for *Bombus* as well, and justified this because the means for flying are almost identical (see above).

When calculating the metabolic rate of a bee in flight we must also consider the weight of the nectar load being carried (Wolf et al., 1989). The same is probably true for a bee walking, because for the ant *Camponotus herculeanus* the metabolic cost of transporting a load is the same as for additional body mass (Nielsen et al., 1982). The mean weight of *A. mellifera* foraging on 'Grosso' lavender is 0.097g (Balfour et al., 2013). Because, foraging *A. mellifera* gain c. 0.03g on a foraging trip (Dukas and Visscher, 1994) we estimate 0.015g (a half-load) of the weight of our honey bees (0.097g) to be nectar. But, we do not expect load weight to influence the energetics of flower handling, when a bee is on a flower and not moving its whole body. For these calculations we estimate *A. mellifera* to weigh 0.082g (0.097 minus 0.015g) and *Bombus* 0.193g, mean weight of foraging *B. terrestris*, 0.227g (Balfour et al., 2013), minus 15%.

We could not identify any studies that measured the energy expended by *A. mellifera* or *Bombus* foragers engaged in flower handling. Therefore, we use measures of 'resting', although we expect this to be a slight underestimate. Again we use the mean of ten figures: six 'resting' and one 'grooming' estimate taken from six *A. mellifera* studies (Table A) and three *Bombus* 'resting' or 'resting/walking' estimates (Table A) in our calculations of honey bee and bumble bee flower handling energetics. Given that we expect flower handling to be more energy expensive than 'resting', we have increased this number by 25% to 0.077 J/g/s. Whether a correction of 0%, 25% or 100% was applied had no effect on our overall conclusions, Fig. 4.3.

Lastly, we need to consider the possible effect that flower discrimination (i.e. the acceptance or rejection of individual flowers) has on the nectar volumes collected by honey bee and bumble bees foraging on lavender. Because the accuracy of this discrimination remains unknown for our bees, we use the only appropriate estimate in the literature (Wetherwax, 1986), which measured 24% more nectar in *Lotus corniculatus* flowers 'accepted' by honey bees than flowers sampled at random. Although this estimate is taken from another flower species, *L. corniculatus* and 'Grosso' lavender both have a similar proportions of empty flowers (both c. 50%) and

nectar volumes (both c. 0.01- 0.02µl per flower). For our energy budget calculations we have increased the mean lavender nectar volumes (which were randomly sampled) on our patches by 24% for honey bees and by 6% for bumble bees. The bumble bee correction is 25% that of the honey bee because *B. terrestris* rejected approximately four times fewer lavender flower than did *A. mellifera*. Again, whether a correction of 10%, 25% or 50% was used had no effect on our overall conclusions, Fig. 4.3. Furthermore, as we found 44% of Grosso flowers without detectable nectar in our bumble bee excluded patch, we infer honey bees can access all the available nectar in a Grosso flower that it is visiting.

A1. Energy reward per lavender flower in BBE (bumble bees excluded), HBE (honey bees excluded) and CON (control) lavender patches

Assumptions

Mean nectar volume/flower from random sample: BBE: 1.92×10^{-5} ml, HBE: 0.74×10^{-5} ml, CON: 0.67×10^{-5} ml (see Results)

Equation Used

The energy (N) of the nectar of one average lavender flower in joules (J) is the product of the mean volume (v in ml) of nectar per lavender flower, the sugar concentration (s) of nectar lavender (39%; see Results), the specific gravity (g) of a solution which is 39% sugar (1.148 Brix) and the energy content of sucrose (c; 16480 J/g; Kearn and Inouye 1993):

$$N = vsgc \quad (1)$$

Energy per flower in each treatment patch using Equation 1

Parameter	**Calculation**
Energy per flower (BBE patch)	$(1.92 \times 10^{-5}) \times (0.39) \times (1.148) \times (16480) = 1.42 \times 10^{-1}$ J
Energy per flower (HBE patch)	$(0.74 \times 10^{-5}) \times (0.39) \times (1.148) \times (16480) = 5.46 \times 10^{-2}$ J
Energy per flower (CON patch)	$(0.67 \times 10^{-5}) \times (0.39) \times (1.148) \times (16480) = 4.94 \times 10^{-2}$ J

Increased energy per flower on lavender patches due to bee discrimination among flowers

Honey bees (+24%): BBE: 1.76×10^{-1} J, CON: 6.13×10^{-2} J

Bumble bees (+6%): HBE: 5.79×10^{-2} J, CON: 5.24×10^{-2} J

A2. Energy expenditure per lavender flower for honey bees (*Apis mellifera*)

Assumptions

Metabolic rate of 'flying' honey bee = 0.372 J/g/s (mean of 9 *A. mellifera* values)
Metabolic rate of 'walking' honey bee = 0.244 J/g/s (mean of 4 *A. mellifera* values)
Metabolic rate of 'flower handling' honey bee = 0.077 J/g/s (mean of 7 *A. mellifera*/*Bombus* values)
Mean weight of honey bees foraging on 'Grosso' lavender = 0.097g (Balfour et al., 2013)

Time spent in different foraging activities per lavender flower (this study, Table A)

Equations Used

The mean energy expended (E) by a bee per probed lavender flower is the sum of the energy expended in all foraging activities: flying (f), walking (w) and flower handling (h):

$$E = f + w + h \quad (2)$$

Energy (in Joules, J) expended per activity (f, w and h) is the product of the metabolic rate (r) of activity in J/g/s, the mean bee weight (b, in grams, g) and the mean time (t) spend engaged in this activity per lavender flower, e.g.:

$$f = rbt \quad (3)$$

The energy expended per flower by A. mellifera on Bumble bee excluded (BBE) patch, using Equation 2 & 3

Honey bee energy/flower (flying)	$(0.372) \times (0.097) \times (0.407) = 1.47 \times 10^{-2}$ J
Honey bee energy/flower (walking)	$(0.244) \times (0.097) \times (0.663) = 1.57 \times 10^{-2}$ J
Honey bee energy/flower (handling)	$(0.077) \times (0.082) \times (2.130) = 1.34 \times 10^{-2}$ J
Total	$(1.47 \times 10^{-2}) + (1.57 \times 10^{-2}) + (1.34 \times 10^{-2}) = 4.38 \times 10^{-2}$ J

The energy expended per flower by A. mellifera on Control patch (CON) patch, using Equation 2 & 3

Honey bee energy/flower (flying)	$(0.372) \times (0.097) \times (0.947) = 3.42 \times 10^{-2}$ J
Honey bee energy/flower (walking)	$(0.244) \times (0.097) \times (0.533) = 1.26 \times 10^{-2}$ J
Honey bee energy/flower (handling)	$(0.077) \times (0.082) \times (1.890) = 1.19 \times 10^{-2}$ J
Total	$(3.42 \times 10^{-2}) + (1.26 \times 10^{-2}) + (1.19 \times 10^{-2}) = 5.87 \times 10^{-2}$ J

A3. Energy expenditure per lavender flower for bumble bees (*Bombus terrestris*)

Assumptions

Metabolic rate of a 'flying' bumble bee = 0.365 J/g/s (mean of 8 *Bombus* values)

Metabolic rate of 'walking' bumble bee = 0.244 J/g/s (mean of 4 *A. mellifera* values)

Metabolic rate of 'flower handling' bumble bee = 0.077 J/g/s (mean of 7 *A. mellifera*/*Bombus* values)

Mean weight of bumble bees foraging on 'Grosso' lavender = 0.227g (Balfour et al., 2013)

Time spent in different foraging activities per lavender flower (this study, Table A)

The energy expended per flower by B. terrestris on Honey bee excluded (HBE) patch using Equations 2 and 3

Bumble bee energy/flower (flying)	$(0.365) \times (0.227) \times (0.054) = 0.45 \times 10^{-2}$ J
Bumble bee energy/flower (walking)	$(0.244) \times (0.227) \times (0.286) = 1.58 \times 10^{-2}$ J
Bumble bee energy/flower (handling)	$(0.077) \times (0.193) \times (0.810) = 1.20 \times 10^{-2}$ J
Total	$(0.45 \times 10^{-2}) + (1.58 \times 10^{-2}) + (1.20 \times 10^{-2}) = 3.23 \times 10^{-2}$ J

The energy expended per flower by B. terrestris on Control (CON) patch using Equations 2 and 3

Bumble bee energy/flower (flying)	$(0.365) \times (0.227) \times (0.067) = 0.56 \times 10^{-2}$ J
Bumble bee energy/flower (walking)	$(0.244) \times (0.227) \times (0.284) = 1.57 \times 10^{-2}$ J
Bumble bee energy/flower (handling)	$(0.077) \times (0.193) \times (0.770) = 1.14 \times 10^{-2}$ J
Total	$(0.56 \times 10^{-2}) + (1.57 \times 10^{-2}) + (1.14 \times 10^{-2}) = 3.27 \times 10^{-2}$ J

Table A Bee respiration rates (O_2 consumption or CO_2 production) used to calculate the energy expended by honey bees (*Apis mellifera*) and bumble bees (*Bombus* sp.) whilst flying, walking and flower handling. † in Wolfe, Ellington and Begley, 1999; * in Wolf et al., 1989; ** in Rothe and Natchigall, 1989.

Study	Study Species	Caste	Activity	J/g/s
Hocking 1953	*A. mellifera*	Workers	Flying	0.351
Sotavalta 1954	*A. mellifera*	Workers	Flying	0.311
Scholze et al. 1983	*A. mellifera*	Workers	Flying	0.475
Rothe 1983*	*A. mellifera*	Workers	Flying	0.360
Rothe 1983*	*A. mellifera*	Workers	Flying	0.300
Wolf et al. 1989	*A. mellifera*	Workers	Flying	0.546
Rothe and Nachtigall 1989	*A. mellifera*	Workers	Flying	0.240
Joos et al. 1997	*A. mellifera*	Workers	Flying	0.340
Harrison and Fewell 2002	*A. mellifera*	Workers	Flying	0.429
Heinrich 1975b	*B. edwardsii*	Queens	Flying	0.448
Heinrich 1975b	*B. vosnesenskii*	Queens	Flying	0.372
Bertsch 1984	*B. locurum*	Drones	Flying	0.436
Sivola1984	*B. terrestris*	Queens	Flying	0.244
Ellington et al. 1990	*B. locorum*	Workers	Flying	0.348
Ellington et al 1990	*B. pascuorum*	Workers	Flying	0.305
Cooper 1993†	*B. locurum*	Workers	Flying	0.325
Wolfe, Ellington and Begley 1999	*B. terrestris*	Workers	Flying	0.445
Rothe and Natchtigall 1989	*A. mellifera*	Workers	Walking	0.167
Wolf et al. 1989	*A. mellifera*	Workers	Walking	0.247
Stabentheiner et al 2003	*A. mellifera*	Workers	Walking	0.262
Stabentheiner et al 2003	*A. mellifera*	Workers	Walking	0.298
Hocking 1953	*A. mellifera*	Workers	Resting	0.018
Cahill and Lustick 1976	*A. mellifera*	Workers	Resting	0.117
Withers 1981**	*A. mellifera*	Workers	Resting	0.071
Rothe and Natchigall 1989	*A. mellifera*	Workers	Resting	0.006
Goller and Esch 1991	*A. mellifera*	Workers	Resting	0.016
Stabentheheiner et al. 2003	*A. mellifera*	Workers	Grooming	0.167
Stabentheheiner et al. 2003	*A. mellifera*	Workers	Resting	0.013
Kammer and Heindrich 1974	*B. vosnesenskii*	Queens/workers	Resting	0.046
Bertsch 1984	*B. lucorum*	Drones	Resting	0.116
Sivola 1984	*B. terrestris*	Queens	Resting	0.046

Appendix B: Generalized Linear Model Analysis of Chapter Five Survey Data

Table B Results of backwards elimination Generalized Linear Model (GLM) analysis. Explanatory variables are by columns and response variables by rows. p- and F-values are model comparisons using ANOVA. The plus symbol following F-values denote a positive correlation between variables, and a minus symbol a negative correlation. NNR = National Nature Reserve, FVI = flower-visiting insects, df = 310, * = significant p-values ϯ honey bee data were removed from the response variable.

	Flower species richness	Flower abundance	Habitat	Sub-habitat	Distance to NNR	Honey bee abundance
Honey bee abundance	***p <0.001*** F = 18.95 (+)	p = 0.472 F = 0.51	***p = 0.010**** F = 3.88	p = 0.152 F = 1.89	***p <0.001*** F = 19.35 (+)	n/a
Bumble bee abundance	p = 0.360 F = 0.84	p = 0.572 F = 0.32	p = 0.122 F = 1.95	p = 0.908 F = 0.09	p = 0.268 F = 1.23	***p <0.001 ***** F = 42.54 (+)
Other bee abundance	p = 0.735 F = 0.12	***p = 0.014**** F = 6.15 (+)	***p <0.001*** F = 10.76	p = 0.385 F = 0.96	p = 0.292 F = 1.12	p = 0.595 F = 0.29
Lepidoptera abundance	p = 0.419 F = 0.66	p = 0.312 F = 1.02	p = 0.128 F = 1.91	p = 0.420 F = 0.87	***p <0.001 ***** F = 15.71 (−)	p = 0.153 F = 2.06
Wasp abundance	p = 0.595 F = 0.28	***p <0.001*** F = 26.20 (+)	***p <0.001*** F = 18.25	***p = 0.023**** F = 3.80	p = 0.828 F = 0.05	p = 0.169 F = 1.90
Hoverfly abundance	***p = 0.034**** F = 4.54 (+)	***p <0.001*** F = 43.27 (+)	***p = 0.043**** F = 2.75	***p = 0.004**** F = 5.58	***p <0.001*** F = 33.10 (+)	p = 0.419 F = 0.66
FVI abundance	p = 0.242 F = 1.37	***p <0.001*** F = 18.52 (+)	***p = 0.004**** F = 4.52	p = 0.330 F = 1.11	***p = 0.030**** F = 4.74 (−)	***ϯp = 0.001**** F = 8.33 (+)
FVI species richness	***p = 0.001**** F = 10.40 (+)	***p <0.001*** F = 19.62 (+)	***p = 0.012**** F = 3.73	***p = 0.015**** F = 4.23	p = 0.882 F = 0.02	p = 0.069 F = 3.33 (+)
Flower species richness	n/a	n/a	***p <0.001*** F = 23.17	p = 0.126 F = 2.09	***p = 0.003**** F = 9.16 (−)	n/a
Flower abundance	n/a	n/a	***p = 0.002**** F = 5.22	***p <0.001*** F = 17.37	p = 0.504 F = 0.45	n/a

B1. Pair-wise Comparisons Results Following GLM Analysis

Honey bee abundance was significantly different among habitats (GLM, Table B). However, no pair-wise comparisons (HSD) were significant, the lowest p-value being Field Margin/Hedgerow > Set-Aside (p = 0.078). Other bee abundance: Field Margin/Hedgerow > Pasture (p<0.001) and Reserve (p = 0.016); Set-Aside (p = 0.001) and Reserve (p = 0.037) > pasture. Wasp abundance: Field Margin/Hedgerow > Pasture

($p<0.001$) and Reserve ($p<0.001$); Set-Aside > Pasture ($p = 0.002$) and Reserve ($p = 0.001$); long grass > short-grass ($p = 0.044$). Hoverfly abundance: Field margin/Hedgerow > pasture ($p = 0.050$); long grass > short grass ($p = 0.013$) and scrub ($p = 0.034$). FVI abundance: Field Margin/Hedgerow > Pasture ($p = 0.001$). Other FVI species richness: Field Margin/Hedgerow > Pasture ($p = 0.035$); long-grass > short grass ($p = 0.009$).

Flower abundance: Field Margin/Hedgerow > Set-Aside ($p<0.001$), Pasture ($p = 0.046$) and Reserve ($p = 0.063$); Reserve > Set-Aside ($p = 0.046$); long grass > short grass ($p<0.001$); scrub > short grass ($p<0.001$). Flower species richness: Pasture > Field Margin/Hedgerow ($p<0.001$); Reserve > Field Margin ($p<0.001$), Pasture ($p = 0.004$) and Set-Aside ($p<0.001$).