Maximising a mutualism: sustainable bumblebee management to improve crop pollination

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“The last word in ignorance is the man who says of an animal or plant, "What good is it?" If the land mechanism as a whole is good, then every part is good, whether we understand it or not. If the biota, in the course of aeons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering.”

Aldo Leopold, *Round River: From the Journals of Aldo Leopold*

General Abstract

Over 80% of wild angiosperms are reliant upon animal pollination for fruit and seed set and bees and other insects provide a vital pollination service to around a third of the crops we produce. Habitat loss, climate change and disease spread all threaten pollinator populations, with local declines and range contractions in honeybees and bumblebees leading to concerns that crop production may suffer as a result of pollinator shortages.

Whilst agriculture and wildlife are often presented as being at odds with one another, the relationship between farmers growing pollination dependent crops, and the bees and insects that service them could be mutualistic. Flowering crops planted by farmers can provide an important source of forage to wild bees, whilst in return wild bees can contribute to ensuring farmers achieve adequate yields of marketable crops. The potential of this mutualistic relationship can be maximised by farmers by adopting management practices that reduce harm to, and enhance the wellbeing of, the wild bees around their farm.

A group of common pesticides (neonicotinoids) used by farmers have recently been linked to pollinator ill health. Sub-lethal effects resulting from exposure to the neonicotinoid imidacloprid have been reported in honeybees and bumblebees, with bumblebee reproductive success found to diminish as a result of exposure to field realistic doses of this agrochemical. Here, the mechanism behind the reduced queen production in bumblebee colonies is suggested, with bees exposed to imidacloprid showing reduced efficiency in foraging for pollen. Farmers dependent upon pollinating insects for crop production can opt to avoid the use of pesticides known to harm these insects, however future studies are needed to identify safer alternatives that can be use in their place.

Farmers can choose to increase the number of bees at their farms by utilising domesticated honeybees and purchasing commercially reared bumblebees. The use of these pollinators can ensure a minimum number of bees in the vicinity of a crop, and facilitate the production of crops at times when wild bee numbers are low. Concerns have been raised, however, regarding the use of commercially reared bees, mostly in regard to pathogen and parasite transmission, but also in respect to the possibility of outcompeting native species. Here the frequency and severity of attacks on commercial *Bombus terrestris* colonies, by the wax moth, an understudied bumblebee pest, are examined. Wax moths were found to infest almost half of the bumblebee nests deployed at fruit farms, with around a third of infestations resulting in nest destruction. Farmers investing in commercial bees will want to reduce the impact of harmful pests that may result in a reduced pollination service being delivered. Wax moth infestation rates at the study farms using commercial bees were high and the potential of a ‘spill-back’ effect on wild bees was examined. No evidence was found to suggest that nests in close proximity to these farms were any more or less likely to suffer from an attack than nests situated further away. Nest size was found to be the most significant predictor of an infestation, with larger nests more prone to wax moth attacks.

Whilst farmers can utilise domesticated and commercially reared bees, relying on one source of pollination is inherently risky, and the most robust service will likely be provided by a range of pollinators. As well as reducing the use of chemicals known to harm beneficial insects, farmers can improve the habitat around their farms to help encourage and sustain wild pollinator populations. Sowing flower strips has been found to increase the abundance and diversity of pollinating insects, however, studies linking the use of these strips to crop production are lacking. Here we demonstrate for the first time that sowing small flower strips, adjacent to strawberry crops serviced by both wild and managed bees, can increase the overall number of pollinators foraging on the crop.

This thesis contributes to our understanding of the implications of farm management decisions on pollinator health. It provides experimentally based evidence to guide farmers in making informed decisions regarding the future of crop pollination services and highlights the need for an integrated approach to managing pollination services for sustainability.

DECLARATION OF AUTHORSHIP

I, Hannah Louise Erin Feltham, declare that this thesis has been composed by myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

Signed …………………………………………

Date ……………………………………………

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General Introduction

"Natural selection cannot possibly produce any modification in a species exclusively for the good of another species; though throughout nature one species incessantly takes advantage of, and profits by, the structures of others. If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection."

Charles Darwin, Origin of the Species

1.1 Mutualisms

Mutualisms can be defined as reciprocally beneficial or reciprocally exploitative relationships between organisms where each partner receives an overall net benefit (Herre *et al.,* 1999). They are fundamentally important in all ecosystems (Chapin *et al.,* 2011; Kiers *et al.,* 2010; Power, 2010; Doebeli & Knowlton, 1998) and often involve the direct exchange of goods and services. Most mutualisms revolve around one species gaining a much needed service such as pollination or protection from predators, whilst the other species gains a reward, like food or somewhere to live.

Many evolutionary opportunities have been made possible by the development of mutualistic relationships (Boucher *et al.,* 1982) however, interdependent mutualisms carry a risk: the fate of one species is often tied to that of another, with the loss of one potentially resulting in the loss of both. The mutualism between reef building corals and the photosynthetic dinoflagellates that help power them are threatened by warming oceans and other local stresses, resulting in 'coral bleaching', a visible symptom of the disruption in the partnership between the two (Hoegh-Guldberg *et al.*, 2007).

Mutualisms occur at all levels of biological organisation from the oxpeckers that remove parasites on rhinos, receiving for themselves a food source, to countless nutritional symbionts that fix nitrogen and aid digestion (Herre *et al.,* 1999). A diversity of mutualisms exists between insects and plants alone, with insects providing a range of services from pollination to protection to seed dispersal (Whittaker, 1975; Kearns *et al.,* 1998; Bronstein *et al.*, 2006), in return gaining a food source. Habitat loss and fragmentation have led to disruptions in plant pollinator interactions (Kearns *et al.,* 1998; Winfree *et al.,* 2009; Potts *et al.*, 2010) and there are fears that the breakdown of mutualisms could accelerate the effects of global change on biodiversity loss (Kiers *et al.,* 2010).

1.2 Ecosystem services

Ecosystem services can broadly be defined as "*the benefits people obtain from ecosystems*" (Millennium Ecosystem Assessment, 2005). Mutualists provide essential ecosystem services such as pollination and seed dispersal (Potts *et al.*, 2010; Terborgh *et al.,* 2008) and contribute critically to global carbon and nutrient cycling (Wilson *et al.,* 2009).

Natural systems provide climate regulation, water purification, soil production, pest control and crop pollination, which are crucial to human survival (Daily, 1997). Whilst humans frequently manage natural systems to produce ecosystem goods such as wood and fish, few ecological systems are managed in recognition of the services, rather than the products, that they provide.

Estimating the monetary value of ecosystem goods and services can help provide a rationale for assigning more resources towards conserving natural 'capital', however there are risks involved in placing a value on one or a few component parts of interwoven and complex systems.

1.3 Food security

The overarching challenge facing humankind is to reconcile the growing demands of the human population with sustainable management of ecosystems. The global population is likely to plateau at around 9.6 billion people by 2050 (United Nations, 2013). Currently around 925 million people suffer from hunger (a lack of access to sufficient protein and energy in their diets), with a further billion believed to suffer from what has been called ‘hidden hunger’; a lack of access to important macro and micro nutrients (e.g. vitamins and minerals) that facilitate good heath (Foresight, 2011).

Food preferences are shifting with more affluent populations increasing the demand for processed food, meat, dairy and fish (Godfray *et al.*, 2010). Food producers face mounting competition for water, land and energy with the effects of climate change offering a further challenge to food security (Parry *et al.*, 2007; Schmidhuber & Tubiello, 2007). It is becoming increasingly clear that we need to curb the plethora of negative effects that our food production systems are having on the natural environment (Godfray *et al.*, 2010; Bommarco *et al.*, 2013), with some arguing that sustainable intensification (producing more food from the same area of land whilst reducing our environmental footprint) will be necessary to avoid bringing more land into production (Royal Society of London, 2009).

1.4 Agriculture and environmental degradation

The activities of humanity are driving global environmental degradation at an alarming and unprecedented speed (Brook *et al.*, 2008). The loss of natural habitats and increasing habitat fragmentation have, for some time now, been regarded as key threats to biodiversity (Wilcox & Murphy, 1998; Tilman *et al.*, 1994; Laurance *et al.*, 2002).

Declines in biodiversity within agricultural landscapes have been well documented, with the ongoing intensification of farming practices leading to natural habitat loss and the increased use of agrochemicals (Green, 1990; Kremen *et al.,* 2002; Tilman *et al.,* 2008). Approximately half of the global area of land that is suitable for agricultural production is currently used for this purpose (Tilman *et al.,* 2001). Specialisation, which has allowed farmers to increase their yields by tailoring their practices to the production of one or a few commodities, has led to large expanses of land being stripped of 'wildlife friendly' habitat, and replaced with more homogenous landscapes of crop monocultures (Stoate *et al.,* 2001). Agricultural practices can reduce the ability of ecosystems to provide both goods and services and the area intensive nature of this sector often results in consequences being widely felt. Large quantities of pesticides and fertilizers applied to increase yields can result in unintended consequences as nutrients and toxins effect non-target organisms and systems (Krebs *et al.*, 1999; Sparling *et al.,* 2001; Goulson, 2014). Agriculture can reduce the ability of ecosystems to provide goods and services yet its success and sustainability remains ubiquitously tied to functionally intact systems and the services they provide (Godfray *et al.*, 2001).

1.5 The importance of pollinators for crop production

Approximately 84% of European crops are reliant upon animal pollination to some extent (Williams 1994) and diets would be greatly impoverished, both nutritionally and culturally, without the services of pollinating insects (Steffan-Dewenter *et al.,* 2005; Klein *et al.*, 2007). The proportion of land cultivated with pollinator dependent crops is growing (Aizen *et al.,* 2008) and there are concerns over whether increasingly intensive agricultural systems have the ecological capacity to sustain reliable pollination services (Aizen & Harder, 2009). Limitations in pollination can result in the full reproductive potential of crops not being realised, either in terms of fruit produced or seeds set (Aizen *et al.,* 2008). The degree to which a crop is pollinated not only affects the quantity of production but also the quality; influencing size, shape and the classification of fruit for marketing purposes (Garratt *et al.,* 2014) and shelf life (Klatt *et al.,* 2014).

Whilst an array of animals (birds, bats, rodents) can be vectors of pollen, insects are especially suited to this role (Goulson, 2010). Social and solitary bees, wasps, beetles, flies, butterflies and moths constitute the vast majority of the world's pollinators (Vanbergen 2013), with bees in particular being of primary importance to the pollination of many agricultural crops (Klein *et al.,* 2007).

1.5.1 Honeybees

The honeybee, *Apis mellifera*, is the insect most commonly managed for the purpose of crop pollination, and is the most well studied of all insect pollinators. They are excellent generalists and that have been introduced to almost every country on the planet (Goulson, 2003b).

Whilst honeybees are renowned crop pollinators they are not native to many of the areas they now occupy, and are known to compete with native insects, deterring them from utilising the richest sources of forage (Gross, 2001). And while honeybees are often credited with sustaining many pollination dependent crops, wild bees including bumblebees, offer an equally important, often superior, service (Garibaldi *et al.,* 2013; Javorek *et al.,* 2002; Wilmer *et al.,* 1994).

1.5.2 Bumblebees

There are approximately 250 species of bumblebee (*Bombus* spp.) across the globe (Goulson, 2010). The majority of species have an annual lifecycle with queens emerging in the spring to found colonies. These queens produce workers who take over the role of foraging, brood care and the maintenance of the nest. Reproductive offspring in the form of males and new queens are generally produced towards the end of the colony cycle. Once mated, new queens forage and find a hibernation site whilst the old colony founding queen perishes along with the workers and males (Alford 1975).

Bumblebees are hardy and efficient pollinators and compared with honeybees forage over substantially longer periods of the day, in poorer weather conditions, carry more pollen on their bodies and visit more flowers per minute (Wilmer *et al.,* 1994). Bumblebee body size varies greatly both within and between species (Sladen, 1912), and different species exhibit different foraging ranges, e.g. *Bombus terrestris* (Linnaeus 1758), are known to travel distances of up to 1750m on a regular basis (Walther-Hellwig & Frankl, 2000) and less frequently further (Goulson & Stout 2001), whilst *B. pascuorum* (Scopoli, 1763) are believed to remain closer to their nesting site (Knight *et al.,* 2005). Bumblebees can exploit an array of different flowers due to varying inter species tongue lengths. Their ability to buzz pollinate (by rapidly vibrating their flight muscles to shake the anthers of a plant to release pollen) makes them vital for crops such as tomatoes (Van dan Eijende *et al.,* 1991).

Advancements in techniques for rearing bumblebee colonies have resulted in five species of bumblebee being commercially bred for the purposes of crop pollination (Velthuis & van Doorn, 2006). An international market has developed for these bees and they are transported around the globe for use on an increasing number of crops.

Whilst honeybees and bumblebees are key crop pollinators a myriad of other pollinating insects are known to contribute to successful crop yields (Garibaldi *et al.*, 2013). Whilst the relative merits of different pollinating species can be debated, it is likely that in most systems the most resilient pollination service in the long term will be one provided by a range of insects (Garibaldi *et al.*, 2014).

1.6 Pollinator declines

There is evidence that both wild and domesticated pollinator populations are declining (Biesmeijer *et al.*, 2006; vanEngelsdorp *et al.*, 2008). These declines are considered to be caused by a range of interacting effects which include: habitat loss; climate change; an increased use of agrochemicals and the accidental introduction of non-native pests and diseases (Williams & Osborne, 2009; Decourtye *et al.*, 2010; Neumann & Carreck, 2010). Globally over 80% of wild angiosperm species are reliant upon animal pollination for fruit and seed set (Ashman *et al.*, 2004) with a greater proportion of animal pollinated flowering plant species in tropical communities (94%) compared with temperate-zone communities (78%) (Ollerton et al., 2011). Whilst the scope of this thesis does not allow a detailed discussion on the importance of pollinating insects to wild flowers it would be remiss not to mention the implications of declines in this regard. Whilst some farmers are able to increase the number of pollinators servicing their crops by utilising domesticated/commercially reared bees, pollination dependent wild flowers must rely solely on the availability of wild insects. Pollinator losses will likely, therefore endanger many of the plant-pollinator mutualisms that currently exist (Kearns *et al.*, 1998).

Concerns over declining pollinator populations have stimulated debates regarding whether or not we are facing a pollination crisis (Aizen & Harder, 2009; Ghazoul, 2005;

Westerkamp & Gottsberger, 2002) and have led to worries over how diminishing bee numbers could impact food production (Aizen *et al.*, 2009).

Whilst global honeybee stocks have increased (Aizen & Harder 2009) marked declines in wild and domesticated honeybees have been reported in certain regions (vanEngelsdorp *et al.,* 2008; Potts *et al.,* 2010) and recent research has suggested that parasites, diseases and pesticides can interact to have strong negative effects on managed colony health (vanEngelsdorp *et al.,* 2008; Cornman *et al.,* 2012). Regional declines in managed honeybees, coupled with the increased production of pollinator dependent crops have led to mounting concerns that local requirements for pollination services may exceed the supply of honeybees available (Breeze *et al.,* 2014).

Bumblebee range contractions have been reported since the 1950's (Free & Butler 1959) with declines in distribution now widely reported (Colla & Packer, 2008; Grixti *et al.,* 2009; Williams & Osborne, 2009). In the UK, two species have gone extinct, with only 6 of the 19 true bumblebees (in the UK there are also 6 cuckoo species) remaining common and widespread (Goulson, 2010). Bees feed exclusively on flowers and are dependent on pollen and nectar for survival, however floral resources in the UK and Europe have declined as a result of agricultural intensification (Biesmeijer et al., 2006). Hedgerows, woodlands and tussock grasslands are often casualties of agricultural expansion. These are areas that tend to attract small mammals and birds, whose deserted holes and hollows provide suitable bumblebee nesting sites (Sladen, 1912; Fussell & Corbet, 1992; Rasmont *et al.,* 2008). The loss of such natural and semi-natural habitats are likely to have contributed reductions in available forage and nesting sites within agricultural landscapes (Kells & Goulson, 2003).

1.7 Anthropogenic causes of pollinator decline

1.7.1 Habitat loss and fragmentation

Concerns over pollinator declines have led to numerous studies which attempt to quantify how natural habitat availability, landscape heterogeneity and organic farming methods can impact on pollinator abundance and diversity. Some studies have found a positive correlation between the extent and diversity of natural habitat surrounding a farm and the pollination service provided to crops (Ricketts *et al.,* 2008; Rundlof *et al.,* 2008), whilst others have shown a link between organic farming methods and an increased abundance and diversity of bees (Holzschuh *et al.,* 2008).

Brosi *et al.* (2008) determined that optimal farm configuration for the management of crop pollination services comprises small 'parcels' of service-providing habitat interspersed throughout working landscapes. Remnants of semi-natural habitats within an otherwise intensive landscape can act as corridors, along which pollinators can colonise, re-colonise and maintain small fluctuating populations within safe parcels of favourable habitat (Kearns *et al.,* 1998). The importance of some level of habitat heterogeneity is unsurprising given that many pollinating insects rely on a continuity of forage through the spring and summer (Rundlof *et al.,* 2008), and some on the availability of suitable nesting sites (Richards, 2001); these resources are more likely to be provided in a more diverse mosaic landscape than a homogenous one. However, the minimum area requirements needed to sustain functioning pollinator communities is little studied and an area where future research could be directed.

1.7.2 Pesticide use

Pesticides make a significant contribution to maintain world crop production today (Pimentel *et al.,* 1992), yet the spill-over effects they can have on non-target and even beneficial organisms has long been known (Ware, 1980; Flexner *et al.,* 1986, Desneux *et al.,* 2007). When looking at 13 components of agricultural intensification, Geiger *et al.* (2010) found that the use of insecticides and fungicides had consistent negative effects on biodiversity as well as reducing the potential of agricultural lands to support biological control.

There has been a recent surge of research examining the effects of systemic pesticides on pollinating insects, in particular bumblebees and honeybees (summarised in van der Sluijs *et al.,* 2014). Concerns are growing over the use of some pesticides with mounting evidence that neonicotinoids in particular are having sub-lethal effects on honeybees (Bortolotti *et al.,* 2003; Aliouane *et al.,* 2009; Decourtye *et al.,* 2004; Henry *et al.,* 2012) and bumblebees (Gill *et al.,* 2012; Whitehorn *et al.,* 2012; Elston *et al.,* 2013).

When investigating how crop pollination exposes bees to pesticides, Pettis *et al.* (2013) found that the majority of the bees in their experiment fed primarily on weeds and wildflowers around the crops. Despite largely feeding on wildflowers the pollen loads of bees monitored during the study contained high levels of pesticides and fungicides. This highlights that the sub-lethal and unintentional effects of agrochemicals are unlikely to be limited to pollinators and other insects foraging predominantly on the crop (i.e. managed pollinators placed within the crop vicinity), and may well spill-over to insects living in field margins and semi-natural areas nearby that occasionally utilise the crop as a resource.

Calls for more biodegradable and less persistent pesticides that better target specific pests are not new (Kevan, 1975) and it is important to properly understand and thoroughly test the chemicals that we use in our food production. Whilst recent concerns over the widespread use of neonicotioinds has led to a temporary moratorium on the use of some of these chemicals on mass flowering crops, their use in general remains wide spread and more studies are required in order to further clarify their effects and inform future decisions on their use.

1.8 Maximising a mutualism? Managing pollinators for crop production

Whilst modern agriculture as a whole may represent a hostile environment for some pollinators, the number and extent of crops requiring their service has increased (Aizen *et al.*, 2008). Although agriculture and biodiversity conservation are often regarded as being incompatible (Tscharntke *et al.*, 2005), in the case of crop pollination, a mutualistic relationship between the two can exist. In an often otherwise inhospitable landscape pollinators such as bumblebees can benefit from the floral resource provided by crops sown by farmers whilst farmers benefit economically from selling the fruits or vegetables that arise from well pollinated flowers.

In 2005 Shuler *et al.* reported how farming practices influence pollinator populations on squash and pumpkin crops. They found that all of the farmers that they made contact with were aware of the need for insect pollinators in the production of their crop, with most believing that honeybees played the most important role in pollination success. Shuler *et al.* (2005) however found that the squash bee (*Peponapis pruinosa*) was in fact the most abundant pollinator observed on the crop in this study system, demonstrating that farmers, whilst often aware of the importance of pollinators do not necessarily realise the relative contribution of managed and wild species. Whilst commercially reared bumblebees and domesticated honeybees are relatively cheap, convenient and controllable, they can be less efficient than a combination of wild pollinating species that can provide a diverse pollination service to crops (Javorek *et al.,* 2002; Bosch *et al.,* 2006).

Regardless of whether a bee is wild or managed, it requires adequate supply of food and a safe and suitable habitat in which to spend its non-foraging time (i.e. a safe dwelling/nesting site). For a farmer it perhaps does not matter if a bee is wild or commercially reared, so long as the pollination service to their crop is sufficient to ensure high yields that are sustainable through time. If the potentially mutualistic relationships between farmers that grow pollinator dependent crops and the bees that feed on them is to flourish then pollinator management initiatives will help maximise this relationship.

A recent study looking into the types of pollinators visiting two widespread crops (oilseed rape and field beans) revealed that crop specific conservation strategies were beneficial in boosting pollination as the composition of pollinators visiting each crop were found to differ (Garratt *et al.,* 2014b). Field beans would benefit from targeted conservation strategies that boost several key taxa, whilst oilseed rape would benefit from strategies designed to augment overall pollinator abundance and diversity within the crop vicinity.

On farm practices are known to influence pollinator populations (Shuler *et al.,* 2005) and management of farm systems rather than of pollinators themselves will likely, given time, allow farmers to develop a more resilient service for their crops. There are currently sizeable gaps in our understanding of pollination service supply and demand and more research is needed to help determine crop specific requirements and how they vary in space and time. To maximise the pollination of their crops farmers may have to encourage and exploit more than one ‘type’ of pollinator, investing in a range of management strategies to ensure high crop yields throughout the season.

1.8.1 Commercial and domesticated bees

The production of pollinator dependent crops at a commercial scale is increasingly reliant upon managed pollinators (Richards, 2001). For a long time the only widely managed pollinator was the honeybee, *A. mellifera*. However, honeybees are a generalist species that cannot successfully pollinate all crops (e.g. field beans require long tongued bees and tomatoes require buzz pollination). Techniques for mass rearing bumblebees were perfected in the 1980s and since then a multi-million pound industry has grown that supplies commercially reared bees for the production of around 30 different crops (Velthuis & van Doorn, 2006). Whilst the predictability and reliability of commercially reared bumblebees has endeared them to farmers, concerns have been expressed about the ecological risks that surround their use (Meuss *et al.*, 2011; Goulson, 2010b). In Europe there are currently no established standards for screening imported bees in order to detect diseases or parasites. This means that there is potential for the accidental introduction and spread of parasites and diseases to native populations (Greystock *et al.,* 2013). In North America circumstantial evidence suggests links between the decline of three once common bumblebee species and the transportation of bees between Europe and America (Thorp, 2005; Thorp & Shepherd 2005), however empirical evidence is needed to determine if pathogen spill-over could be the cause (Meuss *et al.*, 2011). There is also the potential for commercially reared bees to escape and establish populations in the wild, which may displace or interbreed with wild populations (Ings *et al.*, 2006). Commercially reared bees have escaped and become naturalised in Japan, Tasmania, Chile, Argentina and Mexico (Goulson, 2003).

The contribution commercially reared bumblebees make to crop pollination is understudied and the extent to which they are required to ensure high levels of pollination has not been fully quantified.

1.8.2 Wild bees

Whilst some research has found that commercial pollinators substantially increase yield i.e. blueberries (Desjardins & De Oliveira, 2006), Raspberries (Lye *et al.,* 2011), others have shown that crop pollination is predominately carried out by wild bee species i.e. tomatoes (Greenleaf & Kremen, 2006). Whilst not empirically verified, in many cases the relative contribution of wild and managed pollinators will likely change throughout the season, for example, in the UK commercial bumblebees play a greater role in the pollination of early (pre) season fruit, when wild species have not yet emerged. The contributions of commercial and wild bees may also vary in response to farm characteristics. Lower intensity agricultural systems can support larger populations of wild bees, as less intensive land use can result in a greater range of floral resources and suitable nesting sites about the farm (Aizen & Harder, 2009).

A study by Allsopp *et al.* (2008) estimated the total value of wild insect pollination services and managed pollination services for the deciduous fruit industry of the Western Cape region of South Africa. They estimated the value of wild and managed pollination for this sector to be $358.4 and $312.1 million respectively. At the time of the study only $1.8 million was being paid for the service provided by managed bees and there was no direct payment from producers for the service provided by wild pollinators (Allsopp *et al.,* 2008). This study suggests that there should perhaps be an emphasis on integrated management of wild and managed pollinators and whilst this approach is increasing in popularity in North America, it has not been widely adopted in Europe.

1.8.2.1 Enhancing provisions for wild bees

Flowering crops can provide an important resource for bees and can support an abundance of pollinating insects (Westphal *et al*., 2003) and can have a positive effect on size (Westerphal *et al.,* 2009) and density (Knight *et al.,* 2009) of bumblebee colonies nearby. However, evidence suggests that increases in abundance and diversity correlates only with crop flowering periods (Hanley *et al.,* 2011) and do not represent an overall increase in sexual reproduction within nests (Westphal *et al.,* 2009).

Pollinators will benefit from a diversity of forage and from the provision of a stable supply of flowering plants throughout the season (Bluthgen & Klein, 2011; Mandelik *et al.,* 2012) this is not always provided by crops that flower *en masse*. For mango and sunflower crops, enhancing the richness of non-crop floral resources within fields can benefit levels of pollination (Nicholls & Altieri, 2013), suggesting that providing additional floral resources may not lead to competition with the crop. A number of studies have shown that flower strips are effective at providing forage for a range of bumblebee species throughout the season (Carvell *et al.,* 2007; Carreck & Williams, 2002; Pywell *et al.,* 2005). Schemes found to be most successful in attracting a diversity and abundance of foraging workers are the sowing of either annual or perennial wildflowers or a pollen and nectar mix consisting of agricultural cultivars of legume species (Carreck & Williams, 2002, Meek *et al.,* 2002, Carvell *et al.,* 2004 and 2007; Pywell *et al.,* 2005 and 2006). Habitat improvements targeted at pollinators are likely to increase species richness and abundance (Garibaldi *et al.,* 2014). However, it is likely that investing in environmental services will be most common when farmers can set aside the smallest area required to realise the ecosystem service benefits (Brosi *et al.,* 2008). On-farm management of environmental service provision will prioritise strategies that require minimum effort for maximum gain, for example the widespread implementation of small scale measures within working agricultural areas (Brosi *et al.,* 2008, Bodin *et al.,* 2006).

Little information exists on the ways in which local pollinator management influences agricultural production (Richards, 2001). Whilst studies measuring the impact of wild pollinator enhancement strategies are uncommon, those relating their effects to the success of pollinator dependent crops are rarer still (Garibaldi *et al.,* 2014). It is therefore important to determine to what extent wildflower plantings can increase the pollinator carrying capacity of agricultural landscapes. A diversity of wild pollinators cannot just be added to the agricultural systems the way that domestic honeybee hives and commercial bumblebee nests can. If farmers want a stable and reliable wild pollination service they must in turn ensure a stable and reliable continuity of forage for bees and other insects throughout their active period (Shuler *et al.,* 2005).

In a recent study, Blaauw and Isaacs (2014) created wildflower plantings adjacent to blueberry fields in order to determine their effect on the crop, and found that the use of medium to large flower strips increased the number of pollinators observed on highbush blueberries. In order to maintain and restore wild pollinator communities, farmers are often advised to boost floral abundance within agricultural landscapes, however this management choice is often poorly implemented (Carvalheiro *et al.*, 2011) possibly due to the costs involved, e.g. loss of agricultural land, cost of seed and management (Ghazoul, 2007) or fears that alternative forage may provide competition for crop pollination (Weiss, 1983).

1.8.3 Managing pests and diseases

Predators, parasites, parasitoids and diseases can impact bees at any stage of their life cycle. The effect of parasites and predators is perhaps best demonstrated by looking at the success of pollinators when these threats are absent. For example, bumblebee species close to, or extinct, within the UK (*B. subterraneous* and *B. ruderatus*) are thriving in New Zealand where they were introduced in the 1880s and have since been living in the relative absence of the parasites that usually play a role in stemming population growth (Goulson, 2010).

Emerging infectious diseases (EID) threaten pollinator populations and honeybee keepers have to actively manage hives to reduce the risk of them contracting exotic and high impact pathogens (e.g. the Varroa mite, *Varroa destructor*) (Furst *et al.,* 2014). There are concerns that wild bee declines may be linked to the use of managed pollinators (Evison *et al.,* 2012; Genersch *et al.,* 2006; Meeus *et al.,* 2011), with a recent study showing that domestic honeybees are the likely source of at least one EID in other wild bee species (Furst *et al.,* 2014).

An increased susceptibility to harmful parasites has been linked with the use of agro-chemicals and exposure to sub-lethal doses of pesticides may alter the susceptibility of bees to parasites and pathogens (Pettis *et al.,* 2013; Di Prisco *et al.*, 2013). Whilst most species have natural enemies, an increase in their prevalence is particularly concerning when it is brought about as a consequence of human activities and management decisions.

The threat of parasite spread between commercial bumblebees and wild bumblebees arises when both share a common resource, e.g. the wildflowers surrounding a crop, where diseases can be transmitted (Morandin *et al.,* 2001; Whittington *et al.,* 2004).

In the UK, Whitehorn *et al.* (2012) found that the use of commercial bees did not lead to pathogen spill-over into wild bumblebee populations nearby, however circumstantial evidence from North American suggests that disease and parasite transmission may occur between commercial colonies and wild bees (Thorp, 2005; Thorp & Shepherd 2005; Winter *et al.*, 2006). Recent work by Greystock *et al.* (2013) has revealed the high parasite loads within some commercial bumblebee nests upon arrival from suppliers. This highlights the need for increased pathogen control in managed bees and for more research to facilitate a better understanding of how the use of commercial bumblebees may be impacting the health of wild populations.

1.9 Current pollinator management options

Farmers who grow pollination dependent crops have three broad choices in regards to pollinator management. One, they can do nothing and rely on whatever service they receive from the pollinators that are naturally resident within the vicinity of their crops. Two, farmers can increase the number of domesticated/commercially reared bees at their farm by renting or purchasing hives or colonies. Three, farmers could invest in strategies which will increase the number of wild pollinators around the farm in the hope that these pollinating insects will then service their crops.

There are known links between ecosystem stability and local species richness (Hooper *et al.*, 2005; Loreau *et al.*, 2001). The long term management of pollination dependent crops needs to focus on creating a resilient pollination service as fluctuating populations can result in poor or unpredictable yields.

1.10 Thesis overview

This thesis seeks to explore how the mutual relationship between farmers and the insects that pollinate their crops can be maximised, to ensure healthy and sustainable pollinator populations and crop yields. Throughout these chapters the results of research conducted at raspberry and strawberry farms in Scotland is presented and discussed. Whilst this thesis focuses on the pollination service provided to two particular soft fruit crops, some of the findings may transfer to other flowering crop systems that rely up insect pollinators. Work presented also comments on how the intensification of farming practices i.e. the increased use of pesticides and commercially reared pollinators maybe impacting the health and sustainability of wild bee populations more generally.

Chapter 2 begins with an investigation designed to determine the pollinator requirements of raspberry and strawberry crops, by detailing the contribution of various pollinating insects throughout the season and relating these to yields of marketable fruit produced. Leading on from this, Chapter 3 provides experimental evidence on the benefit to farmers of sowing wildflower strips adjacent to their crops, and discusses how such plantings may also contribute to the creation of sustainable pollination services. Chapter 4 details how novel technology was used to examine the impact of a common agricultural pesticide on the foraging ability of *B. terrestris*, in an attempt to reveal the mechanism behind reduced queen production in bumblebee nests exposed to the neonicotinoid: imidacloprid. Chapters 5 and 6 present research that seeks to shed light on the impact of an understudied bumblebee pest, the wax moth. The frequency and severity of attacks on commercial nests used by farmers within the study system are reported along with an exploration of how the use of factory reared bees might be impacting upon the prevalence of wax moths in wild bumblebee nests within agricultural settings.

1.11 Aims and objectives

This thesis aims to provide insight into how bees are managed for the purposes of crop pollination and to examine how farmer action and management can shape the health, diversity and resilience of the pollinator assemblages that service their crops. The following chapters hope to provide useful management advice to farmers who wish to maximise the benefits they gain from pollinating insects by providing evidence upon which they can base farm level decisions.

The specific aims of each chapter are:

1. To determine the relative pollination contribution of different species to strawberry and raspberry production; and examine if complementarity exists between different pollinator groups to facilitate the pollination of crops across the season.
2. To test the prediction that the presence of wild flower strips can increase the number of pollinators visiting adjacent strawberry crops, whilst accounting for potential confounding effects.
3. To examine the effect of a field realistic dose of imidacloprid on the foraging ability of *B. terrestris* workers.
4. To investigate the frequency and severity of wax moth attacks on commercial *B. terrestris* colonies.

5. To assess experimentally if nests close to fruit farms where commercially reared bumblebees are in use, are at a greater risk of wax moth attacks as a result of their proximity to these farms.

Each chapter is presented as a stand-alone paper so that reference to this general introduction should not be required for interpretation of the work.

Chapter 2- Functional diversity matters: Seasonal complementary of pollinators ensures fruit production through the growing season

The work presented in this chapter is taken from the paper Ellis, C., Feltham., H., Park, K., Hanley, N. & Goulson, D. Functional diversity matters: Seasonal complementary of pollinators ensures fruit production through the growing season. Submitted for publication in Ecology and Evolution.

The remit of this section of my project required additional help from another researcher therefore Ciaran Ellis and I undertook the research as a joint study. Work was conducted by us jointly and in equal measure, with C.Ellis leading on the modelling analysis and H.Feltham leading on the spatial analysis. This chapter will also be found in the PhD thesis of C. Ellis entitled: 'Valuing wild pollinators for sustainable pollination services'.

K.Park, N. Hanley and D.Goulson supervised the project and all authors commented on draft versions of the manuscript.

2.1 Abstract

Understanding the relative contributions of wild and managed pollinators, and the functional contributions made by a diverse pollinator community, is essential to the maintenance of yields in the 75% of our crops that benefit from insect pollination. Through a field study and pollinator exclusion experiments on two soft-fruit crops in a system with both wild and managed pollinators we have linked seasonal differences in the abundance of pollinator groups to yields across the growing season. The seasonal complementarity provides evidence for the need to manage multiple species groups and highlights the risks of replacing all pollinators with managed alternatives. Pollinators responded differently to weather and habitat variables suggesting that diversity can reduce the risk of pollination service shortfalls. The functional approach taken here shows that low efficiency pollinators such as flies may be more important to pollination than expected. Understanding how differences between pollinator groups can enhance pollination services to crops strengthens the case for multiple species management and provides evidence for the link between increased diversity and function in a real crop system.

2.2 Introduction

Insect-mediated pollination increases yield in around 75% of world food crops, which represent ~35% by volume of primary food production (Klein *et al.,* 2007). The importance of insect pollination has led to the commercialisation of not only the honeybee, but also several species of bumblebee and various solitary bees (Pitts-Singer & Cane, 2011). Nonetheless the role of wild pollinators is likely to be greater than was previously assumed: a meta-analysis of pollination data from 41 crop systems suggests that honeybees supplement wild pollinator numbers, rather than the other way around (Garibaldi *et al.,* 2013) and wild pollinators play a significant role in varied crop systems (e.g. Winfree *et al.,* 2008; Breeze *et al.,* 2011; Rader *et al.,* 2012).

Wild species are also important for their contribution to pollinator diversity, which has been shown to positively influence crop success (Klein *et al.,* 2003). Increased diversity increases ecosystem service provision when species contribute slightly different functions (Cadotte *et al.,* 2011). Particularly, functional diversity is increased when species (or species groups) are complementary in the services they provide so the overall scope of service provision is increased when more species are present. For example, pollinator species may be complementary in the heights at which they forage; honeybees and wild bees are complementary in their use of space on almond trees, so having both groups present increases yield overall (Brittain *et al.,* 2013). Likewise seed set in pumpkins grown at different heights was increased when more pollinator groups with different preferred pollinating heights were available (Hoehn *et al.,* 2008). For crops with long growing seasons, one species or group of species may not be active for the entire growing season, and so complementarity in abundance or activity across time (seasonal complementarity) will be important (Bluethgen & Klein, 2011).

Species or species groups that overlap in functional contribution may respond slightly differently to changing conditions, thus buffering the overall service over multiple years (Winfree & Kremen, 2009; Brittain *et al.*, 2013). Differential responses to landscape context or management practices could ensure the service provision continues after land-use alterations (Steffan-Dewenter *et al.,* 2002; Jha & Vandermeer, 2009; Tuell & Isaacs, 2010). Maintaining both complementarity functions and response diversity will ensure that future pollination needs are met under a range of circumstances (Elmqvist *et al.*, 2003).

The soft fruit industry in Scotland produces 216,000 tonnes of strawberries (5% of the global total) and 3,000 tonnes of raspberries per year (FAOSTAT). Both crops are highly reliant on insect pollination for marketable fruit. The pollinator requirements of raspberries and strawberries differ: raspberries are highly attractive to bees and the peak of flowering coincides with the seasonal peak in bee numbers. Strawberries, on the other hand, have a long growing season which may require multiple pollinator groups to ensure pollination across the season. This study examines the importance of diversity in the pollination of these two soft-fruit by asking the following questions:

1. What are the relative levels of pollination contributed by different species?
2. Is there seasonal/temporal complementarity between different pollinator groups enabling strawberry pollination across the season?
3. Are there differences in the response of different pollinator groups to weather and habitat variables which could be important for the continued pollination of these crops?

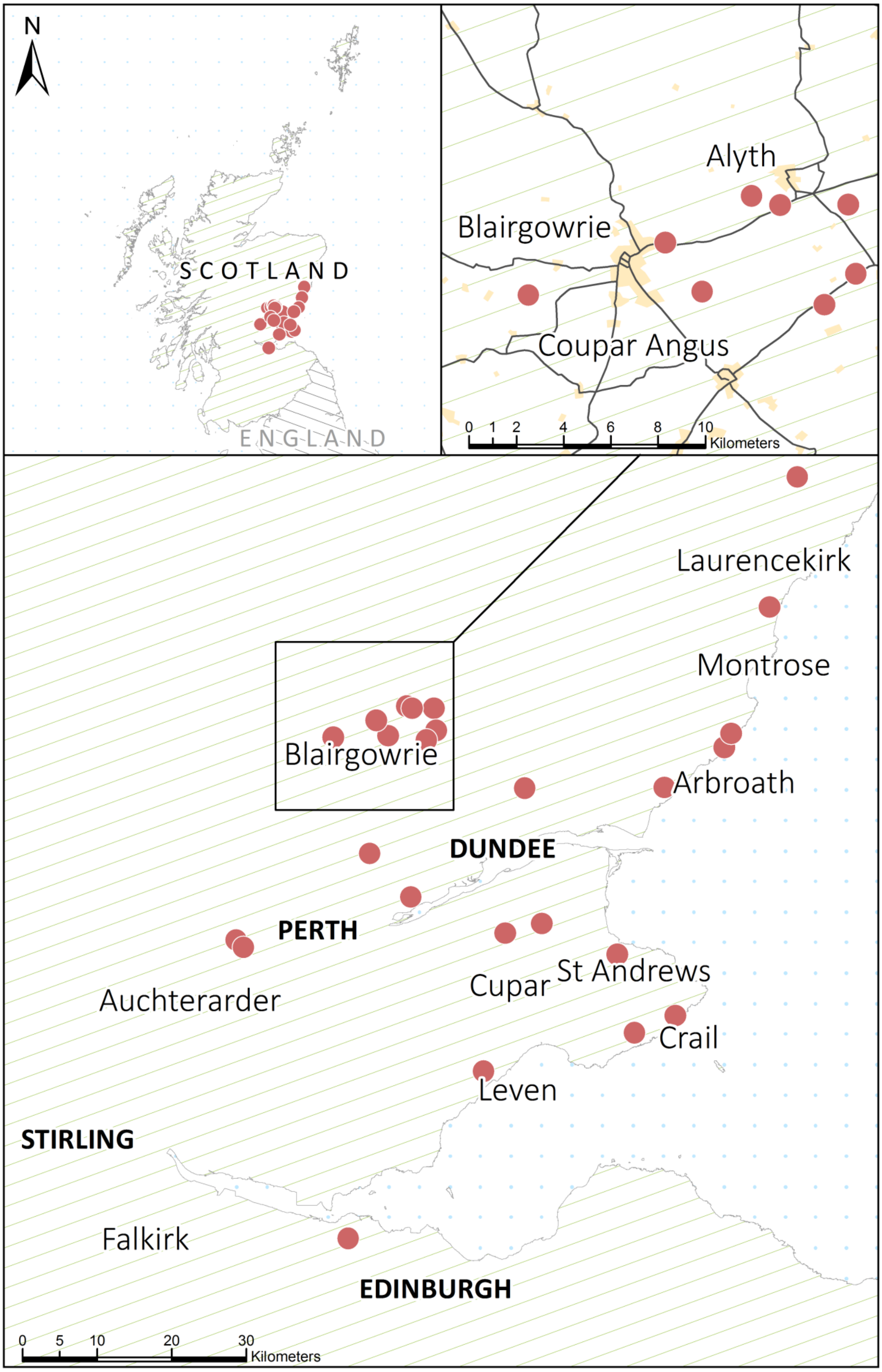

Figure 2.1 Location of study sites within east and central Scotland

2.3 Materials and Methods

2.3.1 Sites and survey

The main domesticated pollinators on soft-fruit farms are commercially-reared bumblebees. Honeybees are also sometimes present though they are not ubiquitous in UK crop systems (Breeze *et al.* 2011). Seven species of wild bumblebees are common in the study area as well as other pollinators including solitary bees, hoverflies and other flies (Lye *et al.* 2011). Contact was made with soft-fruit farms in Autumn 2010 and 29 farms were visited in early 2011. Farm managers were asked about commercial pollinator management; how many bumblebee colonies were used and whether, to their knowledge, honeybees were kept within flight distance of the farm. They were also asked about wild pollinator management e.g. whether wild flower strips were grown. Twenty-five farms spread through Angus, Perthshire and Fife (Fig. 2.1) were then chosen for inclusion in the field study. Of these nine grew only strawberries, four only raspberries and twelve grew both. Most soft-fruits were grown undercover in polythene tunnels (polytunnels), all of which were open-ended, some were open-sided while others had closed sides.

2.3.2 Pollinator Activity Transects

For each transect, a tunnel was picked at random from those with flowering crops and walked along at a slow walking pace, recording all pollinator visits to flowers. Transects on each farm ran for a total of 300m and included between two and four adjacent tunnels. *Bombus* species were classified to species level where possible; workers of domesticated *Bombus terrestris,* wild *B. terrestris* and wild *B. lucorum* cannot be reliably distinguished by eye. To split the counts of these species into wild and domesticated classifications, we used the average number of *B.terrestris/B.*

lucorum observed at farms not using commercial bees divided by the average number of *B. terrestris/B. lucorum* seen at farms using commercial bees to estimate the proportion of *B. terrestris/B. lucorum* observed, that could be attributed to wild sources. These proportions (for each fruit and time period) were then applied to the overall counts on farms using commercial bees, to obtain an estimate of the number of *B. terrestris/B. lucorum* from wild populations, and *B. terrestris/B. lucorum* from commercial sources.

Other pollinators were assigned to broad grouping, i.e. solitary bees were all grouped together, as were flies (including hoverflies). Three replicate flowers counts were taken in 1m^2 areas within each tunnel to estimate floral resources provided by the crop. Cloud cover was estimated as a percentage. Wind speed was measured on a three point scale (0 = still, 1 = light breeze, 2 = strong breeze), as was rain (0 = no rain, 1 = light rain, 2 = heavy rain). Days with heavy rain were avoided where possible, but if rain began during a visit the transect was completed. Weather stations closest to each farm were used for daily temperature and humidity data. Transects were all walked between 10 am and 5 pm. The time and type of polythene tunnel (closed-sided or open-sided) were also recorded. Farms were visited six times throughout the season, with approximately three weeks between each visit.

2.3.3 Exclusion experiment

The effect of pollinator visits on fruit quality and weight was evaluated at a subset of the farms (9 raspberry-growing farms and 11 strawberry-growing farms). Pollinators were kept away from flowers using polythene mesh netting (holes 1.35mm^2, Harrod

Horticultural Ltd, Lowestoft, UK). The net was chosen to exclude insects without allowing moisture to build up within the net, and to allow wind-blown pollen through.

For raspberries, 6 plants were used in each of 3 different polytunnels per farm; on each plant a bunch of approximately 9 unopened flowers were covered with the netting which was secured to the branch with covered wire. The bunches were marked with coloured tape along with a control bunch from the same plant. Strawberry plants were entirely covered with the exclusion mesh which was supported by arches of flexible garden wire and fixed to the bed with metal staples and duct tape. The plants were covered in groups of four (two groups of four were covered in each of two polytunnels). Each group was matched with a group of control plants which were marked with tape and signposted. Excluded and control fruits were picked when ripe. The picked berries were categorised into class I and class II fruit based on European marketing criteria and weighed (European Commission, 2011).

2.3.4 Habitat data

Landscape data were obtained from the OS MasterMap Topography Layer (EDINA Digimap Ordinance Survey Service) and ArcGIS 9.2 was used to create circles 1km around each study site. The feature classes from the topography layers were reclassified into five categories; (i) urban areas (buildings and structures), (ii) farmland, (iii) water (inland and tidal), (iv) linear man-made structures (roads, tracks and paths); and (v) semi natural habitat (rough grassland, scrub and woodland). The proportions of land cover for each of the five categories within each 1km buffer were calculated and used in subsequent analysis.

2.4 Statistical Analyses

2.4.1 Exclusion experiment

Statistical analyses were conducted using the statistical software R version 2.15.1 using packages lme4 and MASS (R Development Core Team, 2011). Models were fitted to the strawberry and raspberry data sets with fruit quality (with binomial errors) or fruit weight (with Gaussian errors) as response variables. Strawberries had high inter-farm variation and so farm identity was fitted as a random factor within a generalised linear mixed model (GLMM). For the raspberry data the residual deviance after fitting a GLM was approximately equal to the remaining degrees of freedom; there was little remaining variation to explain through random effects and so a GLMM was not used (Crawley, 2002). For all models, treatment (insects excluded vs. not excluded) and the average number of pollinators in the transects walked during the ripening period were included as factors, the ripening period was taken as the five weeks prior to picking (Lye *et al.*, 2011). To take into account the differences in ability to transfer pollen and the speed at which pollinators work, the abundance counts were multiplied by efficiency factors to provide efficiency-adjusted counts (Isaacs & Kirk, 2010). Honeybee numbers was reduced by a factor of 0.5 relative to bumblebees (Willmer *et al.*, 1994) and fly numbers were reduced by a factor of 0.2 (Albano *et al.*, 2009; Jauker *et al.*, 2012)

2.4.2 Pollinator activity

Counts of each pollinator group were summed along transects for each time period. With abundance of each pollinator group as the response, GLMM models with Poisson errors were fitted to the data with farm identity as a random factor. Data were overdispersed and so observation-level random effects were included in addition to the farm level random effects (Maindonald & Braun, 2010). Potential explanatory variables were split into three sets; observation variables (those variables available for each observation including weather variables, date etc), management variables and habitat level variables (Table 2.1). The analysis therefore took a hierarchical approach, with observation level variables and farm level variables (habitat and management variables) (Gelman & Hill, 2007). A full observation level model was fitted to each pollinator group on each soft-fruit. This model was reduced by removing non-significant terms ($p>0.10$) and comparing the Akaike Information Criterion (AIC) between models until the model with the lowest AIC was achieved. The management variables and habitat variables were then fitted separately to the most informative observational level model and the two-level models were reduced as before.

2.4.3 Complementarity

Species groups show seasonal complementarity if the peaks of their distributions across the season tend to occur separately. This can be tested for using a variance ratio test (1) (Schluter, 1984; Stevens & Carson, 2001; Winfree & Kremen, 2009), which is based on the relationship between total variance of M elements and the covariances between them (2). In this case the elements (X) are the abundances of the four pollinator groups through time.

Table 2.1 List of variables used in GLMMs to explain pollinator visitation to strawberries and raspberries

Observation level	***Farm Level***	***Farm Level***
	Management variables	**Habitat variables**
Day	Honeybees within 1km of farm (Yes or No)	% Woodland and scrub within 1km
Day squared	Number of bumblebee colonies used on crop per year	% Urban area within 1km
Time of day	Wild flower strips planted (Yes or No)	% Roads within 1km
Polytunnel type	Field margins left unmowed (Yes or No)	
Wind speed (0, 1, 2)		
Cloud cover (%)		
Humidity (%)		
Temperature (^{0}C)		

$$C = \frac{Var(\sum_i^M [\![Si)]\!]}{\sum_i^M Var(Xi)} \quad (1)$$

$$Var(T) = \sum_i^M Var(Xi) + 2 \sum_{i<l}^M Cov(Xi, Xl) \ (2)$$

If the species groups do not tend to covary positively or negatively, the total variance will be equal to the sum of the variance of each element, and hence the test statistic (C) will be close to 1. Test statistics less than 1 implies negative covariance and thus that the pollinator groups have different peaks throughout the season. A test statistic (C) across all the farms was calculated from the raw data. Per farm test statistics were not calculated as only one visit per farm was made per time period and weather impacted abundance. We generated farm level complementarity figures by simulating pollinator abundances by group for 6 time periods throughout the season. To control for effects of weather we took the average weather variables for each of 6 time periods and used these to generate 1000 random weather scenarios. These scenarios were used as inputs to the best fitting two-level GLMM model for each pollinator group. The complementary figures for each simulated set of pollinator abundances were then calculated. As in Winfree & Kremen (2009) we then compared the complementarity results for the simulated data using the full model, versus the results from the same models but with the day and day squared terms eliminated (the null model) using Wilcoxon signed rank test.

2.4.4 Impact of complementarity on yield

To assess the importance of different pollinator groups to fruit yield across the season, the GLMM models for wild bumblebees, honeybees and flies were used to simulate pollinator numbers across the season under average conditions. The outputs were totalled and adjusted for pollinator efficiency and the total adjusted pollinator numbers at each time point were then used as an input for the fruit quality GLMM. On the basis of discussions with farmers, the threshold for profitability was taken to be an average of 80% first class fruit. Pollinator groups were then deleted one by one from the total set, and fruit quality across the season re-evaluated.

2.5 Results

2.5.1 Pollinator Activity Transects

From 15 April to 19 August 2011, we observed 2,478 pollinators visiting strawberries in 129 transects at 21 farms and 4,464 pollinators visiting raspberries in 80 transects at 16 farms. Transects took on average 43 minutes to walk. Pollinators were observed on raspberry transects from mid-May to late July, and on strawberries from mid-April to mid-August. On average four (three to five) raspberry transects were walked on each farm with raspberries, and six (four to six) strawberry transects were walked on each farm with strawberries. Strawberry plants were considerably less attractive to pollinators than raspberry plants, with an average density of 6.4 pollinators per 100m^2 (mean ± s.d. = 3,556 ± 24 flowers), compared to an average of 18.6 pollinators per 100m^2 (mean ± s.d. = 1,934 ± 23 flowers) on raspberries. These figures are the equivalent of 0.91 pollinators per 500 flowers for strawberries, and 4.89 per 500 flowers for raspberries. Of 21 farms growing strawberries, 18 (86%) used commercial bumblebees on this fruit. While the majority purchased bumblebees for pollination early in the season (late April to June), 3 out of 18 farms restocked with additional colonies mid-way through the season. In contrast, nine of the 16 farms (56%) growing raspberries used commercial bumblebees on raspberries and these farms only bought bees once at the beginning of the season.

B. terrestris/B. lucorum, including commercial bumblebees, provided around half the pollinator visits for both crops averaged across all farms (57% of visits to raspberries and 46% of visits to strawberries, see Appendix 2.1). We estimated that around 16% of visits to raspberries and 29% of visits to strawberries were by commercial *B. terrestris*, and honeybees contributed approximately a quarter of visits to both crops (Appendix 2.1). Other bumblebee species together comprised 20% of pollinator visits for raspberries and 10% for strawberries; these included *B. lapidarius, B. pascuorum* and *B. pratorum*. *B. hortorum* was seen on raspberries but not strawberries. Hoverflies and other flies made up around 1% of visits to raspberries and 23% of visits to strawberries. Other pollinators included solitary bees which made 68 visits to strawberries and 23 visits to raspberries, and butterflies which were only observed visiting strawberries (5 visits); these were not analysed further. The pollinator counts were subsequently grouped into wild bumblebees (including our estimate of the number of *B. terrestris/B. lucorum* attributable to wild pollinators), commercial bumblebees (the remainder of *B. terrestris/B. lucorum* visits), honeybees and flies (including hoverflies).

A total of 17 of the 25 farms had wild flower strips on the farm with 11 leaving field margins unmowed to assist pollinators. Neither of these variables predicted the number of wild bumblebees on either raspberries or strawberries (Tables 2.2 and 2.3). Farmer management of commercial pollinators did, however, have an effect; commercial bumblebee numbers significantly increased with the number of colonies used on strawberries. Where farmers indicated that there were honeybees within flying distance of the farm, higher numbers of honeybees were seen on both raspberries and strawberries.

Table 2.2 Coefficients and standard errors for variables in the most informative observational model (lowest AIC) explaining number of visits by pollinator groups to strawberry flowers

Strawberries	***Observation level variables in best fit model***								
Pollinator group	Day	Day squared	Polytunnel	Flowers	Cloud cover (%)	Wind (0,1,2)	Rain (0,1,2)	Temp (°C)	Humidity (%)
Wild bumblebees	0.42±0.17*	-1.31 ± 0.15***	-0.20 ± 0.21	0.27 ± 0.11**	-0.22 ± 0.10*	-0.42 ± 0.13**	-0.84 ± 0.35*	0.20 ± 0.12 .	ns
Commercial bumblebees	-0.98±0.15***	Ns	0.11 ± 0.23	Ns	Ns	-0.28 ± 0.13*	-1.34 ± 0.41**	0.46 ± 0.12***	0.22 ± 0.12 .
Flies and hoverflies	1.69±0.17***	Ns	0.39 ± 0.30	Ns	Ns	0.61 ± 0.17***	0.41 ± 0.26	-0.34 ± 0.14*	-0.40 ± 0.14**
Honeybees (presence)	Ns	-1.34 ± 0.36***	1.28 ± 0.61*	Ns	-0.69 ± 0.28*	Ns	Ns	ns	ns
Honeybees (when present)	0.61±0.18***	Ns	1.10 ± 0.47*	Ns	-0.41 ± 0.14**	Ns	Ns	ns	ns

Strawberries	***Farm level variables in best fit model***	
Pollinator group	Management	Habitat
Wild bumblebees	Ns	Ns
Commercial bumblebees	0.0018 ± 0.000826*†	Ns
Flies and hoverflies	Ns	0.60 ± 0.21**¶
Honeybees (presence)	Ns	-0.16 ± 0.06**§
Honeybees (when present)	1.20 ± 0.56*‡	Ns

† Number of colonies bought. ‡ Honeybees known to be deployed nearby (yes or no). ¶ Proportion of urban area within 1km. § Proportion of natural habitat within 1km.

Table 2.3 Coefficients and standard errors for variables in the most informative observational model (lowest AIC) explaining number of visits by pollinator groups to raspberry flowers

Raspberries	***Observation level variables in best fit model***								
Pollinator group	Day	Day squared	Polytunnel	Flowers	Cloud cover (%)	Wind (0,1,2)	Rain (0,1,2)	Temp (^{o}C)	Humidity (%)
Wild bumblebees	1.48 ± 0.22***	-1.88 ± 0.32***	-0.02 ± 0.20	0.75 ± 0.11***	-0.36 ± 0.11***	Ns	Ns	ns	ns
Commercial bumblebees	Ns	Ns	-4.52 ± 1.26***	1.29 ± 0.59*	Ns	Ns	Ns	ns	ns
Honeybees (presence)	Ns	Ns	1.54 ± 0.71*	0.69 ± 0.44	Ns	Ns	Ns	ns	ns
Honeybees (when present)	1.55 ± 0.54***	Ns	0.19 ± 0.42	1.06 ± 0.26***	-0.52 ± 0.26*	1.15 ± 0.30***	Ns	0.76 ± 0.26**	ns

Raspberries	***Farm level variables in best fit model***	
Pollinator group	Management	Habitat
Wild bumblebees	Ns	Ns
Commercial bumblebees	Ns	Ns
Honeybees (presence)	Ns	-0.19 ± 0.08*§
Honeybees (when present)	1.18 ± 0.58*†	Ns

† Honeybees known to be deployed nearby (yes or no), § Proportion of natural habitat within 1km.

Honeybees were less likely to be found in polytunnels with closed sides than open sides. Commercial bumblebees, on the other hand, were more abundant in closed sided tunnels, likely reflecting the fact that commercial bees (when used) are more likely to remain within a tunnel with closed sides.

The factors influencing the abundance of pollinators differed between pollinator groups (Table 2.2 and 2.3). Wild bumblebees, commercial bumblebees and honeybees had similar responses to weather variables, reducing numbers with increasing cloud, wind and rain, and increasing with temperature. Flies, on the other hand, seemed to respond in the opposite way, increasing in number with increasing wind, rain and decreasing temperature. Numbers of flies visiting strawberries increased with the proportion of urban area within 1km of the farm. The probability of presence of honeybees on a farm declined with an increased proportion of natural habitat within 1km of the farm.

2.5.2 Exclusion Experiment

When pollinators were able to access flowers, a higher proportion of raspberries were first class (Appendix 2.2: mean = 91% first class, s.d. = 0.09), than when pollinators were excluded (Appendix 2.2: 28% first class, s.d. = 0.09) (Fig. 2.2a (i), $Z = 10.28$, $p < 0.001$). Raspberries were also heavier when pollinators were allowed to forage (Appendix 2.2: mean of 3.39g ±0.68 v 4.70g ±1.13) (Fig. 2.2b (i), $t = 2.11$, $p=0.051$). There was no relationship between raspberry quality and the number of pollinators in the transects during the ripening period (Fig. 2.2c (i), $Z = -1.21$, $p>0.05$).

Excluding pollinators from strawberries caused a decline in fruit quality by approximately 50% (0.4 vs 0.8 fruits reaching 1st class) (Fig. 2.2a (ii), $Z = 10.28$, $p < 0.001$). There was no significant difference in the weight of the strawberries grown with or without pollinators (Appendix 2.2: mean = 11.2g ±1.70 v 10.2g ±1.57) (Fig. 2.2a, Z

= -0.29, p>0.05). Total efficiency adjusted pollinator number was a significant predictor of the proportion of first class fruit when pollinators were allowed to forage (Fig. 2.2c (ii), Z = 2.55, p = 0.011).

2.5.3 Seasonal Complementarity

The variance of the abundance over time for all species at all farms ($Var\ (T)$) was 45.3 whereas the sum of the individual variances ($\sum Var(Xi)$) was 80.3, giving a variance ratio of 0.56 (see Appendix 2.3). A test statistic of below 1 supports the hypothesis that pollinator groups peak at different times across the season. The same conclusion was reached when the simulated values for each farm were analysed: comparing the simulated values with and without individual time components, the simulated values from the full model were 0.77 on average for the closed-sided tunnels (compared to 0.96 for the null model; W= 232183, p<0.001) and 0.76 on average for the open sided tunnels (compared to 0.93 for the null model; W = 282753, p<0.001). The results were consistent whether the abundance figures were adjusted for efficiency or not (Appendix 2.4).

2.5.4 Impact of Complementarity on Strawberry Yields

In both closed and open-sided tunnels there were insufficient pollinators for a high proportion of first class fruit early in the season, which coincides with commercial bumblebee use (Fig. 3.3).

The proportion of first class fruit in the mid-season is predicted to be low in closed sided tunnels if wild bumblebees are not present as honeybees (the other pollinator group present in abundance in mid-summer) are not abundant in this type of tunnel.

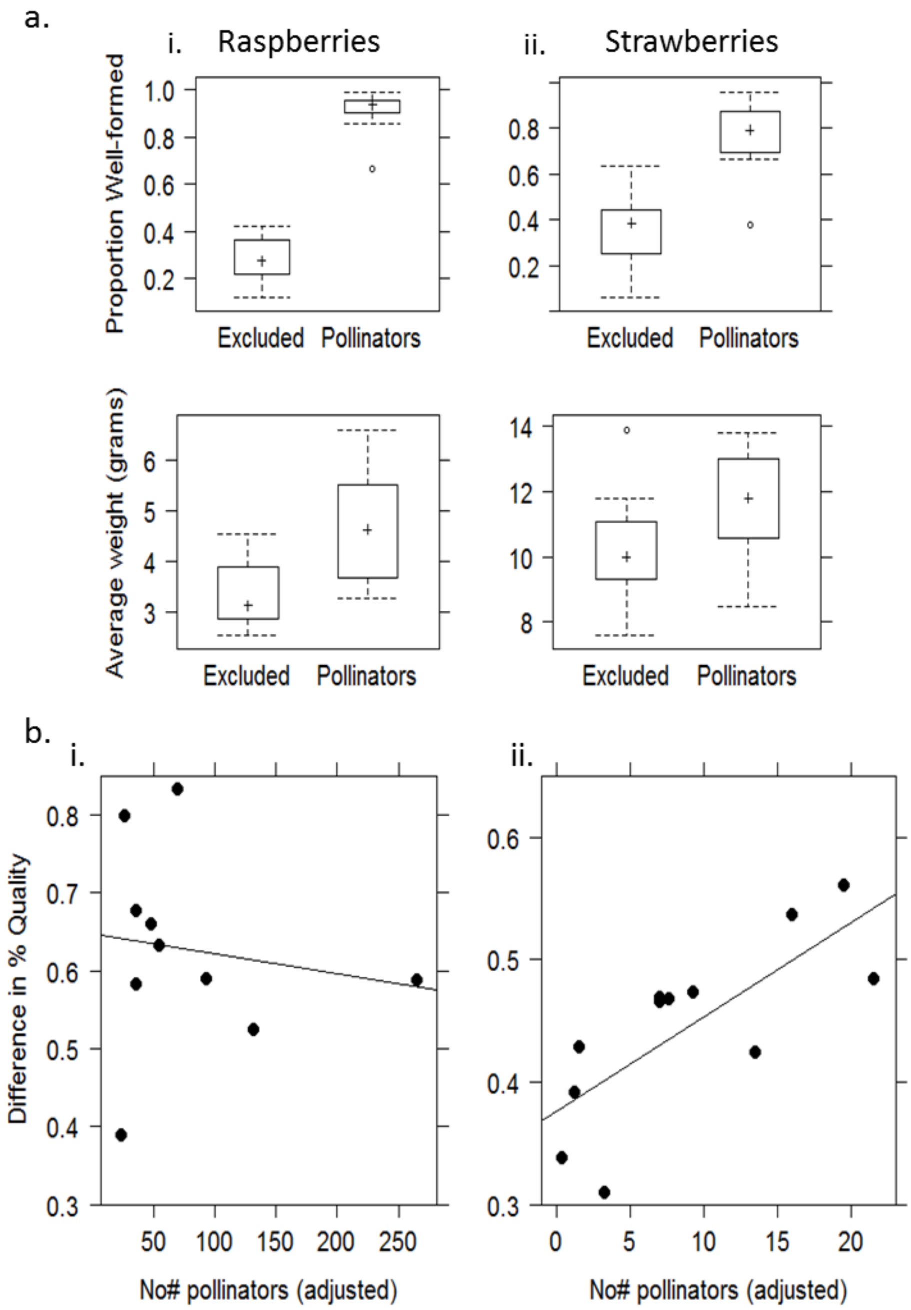

Figure 2.2 Effect of pollinator exposure and numbers of pollinators (adjusted for efficiency) on fruit quality and weight. (a) Proportion of class I fruit was higher when insects could visit flowers of (i) raspberries (t = 10.28, p < 0.0001) and (ii) strawberries (t = 10.43, p < 0.0001), weight of fruit was marginally significantly higher when insects could visit (iii) raspberries (Z = 2.11, p = 0.0513), but insects did not increase weight of (iv) strawberries (Z = 1.60, p > 0.05). (b) Fruit quality increased with the number of pollinators adjusted for efficiency in (ii) strawberries (treatment x adjusted pollinator number, t = 2.55, p = 0.011) but not (i) raspberries were no relationship was observed (t = -1.21, p > 0.05).

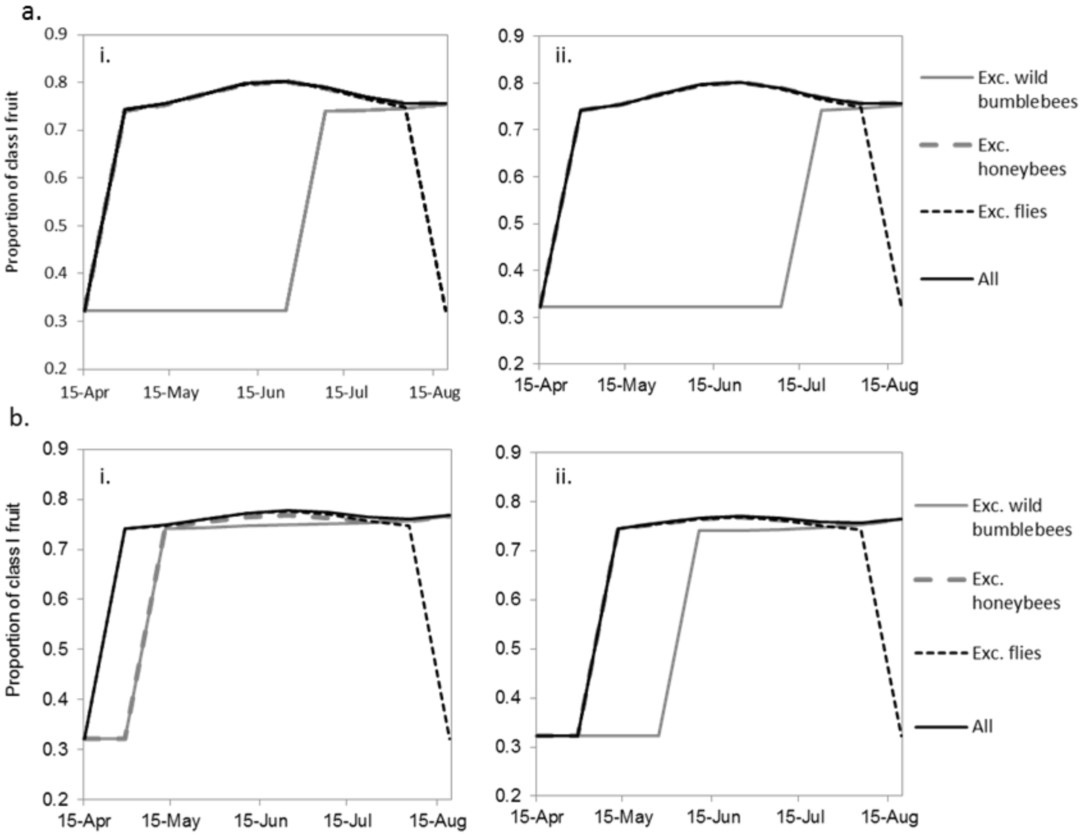

Figure 2.3 Simulated proportions of class I strawberries across the growing season with pollinator groups deleted. (a) closed-sided tunnels (i) Honeybees kept in the vicinity and (ii) honeybees are not kept within the vicinity. (b) Open-sided tunnels (i) honeybees kept in the vicinity (ii) honeybees not kept in the vicinity.

In open-sided tunnels, both honeybees and wild bumblebees pollinate during the middle of the season. Correspondingly the proportion of first class fruit does not drop as severely if wild pollinators are not present.

Flies were important for pollination at the end of the season for both tunnel types, and predicted aggregate yield fell on the removal of this pollinator group. In neither tunnel type are pollination visits sufficient for 80% pollination across the whole season, but with all pollinator groups present this target was more likely to be hit. Simulations were not run for raspberries as the quality and weight of raspberries was consistently high at all farms sampled, suggesting that pollination services are not limiting raspberry production.

2.6 Discussion

The pollination of strawberries throughout the year is facilitated by seasonal complementarity among both wild and commercial pollinators. Honeybees and wild bumblebees can provide pollination through the peak of the season, June and July, after which flies provide the bulk of pollination services. The potential for complementarity in seasonal abundance between different pollinator groups was suggested by Bluethgen and Klein (2011), but to our knowledge this is the first experimental evidence for such an effect. Our data supports the suggestion that species diversity can improve ecosystem services by increasing the functional range of the service provided.

Wild bee numbers were sufficient to provide adequate pollination for raspberries. Raspberries are more attractive to pollinators than strawberries and they have a shorter growing season, which coincides with the peak of wild bee activity. Despite this, commercially-reared bumblebees were used on half of the sites which grew raspberries. While commercially-reared bumblebees may not be necessary every year, there can be high variation in pollinator services between years; Lye *et al.* found that raspberry pollination was limited by lack of wild pollinators in an experiment in the same area in 2009 (Lye *et al.* 2011). The relative abundance of different species can change dramatically between years as observed on watermelon and oil-seed rape (Kremen, *et al.,* 2002). Smoothing out interannual variability in pollination services might be a justification for using domesticated bees on the farms studied.

There is no reliable way to distinguish commercial *B. terrestris* and wild *B.terrestris/lucorum* in the field. The proportions of *B. terrestris/lucorum* observed that were wild bees were estimated based on data from farms which do not use commercial bumblebees. This assumes that commercial bumblebee use itself does not reduce the number of wild bumblebees seen at a farm, and that there are not fundamental differences between farms that do and do not deploy commercial bumblebees that might affect wild bee abundance. We would expect commercial bumblebees to have an impact on wild bumblebees if densities of bees were high enough for competition to occur. This is unlikely on strawberries as the overall pollinator density was low. Densities were far higher on raspberries, so it is possible that the use of commercial bees could have affected the numbers of wild bees observed here. However any competition would presumably also affect *B. pascuorum* and *B. pratorum;* there were no differences in densities of these species between raspberry farms which used commercial bees and those that did not. It is reasonable to conclude that significant competition did not occur on the farms observed and that there are not major differences in wild bee fauna between farms that do and do not deploy commercial bees.

There were differences in the responses of the pollinator groups to weather experienced during the field study. Information on response diversity could be critical to managing pollination services over time; if a species of pollinator were to decline in abundance or reduce activity due to poor weather conditions, pollination may fall below the threshold required for a profitable harvest. In our system, this is particularly important for strawberries; even during May and June, the threshold for a profitable strawberry harvest was only just met by wild pollinators on the average farm. If different pollinator groups respond differently to weather conditions, the risk of pollination falling too low could be reduced by ensuring the presence of a diversity of species (Elmqvist *et al.,* 2003). However the bees in our study responded in the same way to weather variables; both bumblebee and honeybee activity was reduced with higher wind, rain and cloud cover. The ability of these bee groups to buffer the activity of the other in varying weather conditions is therefore limited. Conversely, flies seemed to respond in the opposite way to both *Bombus* and *Apis* bees, and were more likely to be seen on transects in wet weather and higher winds. Both *Bombus* and *Apis* species are sensitive to weather conditions experienced while leaving the nest, and may not forage in unfavourable conditions. *B. terrestris* were observed to cease foraging within tunnels when rain began, despite the polythene covering. In contrast, flies may seek shelter within the tunnels in poor weather (since they have no nest to retreat to), increased numbers of flies on the crop.

Different pollinator groups also responded differently to habitat surrounding the farms. Similar to Steffan-Dewenter and Tscharntke (1999), we found that honeybees were less likely to be observed on a transect with increasing natural habitat in the 1km surrounding the farm. This relationship might be because natural habitat provides floral resources that are more attractive to honeybees. No habitat variable tested influenced the numbers of bumblebees in our study. While some studies have found a declining relationship between social bee abundance and habitat isolation (reviewed by Ricketts *et al.,* 2008), other studies found no relationship between bumblebees and semi-natural habitat at any scale (Westphal *et al.,* 2006). Another study in the same region of Germany found a trend of increasing bumblebee numbers with more natural habitat at large scales, though this was not significant (Steffan-Dewenter *et al.,* 2002). There was also no relationship between wild bee visitation and proportion of natural habitat around watermelon crops across Pennsylvania and California, with the suggestion that other features in the farming landscape can provide bee habitat which can mitigate loss of semi-natural areas (Winfree *et al.,* 2007). Bumblebees also have relatively large home ranges (Knight *et al.,* 2005; Osborne *et al.,* 2008) which could account for their insensitivity to natural habitat within 1km in this study and others (Greenleaf *et al.,* 2007).

Fly abundance was positively related to the proportion of urban areas in the surrounding environment, and whilst come fly species are associated with human activity e.g. breeding in organic waste and refuse heaps (Goulson et al., 2005), the mechanism behind the correlation observed in this study requires further investigation. Gardens within urban areas may also provide floral resources that support pollinators (Goulson *et al.,* 2010), though it was notable that only flies showed a relationship with urban areas in this study.

While farmers could increase the number of commercial pollinators by buying more bumblebee boxes, or keeping honeybees near to the farm, the wild pollinator management prescriptions (wild flower strips and unmowed field margins) did not increase the visitation rate of any of the pollinator groups. Increasing floral resources has been seen to boost queen numbers in some bumblebees (Lye *et al.,* 2009), and is well known to attract large numbers of worker bumblebees (Kells *et al.,* 2001; Carvell *et al.,* 2007), but the link to increased pollination of nearby crops is less clear (Klein *et al.,* 2012). The pollinator management parameters we used were self-reported from the farm managers. Many of the farms that had wild flower strips were part of supermarket schemes to boost pollinators. However, the area requirement was generally very small (~0.2 ha) and it could be far away from the crop, with farmers reporting poor germination of some seed mixes. While such actions, if successful, may contribute to the abundance of pollinators on the farm (Haaland & Bersier, 2011), they are unlikely to significantly boost the number of bees on a crop unless they encompass a sizeable area, establish to provide a flower-rich sward, and are near to the crop plant requiring pollination.

Our data suggest that flies are important pollinators of strawberries in late season. Methods to increase fly populations or those of other non-bee pollinators have rarely been studied (although see Hickman & Wratten, 1996), but there is anecdotal evidence of mango farmers positioning animal carcasses near to crops to encourage flies, suggesting they are important in other fruit systems (Barbara Gemmill-Herren *pers comm*). It would be useful to investigate in more detail the particular species involved in strawberry pollination. For example, provision of breeding habitat for flies (which might include dung heaps for many calyptrate flies or butts of stagnant water for hoverflies such as *Eristalis* sp.) would require little space and minimal maintenance.

Despite not being efficient pollinators of strawberries, flies increase overall pollination services through their unique contribution to functional diversity; they increase the seasonal spread of pollinators and widen the range of weather conditions in which pollination can occur. Developing methods of evaluating the benefit of increasing number of species, which take into account the increase in function diversity will be important if the true contribution of species groups is to be appreciated.

2.7 Chapter 2- Appendix

Table 2.1. Overall number and percentage of total visits observed by different species of pollinator, and different pollinator groups, bold entries are pollinator groups used in analysis

	Raspberries		***Strawberries***	
	Visits to flowers	Percent of total visits (%)	Visits to flowers	Percent of total visits (%)
B. terrestris/B. lucorum	2502	57%	1141	46%
Of which commercial	**697**	**16%**	**706**	**29%**
Of which wild	1805	41%	441	18%
B. pascuorum	160	4%	21	1%
B. pratorum	487	11%	65	3%
B. lapidarius	131	3%	134	5%
B. hortorum	24	1%	0	0%
All wild bumblebees	**2607**	**59%**	**661**	**19%**
A. mellifera	**1029**	**23%**	**477**	**27%**
Solitary bees	15	0%	68	3%
Flies	55	1%	**561**	**23%**
Total	**4403**		**2473**	

Table 2.2. GLM (raspberries) and GLMM (strawberries) for the effect of efficiency adjusted pollinator numbers on percentage of first class fruit and fruit weight. A significant interaction between the treatment (pollinators present or excluded) and the adjusted number of pollinators signifies that increasing pollinator abundance increases the fruit set. This was the case in strawberries, percentage of class I fruit increased with adjusted number of pollinators, but not for raspberries where pollinators did improve fruit set, but sufficient pollination was achieved at all farms.

	Raspberries	Coefficient ± S.E.	Z/t	P=value		Mean ± S.E
% Class I	With pollinators	1.35 ± 0.13	10.28	<0.0001	With pollinators	0.91 ± 0.03
	x adj. pollinator no	0.00 ± 0.00	-1.21	Ns	Pollinators excluded	0.27 ± 0.03
Weight	With pollinators	1.46 ± 0.69	2.11	0.0512	With pollinators (g)	4.70 ± 0.36
	x adj. pollinator no	0.00 ± 0.00	-0.29	Ns	Pollinators excluded (g)	3.39 ± 0.22

	Strawberries	Coefficient ± S.E.	Z/t	P=value		Mean ± S.E
% Class I	With pollinators	1.78 ± 0.17	10.43	<0.0001	With pollinators	0.77 ± 0.04
	x adj. pollinator no	0.04 ± 0.02	2.55	0.0109	Pollinators excluded	0.35 ± 0.05
Weight	With pollinators	0.46 ± 0.66	0.69	Ns	With pollinators (g)	11.5 ± 0.49
	x adj. pollinator no	0.09 ± 0.06	1.60	Ns	Pollinators excluded (g)	10.2 ± 0.45

Table 2.3. Average number of each pollinator group per transect of strawberries, data used to calculate complementarity statistic.

Average number of pollinators per transect

Time Period	C bumblebees	W bumblebees	Honeybees	Flies	Sum
1	12.6	0.8	1.6	0.0	15.0
2	5.5	1.9	1.0	1.5	9.9
3	5.5	10.8	6.4	1.0	23.6
4	6.6	10.6	9.1	3.0	29.3
5	3.1	5.2	3.1	3.9	15.3
6	1.9	1.8	1.9	19.2	24.8
Variance	11.5	17.1	8.6	43.1	
				V of the sums	45.3
				S of the variances	80.3
				Complementarity	**0.56**

Table 2.4**.** Summary statistics for complementary values from 1,000 simulations of pollinators using the full GLMM model, and the null model with day and day squared terms removed. For each tunnel type the mean complementary statistic was lower when pollinators were generated from the full model (implying that pollinator groups did peak at different times). This difference was observable whether or not the abundances were adjusted by efficiency.

	Tunnel type	**Model**	**Mean**	**Variance**	**Wilcox statistic**	**P-value**
Pollinator abundance	Closed-sided	Full model	0.77	0.059	232183	<0.0001
		Null model	0.96	0.037		
	Open-sided	Full model	0.76	0.059	282753	<0.0001
		Null model	0.93	0.056		
Pollinator abundance adjusted by efficiency	Closed-sided	Full model	0.83	0.044	227422	<0.0001
		Null model	0.99	0.028		
	Open-sided	Full model	0.84	0.038	245408	<0.0001
		Null model	0.98	0.027		

Chapter 3- Experimental evidence that wild flower strips increase pollinator visits to crops

A version of this chapter has been submitted to *Ecology and Evolution*:

Feltham, H., Minderman, J., Park, K. & Goulson, D. Experimental evidence that wild flower strips increase pollinator visits to crops.

K. Park and D.Goulson supervised the project and J.Minderman provided advice on the statistical method used. All authors commented on draft versions of this manuscript.

3.1 Abstract

Wild bees provide a free and potentially diverse ecosystem service to farmers growing pollination dependent crops. Whilst many crops benefit from insect pollination, soft fruit crops, including strawberries are highly dependent on this ecosystem service to produce viable fruit. However, as a result of intensive farming practices and declining pollinator populations, farmers are increasingly turning to commercially reared bees to ensure that crops are adequately pollinated throughout the season. Wildflower strips are a commonly used measure aimed at the conservation of wild pollinators. It has been suggested that commercial crops may also benefit from the presence of non-crop flowers however, the efficacy and economic benefits of sowing flower strips for crops remains relatively unstudied. In a study system that utilises both wild and commercial pollinators we test whether wildflower strips increase the number of visits to adjacent commercial strawberry crops by -pollinating insects. We quantified this by experimentally sowing wildflower strips approximately 20 meters away from the crop and recording the number of pollinator visits to crops with, and without, flower strips. Between June and August 2013 we walked 292 crop transects at six farms in Scotland, recording a total of 2,826 pollinators. On average, the frequency of pollinator visits was 25% higher for crops with adjacent flower strips compared to those without, with a combination of wild and commercial bumblebees (*Bombus* spp.) accounting for 67% of all pollinators observed. This effect was independent of other confounding effects, such as the number of flowers on the crop, date and temperature. This study provides evidence that soft fruit farmers can increase the number of pollinators that visit their crops by sowing inexpensive flower seed mixes nearby. By investing in this management option farmers have the potential to increase and sustain pollinator populations over time.

3.2 Introduction

In the past few decades populations of both domestic and wild honey bees have fallen dramatically in some countries such as the UK and USA (Kremen et al. 2004; Potts et al. 2010). Concurrently, some bumblebee species have experienced substantial range contractions across both Europe and North America (Sárospataki et al. 2005; Carvell et al. 2006; Colla & Packer, 2008). Agricultural intensification is believed to be one of the key drivers of these declines (Goulson et al. 2008), but while modern agriculture may represent a hostile environment for pollinators, the number and extent of crops requiring pollination has increased. Approximately one third of global crops by volume and 84% of European crops benefit from animal pollination of some kind (Klein et al. 2007), with limitations in pollinator number likely to result in reduced reproductive potential of crops (Aizen et al. 2008). Insect pollination has been conservatively calculated to be worth around $3.07 billion per annum in the United States alone (Losey & Vaughn, 2006) making pollinator declines particularly concerning when considering the sustainability of our food production systems (Biesmeijer et al. 2006; Aizen et al. 2008; Aizen & Harder 2009; Goulson 2010; Potts et al. 2010; Ollerton, Winfree & Tarrant, 2011).

The soft fruit industry is growing rapidly worldwide, with production quantities of strawberries alone increasing by almost 40% between 2002 and 2012 (FAOSTAT). In Scotland the output value of soft fruit increased from £20 million to £74 million between 2001 and 2011, which coincides with a large scale move towards protected cultivation, e.g. using polytunnels. Strawberries are particularly dependent on insect pollinators to ensure a successful crop and the production of marketable fruit, and bee pollination has been found to improve shape, weight and shelf life of berries, increasing the commercial value of the fruit by 39% relative to wind pollination alone (Klatt et al. 2014).

In Scotland, farmers who produce strawberry crops on a medium to large scale rarely do so without the aid of polytunnels and commercial bees, the latter of which are usually purchased once or twice a season to help ensure adequate levels of pollination. Whilst the purchase of commercial bees represents a significant annual cost to many soft fruit farmers, wild bees provide a free pollination service. However there are concerns over the sustainability of wild pollinator populations due to recent declines. Although the relative contribution of wild and managed bees has been found to vary (Greenleaf & Kremen, 2006, Desjardins & De Oliveira, 2006; Lye et al. 2011), previous work has emphasized the importance of taking an integrated approach to pollinator management (Allsop et al. 2008; Garibaldi et al. 2013).

Financial support by way of agri-environment schemes can encourage farmers to manage their land for the benefit of wildlife, by creating or maintaining habitats favourable for pollinating insects, e.g. sowing wild flower seed mixes in dedicated areas, or strips within cropland. Such wild flower strips can provide forage for a range of pollinating species (Williams 2002; Carreck & Pywell et al. 2005; Carvell et al. 2007) and are thus likely to provide an effective method for increasing the abundance of these pollinators (Marshall et al. 2006). Research has also found that the abundance and diversity of pollinating species visiting crops is positively correlated with the availability of semi-natural habitat nearby (Ricketts et al. 2008), which is unsurprising given the requirements that many species have for suitable nest sites and a continuity of forage through the spring and summer (Richards, 2001). In order to maintain and restore wild pollinator communities farmers are often advised to create areas rich in plant diversity within agricultural landscapes, however this management choice is often poorly implemented (Carvalheiro et al. 2011).

In a recent study Blaauw and Isaacs (2014) created wildflower plantings adjacent to blueberry fields in order to determine their effect on the crop, and found that the use of medium to large flower strips increased the number of pollinators observed on highbush blueberries. Here we aim to test the prediction that the presence of wild flower strips can increase the number of pollinators visiting adjacent strawberry crops, whilst accounting for the potential confounding effects of date, temperature and the abundance of flowers on the crop. The flower strips used here were smaller than those sown in Blaauw and Issacs and will reveal if fairly small areas of land planted with wildflowers can be sufficient to increase the number of pollinators observed on nearby crops. Determining the minimum amount of land required to boost pollination services is likely to be important to farmers who have to pay the opportunity cost associated with not using the land for something else e.g. crop production. Whilst blueberry crops flower for a relatively short period of time the strawberry crop studied here can flower for many months and we seek to add insight into whether planted flower strips can increase crop pollination throughout a longer growing season.

3.3 Methods

3.3.1 Site selection and experimental protocol

We selected six farms in the central Scotland area that were owned by farmers who had previously expressed an interest in sustainable pollinator management, and who produced strawberries in a minimum of 10 polytunnels using a double cropping system. Double cropping involves growing two crops in the same space within the same growing season. In the case of strawberries this means that one seasons’ crop comes from two sets of plants. Crops that are planted and flower in the summer of one season also produce flowers the following spring, before being replaced by new plants. This creates a cycle allowing for continual fruit production from May to September.

We provided farms with 600g of wildflower seed (purchased from Scotia Seed Ltd., Angus, Scotland, UK) which contained a mixture of annual and biennial flowering species known to offer high pollen and nectar rewards (See Table 3.1 in Chapter 3 Appendix). This quantity of seed was sufficient to sow one 6m x 50m flower strip (at a recommended sowing rate of 2g seed/m^2); long enough to span the entrances of the 5 polytunnels containing strawberry plants. Flower strips were situated approximately 20 meters from the crop in order to prevent damage caused by regular vehicle access into the tunnels. One strip per farm was sown in spring of 2012 but three failed to establish sufficiently well due to particularly wet weather conditions and were re-sown in the spring of 2013. At each farm an area containing 5 polytunnels situated at least 500m away from the flower strip was selected to use as a control. An area of the same size and shape as the wildflower strips was marked out adjacent to these tunnels, with both treatment and control strips being located at field edges rather than between tunnel blocks. Treatment and control areas were selected to ensure that the tunnels surveyed at each farm contained the same strawberry variety. All of the farms surveyed stocked commercial bumblebee nest at a density of one nest per 100m tunnel. Nests used at treatment and control tunnels were purchased at the same time from the same company (either Koppert or Syngenta, farm depending) and therefore were at even stages of development upon arrival. Nests were positioned near to the centre of the tunnel and mounted on top of a small crate or suspended from the raised beds containing the crop, in order to prevent contact with the ground.

3.3.2 Pollinator counts

Each farm was visited throughout the growing season between June 12, 2013 and August 7, 2013, with visits commencing when the first flowers on the strip began to open. Three farms were visited six times and two were visited five times depending on the availability of flowers on the crop. One farm was only visited twice during the study because the farmer decided not to double crop and strawberry plants ceased flowering before six visits could be made, data from this farm was still included in all analyses. Farms were visited approximately once every seven days with surveys being carried out during dry weather conditions and when temperature exceeded 15°C. The treatment and control crops and strips at each farm were visited on the same day to try to ensure both were monitored during similar weather conditions and the order of visit randomised to avoid time of day bias.

At each farm, pollinators on the crop were counted using a modified version of the standard line transect method developed for butterfly surveys (Pollard 1977), with each of the 5 tunnels adjacent to the flower/control strip walked once per visit. Where polytunnels were longer than 100m, (20 out of 60 tunnels), only the 100m of crop closest to the strip was monitored. Counts were made by walking slowly through the centre of the tunnel, recording pollinators seen along a 2m wide transect. All bumblebees were visually identified to species and where possible recorded as workers, males or queens. Honeybees, solitary bees and hoverflies were also recorded as a range of insects have previously been found to pollinate strawberry plants(Nye & Anderson 1974; Oliveira *et al.,* 1991 Kakutani *et al.,* 1993). It is not possible to distinguish commercial *B.terrestris* and wild *B.terrestris* in the field and we were therefore unable to differentiate between wild and commercial bees of this species during the transect counts. Due to the difficulties in distinguishing the workers of *B. terrestris* and *B.*

lucorum in the field these species were pooled.. In order to account for variations in crop bloom we also counted the number of open strawberry flowers on each transect.

During each visit the number of bees found foraging on the treatment or control strip adjacent to the polytunnels were also recorded by slowly walking the length of the strip and recording all bees present. In addition to recording the species of pollinator observed, a record was made of the flower species that each individual was foraging on in order to examine the relative attractiveness to pollinators of the different species included in the seed mix. Due to high pollinator abundance on the strips, we were unable to count hoverflies during this survey, however all bumblebees, honeybees and solitary bees were recorded.

In order to monitor forage resources availability at the wildflower and control strips, a simple floristic index defined previously in Carvell et al. (2004) was used. During each visit all flowering species were identified and their abundance scored as (1) rare (approximately 1-25 flowers); (2) occasional (approximately 26-200 flowers); (3) frequent (approximately 201-1000 flowers); (4) abundant (approximately 1001+ flowers) or (5) super-abundant (more than 5000 flowers). A flower ‘unit’ was classed as a single flower or spike, or in the case of multi-flowered stems, one umbel or head (See Table 3.1 in Chapter 3 Appendix).

3.4 Statistical Analysis

Flowering plant abundance scores for the wildflower treatment and control strips were expressed as the median value for each range, to provide an estimate of the number of flowering units present on each visit. The estimated number of flowers available during each visit was then summed to give an overall floral abundance score for each strip per visit. All flowering species present contributed to this score, regardless of the number of pollinators recorded foraging on them during the course of this study.

We separately analysed the total number of pollinators on the crop and the number of bees on treatment and control strips using two Generalised Linear Mixed Effects Models (GLMMs) fitted using the glmmADMB package version 0.8.0 (Fournier et al. 2012) in R version 2.15.2.

First, the number of pollinators counted per visit per tunnel was analysed using a GLMM with a negative binomial error distribution. In addition to “treatment” (tunnel with or without flower strip) as the key fixed factor of interest, we included the year in which the strip was sown (as a fixed factor) and date, temperature (°C), and the number of open strawberry flowers (covariates) to account for potential confounding effects. To test whether the effect of treatment depends on the number of open flowers, date, or sowing year, we tested whether these three interactions were significant by adding each individually to the model. As the aim of the study was to look at the effect of the wild flower treatment accounting for random variation between farms (rather than to estimate farm specific effects) farm was included as a random factor and tunnel was nested within farm to account for the clustering and repeated measures of our design. The second GLMM modelled the number of bees counted on the treatment and control strips during each visit as a function of the key fixed effect of treatment. This was included as a fixed factor while accounting for the confounding effects of date, temperature, year in which strip was sown, the mean number of open strawberry flowers across the adjacent five polytunnels, and the floral abundance score (included as covariates). Farm was included as a random factor. The potential significance of interactions between treatment and year of sowing, treatment and date, and treatment and the mean number of open strawberry flowers was also tested as described above.

We present the results of full models including all main effects and provide a pairwise comparison of the full model and the full model minus each parameter using Likelihood Ratio Tests. Interactions are only included in the full model if significant. Unless otherwise stated all averages are means +/- standard error.

3.5 Results

3.5.1 Pollinators on the strawberry crop

During the course of the study 2,826 individual insects were observed foraging on the strawberry crop; 1,228 on control transects and 1,598 on treatment transects, equivalent to an average of 8.27 +/-0.55 pollinators per 100m transect in controls and 11.10 +/-0.61 on treatment transects. Sixty seven percent of the pollinators observed across all transects belonged to the genus *Bombus* (58% *B. terrestris/lucorum*, 4% *B. lapidarius*, 3% *B. pratorum* and 2% *B. pascuorum*).

Table 1. Parameter estimates and likelihood ratio tests of the GLMM for the abundance of all pollinators found foraging on the strawberry crop

Fixed Effects	***Estimate***	***Standard Error***	***Δ Log Likelihood***	***χ^2***	***χ^2 df***	***P***
Intercept	-332.402	119.201				
Treatment	0.221	0.079	-3.86	7.726	1	0.005
Flowers on crop	0.025	0.003	-31.63	63.272	1	<0.001
Temperature	-0.041	0.016	-3.32	6.638	1	0.009
Date	0.008	0.004	-1.51	3.031	1	0.082
Year of sowing[1]	0.291	0.235	-0.53	1.064	1	0.302
Random effect variance						
Farm	0.039					
Tunnel/Farm	<0.001					

[1] Strips established in second year

Hoverflies were slightly more abundant in treated crop polytunnels (2.84+/-0.46 per 100m transect) than in controls (2.31+/-0.44), with the inverse being true of honeybees, which were more likely to be observed on control transects than treated transects (0.61+/-0.13 and 0.21+/-0.07 per 100m transect, respectively), however, both honeybees and solitary bees were poorly represented on crop transects relative to *Bombus* spp. and *Syrphidae* spp.

On average there were 25% (22-33%) more pollinators on crops with experimentally sown wildflower strips nearby, compared to those without such strips (Fig. 1; Table 1). This effect was independent of date, year of sowing or the number of open flowers (p>0.1 for all interactions and they were therefore removed from the full model), and was found whilst accounting for the effects of a range of potentially confounding variables.

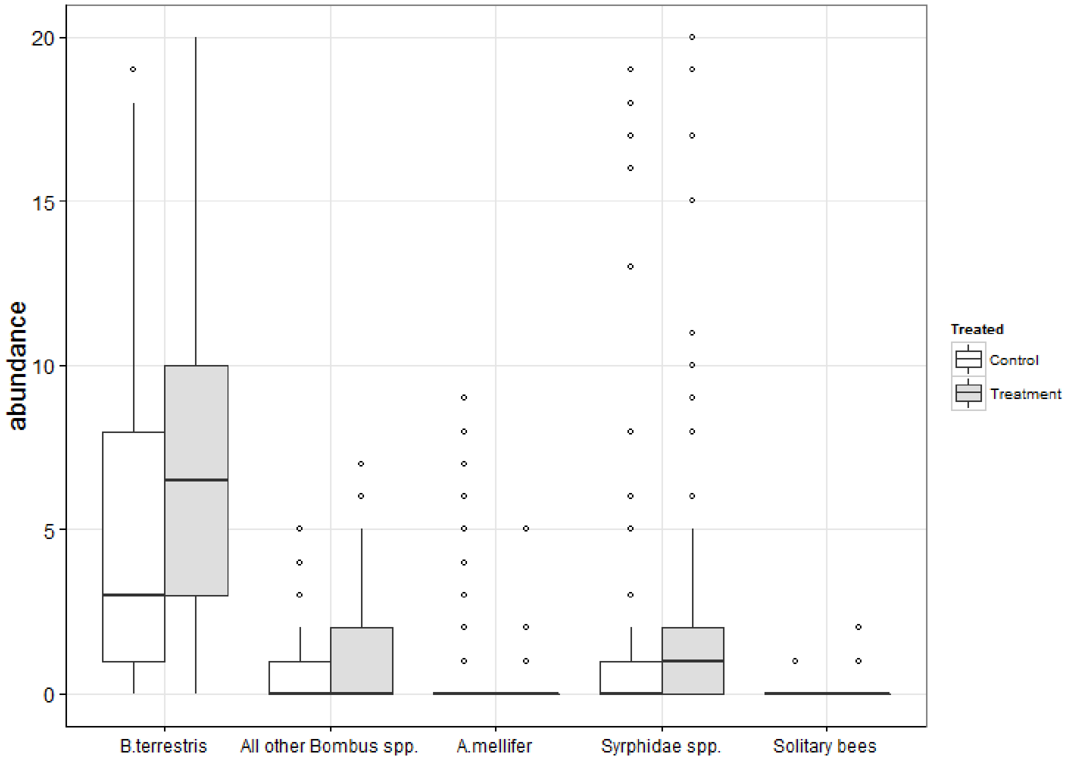

Figure 3.1. The abundance of pollinators on strawberry crops with and without a flower strip treatment. The box plots depict the median and interquartile range, with circles representing outliers. Whiskers represent the highest and lowest values excluding outliers.

Unsurprisingly, the number of pollinators found visiting the crop increased significantly with the number of strawberry flowers available on the transect, with temperature being the only variable to have a significantly negative effect on pollinator numbers.

3.5.2 Pollinators on the wildflower strips

Overall during the course of the study, 22 flowering plant species were recorded at wildflower treatment and control strips, including both sown and unsown species. They were visited by 1,757 pollinators, with 412 bees visiting 14 flowering species on control strips and 1,345 bees visiting 11 flowering species on treatment strips. Across all farms, 96% of bees recorded were *Bombus* spp. (56% *B. terrestris/lucorum*; 18% *B. pascorum*; 10% *B. lapidarius*; 11% *B. hortorum*; 1% *B. pratorum*) and 4% were *A. mellifera*, with 85% of pollinator visits to flowers of just four species: *Trifolium pratense*, *T. hybridum*, *T. repens* (Linnaeus) and *Phacelia tanacetefolia (*Benth). The most frequently visited species on control strips was *T. repens*, with 85% of all visits to this flower, whilst *P. tanacetefolia* when in flower attracted the most bees at treated strips (36%). There were more pollinators on treatment versus control strips (χ^2=22.55, df=1, p<0.001), however the floral abundance score was not a significant predictor of the number of pollinators observed (χ^2=0.002, df=1, p=0.96). Date significantly improved the model fit (χ^2=6.37, df=1, p=0.01) with a general increase in the number of pollinators being seen on strips as the season progressed. The number of pollinators on the strip was not significantly influenced by temperature (χ^2=0.44, df=1, p=0.506) or the number of open strawberry flowers on the nearby crop (χ^2=2.10, df=1, p=0.147). The only significant interaction was between treatment and the number of flowers on the crop, with significantly fewer bees observed on flower strips when the number of flowers on the crop was high (χ^2=9.214, df=1, p=0.002).

3.6 Discussion

The aim of this study was to test the prediction that the presence of wild flower strips can increase the number of pollinators visiting adjacent strawberry crops whilst taking into account other potentially confounding variables. The results presented here suggest that the abundance of pollinators, in particular bumblebees, found foraging on the crop can be significantly increased by the use of planted strips; with the model predicting an increase of pollinator abundance on crops of approximately 25% (22-33%) when flower strips were sown nearby. This effect was independent of date, the number of open crop flowers and year of sowing; suggesting that the presence of flower strips may increase pollination throughout much of the season. The most abundant species observed on both the crop and neighbouring strip was *B.terrestris* which is unsurprising given that *B.terrestris* is the most common bumblebee throughout most of the UK and is also the species used in commercial bumblebee nests stocked at farms. The inability to distinguish between wild and commercial individuals of this species means that we are unable to determine fully to what extent the flower strips sown in this study increased visitation of wild bees to the crop. The increased visitation could reflect more visits by wild insects, increased retention of the commercial bees in the crop area, or increased growth of the commercial bee nests. From a farmer's perspective these distinctions are not important; what matters is that the flower strips resulted in more pollinators on the crop.Highly attractive plants ('magnet-species', Thomson 1978) have been shown to increase the pollinator service to other neighbouring species (Johnson et al. 2003; Molina-Montenegro, Badano & Cavieres 2008; Cussans et al. 2010; Seifan et al. 2014) and it is likely that the flower strips used in this study function in a similar way.

It is likely that to attract bees to the crop area the flower strips used in this study need not have contained all of the species included in the mix. The majority (85%) of bees visiting the flower strips foraged on four species, three species of clover (*T. pratense, T. repens, T. hybridum*) and *P. tanacetefolia*. Whilst the three species of clover included in the mix are native, *P.tanacetefolia* is not and would preferably be replaced by another annual flowering species of native origin. Unsown white clover present within some control strips was effective at attracting bees, which may have reduced the contrast between pollinator counts on treatment and control crops. It is possible, therefore, that had white clover not been present at control strips then an increased effect of the treatment might have been seen.

In large fields, insect pollination of field beans has been found to be inadequate, with seed yields in plants at the edge of the field greater than those at the centre (Free & Williams 1976). Whilst the current study shows that flower strips can indeed boost the pollination service to nearby crops, further studies would be needed to examine how far into fields the effect of the flower strip extends. At large soft fruit farms fields can be sizeable, housing blocks of over 100 polytunnels, and in cases like these it is unlikely that effects of strips sown at the edge of the field will reach the centremost tunnels. However, it is worth noting that at all farms used in this study there were areas of unused land between and around tunnels where flower seed could be used to increase the abundance and diversity of forage around the crop, which may provide similar benefits to the flower strips created here.

Bees that feed on both wildflowers and the crop are likely to be carrying a range of pollen types and it is possible that this could affect quality of pollination they provide (Lopezaraiza-Mikel et al. 2007). Further studies are needed to test if the presence of wildflower strips increases heterospecific pollen transfer to the crop, and to quantify more explicitly how an increased pollinator abundance resulting from the use of flower strips translates into changes in crop yield throughout the season. The flower strips sown in this study did not start flowering until June and as such earlier flowering crops may remain heavily dependent on the service provided by commercial bumblebees to ensure sufficient pollination.

3.6.1 Economic analysis of pollinator management strategies

Over 80% of 29 soft fruit farms surveyed in Scotland purchased commercial bumblebees, with some farms using as few as 6 nests per season and others as many as 500 (Ellis & Feltham, unpublished data). Many farmers' stock bees at a rate of one nest per tunnel and individual nests cost approximately £32. There are additional labour costs involved in deploying the bees and also in the opening and closing of the doors to the nests before and after the application of certain pesticides, as well as disposal of nests after use.

The cost of seeds for sowing a flower strip of the dimensions used in this study is £62.64, and the strips provided an increased pollination service to five tunnels, making the cost per tunnel £12.53. The plant species most favoured by bees at treated strips were also some of the cheapest components of the mix, suggesting that the cost of the flower strips could be reduced with the inclusion of fewer species. This figure refers only to the cost of purchasing the seed for the strip and not to other costs associated with its management and establishment, for example the time and labour needed to prepare the land for planting and the cost of the diesel required to power the machinery needed to sow in the seed.

Whilst commercial bumblebee nests need to be replaced every year, flower strips can last multiple seasons (Carvell et al., 2004) and in this experiment were found to require minimal management (topping once in the autumn). The strips planted in this study were smaller than those used previously by Blaauw and Issacs (2014) and still successfully encouraged an increased number of pollinators onto the crop. In trying to establish the cost effectiveness of the different management strategies available to farmers it is worth noting that in some cases there may be an opportunity cost associated with the land that farmers use for the flower strip; that is the money that the farmers may forfeit by not using the land for something else, e.g. crop production (Morandin and Winston, 2006). Whilst it was possible to find 'spare' areas of land not otherwise being used at all of the farms in this study further research could focus on exploring the costs and benefits of different sized flower strips in relation to the additional crop pollination service they provide.

Bee visitation to strawberry flowers increases the proportion of fertilized ovules (Albano et al. 2009) and thus reduces the proportion of malformed fruit which is less economically valuable (Andersson, Rundlöf & Smith, 2012). Klatt et al. (2014) found that bee pollination increased the commercial value (shape, size, weight, shelf life) of strawberry fruits by 54% compared with self-pollination and 39% compared with wind pollination. Wind pollination of crops housed within polytunnels is likely to be less than those grown in open field situation which could results in a higher dependence on insect pollinators. Ellis et al. (unpublished data) found that without pollinators the yield of first class fruit in strawberry plants housed in polytunnels within the current study system is reduced by 50%. If increased pollinator visits resulting from sowing flower strips boosted the proportion of first class fruit achieved even by just 1% then farmers would be gaining an extra £1080 per hectare or £77.14 per tunnel per annum (based on the £3000/tonne output price for strawberries reported in the Economic Report on Scottish Agriculture, 2012). If the additional pollination increased the proportion of first class fruit by 5% these figures would go up to £5400 and £385.71 respectively. Whilst further work should focus on empirically testing what increase in strawberry yield occurs as a result of planting wildflower strips the inference of such calculations are supported by the work of Blaauw and Isaacs (2014) who found that the increase in revenue achieved as a result of higher yields more than offset the cost of establishing and maintaining the larger wildflower areas used in their study.

The results of our work suggest that sowing flower strips adjacent to crops which require pollination can significantly increase the number of pollinators found visiting the crop. A large number of pollinators were found foraging on the flower strips that were planted in this study suggesting that by investing in relatively cheap flower strips farmers are likely contributing to the creation of a more sustainable pollination service. Whilst the per tunnel cost of planting flower strips is considerably lower than the per tunnel cost of purchasing commercial bees, the economic gain resulting from both management choices needs further assessment, particularly given the difficulties within the current study system in accurately determining the relative abundance of wild and commercial *B.terrestris*.

This study emphasise the importance of considering integrated pollinator management strategies at soft fruit farms, whereby cheap seed mixes comprising clovers and *P. tanacetifolia* can be used to boost pollinator visitation to crops. Investing in flower strips provides a potential way to reduce reliance on commercial pollinators and provide insurance against future supply failure in the commercial bumblebee market. Given that agri-environment funding is often available to support the provision of pollinator friendly habitats this would appear to be a win-win situation for farmers.

Chapter 3 Appendix

Table 3.1. Flowering plant species included in flower mix

Species	*Common name*	*Annual/Perenial*	*Defined flower unit (Umble or head)*	*% of seed mix*
Centaurea cyanus	Cornflower	annual	head	10
Centaurea nigra	Common knapweed	perennial	head	10
Echium vulgare	Vipers bugloss	perennial	spike	4
Knautia arvensis	Field scabious	perennial	head	2
Lamium purpureum	Red Deadnettle	annual	spike	5
Papaver rheoas	Corn poppy	annual	head	10
Phaelia tanacetefolia	Phacelia	annual	umble	15
Prunella vulgaris	Selfheal	perennial	spike	10
Trifolium hybridum	Alsike Clover	perennial	head	10
Trifolium pratense	Red Clover	perennial	head	12
Trifolium repens	White Clover	perennial	head	12

Table 3.2. Species counts on treatment and control transects

	B. terrestris	*B. pascourum*	*B. lapidarius*	*B.pratorum*	*Syrphidae spp*	*A. mellifera*	*A. haemorrhoa*	*C. daviesanus*	*Other flies*	*Total*
Treatment	968	33	78	44	409	31	10	6	19	**1598**
Control	656	32	44	47	336	89	2	1	21	**1228**
Total	**1624**	**65**	**122**	**91**	**745**	**120**	**12**	**7**	**40**	**2826**

Chapter 4- Field Realistic Doses of Pesticide Imidacloprid Reduce Bumblebee Pollen Foraging Efficiency

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4.1 Abstract

Bumblebees and other pollinators provide a vital ecosystem service for the agricultural sector. Recent studies however have suggested that exposure to systemic neonicotinoid insecticides in flowering crops has sub-lethal effects on the bumblebee workforce, and hence in reducing queen production. The mechanism behind reduced nest performance, however, remains unclear. Here we use Radio Frequency Identification (RFID) technology to test whether exposure to a low, field realistic dose (0.7ppb in sugar water and 6ppb in pollen) of the neonicotinoid imidacloprid, reduces worker foraging efficiency. Whilst the nectar foraging efficiency of bees treated with imidacloprid was not significantly different than that of control bees, treated bees brought back pollen less often than control bees (40% of trips versus 63% trips, respectively) and, where pollen was collected, treated bees brought back 31% less pollen per hour than controls. This study demonstrates that field-realistic doses of these pesticides substantially impacts on foraging ability of bumblebee workers when collecting pollen, and we suggest that this provides a causal mechanism behind reduced queen production in imidacloprid exposed colonies.

4.2 Introduction

Around a third of all human food is thought to depend on insect pollination (McGregor, 1976) and many crops benefit from this service, with adequate pollination increasing yields and improving crop quality (Klein *et al.,* 2007). Recently, however, there has been growing concern over the use of neonicotinoid pesticides in agriculture and the sub-lethal effects they can have on pollinators (Decourtye *et al.,* 2004; Desneux *et al.,* 2007; Yang *et al.,* 2008; Aliouane *et al.,* 2009; Henry *et al.,* 2012; Whitehorn *et al.,* 2012; Gill *et al.,* 2012; Williamson & Wright, 2013; Di Prisco *et al.,* 2013; Matsumoto, 2013), which has culminated in an EU-wide restriction on the use of three neonicotinoid pesticides. The ban comes into place in December 2013 and is a temporary, two year measure preventing the use of imidacloprid, clothianidin and thiametoxam until further research can clarify the impact these pesticides are having on bees (European Commission, 2013). Sub-lethal effects do not bring about direct mortality but impair an organism's ability to function in some other way, for example by impacting on activity, fecundity, neurophysiology, learning performance or other aspects of behaviour (Desneux *et al.,* 2007; Laycock *et al.,* 2012). There is mounting evidence that neonicotinoid pesticides, formulated to target neurotransmitter receptors in insects, are negatively impacting the foraging behaviour of bees by inducing memory and learning dysfunctions, and impairing navigational skills (Henry *et al.,* 2012; Aliouane *et al.,* 2009; Yang *et al.,* 2008; Decourtye *et al.,* 2004). The continuing decline of pollinators such as bumblebees and honeybees, coupled with an increased focus on sustainable food production means that a greater understanding of the wider impacts of pesticides on pollinators is required.

Imidacloprid is the second most widely used agrochemical in the world (Pollak, 2011), and is commonly used as a seed dressing to protect crops from insect pests. The pesticides used in these seed dressings are transported throughout the plant via the sap, ending up in both pollen and nectar at concentrations typically ranging from <1 to 10 ppb (parts per billion) (Cresswell, 2011; EFSA, 2012). Oil seed rape is the second most abundant arable crop grown in the UK in terms of area (Garthwaite *et al.,* 2010) and its flowers are known to attract bumblebees, honeybees and other pollinating insects (Hayter & Cresswell, 2006). The majority of growers that produce oilseed rape do so using dressed seeds, with recent figures suggesting that only around 4% of seed sown in the UK remain untreated (Garthwaite *et al.*, 2010). Fields of rape offer a mass flowering crop that blooms for several weeks and bees that forage on these plants are thus exposed over large scales to trace dietary insecticides such as imidacloprid.

A recent study by Whitehorn *et al.* (2012) found that *B. terrestris* colonies exposed to field realistic doses of imidacloprid (0.7ppb in sugar water and 6ppb in pollen), produced 85% fewer queens than control colonies. Bumblebees have an annual cycle, with new queens single-handedly founding the next generation of nests (Goulson, 2010). There is evidence that only the most successful nests produce new queens (Müller & Schmid-Hempel, 1992) and reductions in the number of queens produced one year will likely have knock on effects for the number of colonies founded the next. However, Whitehorn *et al.* (2012) did not attempt to elucidate the mechanisms underlying reduced performance of treated colonies, which might include reduced fecundity of the queen, or reduced foraging efficiency of the workers. Gill *et al.* (2012) provide a possible explanation; they found that exposure to imidacloprid at 10 ppb in sugar water reduced the foraging success of worker bumblebees. However, the highest concentration of imidacloprid found in the nectar of seed-treated oilseed rape to date is 0.8ppb (EFSA, 2012). In this study we examined the effect of a lower, more field realistic dose of imidacloprid to determine what effects it may have on the foraging ability of *B. terrestris* workers.

4.3 Methods

Six commercial *B. terrestris* colonies (Biobest N.V., standard hives) were used to examine the effect of field realistic doses of the pesticide imidacloprid on foraging activity over a four week period. Upon arrival, nests were small, evenly aged and consisted of the queen and up to 65 workers. They contained two internal tanks which supplied the bees with sugar solution during transportation, and these tanks were sealed prior to controlled feeding to ensure that bees only had access to the 'nectar' provided as part of the experiment. Colonies of approximately equal weights were randomly allocated to either a treatment or control group (three in each). Control colonies were fed ad libitum (ad lib) with pollen and a sugar water solution for a period of 14 days in the laboratory. Over the same period colonies assigned to the treated group were fed pollen and sugar water containing 6ug kg^{-1} and 0.7ug kg^{-1} imidacloprid respectively, thus mimicking levels of imidacloprid found in oil seed rape (Bonmatin *et al.*, 2003). During the 14 day period bees were provided with no alternative forage.

After two weeks of controlled feeding in the lab, all colonies were placed out in the field and the foraging behaviour of bees was monitored over a four week period (07.08.2012 – 04.09.12). The study was carried out in domestic gardens in an urban area of Stirling in the Central-belt region of Scotland. The nearest farmed area was over 1km away. Bees were allowed to acclimatise to their surrounding for 24 hours. After this time the first 12 bees exiting each nest that had undamaged wings were collected. In treated nests this first batch of bees would have been individuals that consumed contaminated pollen and nectar during their adult life, however given the 9 day pupation phase they are not likely to have been larvae reared on food contaminated with imidacloprid (Van Der Steen, 2008). We used Radio Frequency Identification (RFID) technology to monitor the foraging duration of individual bumblebees, and an automated system to record the weight of bees entering and exiting the nest. RFID technology is increasingly being used to study the behaviour of insects (Robinson *et al.,* 2009: ants; Streit *et al.,* 2003; Molet *et al.,* 2008: bumblebees; Sumner *et al.,* 2007: paper wasps), and allows an accurate and automated way of monitoring their activity (Ohashi, *et al.,* 2010). A small RFID tag (mic3®-AG64 bit RO, iID2000, 13.56MHz system, 1.0x1.6x0.5mm; Microsensys GmbH, Erfurt, Germany) weighing 3mg (<3% of the weight of the smallest bee tagged) was glued to the dorsal surface of each bee's thorax. The weight of these tags was small relative to the average weight of nectar and pollen carried by bees; bumblebees are known to carry up to 90% of their own body weight (Goulson *et al.*, 2002). The tags were carefully positioned so that they would not hamper wing movement and bees were then released and left to forage independently for a period of four weeks. Treated and control colonies were randomly paired and each pair were monitored for a 24h period every third day (approximately). A fully automated system was set up to record the time and weight of bees departing from and returning to the nest: in a set-up similar to that used by Stelzer *et al.* (2010) a system of 2cm tubes were used to connect the entrance of each colony to a clear plastic box mounted on top of a balance (weighing to 3 decimal places). A small clearance gap was left between the tube system and the weighing box to ensure that only the weight of bees in the box was recorded. In most cases the time it took for bees to traverse the box was sufficient to get a stable reading. However on about a third of occasions there were multiple bees in the box at one time which meant it was not possible to obtain an accurate weight of any one individual bee. If this occurred when a bee was returning to the nest, the trip was excluded from the analysis of weight data. However if it occurred when a bee was leaving the nest then an average weight of that bee was obtained from other departures made during the monitoring period, and this was used as proxy in the analysis. After traversing the box, bees then entered another length of tube leading them to the outside. RFID readers were mounted between the nest entrance and the first length of tube, which recorded the exact time bees entered and exited the nest. A motion detecting camera was set up to record the weight of bees as they passed over the balance, and to determine if bees returned carrying pollen. As in Stelzer *et al.* (2010) any trips that lasted for five minutes or less as well as those flights where bees lost mass were excluded from analysis as the majority of these were likely to have been orientation or defecation flights. These trips only accounted for a small number of the total trips recorded and numbers were similar in treated and control colonies (only 15 trips in total, nine for treated and 8 for control bees). Additionally any trips over four hours in duration (seven in total, three for treated and four for control bees) were excluded from the analysis as these often occurred on rainy days where bees may have been prevented from returning to the nest due to adverse weather.

Fourteen days into the four week data capture period a further 12 bees from each nest were tagged. The development of *B.terrestris* workers in laboratory conditions includes ~14 day of larval development during which larvae are frequency fed, followed by ~9 days as a pupa. Thus in treated colonies, bees tagged at this point were likely to have been those reared on the pollen and nectar dosed with imidacloprid. At the mid-way stage of the experiment one control nest and one treated nest had to be removed from the experiment due to wax moth infestations; therefore no further bees were tagged in each of these colonies.

All statistical analyses were conducted using R version 2.15 (R Development Team, 2012). Generalised Linear Mixed Effects models (GLMM; fitted by maximum likelihood using the lme4 package) with Gaussian errors were used to test the effect of imidacloprid treatment on, trip duration, weight of forage collected and the foraging efficiency (mg of forage collected per hour) of individual bees. The time of day for each trip and the number of days since each bee was tagged were included as covariates along with treatment as a fixed factor. Individual and colony I.D. were included as random factors to account for pseudo-replication between and within colonies. ‘Batch’ (whether the bees were tagged at the start of week one, or the start of week three) was also included as a fixed factor. All two way interactions were included in the starting model. Factors that did not contribute significantly to the model were removed in a stepwise manner, using p=0.05 as a threshold for factor retention or removal. After each simplification step models were assessed using the Akaike’s information criterion (AIC; Akaike, 1974). When modelling both pollen and nectar foraging efficiency the most parsimonious model determined using the stepwise approach matched the model with the lowest AIC value.

A GLM, with quasibinomial errors to allow for over dispersion, was used to determine if there was a significant difference in the proportion of trips in which treated and control bees returned with pollen. The number of trips in which bees returned with pollen over the number of returns without pollen was modelled as the response variable and treatment and batch were included as fixed effects. Nest was included as a fixed effect in this analysis as, due to the relatively small sample size, models including nest as a random effect were unable to correctly separate out nest effects that were not due to treatment.

A Pearson's correlation was used to examine the relationship between time spent foraging and the weight of the load collected during each bout. Unless otherwise stated all averages are means ± standard deviation.

4.4 Results

Between 07 August 2012 and 04 September 2012 data were gathered from 256 foraging bouts; 21 foragers from control colonies were recorded making 113 foraging trips (5.4 ± 1.4 trips per bee), and 24 foragers from treated colonies made a total of 142 trips (5.96 ± 1.9 trips per bee). During the course of the study two bees from the treated group and one bee from the control group failed to return to the nest. There was no difference in the lifespan of bees from treatment and control groups, with all tagged bees (with the exception of the three that failed to return to the nest) surviving until the end of the study.

Control bees spent on average 25.44 ± 6.1 minutes foraging for nectar, with a mean weight of 42.6 ± 9.86 mg collected per bout, resulting in a nectar foraging rate of 101 ± 10.68 mg/hr^{-1}. This was not significantly different from the nectar foraging rate of treated bees (GLMM: χ^2=0.534, d.f=1, p=0.464; Fig. 4.1a) who spent on average 27.26 ± 8.4 minutes foraging for nectar, bringing back 44.7 ± 12.49 mg of nectar per bout resulting in a foraging rate of 99.24 ± 9.67 mg/hr^{-1}. Neither treatment nor any of the other proposed explanatory variables; time of day, batch and number of days since the bee was tagged, were significant in explaining trip duration, weight of nectar collected or nectar foraging efficiency.

The average length of time spent on pollen foraging trips (trips in which the returning bee had visible pollen loads) was 73.8 ± 14.38 minutes for control bees with a mean weight of 57.32 ± 11.22 mg being collected per bout, resulting in a pollen foraging rate of 47.71 ± 7.62 mg/hr^{-1}. The mean length of time spent on pollen foraging trips in treated bees was 77.85 ± 24.96 minutes, with the minimal model for trip duration including treatment, the number of days since the bee was tagged and the interaction between the two (GLMM: χ^2=9.99, d.f=1, p<0.01). Trip duration in control bees remained approximately constant throughout the experiment, however in treated bees the duration of trips increased with time from tagging. The mean weight of pollen collected per bout by treated bees was 41.07 ± 12.72mg, with treated bees bringing back significantly less pollen than control bees (GLMM: χ^2=4.76, d.f=1, p <0.01), with no other factors remaining in the minimal model. This resulted in a mean foraging rate for pollen of 32.97 ± 9.43 mg/hr^{-1}, a 31% reduction compared to control bees (GLMM: χ^2=18.06, d.f=1, p <0.001; Fig. 4.1b).

There was no significant effect of time of day, batch and number of days since individual bees were tagged in explaining pollen foraging efficiency. Treated bees were also significantly less likely than control bees to return to the nest carrying pollen (41% vs 65% of foraging bouts respectively; t= -2.135, n=42, p<0.05).

The positive correlation between time spent foraging for pollen and the amount of forage collected was significant in both treated bees (r=0.576, n=57, p<0.001) and control bees (r=0.729, n=71, p<0.001).

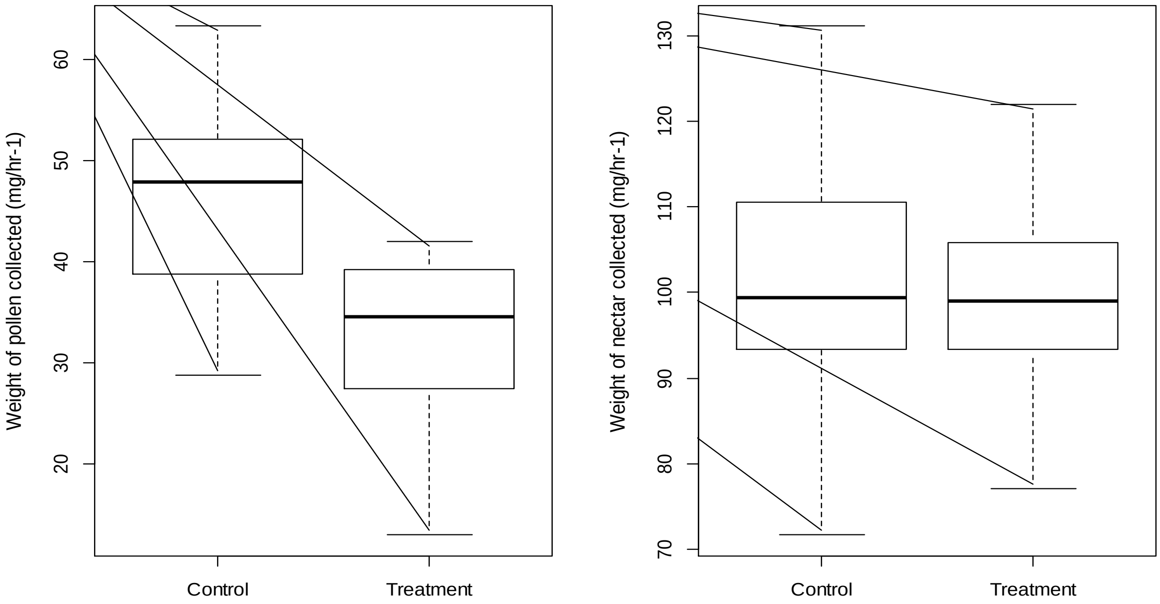

Figure 4.1 The efficiency of foraging on pollen gathering trips was significantly lower for treated bees than untreated bees, whilst no significant difference was found in foraging efficiency on nectar gathering trips. The box plots depict median and interquartile range, with the bars representing the means of treated and untreated bees and their 95% confidence interval.

In accordance with Whitehorn *et al.* (2012) treated nests gained less weight than control nests and also produced fewer workers and queens, but the number of nests used in our experiment was too few to permit meaningful statistical analysis of these differences.

4.5 Discussion

This study strongly corroborates the findings of previous studies, and shows that the neonicotinoid imidacloprid can have sub-lethal effects on free-flying worker bumblebees, and thus is likely to reduce colony success. Here we were able to quantify for the first time the change in bumblebee foraging efficiency as a result of field-realistic measures of imidacloprid exposure showing that, on pollen gathering trips, treated bees brought back 31% less forage per hour than controls, representing a significant reduction in efficiency. This is in accordance with the findings of Gill *et al.* (2012) who ranked the pollen loads of bumblebees returning to the nest as small, medium or large and found that imidacloprid exposed bees brought back proportionally more small loads than unexposed bees. Gill *et al.* (2012) also found that imidaclopid exposed bees collected pollen on 59% of their foraging bouts, versus control bees that collected pollen on 82% of occasions (a 28% decrease). Using a lower, field realistic dose we found that bees exposed to imidacloprid showed a 23% reduction in the frequency of pollen-collecting trips, compared to controls.

Pollen is the main protein source for bumblebees and is particularly important for the rearing of young to replace older workers (Harder, 1990). It has been suggested that foraging for pollen is more challenging than foraging for nectar (Raine & Chittka, 2007), and it is usually restricted to dry, sunny weather, whereas nectar can be collected in most conditions except heavy rain (Peat & Goulson, 2005), so that pollen rather than nectar shortages are more likely to limit colony success (Goulson, 2010). This is reflected in the lower foraging efficiency of bees when gathering pollen versus nectar (Peat & Goulson, 2005). Using the same concentrations of imidacloprid as the current study (6ppb pollen and 0.7ppb nectar), and the same two-week exposure period, Whitehorn *et al.* (2012) found an 85% reduction in queen production in colonies exposed to imidacloprid. Developing queens are known to require more food during their developmental period and thus queen production is likely to suffer as a result of lower provisions of pollen. Whitehorn *et al.* (2012) also found that colonies exposed to imidacloprid gained significantly less weight over time than control colonies. In previous work studying *B. lucorum*, a species closely related to *B. terrestris,* a positive correlation was found between nest size and queen production (Müller & Schmid-Hempel, 1992). Hence our data provide a simple mechanism for the dramatic declines in queen production described by Whitehorn *et al.* (2012); a substantially reduced pollen supply to the colony.

In this experiment we made the assumption that if a bee returned with a visible pollen load then any increase in weight recorded was due to the pollen it had collected whilst foraging. In some cases however it is likely that bees foraged for a mixture of pollen and nectar. It is thus possible that the lower weight of forage brought back by bees exposed to pesticide was due to reduced nectar collection, or a combination of reduced pollen collection and reduced nectar collection. The former seems less likely since bees which returned only with nectar showed no significant impact of pesticide treatment.

It is worth noting that in the present study bumblebees were kept in the lab for two weeks and treated colonies were given no alternative but to feed upon pollen and nectar dosed with imidacloprid. This is perhaps unrealistic of field conditions as bees would normally be free to forage on a range of contaminated and uncontaminated resources. However, oil seed rape is the third most abundant arable crop grown in the UK (after wheat and barley) with the production area for this crop having increased by 17.75% between 2010 and 2012, representing an increase of 114,000 hectares (Garthwaite 2010; DEFRA 2012). Rape is known to flower for around 3-4 weeks providing an abundant, if short-lived floral resource (Goulson *et al.* 2010). Both bumblebees and honeybees feed on oil seed rape (Hayter & Cresswell 2006) and given the general decline in floral resources in the countryside (Carvell *et al.* 2006) it is likely that, whilst in flower, oil seed rape constitutes a large component of many bees' diets. A recent study by Thompson *et al.* (2013) that examined the effects of three neonicotinoids on bumblebee colonies in field conditions failed to establish a negative control, demonstrating that these substances are widespread in agricultural environments. Furthermore, substantial concentrations of neonicotinoids (up to 9ppb) have been found in wildflowers growing near to treated crops (Krupke *et al.*, 2012), suggesting that exposure to these types of pesticide might not be restricted to bees foraging on the crops themselves. Hence we suggest that the level of exposure used here is likely to approximate that experienced by some wild bumblebee nests under field conditions.

Interestingly, we found no significant difference in foraging efficiency between the first batch of foragers tagged and the second. This suggests that impaired foraging continued to be seen in bees from treated colonies for at least four weeks after exposure. However, it is not clear whether this occurred because bees continued to be exposed to imidacloprid in honey stored within the nests, or whether the reduced performance of the second batch of foragers was due to exposure as larvae. Yang *et al.* (2012) describe impaired learning in honeybees following exposure to imidacloprid as larvae. Further studies could clarify the persistence of imidacloprid within the nest and the effects it may have on subsequent generations of workers. Whatever the mechanism, our data suggest that exposure to imidacloprid may reduce worker performance for at least four weeks after the source of exposure is removed.

Whilst this study has put forward a mechanism for reduced queen production in imidacloprid exposed colonies (Whitehorn *et al.*, 2012) it is not able to fully explain the mechanism behind the reduced pollen foraging efficiency found in treated bees. In both treated and control bees a positive correlation was found between time spent foraging and the weight of pollen collected and no significant difference was found in the number of bees failing to return to the nest between treatments, which, coupled with the fact that there were no differences found in nectar foraging efficiency, suggests that navigation is not likely to have been the issue. Further studies are needed to clarify how imidacloprid impairs bumblebee's ability to forage for pollen, with the evidence gathered in this study suggesting that the pesticide may either reduce motivation to collect pollen, or impair the bees' ability to collect pollen from flowers, rather than affecting their trips to and from their foraging sites.

Agricultural intensification has been proposed as a primary driver behind the decline of bumblebees, with habitat loss as well as increased pesticide use believed to be two important causal factors (Goulson *et al.*, 2008). Whilst this study has focused on the effects of imidacloprid, the uses of other neonicotionoids have also been called into question, since the three most commonly used compounds, imidacloprid, thiamethoxam and clothianidin all have similar modes of action (Nauen *et al.*, 2003). A recent study has reported harmful effects of thiamethoxam on honeybee homing abilities (Henry *et al.*, 2012), whilst another found no significant impact of thiamethoxam on colony initiation in bumblebees (Elston *et al.*, 2013).

If ecologically and economically important pollinator populations are to be maintained then the advisability of any future use of neonicotinoids on flowering crops must be questioned and further work is needed to clarify their impact.

Chapter 5- Frequency and severity of wax moth (*Aphomia sociella*) attacks on bumblebee colonies used for crop pollination

Feltham, H., Whitehorn, P., Park, K. & Goulson, D. Frequency and severity of wax moth (*Aphomia sociella*) attacks on bumblebee colonies used for crop pollination.

P.Whitehorn, K.Park and D.Goulson all supervised the project and P.Whitehorn also assisted with fieldwork and nest dissection. All authors commented on draft versions of this manuscript.

5.1 Abstract

Increasing numbers of farmers are purchasing colonies of commercially reared bees in order to supplement wild pollinators. To date no study has assessed the impact of one potentially important parasite of bumblebee nests: the wax moth. Here we investigate the frequency with which wax moths infest commercial *B. terrestris* colonies, and examine the likelihood that a nest, once infested, will go on to be destroyed by the wax moth larvae. We examine if a biological Lepidopteran larvicide (Certan™) can reduce the likelihood of harmful infestations and investigate the relationship between density of commercial nest use and frequency and severity of wax moth attacks. Almost half (44%) of all nests used in the study were infested with wax moth larvae and 34% of infestations led to nest destruction. The use of Certan™ did not significantly reduce the likelihood of an attack but did reduce the probability that an infested nest would go on to be destroyed. We found that the relative frequency of wax moth infestations increased with the density of commercial nest use and therefore suggest that management be put in place, in order to reduce infestations and maximise the pollination service provided by commercial bees.

5.2 Introduction

Approximately one third of global crop production is believed to depend on insect pollination (Klein *et al.*, 2007) with an adequate pollination service often increasing both the yield and quality of crops (Dimou *et al.*, 2008). The health of pollinators has caused much concern in recent years with declines being reported in both wild and domesticated bee populations across the globe (Biesmeijer *et al.*, 2006; Grixti *et al.*, 2009; Potts *et al.*, 2010). Bumblebees are particularly efficient pollinators due to their tolerance of low temperatures (Corbet *et al.*, 1993), and their ability to buzz-pollinate (de Luca & Vallejo-Marin, 2013) and thus provide an important ecosystem service for both wild and managed systems (Kevan *et al.*, 1991; Memmott *et al.*, 2004).

Land-use change, habitat loss and the resulting decreases in the availability of suitable forage and nesting sites are often blamed for reduced wild bumblebee numbers (Goulson *et al.*, 2008; Potts *et al.*, 2010) and although many studies have focused on the causes of bumblebee declines, relatively little attention has been paid to the impact of predators and macro-parasites on bumblebee health. Birds and spiders are thought to be the main predators of bumblebees in temperate regions, but there have also been reports of badger, fox, mole, weasel, shrew and vole attacks on nests (Goulson, 2010). Social insects are prime targets for parasites due to their abundance, family structure and persistent colonies (Schmid-Hempel, 1998). Bumblebees play host to a number of bacterial, viral and protozoan diseases, and individuals can be parasitised by mites, nematodes and parasitoids (Liersch & Schmid-Hempel, 1998).

The wax moth (*Aphomia sociella* Linnaeus, 1758), is a specialist parasite of bumblebee colonies (Cumber, 1949; Goulson, 2010), and was regarded by Hoffer (1882-1883) as being one of their most serious enemies (note that honeybees are attacked by two unrelated ‘wax moths’, *Galleria mellonella* and *Achroia grisella* Linnaeus, 1758). Despite being known to cause substantial damage to bumblebee nests, *A. sociella* have, however, received only a relatively brief mention in a handful of publications (Goulson *et al.*, 2002; Pelletier & McNeil, 2003; Spiewok & Neumann, 2006). Almost nothing is known of the frequency with which nests are infested, and there are no data quantifying the damage that they inflict.

Adult *A. sociella* are believed to emerge in June (Alford, 1975), although individuals have been found in Scotland as early as May (P. Lintott pers. comm). Mated *A. sociella* females lay their eggs inside the nests of bumblebees and the larvae that hatch develop within the host nest, consuming nest materials and food stores as well as waste products (Gambino, 1995; Goulson, 2010). They create silken tunnels to move through the nest and the webbing they spin is dense and difficult to penetrate, presumably protecting the larvae from adult bees. Larger larvae can consume bee pupae (pers. obs) and heavy wax moth infestations have been known to completely destroy healthy bee colonies, with the bumblebees appearing to have little defence. When fully grown, the larvae then overwinter in tight clumps of pupae close to the nest they inhabited, emerging as adults the following spring when the cycle begins again. Wax moths do not kill their host as a pre-requisite for successful development (this distinguishes them from parasitoids), however their presence within a nest has the potential to substantially reduce colony fitness and will likely lead to an increased rate of nest mortality.

In a study examining floral resource availability and growth rates of bumblebee colonies, Goulson *et al.* (2002) found that the nests in urban areas were significantly more prone to wax moth attacks than those in agricultural settings (80% and 17%, respectively). They speculate that this is likely to reflect the comparatively high density of bumblebee nests found in urban areas (Osborne *et al.*, 2007), enabling wax moths to persist at elevated densities. Bumblebee nest density may be artificially increased in certain agricultural areas due to the use of commercially reared bumblebees. Such commercial colonies have been used since the 1980s to supplement pollination of crops such as strawberries, raspberries, tomatoes, melons and cucumbers (Velthuis & van Doorn, 2006) and more than 1 million nests are produced each year globally (Greystock *et al.*, 2013). Classical models by Anderson and May (1978) predict a positive correlation between host density and parasite prevalence, which has been supported by a number of empirical studies (Lloyd & Debas 1966; Arneberg et al., 1998; Krasnov et al., 2002).

If wax moths are indeed more common where their hosts are at higher densities (Goulson *et al.*, 2002) then the use of commercial bumblebee colonies over the years may have led to a local increases in wax moth populations. This could result in a higher frequency of wax moth attacks in areas where commercial nests are used at high densities, with knock-on effects to pollination services leading to a reduction in crop yields or the need to buy more colonies. Farmers may therefore wish to manage their colonies to reduce the likelihood of wax moth infestations. The wax moths of honeybees (*G. mellonella* and *A. grisella*) can be controlled with Certan™, a product containing the microorganism *Bacillus thuringiensis* (Burges & Bailey, 1968; Ahmad *et al.*, 1994). To date no study has examined if this Lepidopteran larvicide could be used to manage infestations in commercial bumblebee colonies, and whilst Ings *et al.* (2006) did use Certan™ to treat the commercial nests that were being used in their research, they did not report on its efficacy. As well as potentially resulting in a diminished pollinator supply to crops, an increase in wax moth attacks could lead to spill-back effects on wild bumblebee populations. It is therefore important to get a better understanding of the impact that these predators have on bumblebee nests and colony survival.

The aims of this study were:

1. Determine the frequency with which bumblebee nests at soft fruit farms become infested with wax moth larvae;
2. Determine if the intensity of an infestation impacts the probability of premature nest destruction or reduced reproductive success;
3. Investigate the relationship between the density of commercial nests and frequency and severity of wax moth attacks and,
4. Examine whether using Certan™, a biological control agent, can reduce the frequency or severity of wax moth attacks.

5.3 Methods

Seven soft fruit farms that import commercial bumblebee colonies in East and Central Scotland were selected for this study, selected farms were at least 5km from each other.

Twenty colonies were randomly selected at each farm upon delivery and weighed. Colonies used in the experiment came from one of two suppliers; Koppert (Natupol Beehive, Koppert Biological Systems) and Syngenta (Beeline, Syngenta) and on arrival each nest included a queen and between 50 and 100 workers. Nests of similar weights were paired and one of each pair was randomly assigned to a treatment or control group. Nests allocated to the treatment group were sprayed with B401®/CERTAN^{TM} and spraying took place between 10 April and 10 May 2013, with the date varying in accordance with when nests arrived at farms.

As recommended by the supplier, the B401® solution was diluted to 5% with water and each nest was sprayed twice on top of the nest and twice underneath (a total volume of 5ml per nest). Control nests were sprayed with an equivalent quantity of water. Treatment and control nests were then randomly assigned throughout a block of polytunnels containing the first flowering strawberry crop and allowed to forage naturally for the duration of their lifespan. Nests were checked every two weeks and collected in when they had five or fewer workers remaining (between 15 July and 17 August 2013).

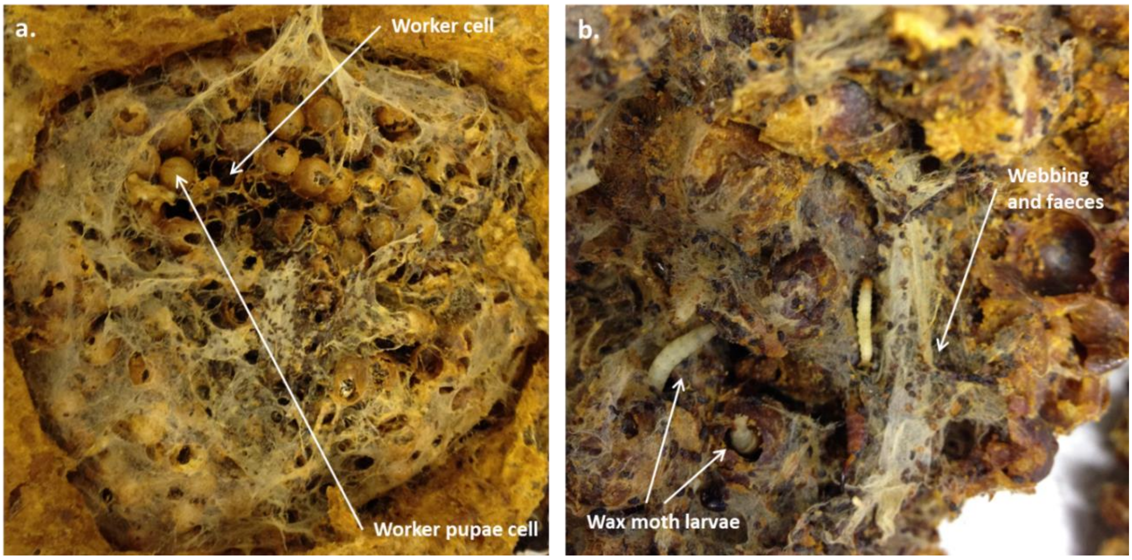

Figure 5.1 a) Bumblebee nest showing the silk webbing typically present as a result of a wax moth infestation. This nest was not classified as destroyed as worker cells and pupae are clearly distinguishable. b) Section of a severely infested nest with wax moth larvae and faeces clearly visible. Here most nest parts were damaged to the point that they were no longer identifiable and the nest was thus classified as destroyed.

After collection, each nest was placed in a -80°C freezer to humanely kill any remaining bees and then moved to a -40°C freezer for storage. Each nest was then dissected and the following information recorded: final weight of nest, number of empty worker cells, number of empty queen cells, number of worker pupae, number of queen pupae, number of wax moth larvae present and weight of wax moth larvae present. A nest was classified as destroyed if the damage inflicted was so severe that it prevented the elements of the nest from being counted (in heavily damaged nests there are few entire cells or pupae, and the wax structure is highly fragmented, see Fig. 5.1).

Farmers were asked to supply information on the number of nests in use at a farm, whilst knowledge of the local area and ArcGIS (version 10) were used to determine the number of farms using commercial bumblebees for pollination within a 5km radius of each farm. As little is known about the dispersal of wax moths a conservative radius of 5km was used to assess the effect of commercial nest density on infestation.

Of the 140 nests at the start of the experiment, 45 were damaged by farm machinery or accidentally disposed of before collection by farm workers, reducing the overall number of nests that could be included in the analysis to 95.

5.3 Statistical analysis

The likelihood of a nest becoming infested was analysed using a GLMM with binomial errors, with treatment included as a fixed factor and initial nest weight, date of deployment, the amount of time a nest spent in the field, the number of nests in use at the farm and the number of farms within 5km using commercial bees, included as covariates. Prior to inclusion in the model all variables were tested for colinearity. In all models farm was included as a random factor.

The likelihood of an infested nest then going on to be destroyed (or not) was examined using a GLMM with binomial errors and the same variables previously listed, with the number of wax moth larvae within each nest included as an additional covariate.

The number of queens, pupae and worker bees within each nest were each, in turn, modelled as the response variable to examine the effect of wax moth abundance on reproduction. The glmmADMB package was used to account for over dispersion and zero-inflation, and in each case treatment was included as a fixed effect, with start weight and the date that a nest was deployed included as covariates.

Finally the number of wax moth larvae within nests was modelled as the response variable to examine potential effects of nest density: the number of nests used at each farm and the number of soft fruit farms within a 5km radius using commercial bees were included as fixed effects. The initial weight of each nest and the number of days it spent in the field were included as covariates and farm as a random effect. The glmmADMB package was again used to address over dispersion and zero-inflation within the data.

The MuMIn package (Barton, 2014) was used to generate pseudo-R^2 values (hereafter R^2 values); to enable the assessment of model fit (Nakagawa & Schielzeth, 2013). We present the results of full models including all main effects and provide a comparison of models excluding each parameter in turn using Likelihood Ratio Tests. Unless otherwise stated all averages are means ± standard error.

5.4 Results

5.4.1 Frequency of infestation

Of the 95 nests that were collected in at the end of the study, 42 had been attacked by wax moths, with 44% of nests treated with Certan™ and 45% of untreated nests suffering from infestations. The mean number of wax moth larvae found within infested nests was 102.98±3.15 and there was a significant increase in the likelihood of a nest being infested when higher numbers of commercial bees were in use at a farm (P =0.005, Table 5.1, Fig. 5.3b). The number of farms stocking commercial bees within a 5km radius was also significant (P=0.047, Table 5.1) with the model predicting that the likelihood of an infestation occurring doubles as the number of farms nearby increases from 0 to 1 (Fig. 5.3a). The initial weight of a nest was marginally significant in explaining infestation, with heavier nests being more likely to become infested (P=0.055, Table 5.1) and nests deployed later in the season were significantly more likely to suffer from an attack (P=0.041, Table 5.1).

Table 5.1 Parameter estimates and likelihood ratio tests of the GLMM for nest infestation.

Fixed Effect	***Estimate***	***Standard Error***	***Δ Log Likelihood***	χ^2	**df**	**P**
Intercept	-0.203	0.418				
Treatment[1]	-0.872	0.620	-1.049	2.097	1	0.148
Days in field	0.015	0.565	-0.001	<0.001	1	0.979
Date deployed	0.826	0.441	-4.196	4.196	1	**0.041**
Initial weight	-0.757	0.397	-1.843	3.686	1	0.055
Number of farms 5km	0.787	0.383	-1.973	3.945	1	**0.047**
Number of nest at farm	1.082	0.323	-4.014	8.027	1	**0.005**
Random effect variance						
Farm	<0.001					
Maximal model R^2 value	0.58					

[1] treated nests

5.4.2 Nest destruction and reproductive success

Of the 42 nests that were infested, 15 nests (36%) went on to be destroyed by the wax moth larvae. The number of pupal cells (GLMM: χ^2=9.134, d.f=1, p=0.003) and worker cells (GLMM: χ^2=5.272, d.f=1, p=0.022) were significantly higher in nests with large wax moth infestations, indicating that larger nests have heavier infestations. The only significant predictor of queen production was date of nest deployment, with nests put out later in the season ultimately producing more queens (GLMM: χ^2=5.346, d.f=1, p=0.021), however it is worth noting that the number of queens, worker cells and pupae could not be counted in the most heavily damaged nests.

Table 5.2 Parameter estimates and likelihood ratio tests of the GLMM for the abundance of wax moths within nests.

Fixed Effect	*Estimate*	*Standard Error*	*Δ Log Likelihood*	*χ^2*	*df*	*P*
Intercept	5.114	5.134				
Treatment[1]	-0.205	0.432	-0.12	0.226	1	0.635
Days in field	-0.576	0.321	-1.31	2.604	1	0.021
Initial weight	-0.003	0.013	-0.03	0.070	1	0.791
Number of farms 5km	2.012	0.256	-8.48	16.944	1	**<0.001**
Number of nest at farm	0.026	0.004	-5.9	11.792	1	**<0.001**
Random effect variance						
Farm	<0.001					

[1] treated nests

5.4.3 Commercial nest use and the severity of wax moth attacks

Both the number of nests in use at a farm and the number of other farms using commercial bees within 5km were highly significant in predicting wax moth abundance (Table 5.2), with increased densities of nests or farms resulting in increased wax moth numbers in nests.

5.4.4 Effectiveness of Certan™ *treatment*

The mean number of wax moth larvae in nests treated with Certan™ was 29.72±11.17 compared with 56.54±15.765 in control nests, however due to high variability in the data (Figure 3a) the effect of treatment on the likelihood of infestation (Table 5.1) and wax moth abundance (Table 5.2) was not significant. Treatment was marginally significant in reducing the likelihood of destruction (P =0.064, Table 5.3), with 21% of control nests being destroyed compared to only 10% of treated nests.

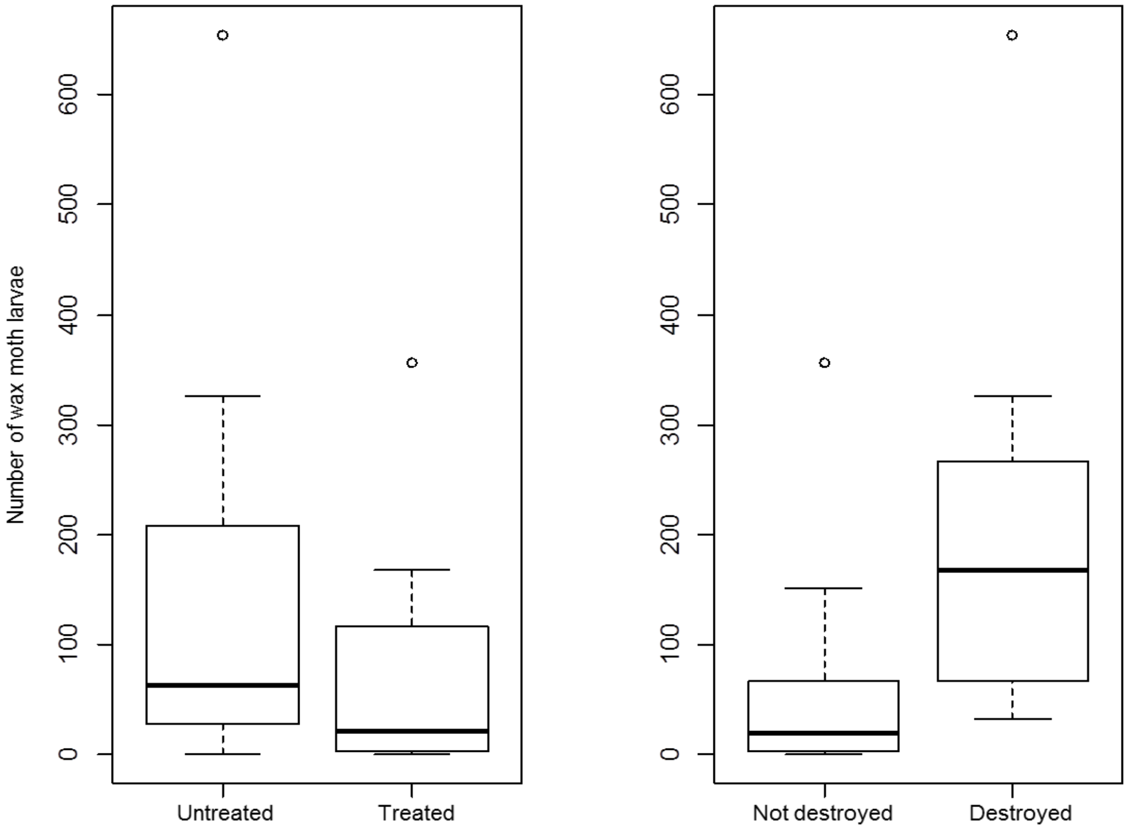

Figure 4.1 The number of wax moth larvae found in infested colonies was not significantly different in nests that had been treated with CERTAN™ from those that had not (a); nests that were classified as destroyed contained a significantly higher number of wax moth larvae than those that survived (b). The box plot depicts the median and interquartile range, with the bars representing the mean number of wax moths found, with circles representing outliers.

Table 5.3. Parameter estimates and likelihood ratio tests of the GLMM for nest destruction.

Fixed Effect	*Estimate*	*Standard Error*	*Δ Log Likelihood*	χ^2	*df*	*P*
Intercept	3.190	10.713				
Treatment[1]	-1.754	0.999	-1.713	3.428	1	0.064
Days in field	-0.627	1.229	-0.137	0.276	1	0.599
Date deployed	1.074	8.443	-0.022	0.045	1	0.832
Initial weight	0.685	0.828	-0.339	0.673	1	0.412
Number of farms 5km	1.667	7.530	-0.199	0.399	1	0.527
Number of nest at farm	1.941	5.806	-1.054	2.109	1	0.146
Number of wax moths	1.340	0.554	-4.589	9.178	1	**0.002**
Random effect variance						
Farm	<0.001					
Maximal model R^2 value	0.73					

[1] treated nests

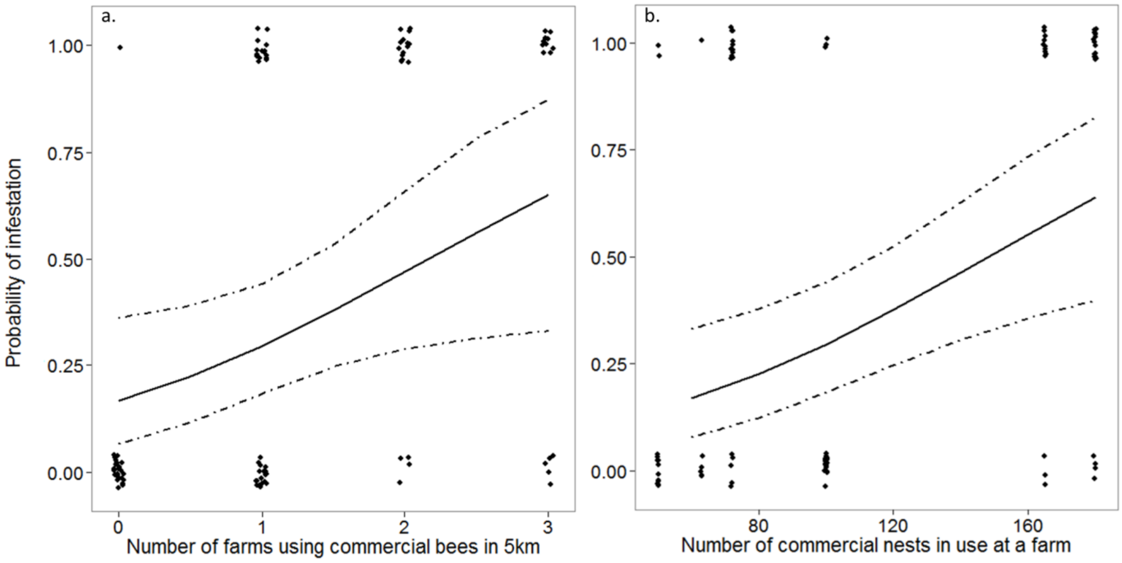

Figure 5.3. The predicted probability of infestation as a result of number of farms using commercial pollinators within 5km of a nest (a). The predicted probability of infestation as a result of number of commercial nests in use at a farm (b). Circles represent the raw data jittered for clarity with the solid line showing the predicted effect and the dot-dash line showing the upper and lower 95% confidence intervals. Predictions are based on model 1 (Table 2) and are made from the median observed values for other parameters in the model.

5.5 Discussion

As dependence on pollinators for our food production increases (Aizen *et al.* 2006), it is important to determine factors contributing to pollinator declines and reduced pollination efficiency. It is particularly important to understand how our management of pollinators, both wild and commercial, can influence the sustainability of the service that they provide. In this study we found that a high proportion of commercial nests used to increase crop pollination at soft fruit farms suffer from wax moth larvae infestations. In total, 44±9.98% of nests became infested, a higher proportion than the 17±7.88% previously found in agricultural settings in the south of the UK (Goulson *et al.*, 2002). Theory predicts wax moths will be more common in areas where their host are also more common (Anderson & May, 1978, Arneberg 2001), so it is therefore possible that farms which utilise commercial bumblebee colonies are creating wax moth ‘hot spots’ by providing an abundant and predictable host supply. Previous work has linked the prevalence of bumblebee nests parasites (e.g. the cuckoo bumblebee *B. vestalis*) to host density, with parasite free zones occurring when host numbers were low (Antonovics & Edwards 2001) and a high infestation rate recorded when bumblebee colonies were proximate to abundant foraging resources (Carvell et al., 2008).

Wax moth infestations can lead to nest destruction, and in this study 36% of infested nests were entirely destroyed by wax moths. The number of wax moth larvae present in nests was positively associated with the number of other farms using commercial colonies within a 5km radius of the site, as well as the number of nests in use at a farm. Commercially produced bumblebee colonies have been used in agriculture for approximately 25 years, creating an artificially high number of nests in some areas. Parasites such as wax moths can contribute significantly to host mortality (May, 1983) and in some cases parasites provide the ultimate determinant of population densities (Lloyd & Dybas, 1966). In the case of commercial bumblebee colonies, elevated wax moth populations may have knock-on effects for pollination services, reducing yields or resulting in the need to purchase replacement commercial nests.

In this experiment the effectiveness of Certan™, a biological control, was examined to see if it could potentially be used to reduce the frequency or severity of attacks on bumblebee nests. Our results were equivocal; the number of wax moth larvae found in treated nests was 48% lower than control nests, but numbers were highly variable and the difference not significant. Twice as many control nests were destroyed by wax moths as treated nests, but the difference was only marginally significant. We tentatively suggest that treatment may be having some effect, but clearly this requires further investigation. It is likely that the effectiveness of this product was limited by our lack of ability to treat the entire nest, as in this study we were only able to spray the outer parts with the Certan™. If the whole of the nest area could be treated, it is possible that greater effects would be seen. It may be feasible to treat the nests more effectively in the factories where the bees are reared, perhaps by spraying them on multiple occasions as the nest grows, or by lacing the insulation material provided to keep the nests warm.

Our results highlight the potential danger of a spill-back effect from commercial to wild bee nests, which may reduce the number and fitness of wild colonies close to soft fruit farms. Parasite spill-back can occur when a nonindigenous species is a competent host for a native parasite, with the presence of the additional host increasing the parasite burden in native species (Kelly et al., 2009). Whilst parasite spill-over is well studied within the sphere of invasion ecology, the concept of parasite spill-back has been relatively neglected. Despite this numerous empirical studies have recently emerged demonstrating the harmful impact of parasite and pathogen spill-back on native fauna (Mastitsky & Veres 2010; Poulin et al., 2011; Patterson et al., 2013).

Further research is needed to determine if wax moth spill-back could be negatively impacting upon native bumblebee heath or if in fact a ready supply of commercial bees could be reducing the parasite burden in wild nests. Commercial colonies are potentially easier for the wax moths to locate, due to their large size and prominent location and they may in fact act as a decoy for wax moths, thus reducing the number of attacks on wild nests. It is possible that the design of commercial colonies make them particularly vulnerable to wax moth attacks, as the plastic casing containing the colony offers many places where the relatively small moth can enter the nest. Future studies could help to determine if wild nests are negatively impacted by high commercial nest density, or if indeed, a 'decoy' effect occurs. If wax moth are disproportionately attracted to commercial bumblebee nest and nests are properly disposed of, as per supplier guidance (i.e. nests that are frozen at the end of the season) then commercial bumblebee colonies could provide a reproductive 'dead end' for wax moth, thus potentially reducing local populations of *A. sociella*. However personal communications with soft fruit farmers suggests that nests are not always destructively disposed of at the end of the season and are often found discarded within, or close to polytunnels containing the crop.

Commercial colonies have access to internal nectar tanks and are often positioned within a flowering crop and thus grow quickly. In this study we found that larger nests were more likely to suffer from wax moth infestations than smaller nests, and that there was a positive relationship between the number of worker and pupae and the number of wax moth larvae (when excluding the nests that were destroyed). The largest nest in this study produced 2,012 worker bees and 311 queens, considerably more than recorded previously (for nests without supplemented food, Goulson 2010). It is likely that larger colonies will be more attractive to female wax moths because they will offer more resources upon which developing larvae can feed.

The impact that wax moth larvae can have on bumblebee colonies not only depends on the size of an infestation but also on the point at which the bumblebee nest becomes infested. If an infestation occurs when a nest is large and has already produced new queens and males then it is less likely that the wax moth larvae will have a large impact on overall nest success. Co-existence within the nest might be possible if the infestation is light or if it occurs late in the colony cycle (Gambino, 1995). However, if nests are attacked before they reach the point where they produce queens and males, then an infestation might have a significant impact on nest success and survival. Wax moth attacks may place selective pressure on bumblebees to emerge early from hibernation and also to produce new reproductives early in the year. Further studies would be useful to see how often wax moth attacks prevent or reduce queen production and to measure how an infestation impacts the pollination activity of worker bees. If the use of commercial colonies is increasing the number of nest attacks in certain areas, it is important to understand the impact infestations have on reproductive health particularly in regards to wild bumblebees. From a farmer’s perspective wax moths are only a problem if they are reducing the pollination efficiency of the nests and thus impacting crop yields; this seems likely, given that some nests were destroyed, however more experiments would be beneficial to quantify the impact of infestations on pollination.

This is the first study that has investigated wax moth predation of bumblebee nests and is unique in examining the relationship between infestation levels and nest destruction. In addition, key links have been drawn between the density of commercial nest use and the levels of infestations occurring. Nests had significantly more wax moth larvae when densities of commercial nest use at farms and within a 5km radius were high, and nests with large wax moth infestations were significantly more likely to be destroyed. Further research should be targeted at understanding the relationship between commercial nest use and wax moth infestation rates in wild nests, as well as how to effectively manage this economically important pest in commercial bumblebee colonies.

In light of concerns about pollinator declines (Biesmeijer *et al.*, 2006; Pywell *et al.*, 2006; Goulson *et al.*, 2008; Grixti *et al.*, 2009; Winfree *et al.*, 2009; Potts *et al.*, 2010), and an increase in our dependency on the service they provide (Aizen *et al.*, 2008), it is important that we learn more about how one of bumblebees' greatest enemies is impacting their survival.

Chapter 6- Use of commercial bumblebees for crop pollination does not lead to wax moth spill-back

6.1 Abstract

The wax moth *(Aphomia sociella)* is common throughout the UK and is an economically important pest of commercial bumblebee nests used in crop pollination. Wax moths lay their eggs in bumblebee nests and the larvae that hatch consume wax, food stores and waste products, reducing the fitness of the colony and sometimes even destroying it. A reliance on bumblebee nests for the completion of their lifecycle means wax moth populations are likely to be highest where bumblebee nests are common. The use of commercially reared bumblebees for crop pollination has led to artificially high numbers of nests in certain agricultural areas and these nests are particularly at risk of wax moth attacks. However, it is not known whether the high infestation rates recorded in commercial nest 'hotspots' could be affecting the likelihood of wild nests nearby suffering infestations. In this study we placed 50 bumblebee nests at increasing distances away from fruit farms stocking commercial bees, in order to determine if 'spill-back' was occurring. We found no evidence that nests located close to farms using commercial bees were any more likely to suffer from a wax moth infestation than those located further away from such areas. Nest size was the only variable that was found to have any significance in predicting infestations, with larger nests being more prone to attacks. This study suggests that whilst wax moths remain an economically important pest for farmers who rely on commercial bees for pollination, their prevalence in nearby agricultural areas remains low.

6.2 Introduction

There are concerns over declining pollinator populations (Biesmeijer *et al.*, 2006; Grixti *et al.*, 2009; Potts *et al.*, 2010), not least because of our dependence upon insect pollinators for the production of almost a third of the food that we eat (Klein *et al.*, 2007). The area cultivated with pollinator dependent crops has been increasing more rapidly than the area cultivated for non-dependent crops (Aizen *et al.*, 2008). Farmers that are highly reliant on pollinators often choose to actively manage this service by utilising domesticated and commercially reared bees (Garibaldi *et al.*, 2009).

Bumblebees have been reared commercially for over two decades (Velthuis & van Doorn, 2006) and are particularly efficient pollinators due to their large size, their hardiness to cooler weather (Corbet *et al.*, 1993) and their ability to buzz pollinate (de Luca & Vallejo-Marin, 2013). Over 1 million factory reared *B. terrestris* colonies are produced per year globally (Velthuis & van Doorn, 2006) and are used to pollinate crops in countries where *B.terrestris* is native, but also where this species is not indigenous (Matsumura *et al.*, 2004). They are used in the production of many crops from strawberries and tomatoes, to cucumbers, melons and almonds (Velthuis & van Doorn, 2006). Whilst commercial bumblebees helps to ensure a reliable pollination service for crops, their use could have unanticipated ecological consequences. For example, commercially reared bees have the potential to outcompete their native conspecifics (Ings *et al.*, 2006) and the introduction of commercially reared bumblebees in North and South America as well as in Japan have been correlated with declines in indigenous bumblebee species (Goka *et al.*, 2001; Colla *et al.*, 2006; Otterstatter & Thomson, 2008; Meeus *et al.*, 2011).

Another risk associated with the use of commercial bees is the spill-over of pathogens to wild populations (Colla *et al.,* 2006). A recent study by Greystock *et al.* (2013) found that 77% of commercially reared bumblebee colonies carried microbial parasites which had the potential to be harmful to wild bumblebees and honeybees. Whilst in use at farms, commercially produced bumblebees interact with wild pollinators, sharing floral resources on the crop or on nearby margins (Whittington *et al.,* 2004; Murray *et al.,* 2013). Greystock *et al.* (2013) found five different parasites within the sample of bumblebees that were studied and a further three within the pollen that was supplied as a food source for the bees. 'Pathogen spill-over' can occur as a result of a heavily infested host population coming into contact with a non-reservoir host population (Daszak *et al.,* 2000). The potential consequences of pathogen spill-over are particularly alarming given the widespread use of commercial nests and already threatened wild pollinator populations.

To manage any adverse impacts commercially reared bees may have, it is important to first understand how their use affects wild pollinators. Whilst the potential for disease transmission between managed and wild bees has been examined, no work has been carried out to assess whether the use of commercial pollinators can lead to artificially high populations of one bumblebee pest: the wax moth *(Aphomia sociella).* Non-indigenous species tend to acquire parasites from the local fauna (Poulin & Mouillot 2003) and 'spill-back' can occur when introduced species provide a competent host for native parasites (Kelly *et al.,* 2009).

Wax moth can be regarded as a parasite and are an important pest of bumblebee nests (Hoffer, 1882-1883; Goulson, 2010), with severe infestations resulting in the destruction of even large and otherwise healthy colonies.

The wax moth life cycle begins in early summer when wax moth females mate and then lay their eggs inside the nests of bumblebees. The larvae develop within the host nest, consuming nest materials and food stores as well as waste products (Gambino, 1995; Goulson, 2010), creating silken tunnels of dense webbing that protect the larvae as they move through the nest. When fully grown, the larvae overwinter in tight clumps of pupae close to the nest they inhabited, emerging as adults the following spring when the cycle begins again.

Wax moths have been little studied and, whilst they are a known pest of bumblebee nests, the frequency and impact of their attacks is poorly understood. In a study examining bumblebee colony growth Goulson *et al.* (2002) found that 80% of nests located in urban areas suffered from wax moth infestations, compared with only 17% of nests in agricultural settings. In the UK gardens provide an important nesting habitat for bumblebees (Osborne *et al.*, 2008) and it stands to reason that wax moths will be more common where their prey is more readily available. Chapter 5 examined the frequency with which commercial nests became infested with wax moth larvae and found that almost half of the nests in use at farms suffered from an infestation, and that in 34% of cases this led to nest destruction. The frequency of attacks was considerably higher than would otherwise have been predicted within an agricultural landscape (Goulson *et al.*, 2002), and there was a positive correlation between the density of commercial nest use in an area and the frequency and severity of an infestation. It is therefore important to determine if the use of these bees creates wax moth ‘hotspots’ that could increase the likelihood of nearby wild nests suffering from attacks. Wild bees play an important role in the pollination of soft fruit crops (Lye *et al.*, 2011) and it is thus key to determine if commercial bumblebee use is negatively impacting their fitness. It is possible that the commercial nests used by farmers serve as ‘decoys’, attracting wax moths and thus reducing attacks on wild nests. Conversely the use of commercial nests could artificially increase numbers of wax moths and subsequently result in more attacks on wild nests. This study seeks to determine if nests close to fruit farms where commercially reared bumblebees are in use are at a greater risk of wax moth attacks as a result of their proximity to these farms.

6.3 Methods

Fifty commercially reared *B.terrestris audax* nests (Biobest, Standard Hives) were used in the experiment and contained one queen and between 5 and 15 workers when delivered on the 1st May 2013. Each nest contained two internal tanks to supply the bees with nectar during transportation and these were drained before each nest was weighed and labelled. Whilst in the laboratory all colonies were allowed access to the large nectar tank that was stored below the nest and were fed pollen as and when required.

All nests were placed out in the field between the 7th and the 16th May 2013, and prior to deployment the nectar tank below each nest was sealed so that bees were required to forage. Ten soft fruit farms stocking commercial bees were selected from three of the largest soft fruit growing regions in Scotland; Angus, Tayside and Fife. One nest was positioned at each of the ten farms (Fig 6.1), either within or next to an open ended polytunnel containing raspberry crops and three were placed along a transect leading away from each farm. Nests were housed in a plastic box with the lid propped open to allow bees to enter and exit the nest, whilst preventing damage from rain. Nests were placed approximately 500m, 1000m and 1500m away from the focal farm stocking bees, however the exact distances varied according to the availability of suitable locations for the nests (which need to be placed in sheltered locations, out of the way of farm machinery). Where possible, nests were hidden in woodlands, hedgerows or scrubby areas and were placed away from footpaths to prevent disturbance. The final 10 nests were located in agricultural areas at least 2km away from the nearest farm stocking commercial bees (Fig 6.1).

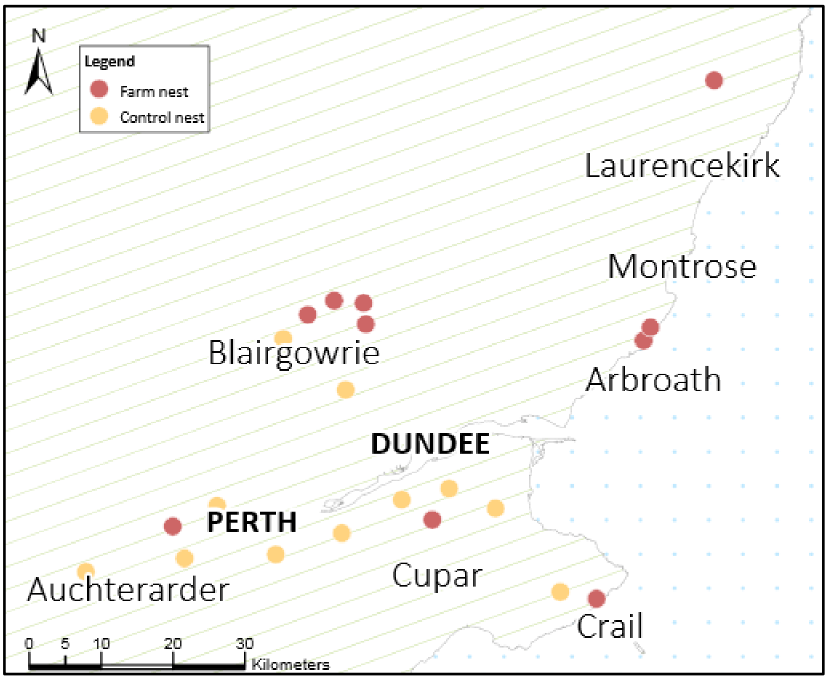

Figure 6.1 Locations of farm nests and control nests

Nests were weighed once every two weeks for a 10 week period and any signs of wax moths recorded. Dead nests or nests that showed signs of heavy wax moth infestations were collected before the end of the 10 weeks of monitoring.

After collection, each nest was placed in a -80^{o}C freezer to humanely kill any remaining bees and then moved to a -40^{o}C freezer for storage. Each nest was then dissected and the following information recorded: final weight of nest, number of empty worker cells, number of empty queen cells, number of worker pupae, number of queen pupae, number of wax moth larvae present and weight of wax moth larvae present. A nest was classified as destroyed if the damage inflicted was so severe that it prevented the elements of the nest from being counted (in heavily damaged nests there are few entire cells or pupae, and the wax structure is highly fragmented).

6.4 Landscape Analysis

The co-ordinates of each nest and all farms using commercial bees within 5km of any nest were recorded using a GPS data logger and imported into ArcGIS 10 (ESRI Inc 2013) as a point layer. The Proximity tool was used to determine the distance from each nest location to the nearest urban village or town and to the nearest fruit farm stocking commercial bees. Buffer and clip tools was used to select the area within a 1km radius of each colony, and data from OS Mastermap topography layer was used to determine the proportion of urban features (buildings, structures and roads) within these radii.

6.5 Statistical Analysis

All statistical analyses were conducted in R version 3.1.0 (R Development Core Team). A General Linear Model (GLM) with binomial errors was used to determine the effect of potential explanatory variables on the likelihood of wax moth infestations in bumblebee nests. Whether or not a nest became infested with wax moth larvae was modelled as a response variable with the distance between a nests and the nearest farm using commercial bees, and the number of farms using commercial nests within a 5km radius of a nest modelled as covariates of interest. As it has previously been identified that urban areas support high numbers of bumblebee nests the distance to the nearest urban area, as well as the proportion of urban features within 1km were also included in the model as covariates, along with the peak weight of each nest. Peak weight rather than final weight was used in our analysis because some infested nests were destroyed or partially destroyed as a result of wax moth attacks and thus lost considerable weight towards the end of the experimental period.

The number of queens, pupae and worker bees within nests were each, in turn, modelled as the response variable to examine the effect of wax moth infestations on reproduction.

Nests that were heavily damaged by wax moth predation were excluded from this analysis as it was not possible within these nests to differentiate between different nest components. The glmmADMB package was used to account for over dispersion and zero-inflation (where appropriate i.e. queens), and the weight of wax moths within a nest, peak nest weight and the date that nests were deployed were included as covariates along with the distance to nearest urban area and the proportion of urban feature within 1km.

Pseudo R^2 values (hereafter R^2 values) for each model were calculated by correlating the fitted values from the model with the observed data (using the MuMIn package in R, Barton, 2014). All results are means ± standard errors unless otherwise stated.

6.6 Results

Of the 50 nests deployed in this study, four were attacked by badgers, three were displaced or vandalised (and so were discounted from further analysis) and 15 were infested by wax moths; five of which were destroyed by the larvae.

The mean peak weight of infested nests was 835.8 ± 68.7g compared with 609.6 ± 34.0g for uninfested nests (Fig 6.2). Nests reached peak weight between weeks 6 and 8 of the study, after which, irrespective of whether a nest became infested, weight tended to fall. The mean difference between peak weight and final weight for infested nests was 247.7 ± 50.1g (a 30% reduction) and for uninfested nests was 83.8 ± 20.5g (a 14% reduction). The mean number of wax moth larvae found in infested nests was 99.9 ± 20.3 and the mean weight of wax moth larvae per nest was 11.2 ± 4.1g.

Table 6.1 Parameter estimates and likelihood ratio tests of the GLM for the probability of a bumblebee nest becoming infested with wax moth larvae.

	Estimate	*Standard Error*	*Δ AIC*	*χ^2*	*χ^2 df*	*P*
Intercept	-0.928	0.455				
Distance to nearest urban area	-0.832	0.533	0.69	2.69	1	0.119
Urban area within 1km	0.133	0.517	-1.934	0.067	1	0.797
Distance to nearest fruit farm	-0.649	0.768	-1.161	0.839	1	0.398
Peak weight	2.013	0.612	15.477	17.477	1	**<0.001**
Date deployed	-0.779	0.779	-0.936	1.064	1	0.317
Number of farms using commercial bees within 5km	0.102	0.577	-1.969	0.031	1	0.86
Maximal Model R^2	0.496					

Nest weight was the only variable included in the model that had a significant impact on whether or not a nest became infested. Larger nests were more prone to wax moth attacks (Table 6.1), with an increase in nest weight from 600g to 800g more than doubling the liklihood of an infestation occuring within a nest (Fig. 6.3a). Neither proximity to fruit farms stocking commercial bees (Fig. 6.3b) or the number of fruit farms within a 5km radius of a nest were significant in explaining infestation (Table 6.1).

Table 6.2 Parameter estimates and likelihood ratio tests of the GLMM examining the number of queens produced by a nest.

Fixed Effect	*Estimate*	*Standard Error*	*Δ Log Likelihood*	*χ^2*	*χ^2df*	*P*
Intercept	0.160	0.326				
Weight of wax moths (g)	-0.638	0.233	-4.087	8.175	1	**0.006**
Distance to nearest urban area	-0.469	0.297	-1.215	2.431	1	0.115
Urban area within 1km	0.268	0.339	-0.34	0.681	1	0.431
Distance to nearest fruit farm	-0.173	0.396	-0.1	0.201	1	0.661
Date deployed	-0.538	0.337	-1.171	2.344	1	0.11
Peak weight	1.526	0.292	-9.963	19.927	1	**<0.001**
Negative binomial dispersion parameter	403.43	2.075				
Zero-inflation	0.58219	0.112				

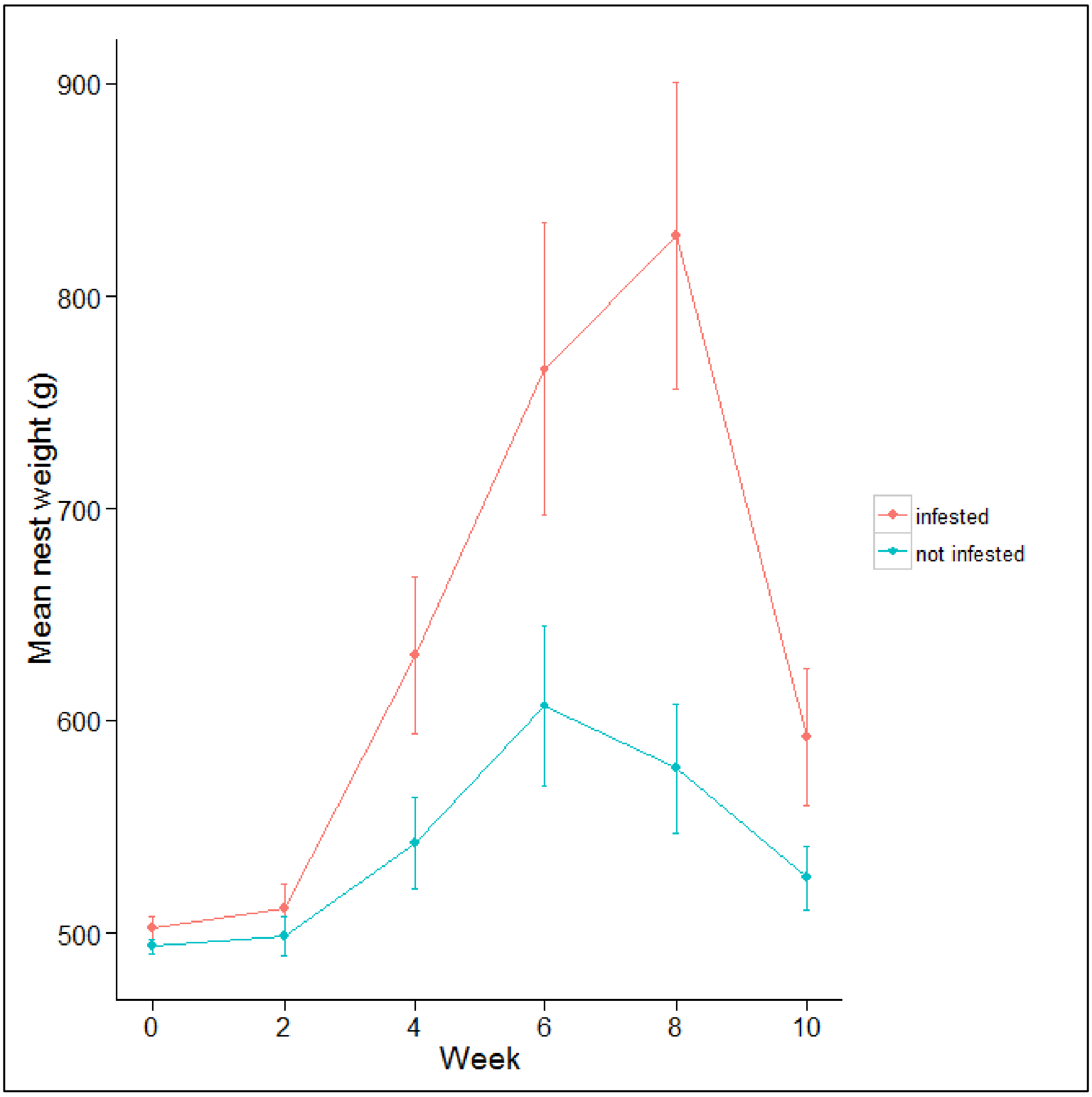

Figure 6.2. Mean observed nest weight for infested and un-infested nests across the ten week monitoring period. Points represent mean nest weight (and their standard errors) and weight includes all accumulated biological material within the nest, including any wax moth larvae present.

The weight of wax moths within a nest along with the peak weight of the nest had a significant influence on queen production (Table 6.2). Unsurprisingly heavier nests produced more queens with the model predicting an 85% increase in queen production with a doubling of nest weight from 600g to 1200g (Table 6.2). Conversely nests containing larger infestations produced fewer queens.

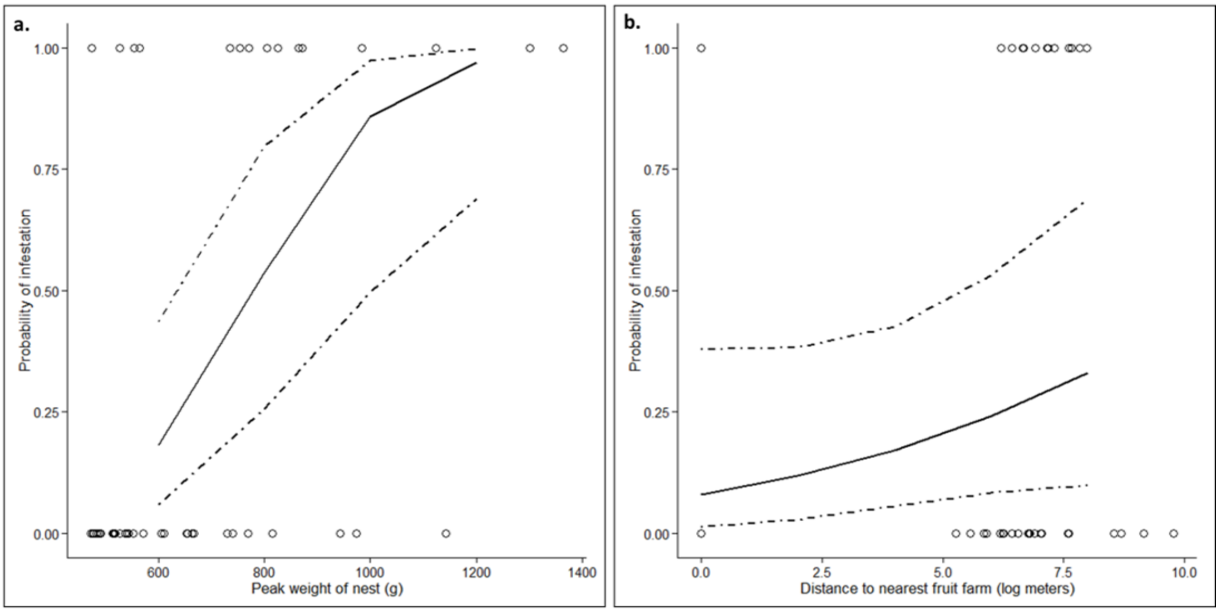

Figure 6.3 The effect of peak weight (a) and distance to the nearest fruit farm (b) on the probability of a wax moth infestation occurring within a nest. Dots are observed data, black lines are the predicted probabilities based on the model shown in Table 6.1, with the dot-dash lines representing the 95% upper and lower confidence intervals. The predictions are made at the median observed values for other parameters in the model.

Whilst the relationship between the weight of a larvae infestation and queen production was significant, the biological effect was small, with the model predicting nests without infestations would produce only 1-2 more queens than those with heavy infestations (Table 6.2). Whilst there was a trend towards more workers and pupae being produced by heavier nests, the weight of a wax moth infestation was not significant in either model (Appendix 6.1 & 6.2).

6.7 Discussion

In order to effectively conserve pollinators such as bumblebees we need to understand both the intentional and unintentional consequences of agricultural management decisions relating to these ecosystem service providers. The use of commercial bees has raised a number of ecological concerns including fears that they might compete with native species, disease transmission and potential interbreeding with wild population (Goulson, 2010b), The previous chapter discussed how commercial bumblebee nests used at fruit farms suffer from high frequencies of wax moth infestations, and demonstrated that severe infestations led to high incidences of nest destruction. The results of chapter 5 raised questions pertaining to how commercial nest infestation rates may impact upon wild bumblebee health. Whilst parasite density can be determined by biotic and abiotic factors not associated with hosts (Antonovics & Edwards 2011), host population density and parasite abundance have been found to be positively connected (Arneberg et al., 1998).

This study set out to determine if the use of commercially reared bumblebee colonies could be increasing the frequency of attacks on nests in nearby areas, or whether they could in fact be acting as 'decoys', thus reducing the number of attacks on wild nests close by.

The link between host density and prevalence of infestation has been supported by empirical studies (Arneberg et al., 1998; Antonovics & Edwards 2011) and correlations have been found between the number of parasites per host and the percentage of hosts infested in a given area (Kraznov et al., 2002). Despite this here we found no evidence to suggest that proximity to fruit farms where commercial bees are used increases the probability or severity of a wax moth attack. Nest weight was the only variable that significantly impacted upon the likelihood on an infestation occurring as wax moths were more likely to infest larger nests, with infestations having a small, but significantly negative impact upon queen production.

Nests with higher peak weights produced significantly more queens, however these were also the nests that were most at risk of becoming infested with wax moth larvae.

Large nests are likely to be particularly appealing to female wax moths as they will contain more resources upon which hatched larvae can feed. In an infested nest the wax moth larvae will consume some of the nest stores and this may result in less food being available for the worker bees to feed new queens. This could explain why infested nests produced significantly fewer queens. Whilst an infestation only reduced queen production by a small amount (1-2 bees), it is possible that if nests were left in the field for longer the difference would be more marked. The effect of an infestation on queen production is likely to be impacted by the timing of an attack and the point at which a nest starts to produce new queens.

It has previously been suggested that if a wax moth attack occurs late on in bumblebee colony development it may have little effect on the success of the nest (Gambino, 1995). In this study we monitored nests every two weeks, which allowed us to examine more closely the impact of an infestation on a nests development. Nests tended to reach peak weight between weeks 6 and 8 of the study period, however subsequent weight loss was greater in infested nests with a 30% reduction in nest weight relative to a 14% reduction in uninfested nests; a difference presumably caused by the larvae consuming the nest stores and material. Whilst too few nests were destroyed to allow meaningful statistical analysis, one of the largest nests used in the study was completely destroyed between one fortnightly visit and the next as a result of a wax moth attack. This demonstrates that large infestations have the ability to inflict fatal damage on otherwise seemingly healthy colonies.

Overall the nests used in this study were in much smaller than the nests being used by farms for the purpose of crop pollination. This could have contributed to the fact that no relationship was found between proximity to these farms and infestation rates, as wax moths seem particularly attracted to larger nests. It could be therefore, that spill-back is occurring, but that any effect of nest density on infestation rate was not detectable here as a result in discrepancies between the size nests in use at farms and size of the nests deployed as part of the experiment. The use of commercially reared bumblebees could provide a useful study system to empirically examine the impact of host density on parasitic infestation, particularly given the likely increase in their use over time.

The desire to produce crops outside of their traditional growing seasons, coupled with concerns over wild bee declines, has increased the demand for commercially reared bees, which can be ordered and delivered to provide a pollination service whenever crops are in flower. Concerns have been raised regarding the ecological consequences of using commercial bumblebees (Goulson, 2010b), however until now no study had examined whether or not high density use has the potential to create a wax moth spill-back effect, leading to an increased frequency of attacks on wild nests. Wax moths are an understudied predator of bumblebees that can destroy or reduce the reproductive success of nests. For farmers reliant on pollinators for successful crop production any pest that reduces the fitness of wild and/or commercial bees is likely to be of economic importance and worthy of management. Whilst we found no evidence that high density commercial bee use is increasing wax moth predation of nearby nests, it is important that commercial bees are managed to prevent wax moth hotspots from forming. Used nests should be disposed of properly at the end of the season in order to prevent any wax moth larvae within them from surviving to hatch the following year. When deployed in polytunnels, commercial nests are often mounted on top of a small crate to prevent dampness caused by being in contact with the ground. These crates should also be checked to ensure they do not harbour hibernating larvae.

Further research is needed to determine the costs and benefits associated with commercially reared bees. Whilst they provide farmers with a predictable and on demand pollination service it is important that we fully understand how their use might impact upon nearby wild populations; particularly since their use looks set to continue and most likely increase. It is key that management methods designed to increase yields do not have negative and unforeseen consequences on natural ecosystems and the services that they provide.

Chapter 6- Appendix

Appendix 6.1 Parameter estimates and likelihood ratio tests of the GLMM examining the number of workers produced by a nest.

Fixed Effect	*Estimate*	*Standard Error*	*Δ Log Likelihood*	*χ^2*	*χ^2df*	*P*
Intercept	4.751	0.226				
Weight of wax moths (g)	0.559	0.624	-0.44	0.876	1	0.349
Distance to nearest urban area	-0.192	0.137	-0.97	1.938	1	0.164
Urban area within 1km	-0.018	0.145	-0.01	0.014	1	0.906
Distance to nearest fruit farm	0.074	0.184	-0.08	0.162	1	0.687
Date deployed	-0.094	0.176	-0.14	0.282	1	0.596
Peak weight	1.336	0.1637	-23.08	46.156	1	**<0.001**
Negative binomial dispersion parameter	2.85	0.692				

Appendix 6.2 Parameter estimates and likelihood ratio tests of the GLMM examining the number of pupae produced by a nest.

Fixed Effect	*Estimate*	*Standard Error*	*Δ Log Likelihood*	*χ^2*	*χ^2df*	*P*
Intercept	3.147	0.133				
Weight of wax moths (g)	-0.29	0.204	-0.91	1.814	1	0.178
Distance to nearest urban area	-0.414	0.155	-3.28	6.552	1	0.01
Urban area within 1km	-0.125	0.177	-0.25	0.49	1	0.484
Distance to nearest fruit farm	-0.229	0.217	-0.55	1.084	1	0.298
Date deployed	0.046	0.214	-0.03	0.046	1	0.830
Peak weight	0.612	0.198	4.88	9.752	1	0.002
Negative binomial dispersion parameter	2.107	0.587				

Chapter 7

General Discussion

A combination of globalised food markets and innovative approaches to farming mean we can grow strawberries in December and eat bananas in Scotland. Contemporary farming techniques have adapted to lessen the environmental constraints on food production allowing crops, particularly those with a high value, to be produced outside of their traditional growing seasons.

In the case of soft fruit production in Scotland, agricultural innovations were needed to overcome two main barriers that previously restricted the times when crops would grow: climate and potential pollination limitations. The traditional season for field-grown strawberries in the UK spans June and July, with production tailing off in August. Delicate fruits such as strawberries and raspberries are easily damaged by exposure to wind and rain with plants growing best in warm conditions. Growing strawberries under protection in polytunnels reduces exposure to adverse weather and increases ambient temperatures in the crop vicinity. Consequently the growing season extends to span May and into September.

Farmers that produce pollinator dependent crops such as strawberries cannot depend on manipulation of local climatic variables alone to extend the growing season. Sufficient bees and other insects are required for adequate crop pollination and these insects simultaneously benefit from the crop as a food source. In order to maximise this mutualistic relationship, careful management of the farm environment is needed. Bumblebees and honeybees are two important crop pollinators whose numbers in certain locals are reducing as a result of human activities. Diminishing habitat quality and availability, disease prevalence and the widespread use of agrochemicals have led to severe regional declines of honeybees as well as bumblebee range contractions throughout Europe and North America (Potts *et al.,* 2010; Williams & Osborne 2009; Steffan-Dewenter *et al.,* 2005). Agricultural expansion and intensification has played a key detrimental role in pollinator health (Goulson *et al.,* 2008; Pywell *et al.,* 2005), yet over a third of the crops that we grow cannot be produced without the help of bees and other insects (Klein *et al.,* 2007).

Insufficient compatible pollen transfer to flower stigmas, otherwise known as pollination limitation, reduces seed set and can result in diminished crop quality and quantity (Szklanowska & Wienlarska, 1993). Pollination limitation can occur in early season flowers, if native bees are not yet on the wing (Baker *et al.,* 2000; McCall & Primack, 1992), and is a likely consequence of decoupling crops and their natural growing seasons. In order to tackle the issue of pollination limitation, farmers are increasingly relying on the use of commercial bumblebee colonies, which can be placed conveniently within early flowering crops before wild pollinators emerge. Whilst honeybees have been domesticated for at least 4000 years (Crane 1990), the domestication of bumblebees is relatively new (Velthuis & van Doorn, 2006). Commercial bumblebees are used in the production of a range of crops from cucumber (Stanghellini *et al.,* 2002) to blueberry (Stubbs & Drummond 2001) to kiwifruit (Pomeroy & Fisher 2002) and are able to provide services that honeybee colonies cannot, for example buzz pollination; making them popular for the production of crops like tomatoes (Velthuis & Van Dorne, 2006).

In the Scottish soft fruit context, personal communications with farmers suggests that purchases of bees are made based on the advice of an agronomist or because of a perceived risk in relying solely on the availability of wild pollinators. Most farmers were aware of pollinator declines, often because of media coverage of honeybee losses, and the majority were also acutely aware of the importance of wild pollinators but lacked confidence in the reliability of the service they can provide. Research has shown that wild bees provide a sufficient pollination service to crops in agricultural areas where their bio-physical needs are met (i.e. sufficient foraging resources and suitable nesting sites are available) however in more intensive agricultural landscapes where a large crop area can flower over a relatively short period of time, pollinator to flower ratios can prove insufficient (Kremen *et al.*, 2004; Winfree *et al.*, 2007).

The benefits of using commercial bees will therefore likely be highest where mass flowering crops are produced intensively and wild bee numbers are insufficient to pollinate all plants (Free and Williams 1976) and lowest in areas where organic cropping is practiced (Andersson *et al.*, 2012) and high levels of habitat heterogeneity within the landscape support a diverse assemblage of wild pollinators (Rundlof *et al.*, 2007).

In light of growing concerns over the ecological consequences of using commercial and domesticated bees e.g. disease spread (Greystock *et al.*, 2013; Furst *et al.*, 2014); competition with native species for resources (Goulson 2003; Ings *et al.*, 2006) it is particularly important to quantify their contribution to crop production and thus determine the necessity of their use. The results of research presented here (Chapter 2) suggest that commercial bumblebees are currently important for strawberry production in Scotland, however findings do not support their use on raspberry crops. These results somewhat contradict those of a previous study by Lye *et al.* (2011) who found that the use of commercial bees significantly increased pollination of raspberry crops in Scotland. Whilst it is possible that differing landscape factors between the two studies could account for this (e.g. the landscape surrounding the study farms in Chapter 2 may have offered bees fewer foraging alternatives than in Lye *et al.* (2011) and thus made the raspberry crop more attractive, it is also possible that pollination services may vary over time. If pollination services vary over time, any research that tries to quantify the benefit of commercial bees to crop pollination needs to take into account cross-year differences in wild pollinator availability.

Inter-annual variability in pollinator numbers in some EU countries could be amplified by the recent two-year moratorium which prevents the use of three common neonicotinoids on flowering crops. This moratorium was passed by the EU in 2013 after a number of studies emerged linking the use of these agrichemicals to bee declines (Yang *et al.,* 2008; Henry *et al.,* 2012; Gill *et al.,* 2012; Whitehorn 2012).

Investigating the impact of one widely use neonicotinoid, Whitehorn *et al.* (2012) found that queen production in *B. terrestris* colonies was significantly diminished as a result of exposure to field realistic doses of the pesticide imidacloprid, however the mechanism behind the reduction was not clear from their study. In Chapter 4 evidence is provided that reveals this mechanism: bumblebees exposed to the same dosage of imidacloprid used by Whitehorn *et al.* (2012) showed a reduced ability to forage for pollen, with treated bees bringing back 31% less pollen per hour than control bees. This finding was supported by Gill *et al.* (2012) who also determined that imidacloprid resulted in bumblebees returning to the nest with smaller pollen loads. Pollen is the main source of protein for bumblebees and is used to rear workers and queens (Harder 1990), if bumblebees are unable to gather this resource effectively then a nests will suffer a diminished ability to reproduce.

If the widespread use of agrochemicals, such as imidacloprid, is partly responsible for wild pollinator declines then it is possible that negative impacts could be reduced as a result of constraints imposed upon pesticide use. Whether bumblebee and honeybee numbers benefit from the temporary restriction depends on many factors, including the length of time that the moratorium remains in place and whether the chemicals that are inevitably used in place of neonicoitinoids have impacts on pollinating species. Recent work has shown neonicotinoids are not just impacting upon bumblebee and honeybee health they might also be linked to declines in bird populations, suggesting the implications of the use of this group of agrochemicals may be broader than previously thought (Hallmann *et al.,* 2014). Further research is needed to ensure that any effects of pesticides on non-target organisms are better understood; failure to do so could result in humanity ‘tinkering’ unwisely with the many cogs and wheels that make up the ecosystem services upon which we depend.

Farmers in England wishing to use *B. t.terrestris* colonies now have to apply to Natural England for a licence in order to do so. This licence stipulates that these non-native bees can only be used in greenhouses or polytunnels and not in open field situations, a limit imposed to help prevent their escape and spread. The study in Chapter 2 was conducted in 2011, at the time farmers were mostly utilising commercial hives that contained non-native *B. t.terrestris*, reared in factories outside the UK. By 2013 most farmers had switched to using nests containing the native *B. t.audax*, a more expensive alternative, but one encouraged by recent restrictions on the use of non-native hives. Whilst farmers in Scotland are not - at present - required to apply for a licence in order to use non-*B. t.terrestris* the new regulation in England signals an increased recognition by policy makers of potential issues concerning the use of commercially reared bees.

For the purposes of strawberry production in Scotland, commercial bees were highly important for pollinating early season fruit, when the abundance of wild pollinators was low (Chapter 2). With commercial bumblebee use becoming a more restricted and more costly practice, farmers may seek alternative management strategies. Future research could examine if farmers could actively promote wild pollinators that emerge earlier in the season in order to determine if a viable alternative to commercial bees exists. The provision of suitable nesting sites has been previously found to augment local bee populations e.g. the alkali bee, *Nomia melanderi* (Parker *et al.,* 1987) and the leafcutter bee, *Megachile rotunda* (Stephan 1961; Peck and Bolton, 1946) and it is possible that the use of artificial domiciles could allow farmers to encourage wild bees to nest close to their crops. It is unknown whether this strategy, coupled with the provision of additional foraging resources early in the season, could increase pollinators in the crop vicinity to a sufficient degree to reduce reliance on commercial bees.

In Chapter 2 the importance of seasonal complementarity was discussed, with findings suggesting that a range of pollinating species, each with a different seasonal niche, provided the most robust pollination service. A body of research has previously detailed the importance of a diversity of complimentary pollinators for the creation of resilient plant pollination services (Bluthgen & Klein 2011; Albrecht *et al.,* 2012) however, experiments showing the importance of *seasonal* functional diversity in real life crop systems were lacking.

Becoming heavily reliant on a single pollinator species is inherently risky, whether wild or commercially reared. An outbreak of disease could decimate the population of a single wild species, or the supply of commercial species, leaving farmers vulnerable. Commercial bumblebees imported to the UK are reared in one of two factories in mainland Europe, so there is a risk of supply failure to the multitude of farms dependent on commercial bees. An accident (e.g. a destructive fire) at a single facility could have a disastrous impact on yield for farms with no alternative pollinator management strategies in place. There is some irony in the fact that many farmers are motivated to buy in commercial bumblebees in order to mitigate risk associated with relying upon wild pollinators, but in doing so are perhaps introducing a new risk: becoming too reliant on the use of commercial bees.

If farmers are to mitigate the risks of pollinator scarcity then an integrated approach to pollinator management is necessary. Chapter 3 showed how the use of relatively cheap wildflower mixes can be used to increase the number of pollinators visiting a nearby crop. Results demonstrated that planting wildflowers adjacent to crops increases pollinator visits, even for crops that are relatively unattractive to bees (Chapter 2). However, given the wide scale use of commercial bees within the study area, we were unable to determine what proportion of the increase visitation was due to increases in the number of wild bees visiting the crop in comparison to commercial bees. Recent work by Scriven *et al.* (2013) has developed non-invasive DNA sampling methods that allow genetic differentiation of *Bombus* species using faecal samples. In the future this method could be adapted to help determine the relative proportion of wild and commercial *B. terrestris* at farms where both are present, helping to reveal further the contribution that both make to crop pollination. In a study system where commercial bumblebee colonies were not in use Blaau and Issacs (2014) also found that wildflower plantings are a successful mechanism for improving the pollination of nearby crops, however the area planted with seed in their study was greater than the 6m by 50m strips detailed in Chapter 3. Farmers are more likely to invest in flower strips if they require smaller areas of land and less seed, it is thus important to determine the minimum investment required to achieve enhanced crop pollination.

Little is known about the long term impact of using wild flower strips to boost pollinator numbers in nearby crops, particularly how these strips might influence the longer term health and diversity of wild bee populations around farms. Does providing an increased abundance and diversity of forage increase pollinator population sizes over time, and could this lead to a more reliable and sustainable crop pollination service?

Sowing wildflower strips close to crops at farms using commercial bees could lead to more interaction between wild and commercial pollinators, both of which will likely feed on flowers provided by the strip. Parasites can be transmitted as a result of shared flower use (Durrer & Schmid-Hempel, 1994) and commercially reared bees are known to interact with other pollinators after importation through the communal use of floral resources (Whittington *et al.,* 2004; Murray *et al.,* 2013). Recent work by Greystock *et al.,* (2013) has shown that commercial colonies often contain various parasites and disease associations have been found between domesticated honeybees and wild bumblebees (Furst *et al.,* 2014). This suggests that creating an attractive floral resource frequently utilised by wild and commercial bees alike could increase disease transmission between factory reared and wild bees.

More work is needed to thoroughly asses the ways in which commercially reared bees may impact upon wild pollinator populations, particularly given the likelihood that their use will continue to increase if demands for pollinator dependent foods rise. In Chapters 5 and 6 some of the first studies on the relationship between commercial bumblebee colonies, wax moths and wild bees were reported. The impact of wax moths on bumblebee nest health had not been previously quantified and, whilst they are mentioned in a small number of studies, until now there had been no experimental work to quantify their prevalence and impact on bees.

Chapter 5 presented research detailing the frequency of wax moth predation of commercial bumblebee nests and provided insight into the proportion of these infested nests that go on to be destroyed as a result of attacks. Results demonstrated that almost half (44%) of nests became infested with wax moth larvae, with 34% of infestations resulting in nest destruction. More needs to be understood about the impact that both lethal and sub-lethal infestations have on pollinator activity and reproductive success. If foraging activity of commercial bees is diminished crop pollination might be negatively impacted. The use of Certan™, a lepidopteran larvicide was investigated as a tool to reduce wax moth infestations within commercial nests. Whilst Certan™ did not significantly reduce the likelihood of nest infestation in our study, results did suggest it was having some effect on reducing wax moth prevalence (albeit a non-significant one). It is possible that a lack of clear impact was caused by limitations in treating the nest successfully (see Chapter 5 discussion) and the effectiveness of this product at protecting nests could easily be tested in a laboratory based experiment. Wax moths are an understudied, economically important pest and further work could focus on determining an effective mechanism to reduce their impact on commercial nests.

Due to the high density use of commercial bees in certain agricultural areas, and personal observations of farmers failing to destroy nests at the end of the crop season (as stipulated by the manufacturers), we set up an experiment to determine if proximity to farms where commercial nest use is high could increase the likelihood of nearby nests becoming infested. The impact of potential negative effects of using commercial bees on nearby populations of wild bees is an area of growing concern (Greystock *et al.*, 2013); however, here we found no evidence that nests located in close proximity to commercial nest 'hot spots' were any more at risk of an attack than control nests that were located far away from such sites. Whilst Chapters 5 and 6 shed light on the relationship between wax moths and bumblebees (e.g. larger nests were found to be significantly more likely to suffer from an attack), there is much scope for further research in this area.

Relatively little is known about the factors influencing the susceptibility of nests to wax moth infestation. It is plausible that disease load or exposure to pesticides could have an impact on a nests susceptibility to a wax moth attack and future work could examine if this is so by testing nest resources for the presence of harmful pesticides and measuring the disease burden of bees from nests with, and without infestations. The nests used in the studies conducted in Chapters 5 and 6 were not screened for wax moth larvae before experiments commenced. The feasibility of detecting wax moth eggs or larvae within these nests without destructive sampling is questionable, however it would nonetheless be beneficial to rule out the possibility that nests arrive already infested with these predators.

This thesis has examined how farm management decisions, such as the use of commercial bumblebee nests, the sowing of wildflowers and the application of pesticides, can impact upon pollinator health and crop pollination; and provides the first experimental evidence of the frequency and severity of wax moth infestations in commercial bumblebee nests. It has highlighted that the relationship between farmers and the insects pollinating their crops can be mutualistic as both can benefit from each other. However, it has also sought to demonstrate that, if this mutualism is to be maximised, farmers need to consider the health and sustainability of beneficial pollinators when making certain management decisions e.g. which pesticides to apply and whether or not to establish wildflower plantings. Whilst pesticides can be a valuable tool in tackling insect crop damage the negative impacts they can have on beneficial, non-target organisms can no longer be ignored. For farmers growing pollinator dependent crops the use of neonicotinoids on their farms, or in neighbouring areas, will likely reduce the abundance and resilience of wild pollinator populations. Conversely, investing in wildflower strips that provide a diversity of year round forage is likely to increase bee numbers around the farm; a benefit that may then spill-over onto the crop. Whilst the use of commercially reared pollinators may be a necessary consequence of decoupling crops from traditional growing seasons, the potential risks involved in their use seem to be gaining attention. If future restrictions are placed on the application of these bees, farmers will benefit from having invested in an integrated approach to pollinator management, whereby they foster and rely on a range of pollinating species, not just one.

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