Multitrophic ecosystem services of hoverflies in strawberry

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To all the people (and insects and flowering plants) who made this project possible. And to my family and friends for humouring me through the hard times and the good.

Acknowledgements

Abstract

Agricultural advances in the last half-century have enabled the production of larger harvests. However, farmland is now at greater risk of pest outbreaks due to losses of genetic diversity within crops rendering crop plants more vulnerable to disease. Moreover, declines in biodiversity in the wider landscape mean that fewer predators of crop pests are present to control pest species. Equally worrying are recent declines in wild and managed insects that are necessary for the pollination of 84% of crop species in Europe. In commercial strawberry (*Fragaria* x *ananassa*), aphid damage is estimated to cost growers at least £2.5 million per year in the UK alone. Moreover, in the absence of pollinating insects, strawberry yields would fall by approximately £112.5 million per year.

In order to counteract these threats, I investigated the pollinator assemblages in commercial strawberry crops; the pollination effectiveness of aphidophagous hoverflies (Diptera, Syrphidae, Syrphinae); the effectiveness of planting wildflowers within strawberry fields to improve pollination and aphid pest control throughout the crop; and the gut contents of potential pest-controlling hoverflies within strawberry crops.

Retail and pick-your-own fruit farms have dynamic pollinator assemblages, including hoverflies. In cage studies, pollination by aphid-eating hoverflies doubled proportions of marketable strawberries when compared to insect-excluded controls. Coriander (*Coriandrum sativum*) planted within strawberry crops reduced aphid infestations and encouraged aphid predators to lay more eggs near aphid colonies. Furthermore, higher counts of pollinators were recorded in coriander plots, though no differences were found in fruit quality across all treatments. Finally, aphid DNA was recovered from the digestive tracts of hoverfly larvae. Prey DNA detection rates were greater near forget-me-not plots than coriander plots.

These findings suggest that hoverflies act as pollinators and pest-controllers in strawberry, and that by integrating coriander within strawberry crops their effects may be enhanced. Future work

should focus on how to augment hoverfly populations in strawberry crops to further enhance their

efficacy so that pesticide use can be further reduced.

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Chapter 1: Introduction

Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are entirely my own. Where I have consulted the work of others, this is clearly stated.

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The global human population is expected to grow steadily through the twenty-first century before reaching a plateau at around 9 to 10 billion (Bongaarts 2009; Godfray et al. 2010). With food resources stretched as it is, feeding an additional 2 to 3 billion people will put significant pressure on our agricultural systems (Godfray et al. 2010; Garibaldi et al. 2017). The issue of food security has been developing gradually for centuries, with the amount of land dedicated to food crops growing by 466% between 1700 and 1980. However, since 1980, the expansion of farmland has slowed in response to the diminishing availability of arable land and agricultural intensification (Meyer and Turner 1992; Garibaldi et al. 2017).

Advances in agricultural methods have improved productivity, often at the expense of biodiversity, by increasing crop density, numbers of crops grown in a season and the use of pesticides and fertilisers, while reducing crop diversity, numbers of small farms, hedgerows and fallow land (Tscharntke et al. 2005; Crowder and Jabbour 2014; Sánchez-Bayo and Wyckhuys 2019). Though the process of agricultural intensification has wrought rewards of larger harvests, huge swaths of farmland are now at greater risk of pest outbreaks (Matson et al. 1997; Crowder and Harwood 2014; Sánchez-Bayo and Wyckhuys 2019). Equally worrying are recent declines in wild and managed pollinating insects (Biesmeijer et al. 2006; Potts et al. 2010; Carvalheiro et al. 2013). Approximately 70% of world food crops rely on animal pollinators (Aizen et al. 2009), with that proportion rising to 84% for crops in Europe (Klein et al. 2007). Moreover, the proportion of global agricultural production that relies on animal-pollinated plants has nearly doubled in the last 50 years (Aizen et al. 2008). Therefore, the loss of pollinating insects would have dramatic and potentially irreversible consequences for biodiversity, agriculture and food security (Meffe 1998; Potts et al. 2010; Sánchez-Bayo and Wyckhuys 2019).

Compounding these concerns is the greater volatility of the global climate, which will likely result in increased frequencies of droughts, flooding and other extreme weather events (IPCC 2018). Climate change may also threaten crop yields by altering pest-predator and plant-pollinator

interactions (Memmott et al. 2007; Hegland et al. 2009; Potts et al. 2010; Burkle et al. 2013; Petanidou et al. 2014; Polce et al. 2014; Welch and Harwood 2014; Sánchez-Bayo and Wyckhuys 2019). As a result, and in order to address these issues and avoid human mass starvation, our society will require a global strategy incorporating radical changes in the way food is produced and distributed (Godfray et al. 2010; Garibaldi et al. 2017).

Crop pollination ecology

Role of wild pollinators

In addition to facing the threat of pest outbreaks, global crop yields are also susceptible to losses as a result of declines in the number and diversity of pollinating insects that visit crop flowers (Steffan-Dewenter et al. 2005; Klein et al. 2007; Potts et al. 2010; Garibaldi et al. 2014). Research by Garibaldi et al. (2013) has demonstrated that contrary to earlier assumptions about the effectiveness of managed pollinators, honey bees, *Apis mellifera* L., cannot replicate the quality of pollination provided by wild insects. Based on surveys conducted in 41 crop systems from around the world, fruit set increased significantly with wild pollinator visitation across all crop types, whereas honey bees enhanced fruit set in only 14% of crops (Garibaldi et al. 2013). Bommarco et al. (2012) quantified the benefit provided by wild pollinators and found that insect pollination contributed 18% to crop yields and 20% to market value in oilseed rape, *Brassica napus* L. Thus, in order to ensure that future crop yields are sustained at levels necessary to feed a growing human population, the maintenance of a healthy wild pollinator community is indispensable (Klein et al. 2007; Potts et al. 2010; Garibaldi et al. 2013).

Not only does the presence of unmanaged pollinating insects improve crop yields, but several studies have shown that a greater taxonomic breadth of flower visitors can also enhance crop pollination services (e.g., Kremen et al. 2002; Klein et al. 2003; Morandin and Winston 2005; Greenleaf and Kremen 2006; Winfree et al. 2007, 2008; Hoehn et al. 2008). On a basic level increased pollinator diversity safeguards against annual fluctuations in populations of individual

species of flower visitors (Kremen et al. 2002; Greenleaf and Kremen 2006; Winfree et al. 2008). Though at first glance annual oscillations in population levels may seem insignificant, Roubik (2001) argued that populations of pollinating insect taxa can double or halve from year to year. Similarly, Kremen et al. (2002) recorded significantly different pollinator assemblages visiting the same coffee crops over two years of sampling. As was the case in the study of coffee plantations, more species-rich communities of pollinating insects compensate effectively for natural variations in individual species abundances (Kremen et al. 2002; Greenleaf and Kremen 2006; Winfree et al. 2008). Furthermore, in a study of apple orchards in northeastern USA, Bartomeus et al. (2013) note that diverse bee assemblages buffer against the effects of differing responses to climate change, which could otherwise have resulted in phenological mismatches between bee emergence and apple peak flowering dates.

Pollinator behaviours on flowers

However, another way in which diverse groups of flower visitors benefit crop pollination is through the varied behaviours that different species exhibit during visits (Chagnon et al. 1993; Hoehn et al. 2008). Hoehn et al. (2008) cite factors such as time of day when different species are most active and time spent on flower heads. The authors separate pollinators into two guilds: early, large bees, which deposit larger amounts of pollen, and late, small bees, which deposit less pollen, but spread pollen grains around the flower head more evenly. Chagnon et al. (1993) view these opposing strategies as complementary in the successful pollination of crops. Therefore, more diverse communities of pollinators offer insurance against annual fluctuations in individual populations (Kremen et al. 2002; Greenleaf and Kremen 2006; Winfree et al. 2008) and against divergent responses to climate change (Bartomeus et al. 2013), and also provide more effective pollination through their varied behaviours in timing and activity patterns on flower heads (Chagnon et al. 1993; Hoehn et al. 2008).

Pollinator diversity

An important question to consider when assessing the diversity of pollinator assemblages is what level of diversity is necessary in order to benefit from the full complement of pollination services mentioned above? An emerging consensus suggests that around 20 species of pollinating insect are sufficient to achieve maximum pollination (Kremen et al. 2002; Klein et al. 2003; Morandin and Winston 2005). In their study of Canadian *Brassica napus* and *B. rapa* fields, Morandin and Winston (2005) discovered that for both crops pollination deficits, defined as higher seed set in hand-pollinated relative to open-pollinated flowers, approached zero as the number of flower visitors rose above 20 species per sampling transect. Similarly, in coffee plantations fruit set increased from 60% to 90% when bee diversity increased from three to 20 species (Klein et al. 2003). Thus, while numbers of species required for full pollination may vary from crop to crop (Greenleaf and Kremen 2006), evidence from these studies suggests that 20 floral visiting species is a relatively consistent minimum level of diversity necessary to maximise the benefits received by pollinating insects (Kremen et al. 2002; Klein et al. 2003; Morandin and Winston 2005).

Other researchers addressing the same question have arrived at a more nuanced interpretation of the relationship between floral visitor species richness and plant reproductive success (e.g., Gómez et al. 2007; Perfectti et al. 2009; Albrecht et al. 2012). Rather than corroborating the notion that around 20 species are required to achieve full pollination potential, these authors argue that increasing pollinator diversity will only enhance seed set if the additional species are more effective pollinators. Gómez et al. (2007) explain that less effective pollinators may deposit more pollen from the same plant, which can be less fertile than pollen from other plants, or alternatively they may exhibit less constancy in their choice of food plant and clog the stigmas with incompatible pollen from other plant species. Building on the work of Chagnon et al. (1993) regarding the complementarity of different guilds of pollinator, recent studies have found functional group diversity more important than pollinator species richness in improving rates of pollination (e.g.,

Albrecht et al. 2012; Gagic et al. 2015). Albrecht et al. (2012) show that seed set in radish plants, *Raphanus raphanistrum sativus* (L.) Domin, increases when flowers are exposed to three pollinator species from three functional groups as opposed to three species from one functional group; however, when nine species (three species from each of the three functional groups) are allowed to visit the flowers, seed set decreases slightly from the maximum reached with a single species from each functional group. Though this research indicates that too many pollinators can begin to inhibit pollination, in empirical studies the slight drop in pollination rate at high levels of pollinator diversity is insubstantial compared to the decrease in pollination rate at low levels of diversity (Albrecht et al. 2012). Moreover, to my knowledge no studies have yet documented crop species that are suffering from a pollination deficit due to very highly diverse pollinator communities (Ghazoul 2005; Aizen et al. 2008).

In an attempt to better understand the drivers that allow more diverse assemblages of wild pollinators to flourish, several researchers have investigated the effect of landscape patterns on wild insect communities in crop systems (e.g., Kremen et al. 2004; Sarthou et al. 2005; Marshall and West 2007; Ricketts et al. 2008; Holzschuh et al. 2010; Andersson et al. 2013; Haenke et al. 2014; Castle et al. 2019). In a review of literature on landscape-scale influences on pollinating insect distributions, Ricketts et al. (2008) noted that the distance from semi-natural habitats negatively impacted pollinator species richness (in 16 out of 19 studies), flower visitation rates (20 out of 22 studies) and to a lesser extent seed set of crops (8 out of 12 studies). In addition to proximity to patches of flower-rich habitat, pollinator diversity has also been positively linked to floral abundance (Winfree et al. 2008) and plant species richness (Klein et al. 2003).

Given that flower abundance and diversity tend to be greater on organic farms (Maeder et al. 2002; Hole et al. 2005; Power and Stout 2011), the results of Winfree et al. (2008) and Klein et al. (2003) predict the outcomes of studies that have shown higher pollinator diversities in organic versus conventional farming systems (e.g., Morandin and Winston 2005; Rundlöf et al. 2008;

Holzschuh et al. 2010; Andersson et al. 2013). In some contexts converting to organic farming may represent the difference between benefitting from full pollination services and experiencing a pollination deficit (Morandin and Winston 2005). However, in spite of the benefits of organic farming, several researchers have highlighted habitat heterogeneity as being more influential than organic farm management regimes (e.g., Benton et al. 2003; Tscharntke et al. 2005; Rundlöf and Smith 2006; Winfree et al. 2008). These authors argue that organic farming confers the benefits documented above only when the surrounding matrix is relatively homogeneous. In landscapes that are already complex mosaics of semi-natural and managed habitats, a switch to organic farming does little to enhance farmland biodiversity because the conditions necessary for species-rich faunal communities to develop have already been met (Benton et al. 2003; Tscharntke et al. 2005; Rundlöf and Smith 2006; Winfree et al. 2008).

Plant pollinators

Pollinating insects include members of the following orders: ants, bees and wasps (Hymenoptera), beetles (Coleoptera), butterflies and moths (Lepidoptera), and flies (Diptera) (Orford et al. 2015; Rader et al. 2016). Though bees (Hymenoptera: Apoidea) constitute the most commonly studied group of flower-visiting insects in agricultural ecosystems, non-bee insects account for 25-50% of crop flower visits worldwide (Rader et al. 2016). Thus, non-bees provide an economically important contribution to global pollination services (Orford et al. 2015; Rader et al. 2016).

Within the Diptera, hoverflies (Diptera: Syrphidae) represent a speciose family of flower-visiting insects with 283 species present in the UK and Ireland (Ball and Morris 2015). In the context of pollination ecology, hoverflies are also the most frequently studied family of dipterans (Orford et al. 2015). However, not only do hoverflies visit flowers as adults, but during their larval stage, species in the subfamily Syrphinae predate aphids (Rotheray and Gilbert 2011). Therefore, syphine hoverflies may be capable of delivering both pollination and pest control services.

Pollinator distributions

A variety of factors likely play a role in determining diversities and distributions of pollinator assemblages; however, the most species-rich communities tend to have abundant and diverse flowering plants and are typically located in complex habitat mosaics within close range of semi-natural habitats (Benton et al. 2003; Klein et al. 2003; Tscharntke et al. 2005; Rundlöf and Smith 2006; Winfree et al. 2008; Garibaldi et al. 2011). In more homogeneous landscapes organic farming management regimes enhance invertebrate diversity and represent a context-specific habitat characteristic that can confer biodiversity benefits (Benton et al. 2003; Morandin and Winston 2005; Ricketts et al. 2008; Winfree et al. 2008; Holzschuh et al. 2010).

For syrphids, species distributions depend, in part, on larval life history, with aphidophagous flies preferring fields with floral resources and hedges, and saprophagous hoverflies favouring fields with forested edges (Sarthou et al. 2005; Marshall and West 2007; Haenke et al. 2014). Haenke et al. (2014) discovered that aphidophagous syrphids were most frequent in fields with hedges that connected forest patches, moderately abundant in fields with isolated hedges and least common in fields with edges dominated by forest. Saprophagous hoverflies, on the other hand, preferred fields adjacent to forests over fields with hedgerows of either variety. The authors attributed the differences in habitat preferences to larval feeding modes (Haenke et al. 2014). Both Marshall and West (2007) and Sarthou et al. (2005) found that numbers of aphidophagous syrphids were highest near fields with flowering crops, though in the autumn Sarthou et al. (2005) documented greater abundances of hoverflies near areas of sheltered habitat irrespective of the amount of nearby floral resources. These trends provide conservationists with the evidence required to begin to formulate management recommendations that foster greater and more diverse populations of pollinating insects in agricultural ecosystems (Tscharntke et al. 2005).

Hoverflies as pollinators of strawberry

Hoverflies were identified as among the most frequent insect visitors to strawberry (*Fragaria* x *ananassa* Duch.) flowers four decades ago (Nye and Anderson 1974). More recently, Kovanci et al. (2007) found that hoverflies were the most abundant aphidophagous predators in organic strawberry crops in Turkey. Despite these discoveries, the extent to which hoverflies provide pollination and pest control ecosystem services in strawberry fields remains largely a mystery (Figure 1.1; Nye and Anderson 1974; Cross et al. 2001; Kovanci et al. 2007).

Figure 1.1 The marmalade hoverfly (*Episyrphus balteatus*) as flower-visiting imago (left) and aphidophagous larva (right) in strawberry crops.

The pollination of strawberry flowers by insects has generated a considerable amount of research interest among ecologists (e.g., Free 1968; Nye and Anderson 1974; Chagnon et al. 1993; Carew et al. 2003; Albano et al. 2009a, b; Roselino et al. 2009; Andersson et al. 2012; Castle et al. 2019). A recurring theme in the studies on strawberry pollination is the role of pollinator visits in determining the frequency of fruit malformations, arising from unfertilised achenes (Nye and Anderson 1974; Chagnon et al. 1993; Carew et al. 2003; Albano et al. 2009b; Roselino et al. 2009; Andersson et al. 2012; Castle et al. 2019). As an aggregate flower, strawberries require that all carpels be evenly distributed and fully pollinated in order to produce high-quality fruit (Carew et al. 2003). Studies have shown that in the absence of insect pollinators, the pollination rate for

strawberry flowers rarely exceeds 60%, with malformations present on around 47% of fruit,

demonstrating a significant reliance on animal-mediated pollen transport for optimal fruit

development (Chagnon et al. 1993; Klatt et al. 2014; Castle et al. 2019). Indeed, research for the UK

National Ecosystem Assessment revealed, in 2007, British strawberry growers relied on insect

pollination for 45% of crop yields, representing around £72 million of the market value in the UK that

year (Smith et al. 2011). Since then, the added value in terms of additional strawberry yields

provided by managed and wild insects has risen to over £110 million/year (Defra 2017).

Strawberry pollinators

Due to their less specialised characteristics, such as radial symmetry, disc shape, easily

accessible nectar and exposed anthers, strawberry flowers are visited by a wide range of insects

(Albano et al. 2009a). Research on the effectiveness of various strawberry pollinators has shown that

several insects are more or less equally important in the creation of high-quality fruit, and indeed

that visits from pollinators with diverse morphologies and behavioural habits tend to produce fruit

more frequently and with fewer malformations (Chagnon et al. 1993; Albano et al. 2009a, b). In

particular, Chagnon et al. (1993) documented behavioural differences between honeybees and small

solitary bees, which led to more complete pollination of strawberry flowers: the large-bodied

honeybees tended to land on the centre of flowers and cause pollination of the apical pistils, while

smaller-bodied solitary bees followed a circular path around the edge of the flower head, pollinating

the pistils that would form the base of the fruit. Subsequent studies, in line with the results from

Chagnon et al. (1993), found that larger-bodied pollinators such as honeybees and large hoverflies

were equally effective at pollinating strawberry flowers on their own, but that no individual species

was more efficient than the combination of multiple species that visited open-pollinated flowers

(Albano et al. 2009a, b; Andersson et al. 2012). In addition, recent research on protected strawberry

crops found that commercially-reared bumblebees were able to boost yields by 17.5% in June-

bearer strawberries (Martin 2018).

In a study of the flower visitor assemblages in strawberry and raspberry fields, Ellis et al. (2017) found that although strawberry flowers attracted fewer visitors per flower than raspberry plants, a wider range of taxa visited strawberry because the flowering period extended longer over the course of the summer. Whereas bees were important flower visitors early on in the growing season, flies became the most numerous flower visitor taxa towards the end of the season. The authors reached the conclusion, therefore, that strawberry is pollinated by a wide range of taxa with different bee and fly species providing important pollination services at different points in the growing season (Ellis et al. 2017). Thus, maintaining a diverse assemblage of wild pollinators is a key component to the production of high-quality strawberries, and hoverflies likely play an integral role in the pollination success of the crop (Albano et al. 2009b; Andersson et al. 2012; Ellis et al. 2017; Castle et al. 2019). However, no studies have quantified the direct contribution that hoverflies make to strawberry yield and quality in the absence of other pollinating insects.

Integrated Pest Management

One technique that has been shown to improve crop yields and economic returns without causing significant harm to the natural environment is Integrated Pest Management (IPM; Dent 1995). Unlike farming practices that have led to greater intensification of agriculture, IPM practices seek to reduce our reliance on pesticides by promoting methods of cultural, mechanical and biological pest control (New 2005; Crowder and Harwood 2014). Most prominent among the tools employed in IPM is the use of predators and parasitoids of pest species (Alford 2011). Given that the natural enemies of pest species are often the most important means of population control in nature, their use in agriculture is a logical solution to the dilemma of how best to manage pest arthropods without degrading the environment (New 2005).

Among the myriad of pest species that damage food crops and threaten farmers with financial ruin, few are more notorious than aphids. Fortunately for farmers, almost equally numerous are the predators and parasitoids that feed on aphids (Alford 2011). One group that has evolved to rely, in

large part, on aphids for their diet are the larval stages of hoverflies (Diptera: Syrphidae) belonging

to the subfamily Syrphinae (Rotheray and Gilbert 2011). Syrphine hoverfly larvae are voracious aphid

predators, with a single larva of the widespread *Episyrphus balteatus* De Geer consuming between

660 and 1140 third-instar aphids during development (Tenhumberg and Poehling 1995). In spite of

their potential as biological control agents (BCAs), most studies of aphid-eating, or aphidophagous,

insects to date have focused on ladybirds (Coleoptera: Coccinellidae) or lacewings (Neuroptera:

Chrysopidae) (Cross et al. 2001; Leroy et al. 2010). Nevertheless, there is growing interest in

hoverflies as evidenced by recent studies on their efficacy at controlling aphid infestations in cereal,

lettuce, broccoli, Brussels sprout, and cabbage crops (van Rijn et al. 2006; Prasad et al. 2009;

Gillespie et al. 2011; Hogg et al. 2011; Ramsden et al. 2014; Raymond et al. 2014).

Research on the efficacy of hoverfly larvae as BCAs has produced mixed results in field studies,

with some workers finding significant reductions in aphid numbers (e.g., Jankowska 2005; van Rijn et

al. 2006; Dib et al. 2011; Wojciechowicz-Żytko 2011), while other studies have yielded more

equivocal results (e.g., Prasad et al. 2009; Gontijo et al. 2013). In organic *Brassica* crops in British

Columbia, Prasad et al. (2009) found that hoverflies represented 75% of aphid predators

encountered in surveys over a four-year period. However, despite their abundance, aphidophagous

syrphid larvae did not effectively suppress aphid populations because the predators appeared one to

two weeks after aphids colonised the crops, and hoverfly populations did not grow rapidly enough to

keep aphid numbers under control. Similarly, Gontijo et al. (2013) discovered that hoverfly larvae did

not respond quickly enough to keep aphid populations in check in Californian apple orchards. These

studies suggest that the effectiveness of hoverflies as BCAs may depend on the crop system involved

or the location of the farm.

In contrast to the evidence above, van Rijn et al. (2006) found that syrphine larvae were able to

limit aphid numbers in Brussels sprout crops in the Netherlands. Moreover, hoverfly larvae were

nearly twice as abundant within four metres of a mixed-flower field margin as in other parts of the

crop, demonstrating the potential of wildflower strips to attract beneficial insects (van Rijn et al. 2006). Other researchers have also documented increased suppression of aphid populations in fields with sown wildflower strips (Bowie 1999; Hogg et al. 2011). For example, Hogg et al. (2011) revealed that planting strips of sweet alyssum, *Lobularia maritima* (L.) Desv., alongside lettuce crops in California boosted numbers of larvae of the hoverfly *Eupeodes fumipennis* (Thomson) to such an extent that aphid colonies were reduced by over 80%. Bowie (1999) documented significantly lower numbers of aphids in areas near wheat field edges planted with canola, which coincided with the regions harbouring the greatest densities of syrphine larvae. Thus, in the right context and with the aid of additional pollen and nectar sources, aphidophagous hoverflies are capable of regulating aphid colonies in at least some crops so that pest populations do not reach economically damaging levels.

Aphid predators in strawberry crops

To date, relatively little research has been conducted on biological control options for aphids on strawberry crops (Easterbrook et al. 2006). However, the need for alternative control methods is great, given that some pest species, such as *Aphis gossypii* Glover and *Macrosiphon euphorbiae* Thomas, are already difficult to suppress with chemicals and others, like *Chaetosiphon fragaefolii* (Cockerell), may soon develop resistance to the insecticides that are currently used for their control (Cross et al. 2001). In addition, the 2019 EU-wide ban on the use of pymetrozine has further restricted the options for farmers who had also previously relied on neonicotinoids to control aphid outbreaks.

In one of the few studies to investigate the role of a wide range of aphid predators in strawberry crops, Kovanci et al. (2007) discovered that hoverfly larvae were the most abundant aphidophagous insect species in strawberry fields, representing a little over a quarter of all aphid predators collected. Moreover, the authors documented a positive correlation between aphid numbers and predator populations, suggesting that the predators played a significant role in keeping

aphids below economically damaging levels. Based on their findings, Kovanci et al. (2007) underscored the potential effectiveness of syrphid larvae as BCAs and recommended the use of habitat management techniques that provide additional pollen and nectar sources to encourage the growth of hoverfly populations. Therefore, despite the comparatively little attention that has been given to hoverflies as BCAs in strawberry crops, the use of their larvae for aphid control may offer strawberry growers an alternative means to keep aphid numbers below economically damaging levels.

Documenting trophic interactions

Establishing trophic interactions in agroecosystems is an important first step in identifying naturally-occurring predators that may be capable of providing effective biological control of aphids. Among the diverse array of predatory arthropods that consume aphids, syrphine hoverfly larvae are voracious predators, consuming up to 168 aphids per day (Hopper et al. 2011). Nevertheless, documenting trophic linkages between species is difficult in a field setting because visual observation is time-consuming and can disrupt normal predator foraging decisions (Gomez-Polo et al. 2015). Microscopic analysis of digestive tract contents can be useful when insect diets contain solid food fragments, but hoverfly larvae are exclusively fluid feeders. Fortunately, molecular analysis is an effective tool for investigating the diet of fluid-feeding invertebrate predators (Piñol et al. 2014).

The development of high-throughput sequencing technologies has allowed the analyses of the dietary breadth of insect predators without the need to predict and develop primers for target prey species, as is the case in polymerase chain reaction (PCR)-based techniques (Pompanon et al. 2012). Additionally, the shorter (157 bp) amplicon used allows for the detection of smaller DNA fragments, typical of the contents of insect predator guts (Gomez-Polo et al. 2015) where DNA is thought to rapidly degrade. Shorter fragments are more likely to be recovered but may impose a limit on taxonomic resolution thus imposing a trade-off (Clare 2014).

Gomez-Polo et al. (2015) used a range of sequencing techniques to analyse the gut contents of hoverflies reared on lettuce crops in Spain. Their analysis found that the aphid *Nasonovia ribisnigri* was the most frequent prey species uncovered in specimens of the hoverfly *Episyrphus balteatus*. Thus, these results suggest that aphidophagous hoverflies may act as biocontrol agents for aphids in lettuce (Gomez-Polo et al. 2015). Although earlier studies looked at the potential of hoverfly larvae to act as BCAs in strawberry, strawberry management and cultivation have changed dramatically and no genetic studies have looked at predation in strawberry crops.

Habitat management for beneficial insects

Among the myriad effects of agricultural intensification, the factors that are most frequently cited as exacerbating pollinator declines are the homogenisation of farmland landscapes and the loss of wildflower-rich meadows, hedgerows and field margins (Marshall and Moonen 2002; Ricketts et al. 2008; Andersson et al. 2013). Given that farmers typically have little control over the landscape context in which their farm is located, most efforts to foster more diverse pollinator communities have focused on within-farm enhancements for beneficial insects, such as switching to organic farming regimes, restoring hedgerows or sowing flower-rich field margins (Holzschuh et al. 2008; Morandin and Kremen 2013; Castle et al. 2019). These three techniques tend to increase the diversity and abundance of flowering plants on farms, which in turn promote the establishment of more diverse assemblages of pollinating insects (Carvell et al. 2006a; Ebeling et al. 2008; Morandin and Kremen 2013; Castle et al. 2019).

Indeed the provision of additional floral resources may be more significant than eliminating the use of agrochemicals in boosting populations of beneficial insects (Winfree et al. 2008; Brittain et al. 2010). Most studies of pollinator communities at conventional versus organic farms have documented more diverse pollinator assemblages at organic farms only if the diversity and abundance of wildflowers were also greater on organic farms (e.g., Morandin and Winston 2005; Holzschuh et al. 2007, 2008; Power and Stout 2011; Andersson et al. 2012). Studies that have

selected organic and conventionally-managed farms with similar flowering plant communities have found no significant differences between the pollinating insect populations at the different farm types (Winfree et al. 2008; Brittain et al. 2010). Thus, evidence from several studies suggests that while switching to organic farming can result in higher arable weed diversity (Menalled et al. 2001; Hyvönen and Salonen 2002; Roschewitz et al. 2005), floral diversity and abundance are more important than the type of farm management regime in fostering speciose pollinating insect communities (Morandin and Winston 2005; Winfree et al. 2008; Brittain et al. 2010).

Sown wildflower strips

In light of the equivocal and context-dependent benefits of organic farming for wild pollinators, creating within-farm semi-natural habitat elements such as sown wildflower strips appears to be the most direct and widely-applicable strategy for bolstering communities of pollinating insects (Rundlöf and Smith 2006; Winfree et al. 2008; Brittain et al. 2010). This view is reflected in current government policy in the UK: the Wild Pollinator and Farm Wildlife Package of the Countryside Stewardship Scheme, which began in 2016, focuses on the establishment of additional floral resources along field edges and on fallow land in order to conserve wild pollinators (Natural England 2013; Defra 2014). As this policy document highlights, uncropped field margins provide not only essential pollen and nectar resources, which may be lacking when crops are not in flower (Garratt et al. 2014b), but also potential nesting sites due to the fact that sown wildflower strips offer undisturbed habitat patches in an environment that is otherwise prone to frequent disturbances (Kells et al. 2001; Marshall and Moonen 2002; Natural England 2013; Defra 2014). Moreover, field edges have become a focus of pollinator conservation efforts in agroecosystems because crop margins are the least productive part of the field, are more prone to drought and shading, and typically possess the greatest floral diversity within the field (Kells et al. 2001; Pywell et al. 2005). Therefore, sowing wildflowers along field boundaries represents the most cost-effective opportunity for farmers to increase the diversity and abundance of arable weeds and thereby encourage the

establishment of greater assemblages of wild pollinators (Kells et al. 2001; Marshall et al. 2006; Haaland et al. 2011; Natural England 2013).

Having identified field edges as the most appropriate part of agricultural fields in which to establish habitat for beneficial insects, the next step is to determine the best method of management to encourage farmland wildlife to visit crops (see Pywell et al. 2006; Carvell et al. 2007). Two contrasting management strategies have been assessed for their potential use in agri-environment schemes: in the first, known as conservation headlands, the outer edge of the crop is not treated with herbicides or pesticides to allow arable weeds and associated insects to flourish, while in the second method, an uncropped and unsprayed margin is either allowed to regenerate naturally or is sown with a wildflower and/or grass seed mixture (Kells et al. 2001; Pywell et al. 2005).

In a study comparing the attractiveness to bumblebees of conservation headlands and uncropped, naturally-regenerated field margins, Kells et al. (2001) found that bumblebee assemblages were consistently more abundant and diverse on naturally-regenerated field edges than in conservation headlands. The authors cited the much higher diversity of entomophilous, or insect-pollinated, plants in uncropped field margins as the most likely driver of the higher abundances of bumblebees. Providing further support to the case that uncropped margins are preferable to conservation headlands for bumblebees, Pywell et al. (2005) documented higher abundances of bumblebees on both naturally-regenerated field edges and margins sown with wildflowers, as compared to conservation headlands, though conservation headlands were more species-rich than conventionally-managed control margins. In this study field edges sown with wildflower seed mixes were found to host the highest number of pollinators; moreover, naturally-regenerated margins tended to support a large number of pernicious weeds (*Cirsium* spp.) which were the primary nectar sources for bumblebees in that habitat type. As a consequence, sown wildflower strips are the preferred method of attracting pollinators to agroecosystems in the UK and

have become the principal management option in environmental stewardship schemes aimed at pollinator conservation (Pywell et al. 2005; Carvell et al. 2007; Natural England 2013; Defra 2014).

Crop quality

Increased abundances of beneficial insects are advantageous to fruit growers because they enhance crop quality and economic value (Blaauw and Isaacs 2014; Garratt et al. 2014a). Several studies have established a link between insect visitation and improved crop quality (e.g., Jauker et al. 2012; Garratt et al. 2014a; Klatt et al. 2014). However, comparatively few studies have managed to document enhanced crop quality following habitat management techniques designed to attract beneficial insects. In a four-year habitat manipulation study in Michigan, USA, Blaauw and Isaacs (2014) found that highbush blueberry crops adjacent to sown wildflower strips produced better quality fruit than plants next to mown field margins. That said, the benefits to crop production were only observed in the third and fourth years after establishment of the wildflower margins. The authors argue that it took beneficial insect communities multiple years to respond to the additional floral resources to such an extent that the insects could then impact blueberry yield and fruit quality measures (Blaauw and Isaacs 2014). Thus, this study offers evidence to suggest that wildflower strips may not only augment populations of beneficial insects, but that these increases may translate to enhanced crop production and economic value.

Wildflower selection

Key to the success of sown wildflower margins at attracting pollinators is the careful selection of flower species mixtures that will establish well on fertile, ex-arable soils; outcompete other weed species; contain a variety of corolla depths to attract a wide range of short- and long-tongued pollinators; include early- and late-flowering species to entice and sustain pollinating insects throughout the spring and summer; and exclude pernicious weed species that may invade other parts of the farm (Pywell et al. 2003; Carvell et al. 2006b; Pywell et al. 2006; Mallinger et al. 2019).

To narrow the enormous array of possible species to include, perennials have been shown to be more attractive to pollinators and more cost effective (Meek et al. 2002; Pywell et al. 2005; Carvell et al. 2006b). In particular members of the Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Ranunculaceae and Rosaceae families have been found to attract a wide range of beneficial insects from several orders (Meek et al. 2002; Pywell et al. 2005; Carvell et al. 2006b; Rotheray and Gilbert 2011). Hence, the majority of studies have focused on encouraging wild pollinators and natural enemies by placing the floral resources on the margins of the crop. As far as I am aware, no studies have utilised wildflowers within strawberry crops to boost pollination and natural enemy abundance.

The main aim of this thesis was to determine the role that hoverflies play in pollination and aphid control in strawberry crops. The following four hypotheses were tested:

H1: Strawberry flowers are visited by a range of insects, amongst which a variety of hoverfly species is included. The abundance, species richness and diversity differ between commercial strawberry plantations.

H2: Aphidophagous hoverflies are crucial to strawberry production because visits to flowers improve pollen transfer and resultant yield and fruit quality. In addition, pollination efficacy can vary according to the species of hoverfly.

H3: The provision of additional floral resources within commercial strawberry crops can improve fruit quality and reduce the incidence of aphids by promoting hoverfly abundance.

H4: It is possible to determine the predation rate of aphids by hoverflies in crops through the detection of aphid genetic material in the digestive tract of hoverfly larvae.

These hypotheses were examined using the methods detailed in subsequent chapters. Chapter 2 covers field surveys of strawberry flower-visiting insects at eight fruit farms in the southeast of Britain with the aim of uncovering management and environmental factors that may impact flower

visitor assemblages. The effectiveness of aphidophagous hoverflies at pollinating strawberry is examined using small cages in multiple replicated experiments (Chapter 3). Then, a field experiment at a commercial fruit farm addresses the possibility of introducing additional floral resources into strawberry rows to improve pollination (through insect visitation frequency) and aphid biocontrol services (Chapter 4). Chapter 5 investigates the ability of high-throughput sequencing to detect aphid DNA in hoverfly larvae collected at the same commercial strawberry farm. Finally, key findings from preceding chapters are summarised and directions for future research are explored in the General Discussion (Chapter 6).

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Chapter 2: The relative frequency of insect flower visitors in open and polythene-clad strawberry crops

Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are entirely my own. Where I have consulted the work of others, this is clearly stated.

Signed: _____

Date: _____

Abstract

Given ongoing declines in managed and wild pollinators, uncovering factors that shape assemblages of crop flower visitors is becoming increasingly important to safeguard the delivery of pollination services. Not only do growing practices such as polythene-clad tunnels have the potential to impede access to crops, but crop flowering times also likely play a role in determining which taxa of flower visiting insects are able to pollinate crops. Field surveys of flower visitors were conducted at commercial strawberry crops grown either under polythene-clad tunnels or in the open at eight fruit farms in the southeast of the UK to investigate whether species assemblages differ by site type. Flower visitor abundance and diversity were analysed using generalised linear mixed models to highlight influential environmental variables. Assemblages of flower visitors were then compared using cluster analysis to group together sites with similar insect assemblages. Finally, pairwise comparisons of sites using the Morisita-Horn dissimilarity index were employed to uncover whether sites with differing farm management practices also attracted more dissimilar assemblages of flower visitors. More diverse assemblages of flower visitors were observed at open sites as compared to polythene-clad sites. Bumblebees were the most numerous functional group in the first two survey rounds; however, hoverflies and honeybees became the most frequent functional groups thereafter. Pairwise site comparisons revealed farm management techniques may influence the variety of insects visiting the crop. The observed week-to-week variation in pollinator assemblages underscores the importance of having a diverse group of pollinators to ensure the consistent delivery of pollination services over the course of the growing season. Moreover, the diverse cast of flower visiting species needed to effectively pollinate strawberries throughout the season highlights the urgency of measures to safeguard wild pollinators in order to avoid future losses in strawberry yields.

Introduction

Global crop yields face future losses as a result of declines in the number and diversity of pollinating insects (Steffan-Dewenter et al. 2005; Klein et al. 2007; Potts et al. 2010; Garibaldi et al. 2014; Hallmann et al. 2017). Research by Garibaldi et al. (2013) demonstrated that contrary to earlier assumptions about the effectiveness of managed pollinators, honey bees (*Apis mellifera* L.) cannot replicate the quality of pollination provided by wild insects. Thus, in order to ensure that future crop yields are sustained at levels necessary to feed a growing human population, the maintenance of a healthy wild pollinator community is essential (Klein et al. 2007; Potts et al. 2010; Garibaldi et al. 2013).

Several studies have shown that a greater taxonomic breadth of flower visitors can also enhance pollination services for a number of crops: e.g., canola, *Brassica napus* L. (Morandin and Winston 2005), coffee, *Coffea arabica* L. (Klein et al. 2003), pumpkin, *Cucurbita pepo* L. (Hoehn et al. 2008), watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Kremen et al. 2002; Winfree et al. 2007, 2008), tomato, *Solanum lycopersicum* L. (Greenleaf and Kremen 2006; Winfree et al. 2008), muskmelon, *Cucumis melo* L., and bell pepper, *Capsicum annuum* L. (Winfree et al. 2008). Increased pollinator diversity can act as a buffer against annual fluctuations in populations of individual species of flower visitors (Williams et al. 2001; Kremen et al. 2002). Roubik (2001) argued that populations of pollinating insect taxa can double or halve from year to year. Furthermore, in a study of apple orchards in northeastern USA, Bartomeus et al. (2013) noted that diverse bee assemblages buffer against the effects of differing responses to climate change, which could otherwise result in phenological mismatches between bee emergence and apple peak flowering dates.

Crop pollination is also enhanced through the varied behaviours that different pollinator species exhibit during visits (Chagnon et al. 1993). In addition, Hoehn et al. (2008) cite behavioural factors such as time of day when different species are most active and time spent on flower heads. Therefore, more diverse communities of pollinators offer insurance against annual fluctuations in

individual populations (Winfree et al. 2008) and against divergent responses to climate change (Bartomeus et al. 2013). Moreover, diverse flower visitor assemblages provide more effective pollination through their varied behaviours on flower heads (Chagnon et al. 1993; Hoehn et al. 2008; Woodcock et al. 2013).

Abundances of wild pollinators in agroecosystems can be affected by a range of large- and small-scale environmental factors, such as the presence of nearby semi-natural habitat, the availability of non-crop floral resources and agricultural field size (Kleijn and Van Langevelde 2006; Steffan-Dewenter and Westphal 2008). In addition, management decisions, such as choosing whether to sow wildflower margins or convert to organic farming, can impact flower visitor abundance and diversity (Kennedy et al. 2013; Jönsson et al. 2015). Pollinating insects drawn in by additional floral resources have been shown to effect enhanced fruit set in crops, thereby establishing a direct link between farm management practices and the delivery of pollination services (Blaauw and Isaacs 2014). Recent work by Ellis et al. (2017) suggests that sown wildflower strips may not lead to more frequent visitation to commercial strawberry flowers; however, the authors note that the wildflower strips in their study were small and sometimes distant from the crops. Furthermore, the sown wildflower species did not always establish well.

With their less specialised characteristics, such as radial symmetry, disc shape, easily accessible nectar and exposed anthers, strawberry flowers are visited by a wide range of pollinating insects (Albano et al. 2009). However, to assess the relative importance of different taxa at different sites, surveys identifying flower-visiting insects in strawberry fields are necessary (Albano et al. 2009; Ellis et al. 2017). The first aim of this study was to determine the relative frequency and diversity of insect strawberry flower visitors in crops covered by polythene-clad tunnels and crops grown in the open. It was hypothesised that polythene would impede access to strawberry flowers and result in fewer insect visits from a smaller range of taxa. A second aim of the project was to investigate whether flower visitor assemblages varied over the life of the crop. Previous work on ever-bearing

strawberry cultivars, which produce multiple blooms of flowers from April to September, has

suggested that the species composition of strawberry flower visitors changes seasonally. This

fluctuation in important flower visiting taxa further underlines the importance of having a diverse

pollinator assemblage (Gibson 2012; Ellis et al. 2017). Thus, I hypothesised that assemblages of

visitors to June-bearing strawberries, which produce one flush of flowers between April and July,

would also change during the course of the flowering period.

Methods

In order to compare the flower visitor assemblages at protected and open strawberry

plantations, eight plantations on different farms were selected in the southeast of England (Fig. 2.1):

four protected by Spanish tunnels covered with polythene cladding and four open (unprotected).

The eight study sites varied in farm management type; four produced fruit solely for retail (hereafter

referred to as 'retail farms') and four were pick-your-own (PYO) farms, where the public was invited

to harvest the crop and purchase fruit directly from the grower. A total of 21 surveys were carried

out during the combined flowering periods, April-July 2015, for the strawberry cultivars 'Elsanta'

(grown at five sites) and 'Malwina' (grown at three sites). 'Elsanta' and 'Malwina' are both June-

bearing strawberry cultivars. Fields varied in size, growing method, management type, strawberry

cultivar and provision of managed bees (Table 2.1).

Table 2.1 Characteristics of the eight study sites. Number of surveys is the sample size (total n = 21).

Farm*	Field size (ha)	Growing method	Manage-ment	Strawberry cultivar	Honeybee hives**	Bumblebee hives***	No. of surveys
C1	1.71	Covered	Retail	'Elsanta'	No	Yes	3
C2	2.53	Covered	Retail	'Elsanta'	No	Yes	3
C3	1.93	Covered	Retail	'Elsanta'	Yes	Yes	4
C4	0.21	Covered	PYO	'Elsanta'	No	No	3
O1	2.60	Open	Retail	'Elsanta'	Yes	No	2
O2	0.19	Open	PYO	'Malwina'	Yes	No	2
O3	0.22	Open	PYO	'Malwina'	Yes	No	3
O4	0.45	Open	PYO	'Malwina'	Yes	No	1

*Farms beginning with "C" were covered by polytunnels, while sites beginning with "O" were open.

**Honeybee hives present within 2 km of the field

***Commercial bumblebee hives (*Bombus terrestris audax*) used in the polytunnel
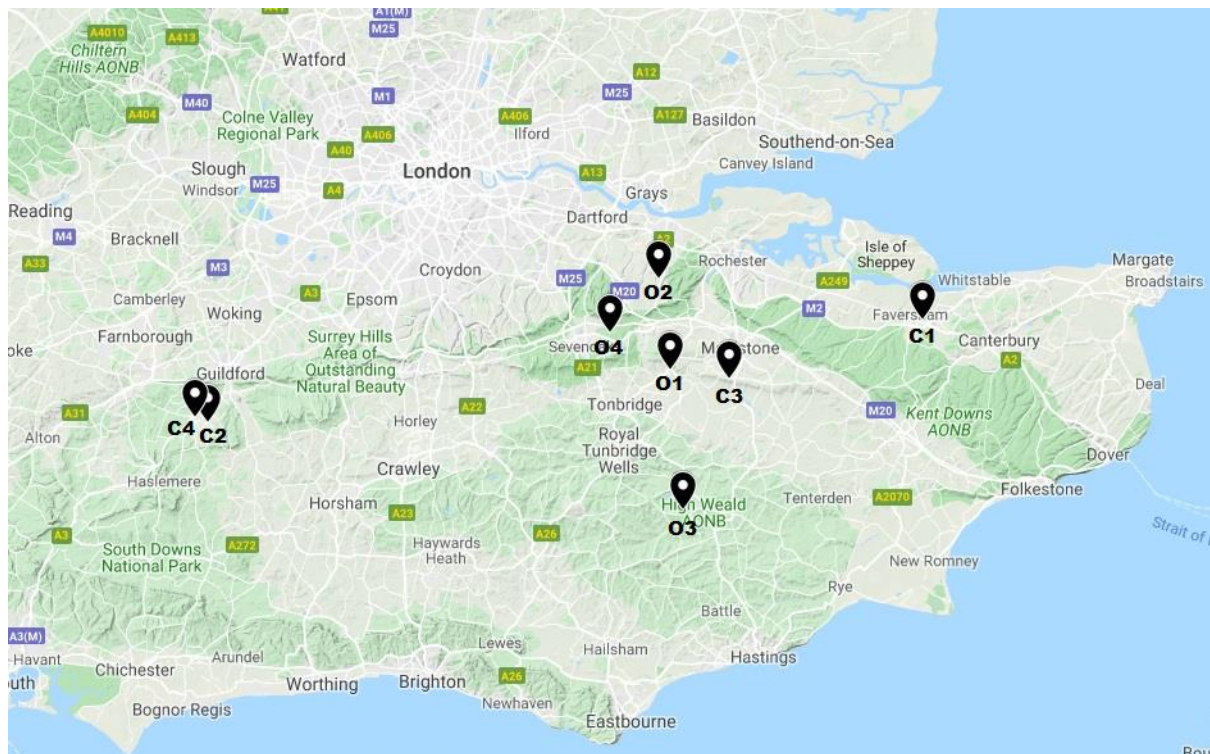
Figure 2.1 Map of the eight study sites. Map data ©2019 Google.

During each round of farm visits, sites were surveyed within a 4-8-day period (mean = 6.4 days), providing the weather conditions were favourable for insect activity as described below. Two study sites were visited per day, with the timing of the surveys for each site (morning or afternoon) alternated from one round of visits to the next in order to reduce bias related to diurnal patterns of insect activity (Rotheray and Gilbert 2011).

To document potential pollinators at each farm, a 100-metre transect along a strawberry row was walked for a period of 30 minutes and insects observed visiting a strawberry flower were recorded. Sites were only surveyed when strawberry plants were in flower. Consequently, the number of surveys per site varied from 1 to 4 (Table 2.1). A sweep net was used to catch strawberry flower visitors that could not be identified in the field (Pywell et al. 2006; Woodcock et al. 2014; Jönsson et al. 2015). Specimens were stored at -20°C for later identification. Cryptic species that could not be identified using morphological characteristics, including *Bombus terrestris/lucorum* workers and *Sphaerophoria* sp. females, were recorded as *B. terrestris/lucorum* and *Sphaerophoria* sp. respectively. Transects were sited nine metres in from the field edge to account for potential edge effects (Chacoff and Aizen 2006).

At the beginning and end of each survey, ambient temperature, relative humidity, wind speed and cloud cover were recorded. Temperature (°C) and humidity (% RH) were measured using a data logger (Omega EL-USB-2, Manchester, UK) situated within 50 m of the transect at open sites and on the side of the polytunnel halfway along the transect at covered sites. Wind speed (km/h) was recorded using a handheld anemometer (Mastech MS6252B, Hong Kong). Cloud cover was assessed in oktas using a circular convex mirror divided equally into eighths. The number of eighths that were covered in cloud was estimated when the mirror was held at waist height. Transect walks were conducted between the hours of 09:00 and 17:00 on dry days. Surveys were undertaken when the temperature was above 10°C, provided that cloud cover did not exceed 4 oktas. On cloudier days, (5-8 oktas), surveys were only conducted if the temperature was above 14°C. Wind speeds had to be

below Beaufort scale 5, or 29 km/h. These weather controls conformed to Butterfly Monitoring

Scheme rules (Pywell et al. 2005). The number of strawberry flowers on each of ten randomly-

selected plants along the transect was also recorded to give an index of floral units available to

potential pollinators at each site.

Data analysis

All analyses were carried out in R version 3.3.3 (R Core Team 2017). Differences in counts of

floral units by growing method, farm management and strawberry cultivar were compared using the

Mann-Whitney *U* test. Mean values, weighted by number of floral units per ten plants per survey,

were calculated for flower visitor abundance, species richness and the inverse Simpson's diversity

index. All averages are presented as mean values ± standard error of the mean.

Generalised linear mixed models were used for modelling flower visitor abundances, species

richness values and inverse Simpson's diversity indices. Plots of residuals against fitted values were

examined for homoscedasticity and normality of the residuals. Fixed effects used in the modelling of

each response variable were separated into survey-level variables that differed for each survey, and

farm-level variables, which differed by farm but remained constant across surveys at the same farm

(Table 2.2). The analyses were then performed using a hierarchical approach, with an optimal model

chosen initially using only survey-level explanatory variables. The optimal model was chosen by

starting with a full model containing all possible survey-level fixed effects and running the 'drop1'

function in R to identify and then remove the least significant fixed effect from the full model. This

process was then repeated, continually removing the least significant explanatory variable, until only

significant fixed effects remained.

Table 2.2 List of variables used as fixed effects in GLMMs to analyse flower visitor abundance, species richness and inverse Simpson's diversity

Survey-level fixed effects	Farm-level fixed effects
Cloud cover* (Oktas)	Farm management (retail or PYO)
Day number (starting with 1 January = 1)	Field area (ha)
Relative humidity* (%)	Growing method (covered by polytunnels or open)
Temperature* (°C)	Strawberry cultivar ('Elsanta' or 'Malwina')
Time of survey* (hour, expressed as a numeric value, midnight = 0.0)	
Time of day (morning or afternoon)	
Wind speed* (km/h)	

*These variables were recorded as the average of readings taken at the start and the end of each survey.

Once an optimal model was derived from the survey-level variables, all farm-level fixed effects were added to the optimal survey-level model and the process of model selection repeated (Ellis et al. 2017). Finally, because the aim of the study was to investigate the effect of management practices while accounting for random variation between farms, farm was included as a random effect term for each model. The significance of the random effect was tested by comparing the final optimal model against a model that only contained fixed effects using the likelihood ratio test.

To compare flower visitor assemblages across sites, strawberry flower visiting species were first assigned to one of five functional guilds: (1) bumblebees: all *Bombus sp.*; (2) honeybees: *Apis mellifera*; (3) hoverflies: all flies in the family Syrphidae; (4) other flies: all dipterans excluding hoverflies; and (5) solitary bees: all members of the superfamily Apoidea excluding honeybees and

bumblebees. The relative frequency of each functional group per survey at the eight sites was then calculated and used to create stacked bar charts to visualise the flower visitor functional group assemblages across the eight sites. Relative frequencies were also weighted by floral units per survey to uncover the influence of floral unit abundance on flower visitor frequencies. Subsequently, a hierarchical cluster analysis was performed using Ward's method and the Morisita-Horn index as a distance measure in order to separate farms into clusters of similar sites based on species abundance per survey and species abundance per survey weighted by floral units. For this analysis, 'species' was defined as the lowest level identification that could be reached for each flower visitor. In some cases this included genus-level identifications, but most were identified to species level.

Dissimilarity among the assemblages of flower-visiting insects at the eight sites was further analysed using the Morisita-Horn dissimilarity index, which is independent of sample size (Baldock et al. 2015). Species totals from transect surveys were divided by the number of surveys conducted at each farm so that abundances were not artificially high at more frequently-surveyed sites. Pairwise comparisons of flower visitor assemblages were then made for all combinations of sites, and each analysis was classed as an open-open site, covered-covered site or open-covered site comparison. Using a beta regression on the Morisita-Horn index values, the mean dissimilarity score for each of the three types of comparison was calculated to reveal whether within-class dissimilarity was greater or less than between-class dissimilarity. When the Morisita-Horn index was equal to one (i.e., when there were no overlapping species between two sites), the value was reduced to 0.9999 so that the beta regression procedure could proceed without errors.

Finally, the abundance of flower visitor functional groups per survey was plotted across the five survey weeks. Generalised linear models were used to analyse abundances of flower visitor functional groups (1-5, as described above) per survey week, employing the same methodology as detailed above for analyses of flower visitor abundance, species richness and Simpson's diversity. The only difference was that the random effect of farm was not included.

Results

Counts of open strawberry flowers at each site revealed that crops under polytunnels reached peak flowering in April or May, whereas open sites reached peak flowering in late May or June (Fig. 2.2). In addition, the median number of open flowers differed significantly according to growing method (covered > open: $U = 25231$, $P < 0.001$), management type (retail > PYO: $U = 11400$, $P < 0.001$) and strawberry cultivar ('Elsanta' > 'Malwina': $U = 24792$, $P < 0.001$).
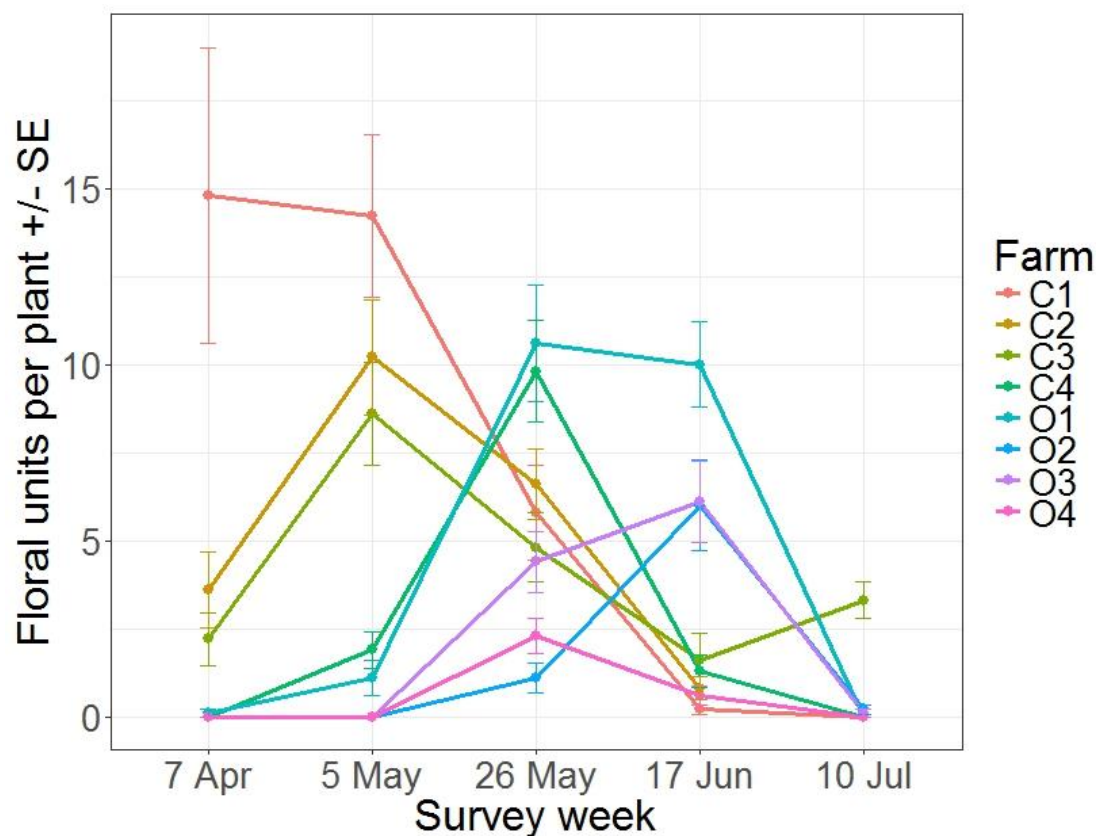
Figure 2.2 Strawberry flower phenology across all five survey weeks. Points represent mean floral units per plant ± standard error. Farms beginning with "C" were covered by polytunnels, whereas farms beginning with "O" were open sites.

Abundance

A total of 157 insects were observed visiting strawberry flowers during 21 surveys across all sites: an overall mean of 15.5 ± 3.0 visitors per hour. Flower visitor abundance was not significantly affected by growing method (open or under polytunnels; $\chi^2(1) = 0.82$, $P = 0.37$). However, counts of

flower visitors were influenced by time of day (morning or afternoon), calendar day and cloud cover.

More insects were observed visiting strawberry flowers in the afternoon than in the morning ($\chi^2(1) =$ 11.41, $P < 0.001$; Fig. 2.3). For each advance of one calendar day, an additional 1.03 ± 0.52 flower visitors were recorded ($\chi^2(1) = 10.48$, $P = 0.0012$). Similarly, as the degree of cloud cover increased by 1 okta, an additional 1.15 ± 0.56 flower visitors were observed ($\chi^2(1) = 7.69$, $P = 0.0056$). Therefore, flower visitor abundance gradually increased through the flowering period and was higher in the afternoon and on days with more cloud cover.
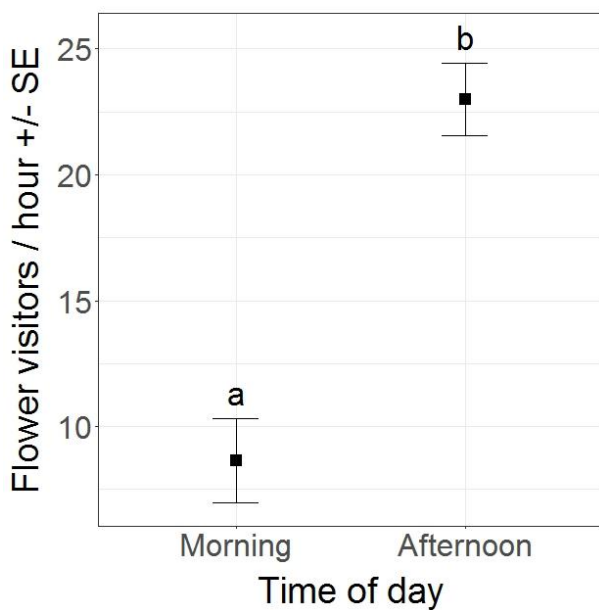
Figure 2.3 Total flower visitor abundance by time of day. Points indicate least square means ± standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

Diversity

Growing method significantly influenced Simpson's diversity but not species richness (Simpson's diversity: $\chi^2(1) = 6.04$, $P = 0.014$; species richness: $\chi^2(1) = 3.65$, $P = 0.056$). Open sites had a greater diversity of insect flower visitors than protected sites (Tukey-adjusted comparisons: Z = -2.66, $P =$ 0.008; Fig. 2.4). No other fixed effects had a significant impact on Simpson's diversity. Afternoon surveys were more species-rich than morning surveys ($\chi^2(1) = 11.16$, $P < 0.001$; Fig. 2.4). For every advance of one calendar day, 1.01 ± 0.52 more species were recorded during transect surveys ($\chi^2(1)$

= 4.64, *P* = 0.03). The hour in which surveys were conducted had a significant impact on species richness. For every additional hour, an extra 0.52 ± 0.83 flower visitor species were observed ($\chi^2(1)$ = 7.38, *P* = 0.007). Similarly, for every increase of 1% relative humidity, a further 1.04 ± 0.53 species were recorded ($\chi^2(1)$ = 5.03, *P* = 0.03).
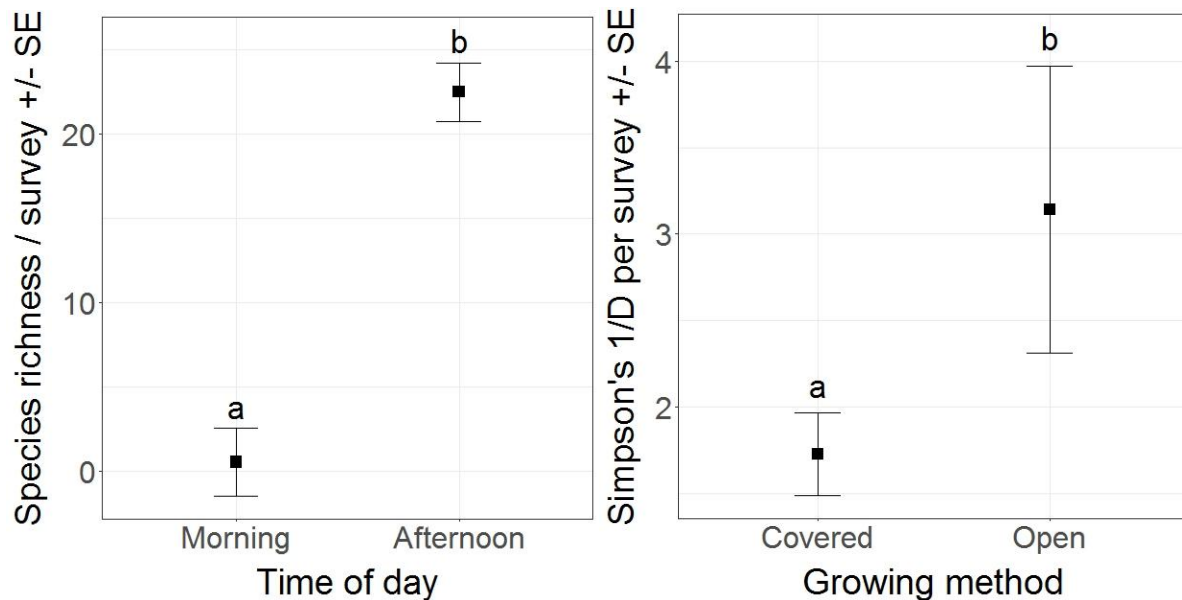
Figure 2.4 Species richness by time of day and inverse Simpson's diversity by growing method. Points indicate least square means ± standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

Species dominance

Hierarchical cluster analysis on species abundance data revealed that sites fell into two broad categories: (1) sites where over 50% of flower visitors were either bumblebees or hoverflies; and (2) sites where over 50% of flower visitors were either honeybees or non-syrphid flies (Fig. 2.5a). When relative frequencies of flower visitors were weighted by the number of floral units per survey, the two site categories remained the same; however, open site 3 (O3) moved from category 2 to 1, and covered site 3 (C3) moved from category 1 to 2 (Fig. 2.5b). These groupings of sites are highlighted in cluster dendrograms, with category 1 in blue and 2 in red (Fig. 2.6).

(a)
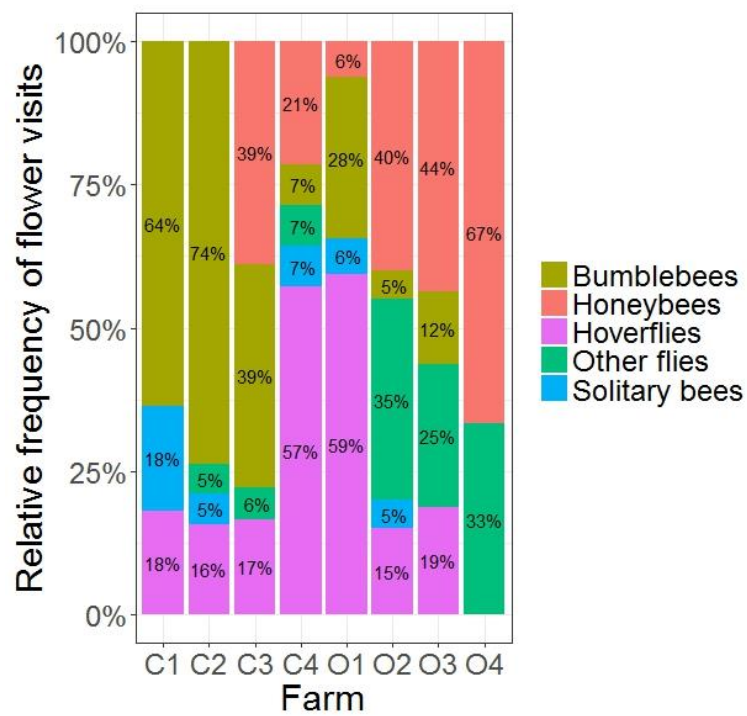
(b)
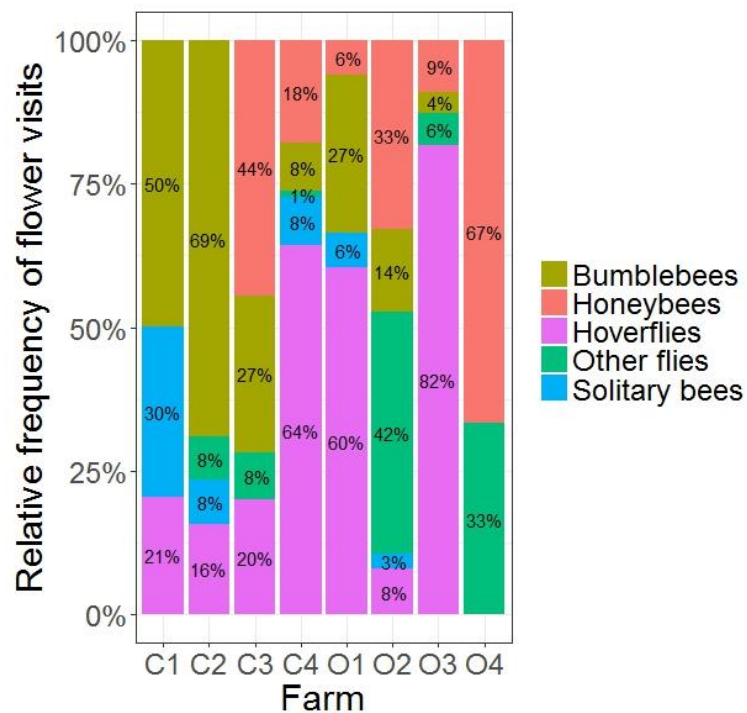
Figure 2.5 Relative frequencies of flower visitor functional groups across all eight sites. Sites beginning with "C" were covered by polytunnels, whereas sites beginning with "O" were open sites.

Figure (a) shows the raw relative frequencies, while (b) shows relative frequencies weighted by floral units per survey.

(a)
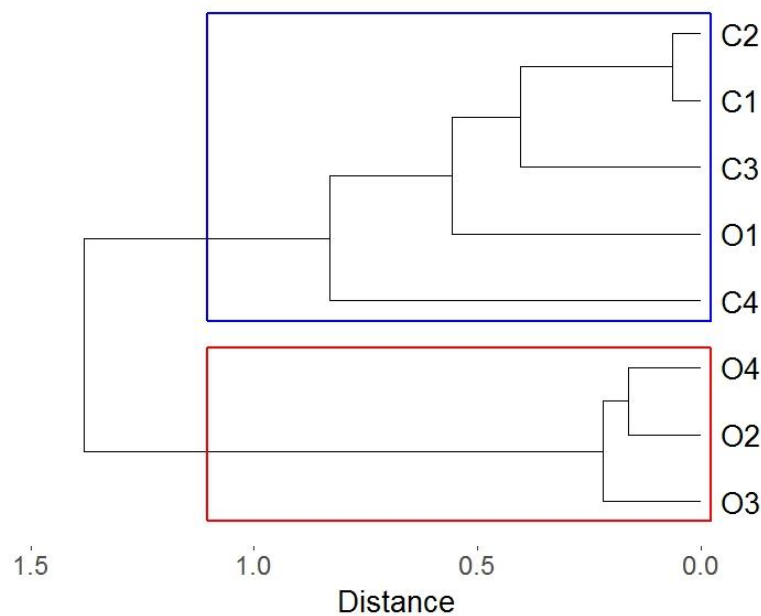
(b)
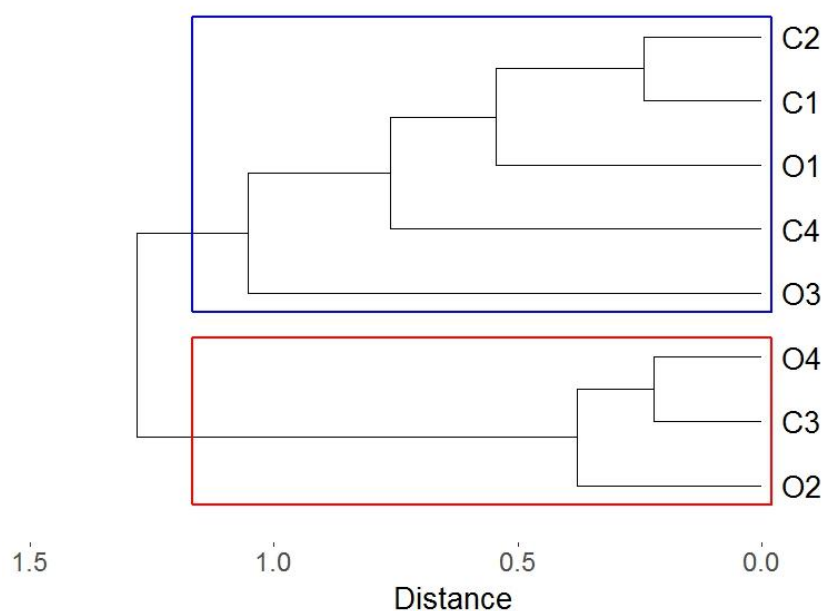
Figure 2.6 Cluster dendrograms based on (a) species abundances per survey and (b) species abundances per survey weighted by number of floral units per survey. Distances between sites were calculated using the Morisita-Horn index and the cluster analysis performed using Ward's method.

Sites beginning with "C" were covered crops and "O" were open crops. Clusters containing a majority of covered sites are highlighted in blue, while clusters with a majority of open sites are highlighted in red.

Mean dissimilarity in flower visitor assemblages between pairs of sites varied according to growing method ($\chi^2(2)$ = 11.93, P = 0.003) and farm management ($\chi^2(2)$ = 23.19, P < 0.001). In each case between-class dissimilarity was greater than within-class dissimilarity (Fig. 2.7). In other words, paired sites had more dissimilar species compositions when the sites belonged to different growing methods or farm management types.
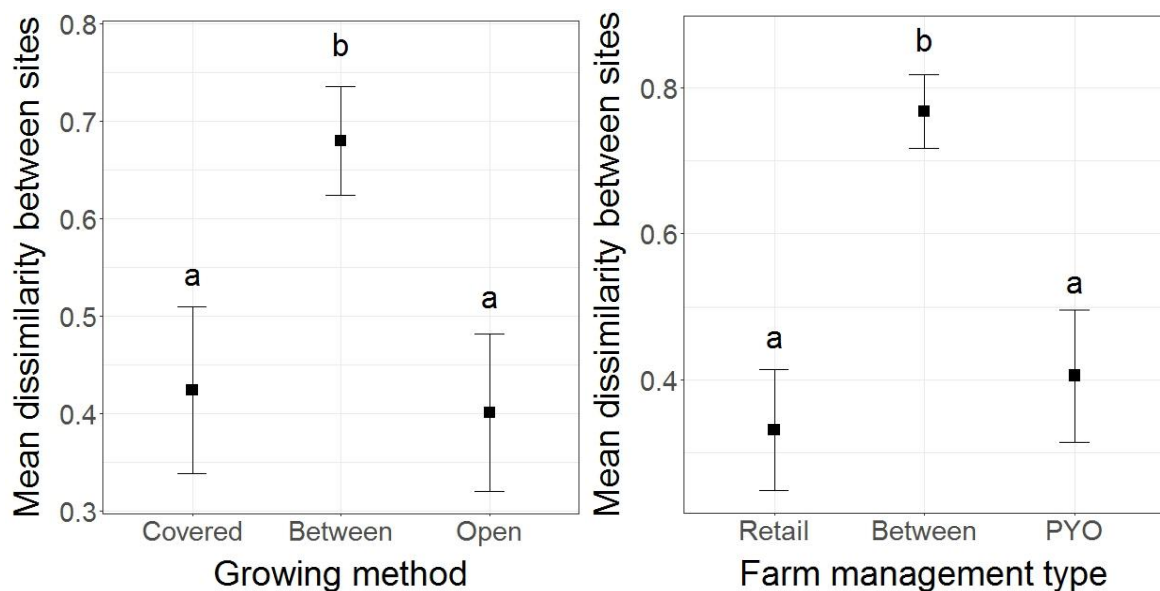
Figure 2.7 Mean Morisita-Horn dissimilarity index values for pairwise site comparisons by growing method and farm management type. "Covered" represents mean dissimilarity of comparisons between two sites with strawberries covered by polytunnels; the same convention applies to "Open," "Retail" and "PYO." "Between" represents mean dissimilarity of comparisons between a covered site and an open site, or a retail site and a PYO site. Points indicate least square means ± standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

When data were pooled from all sites, differences in flower visitor counts were significantly explained by functional group membership in each survey week (Table 2.3; Fig. 2.8). Functional group abundances across survey weeks revealed that bumblebees were most numerous in the first

two weeks, but then hoverflies and honeybees became the most frequent strawberry flower visitors (Fig. 2.8). Non-syrphid flies were the second most common flower visitor functional group at three of the four open sites and their numbers increased slightly as the season progressed. However, at sites under polytunnels, their relative frequency remained below 10% (Fig. 2.5a). Solitary bees were the least frequent strawberry flower visitor group overall and their abundance changed little during the course of the survey period (Fig. 2.8).

Table 2.3 Results of GLMs analysing flower visitor abundance per week. Likelihood ratio tests were performed on models with and without flower visitor functional group as a fixed effect.

Survey Week	Likelihood ratio test	P-value
1	$\chi^2(4) = 48.50$	$P < 0.001$
2	$\chi^2(4) = 45.44$	$P < 0.001$
3	$\chi^2(4) = 11.00$	$P = 0.027$
4	$\chi^2(4) = 16.85$	$P = 0.0021$
5	$\chi^2(4) = 297.91$	$P < 0.001$
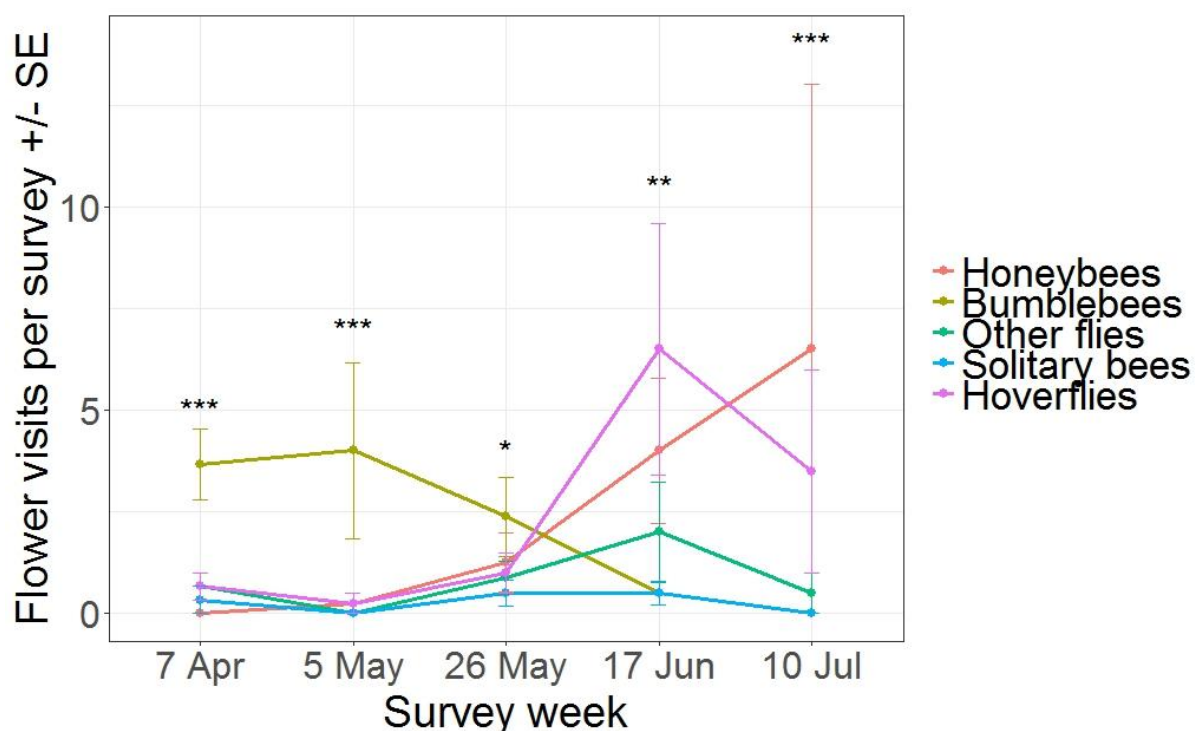
Figure 2.8 Overall frequency of flower visitor functional groups by survey week. Points represent mean visitors per survey ± standard error. Functional group has a significant effect on mean visitor abundance according to the following code: '*' = $P < 0.05$, '**' = $P < 0.01$, '***' = $P < 0.001$.

Discussion

This study compared the flower visitor assemblages in June-bearing strawberries at eight fruit farms in southeast England. Strawberry flower visitor abundance was not affected by the presence or absence of polytunnels, and this factor had no significant effect on flower visitor species richness. However, Simpson's diversity was significantly lower at sites with polytunnels. Both flower visitor abundance and species richness increased as the growing season progressed and were higher in afternoon surveys. Not only was species richness higher in afternoon surveys, but there was also a positive relationship between number of species recorded and the hour of the day in which the survey was conducted. Relative humidity was positively correlated to species richness. Interestingly, the abundance of flower visitors had a significant positive relationship with cloud cover.

The observed difference in Simpson's diversity between open and covered sites could have been due to the fact that unprotected crops presented no physical obstacle for potential pollinators

to reach the strawberry flowers. The presence of polytunnels may have impacted flower visitor diversity by impeding access to the crop. However, additional studies would need to be conducted to confirm this possibility. The effect of time of day on flower visitor abundance and species richness may be explained by the fact that temperatures tended to be higher in the afternoon, which encourages pollinator activity (Willmer 2011).

A qualitative comparison of flower visitor assemblages across the eight sites uncovered two main classes of functional group compositions: (1) sites dominated by bumblebees or hoverflies; and (2) sites with significant proportions of honeybees and non-syrphid flies. These results suggest that flower visitor assemblages are somewhat site-specific. Analyses of dissimilarities between pairs of sites revealed that in the case of both farm management type (retail or PYO) and growing method (covered or open), sites belonging to the same class tended to have more similar flower visitor assemblages than sites belonging to different classes. Therefore, species compositions appear to have been influenced by farm management type and growing method, further corroborating the view that flower visitor assemblages vary from site to site.

Finally, flower visitor functional groups varied in their abundance from week to week. Bumblebees were the most common flower visitor taxon at the beginning of the season but hoverflies and honeybees became more prevalent from May onwards. Covered strawberry crops in the UK are often provisioned with commercial bumblebee (*Bombus terrestris audax*) colonies as soon as flowers appear. Three of the four retail farms in this study were provisioned with bumblebees throughout the growing season. Using the formula from Ellis et al. (2017), commercial *B. terrestris audax* workers accounted for an estimated 65% of all flower visitors recorded in the first two survey weeks. This figure should be interpreted with caution, however, as only one of the seven surveys carried out in the first two weeks was conducted at a farm that had not been provisioned with commercial bumblebees. Had crops at the four farms without commercial bees been in flower in the first two weeks, then the number of flower visitors coming from commercial hives would have

represented a much smaller proportion of the total. Nevertheless, the fact that nearly two thirds of all flower visitors in the first two weeks were commercial bees suggests that, at these three sites at least, commercial bumblebees were providing a much-needed boost to the assemblages of flower visiting insects.

Considering data from all five weeks, these findings underscore the importance of bumblebee provision to early protected strawberry crops to provide pollen transfer. As previous work on ever-bearer strawberry varieties has highlighted, over the course of the flowering season, different taxa may rise and fall in significance as strawberry pollinators (Albano et al. 2009; Gibson 2012; Ellis et al. 2017). In the present study, flower visitor assemblages clearly varied from week to week in line with previous research. However, this study also revealed differences between pollinator assemblages at covered and open sites. Based on the fact that strawberries at open sites tended to flower later in the season when abundances of wild pollinators were greater, commercial bumblebees and honeybees appear to be unnecessary at these sites. Therefore, these data, coupled with supporting evidence from earlier studies, suggest that even in June-bearing strawberry crops, relying solely on commercial bumblebees or honeybee hives may leave crops susceptible to pollination deficits later in the growing season.

The main limitations of this study are the relatively small number of sites surveyed, the two different berry cultivars and the fact that transects were conducted during one growing season only. Future research could build upon my work by sampling from a larger number of study sites. Additionally, different strawberry cultivars have been shown to vary in their attractiveness to flower-visiting insects (Free 1993). Therefore, observing flower visitors to the same strawberry cultivar at all sites would reduce bias caused by the relative attractiveness of different crop cultivars. In this study, different cultivars were surveyed because no single strawberry cultivar was grown both under polytunnels and in unprotected fields. Lastly, surveying strawberry flower visitors over multiple growing seasons would allow researchers to uncover any annual variation in flower visitor

abundances. Such research would reveal whether flower visitor assemblages remain constant year on year, or whether different functional groups become more important depending on annual fluctuations in environmental factors.

Previous work on ever-bearer strawberries has found that the relative importance of hoverflies grows dramatically in the latter stages of the summer when bees are less numerous (Gibson 2012; Ellis et al. 2017), yet our results did not cover this period of the year, as crops at the eight field sites finished flowering by the end of July. Nevertheless, this research has generated useful information on the variation of flower visitor assemblages at different sites and across time, which will help inform management practices for strawberry growers. In particular, that functional group abundances varied across the flowering period suggests that strawberry growers require wild pollinators, in addition to managed bees early in the year, in order to sustain the delivery of pollination services. The diverse cast of flower visiting species needed to effectively pollinate strawberries throughout the season highlights the urgency of measures to safeguard wild pollinators in order to avoid future losses in strawberry yields.

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Chapter 3: Syrphine hoverflies are effective pollinators of commercial strawberry

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Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are entirely my own. Where I have consulted the work of others, this is clearly stated.

Signed: _____

Date: _____

Abstract

Recent declines in wild pollinators represent a significant threat to the sustained provision of pollination services. Insect pollinators are responsible for an estimated 45% of strawberry crop yields, which equates to a market value of approximately £99 million per year in the UK alone. As an aggregate flower with unconcealed nectaries, strawberries are attractive to a diverse array of flower-visiting insects. Syrphine hoverflies, which offer the added benefit of consuming aphids during their predatory larval stage, represent one such group of flower visitor, but the extent to which aphidophagous hoverflies are capable of pollinating strawberry flowers remains largely untested. In replicated cage experiments we tested the effectiveness of strawberry pollination by the aphidophagous hoverflies *Episyrphus balteatus* and *Eupeodes latifasciatus*, and a mix of four hoverfly taxa, when compared to hand pollination and insect pollinator exclusion. Hoverflies were released into cages, and the strawberry fruits that resulted from pollinated flowers were assessed for quality measures. Hoverfly visitation increased strawberry yields by over 70% and doubled the proportion of marketable fruit, highlighting the importance of hoverflies for strawberry pollination. A comparison between two hoverfly species showed that *Eupeodes latifasciatus* visits to flowers produced marketable fruit at nearly double the rate of *Episyrphus balteatus*, demonstrating that species may differ in their pollination efficacy even within a subfamily. Thus, this study offers compelling evidence that aphidophagous syrphine hoverflies are effective pollinators of commercial strawberry and, as such, may be capable of providing growers with the dual benefit of pollination and aphid control.

Introduction

Compounding pressures from rising global food demand and recent declines in managed and wild pollinators pose a significant threat to the production of insect-dependent crops, which comprise 87 of the 115 leading crop species (Williams 1994; Klein et al. 2007; Ellis et al. 2010; Potts et al. 2010). Globally, the proportion of agricultural land devoted to pollinator-dependent crops has grown steadily over the last 50 years (Aizen et al. 2008), and animal-pollinated crops account for 35% of total crop yields worldwide (Klein et al. 2007). Thus pollination represents a vital ecosystem service, contributing an estimated £121.8 billion to the global economy annually (Gallai et al. 2009).

Insect pollination not only boosts yields, but also enhances crop quality (Garibaldi et al. 2014). In commercial strawberry, *Fragaria x ananassa* Duch., open pollination by a range of wild bee species has been shown to result in fruit with fewer malformations, lower sugar-acid ratios, a more intense red colour, heavier berry weight and a longer shelf life than fruit from pollinator-excluded plants (Klatt et al. 2014). Thus, insect pollination can confer the dual economic benefits of larger yields and better-quality produce.

Research for the UK National Ecosystem Assessment has revealed that strawberry growers rely on insect pollination for 45% of crop yields (Smith et al. 2011), which equates to approximately £99 million/year in the UK alone (Defra 2015). With global strawberry production ballooning from 3.4 to 8.1 million tonnes/year between 1994 and 2014 (FAO 2017), the service provided by insect pollinators is becoming an increasingly vital natural resource. Therefore, gaining a clearer understanding of the species involved in this indispensable ecosystem service is paramount to ensuring that future strawberry harvests meet growing demands.

Strawberries are aggregate fruits with each flower receptacle containing multiple carpels (Free 1993). During fruit development the flesh around each achene, or seed, only expands once the achene has been fertilised with a pollen grain (Carew et al. 2003). Thus, poor pollination is one of the main reasons for malformations to occur. Carew et al. (2003) suggest that for fruit to develop properly, at least 70-80% of carpels must be pollinated. Due to their less specialised characteristics,

such as radial symmetry, disc shape, easily accessible nectar and exposed anthers, strawberry flowers are visited by a wide range of pollinating insects (Nye and Anderson 1974; Albano et al. 2009a). Research into the effectiveness of various strawberry pollinators has shown that several insects are more or less equally important in the creation of high-quality fruit, and indeed that visits from pollinators with diverse morphologies and behavioural habits tend to produce fruit more frequently and with fewer malformations (Chagnon et al. 1993; Albano et al. 2009b). Therefore, multiple visits from insect pollinators are necessary in order to achieve full pollination (Free 1993).

To date most pollination research in agroecosystems has focused on bees, with comparatively few studies aimed at other insect pollinator taxa (Ssymank et al. 2008; Ssymank and Kearns 2009). Nevertheless, a growing body of research suggests that hoverflies, specifically honeybee-mimicking drone flies (*Eristalis spp.*), are among the most efficient pollinators of strawberry flowers (Nye and Anderson 1974; Albano et al. 2009b; Ssymank 2009; Gibson 2012). However, *Eristalis* hoverflies, which feed on decaying organic material as larvae, represent a tiny fraction of the Syrphidae family in Britain, and several other species may be equally, or indeed more, effective strawberry pollinators.

This study focused on the pollination effectiveness of a cohort of syrphine hoverflies, which possess aphid-eating larvae and are commonly found in strawberry fields. A series of cage trials was conducted to determine whether these syrphines are effective pollinators of strawberry flowers and if they differ between species in their pollination efficacy.

Methods

Pollination effectiveness of a mix of hoverfly species on strawberry flowers

To determine the pollination effectiveness of a mixture of aphidophagous hoverfly species, 18 nylon mesh cages (47.5 x 47.5 x 93.0 cm; BugDorm, Taichung, Taiwan) were constructed and arranged on the ground in a 3 x 6 grid under a polytunnel at the NIAB EMR research institute, Kent, UK (51.286034° N, 0.449165° E, elevation: 35 m; Fig. 3.1). The study site was surrounded by

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horticultural land which was comprised of other strawberry crops and arable fields, with mixed native hedgerows. Given that the cages were arranged in columns of six on each of three longitudinal drip irrigation lines, two sets of 3 x 3 randomly-generated Latin square designs were used to allocate treatments to the cages, with six cages, or replicates, per treatment. This method ensured that each treatment was represented in every row and twice in each column, reducing bias that may have resulted from distance from the drip irrigation source or from the sides of the tunnel. Ten cv. 'Finesse' strawberry plants in black plastic pots (11 x 11 x 12 cm; Soparco, Condé-sur-Huisne, France) were placed in each cage. All plants were watered and supplied with fertiliser (Ferticare 22-4-22, NutriAg Ltd., Toronto, Canada) at 06:00 and 18:00 daily for five minutes with individual drippers for each pot. The pollination period was started as soon as open flowers were present in each cage: 2 September – 9 October 2015.

Figure 3.1 Experimental cages in situ at NIAB EMR.

The experiment had three treatments: (1) hand pollination (positive control, optimal pollination); (2) insect-exclusion (negative control); and (3) hoverfly visitation. For the hand pollination treatment, a size 12 paintbrush (Major Brushes Ltd., Cardiff, UK) was used to transfer

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pollen from dehisced strawberry anthers onto the entire receptacle of each open flower in the hand pollination cages. Hand-pollinated cages were visited ten times, approximately twice weekly, over the course of the pollination period and all open flowers were brushed once with pollen on each visit. Pollinator-excluded cages were left undisturbed throughout the experiment to allow only self- or wind-pollination to occur.

A combination of four taxa of wild-caught aphidophagous hoverflies was used for the hoverfly visitation treatment. Nine hoverflies were released into each hoverfly-pollinated cage on 2 September, with at least one individual from each of the four groups. Subsequently, additional hoverflies were added to each cage on 17, 23 and 30 September once six individuals belonging to the same taxon were collected. This procedure ensured that the flower visitor assemblages remained consistent across the cages. Dead hoverflies were removed and frozen for identification to species level.

All four taxonomic groups had previously been observed visiting strawberry flowers in surveys at fruit farms in the southeast of England (unpublished data) and were released into cages in the following quantities: (1) five individuals of large-bodied (5.0 – 11.5 mm) species in the genera *Eupeodes* and *Syrphus*; (2) three individuals of large-bodied (6.0 – 10.3 mm) *Episyrphus balteatus* (De Geer); (3) five individuals of smaller (4.3 – 7.0 mm) species in the genus *Sphaerophoria*; and (4) eight individuals (4.5 – 8.0 mm) of the tribe Bacchini, which, in this study, were *Melanostoma* and *Platycheirus*. The first three hoverfly categories all belong to the tribe Syrphini, and all four groups include only species whose larvae predate aphids on herbaceous plants (Ball and Morris 2015). A species list can be found in Appendix 1.

Comparison of pollination effectiveness of hand pollination and two hoverfly species

Because the hand-pollinated plants in the mixed-species experiment did not yield better-quality fruit than the hoverfly visitation treatment (see Results), we set up an experiment to determine the

optimum frequency of hand pollinating strawberry flowers. Four nylon mesh cages were constructed and arranged on the ground in a single column under a small polytunnel at NIAB EMR to exclude insects from visiting the strawberry flowers. Ten 'Finesse' strawberry plants were arranged in each cage, following the procedure in the mixed-species experiment. Four pollination treatments were compared: (1) control, in which no flowers were pollinated by hand; (2) one brush, in which open flowers were brushed with a paintbrush once; (3) two brushes, in which flowers were brushed twice, with 24-48 hours between brushes; and (4) three brushes, in which flowers were brushed three times, again with 24-48 hours between brushes. Plants in each cage were assigned to the four treatments, so that each treatment was represented in every cage. When a flower was brushed, a felt-tipped marker was used to mark the peduncle so that the number of brushes could be tallied for each fruit.

The same general experimental design as the mixed-species experiment was then used to determine whether single species of hoverfly were effective 'Finesse' strawberry pollinators. Twenty cages were constructed to accommodate five replicates for each of four treatments: (1) *Episyrphus balteatus*; (2) *Eupeodes latifasciatus* (Macquart); (3) hand pollination (based on the results from the hand pollination experiment described above); and (4) pollinator-excluded. A randomised block design was employed, with the 20 cages split into five blocks of four cages, with each treatment represented in each block. Both *Episyrphus balteatus* and *Eupeodes latifasciatus* are common visitors to strawberry flowers, and are common in the southeast of England, where the study took place (Ball and Morris 2015).

The pollination period for the trial was 16 – 30 August 2016. Based on experience from the hand pollination study, the hand pollination procedure was modified so that each open flower was brushed with pollen on only two occasions. Each time an open flower was brushed with pollen, a mark was made on the peduncle with a felt-tipped marker.

Fruit quality assessments

At the end of the pollination period, all plants were transferred to a glasshouse to allow the fruit to ripen and to facilitate fruit collection. In the mixed-species experiment, berries from all cages were picked once at least 75% of the fruit surface was red (Klatt 2013). For the latter experiments, strawberries were picked when approximately 25-75% of the fruit surface area had turned pinkish-red to reduce losses to pests. As each berry was picked, a note was made of the cage it came from and its position on the fruit truss, hereafter referred to as "growth position:" primary, secondary or tertiary, following the nomenclature used in Darrow (1929). To compare fruit quality across the treatments, the following variables were recorded for each strawberry: fruit shape class, diameter, fresh weight, maximum firmness, dry weight, Brix (using soluble solids content as an index of Brix), number of fertilised achenes and marketability (Klatt et al. 2014).

Strawberries were given a shape score, ranging from 1-4 (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations). The diameter of each fruit was measured to the nearest tenth of a millimetre using calipers. Berries were then weighed on a scale (Sartorius, Göttingen, Germany) and the mass recorded to the nearest tenth of a gram. Firmness (maximum force in Newtons) was assessed for each fruit in the mixed-species experiment only using a texture analyser (Lloyds Instruments, Ametek, Berwyn, USA) with an 8 mm probe. Each berry was evenly sliced in half and one half was weighed again on the scale and reserved for drying overnight in an oven at 60°C. The following day the dried strawberry halves were weighed a second time and the dry weight recorded.

The other half of each berry was used for Brix measurement and counts of fertilised achenes. To measure the Brix, 1-2 drops of juice were squeezed onto a digital refractometer (Palette, Atago, Tokyo, Japan) and soluble solids concentration recorded to the nearest tenth of a percent. To separate achenes from the flesh of the fruit, each berry was placed in a blender (Minipro, Tefal, Rumilly, France) with 200 ml of water and blended for 20 seconds. The contents were then

transferred to a 500 ml beaker and allowed to settle. All floating achenes were removed by gently

pouring away the supernatant. The sunken achenes were collected by pouring the remaining

contents through a sieve. These achenes were then transferred to a petri dish and dried overnight in

an incubator at 20°C. The following day, the number of fertilised achenes per fruit half was counted

and recorded for each strawberry. In the latter two experiments, rather than pouring out

unfertilised seeds and drying the fertilised achenes in a petri dish, sunken fertilised seeds were

simply counted by lifting the glass beaker and counting the achenes that had collected at the

bottom. Lastly, strawberries with a minimum diameter of 18 mm and a shape score of 1 or 2 were

classed as marketable (Conti et al. 2014; Klatt et al. 2014).

Data analysis

All analyses were carried out in R version 3.3.3 (R Core Team 2017). Average values were

calculated for all fruit quality measurements and are presented as mean ± standard error. For

fertilised seed counts from fruit halves, the mass of the fruit half divided by the mass of the whole

fruit was calculated and used to weight the calculation of mean seed counts. Linear mixed models

were then used on all normally-distributed fruit quality measurements in hoverfly experiments.

Response variables were transformed where necessary. When transformations failed to produce

normally-distributed data and in the case of fruit marketability, generalised linear mixed models

were used instead. For continuous variables, a gamma distribution was used, and for marketability, a

binomial distribution was chosen. Fruit shape score frequency distributions were analysed using

cumulative link mixed models with a probit link function, as degree of misshapenness in strawberries

is a latent continuous variable that was artificially separated into the four shape scores (Christensen

2015).

For all fruit quality measures apart from fruit yield, cage column, cage row, and the interaction

between fruit growth position and pollination method were selected as fixed effects for the full

model of the mixed- and single-species hoverfly pollination experiments. The optimal model was

chosen by sequentially removing the least significant fixed effect from the full model and running the 'drop1' function on the reduced model to test the significance of the fixed effects (Ekstrom 2012). The optimal model was obtained once the reduced model contained only statistically significant fixed effect terms. The nested random effect for each model was growth position nested within cage, or when this term did not significantly influence the response variable, the random effect was simplified to cage. The significance of the random effect was tested by comparing the optimal model against an identical model that only contained fixed effects using the likelihood ratio test. To determine where the differences lay among levels of a fixed effect, least-square means were calculated with the 'lsmeans' function and Tukey-adjusted comparisons were made to reveal any significant differences among factor levels.

For the analysis of fruit yield per cage, general linear models were used in the mixed-species experiment, with cage column, cage row and pollination method as fixed effects. In the single-species hoverfly experiment, generalised linear models were chosen instead using a gamma distribution to account for non-normality in the fruit yield data. The fixed effects of the full model remained the same as those used in the mixed-species experiment. In both cases the 'drop1' function was used to select the optimal model.

Finally, for the hand pollination efficacy experiment, generalised linear models were used to account for the unbalanced number of fruit per treatment. Unlike in the hoverfly pollination experiments, 'cage' was used as a blocking factor in the randomised block design of the hand pollination trial. Therefore, the fixed effects for this experiment were cage and pollination treatment. Response variables were transformed where necessary, and a binomial distribution was used for fruit marketability. Fruit shape score frequency distributions were compared using cumulative link models with a probit link function. Model selection was again performed using the 'drop1' function.

Results

Pollination effectiveness of a mix of hoverfly species

Pollination by the mixed group of hoverflies had significant positive impacts on a range of strawberry quality measures. Across 215 strawberries, fruit diameter varied according to pollination treatment ($\chi^2(2)$ = 12.67, *P* = 0.0018) and growth position ($\chi^2(2)$ = 21.55, *P* < 0.001). Hoverfly-pollinated fruit had the largest mean diameter (28.5 ± 0.84 mm), compared to hand-pollinated fruit (26.7 ± 0.84 mm) and pollinator-excluded (24.2 ± 0.82 mm; Fig. 3.2). Primary fruit diameter averaged at 29.4 ± 0.64 mm, compared to 26.5 ± 0.60 mm for secondary fruit and 23.6 ± 1.24 mm for tertiary fruit. The interaction between pollination treatment and growth position was not significant.

Pollination method also had a significant effect on fruit weight ($\chi^2(2)$ = 17.08, *P* < 0.001). Hoverfly-pollinated fruit weighed 9.7 ± 0.79 g, compared to 7.2 ± 0.68 g for hand-pollinated fruit and 5.3 ± 0.57 g for pollinator-excluded fruit (Fig. 3.2). Growth position similarly influenced fruit weight ($\chi^2(2)$ = 21.11, *P* < 0.001), with primary fruit averaging at 9.7 ± 0.60 g, compared to 7.1 ± 0.48 g for secondary fruit and 5.3 ± 0.85 g for tertiary fruit. Again, the interaction between the two variables was not significant.

Fruit Brix was 6.2% ± 0.11% across the 194 berries that were assessed, but Brix varied according to pollination treatment ($\chi^2(2)$ = 16.61, *P* < 0.001), cage column ($\chi^2(2)$ = 8.56, *P* = 0.014) and cage row ($\chi^2(5)$ = 21.86, *P* < 0.001). Pollinator-excluded fruit was higher in soluble solids (6.6% ± 0.24%) than hoverfly-pollinated fruit (5.9% ± 0.21%) and hand-pollinated fruit (5.5% ± 0.19%; Fig. 3.2). Fruit from columns 1 and 3 possessed a higher Brix (6.2% ± 0.21% and 6.2% ± 0.22%, respectively) compared to column 2 (5.6% ± 0.21%). Finally, Brix generally decreased as cage row number increased with the largest mean Brix of 6.7% ± 0.31% for row 2 and the smallest mean of 5.0% ± 0.23% for row 6.

The mean number of fertilised seeds per fruit half (215 berries) was 54.4 ± 2.37 seeds. Pollination method significantly influenced fertilised seed counts ($\chi^2(2)$ = 31.19, *P* < 0.001). Hoverfly-pollinated fruit had the highest seed count (67.3 ± 4.43 seeds) followed by hand-pollinated (46.9 ±

3.56 seeds) and pollinator-excluded fruit (32.3 ± 3.09 seeds; Fig. 3.2). Cage row also affected the

number of fertilised seeds ($\chi^2(5) = 17.82$, $P = 0.003$), which was lower as row number increased and

ranged from 59.4 ± 4.72 to 37.4 ± 5.38 seeds.
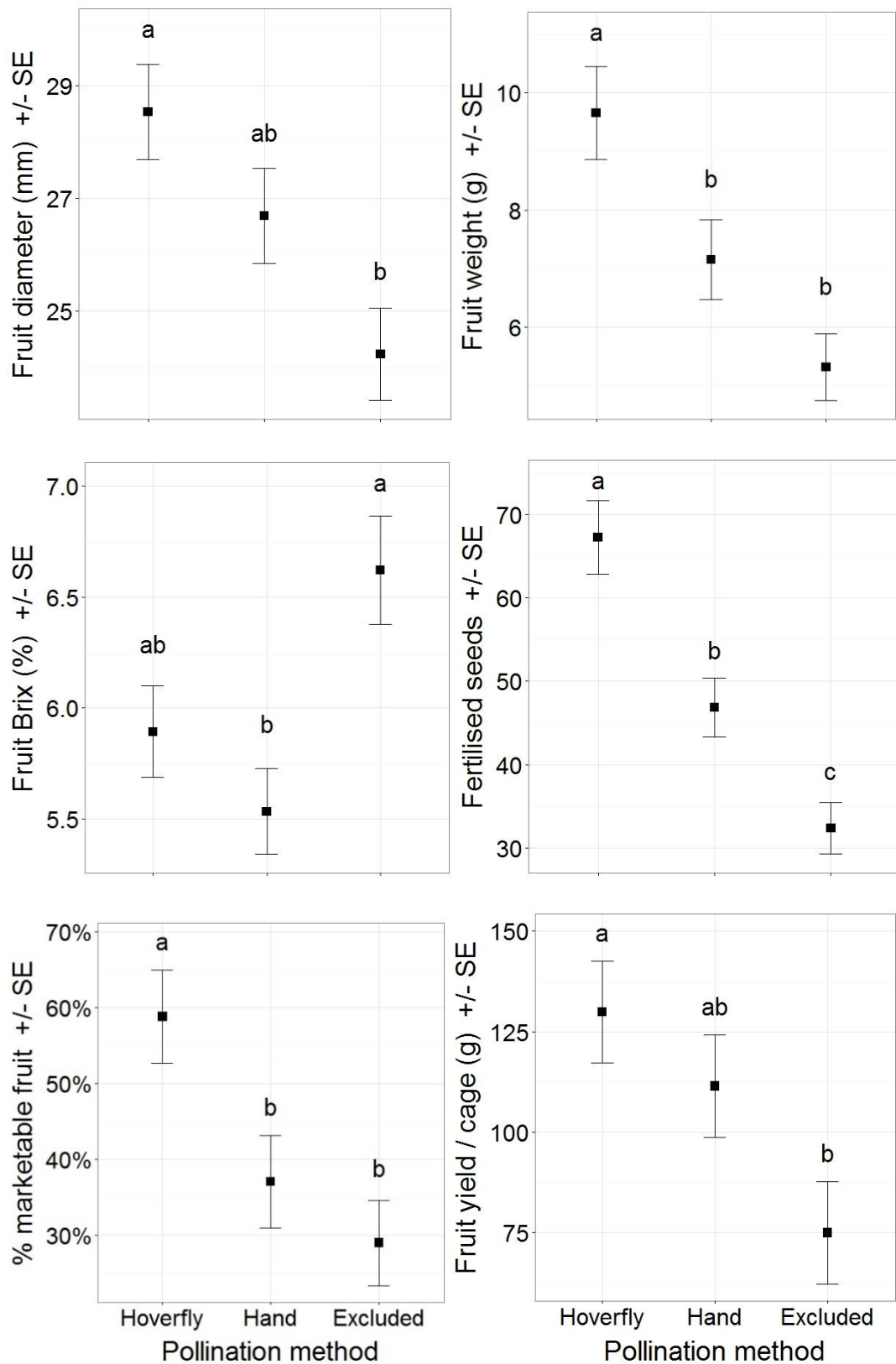
Figure 3.2 Mean fruit diameter, fruit weight, Brix, fertilised seeds per fruit half, proportion of marketable fruit and yield per cage by pollination method. Boxes indicate least square means ±

standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

A total of 215 strawberries were placed into one of four shape categories (ranging from 1-4). Pollination method was the only fixed effect to have a significant effect on the frequency distribution of shape scores ($\chi^2(2) = 14.60$, $P < 0.001$). Compared to the hand and insect-excluded treatments, plants in the hoverfly-pollinated cages tended to produce the least-misshapen fruit (mean shape score = 2.38 ± 0.09), compared to hand-pollinated and pollinator-excluded fruit (mean shape score = 2.77 ± 0.10 and 3.07 ± 0.10, respectively). Moreover, the frequency distribution of shape scores for hoverfly-pollinated fruit was significantly different to the frequency distributions of both hand-pollinated ($Z = 2.63$, $P = 0.02$) and pollinator-excluded fruit ($Z = -4.62$, $P < 0.001$). The shape score frequency distributions of hand-pollinated and pollinator-excluded fruit did not differ significantly from each other ($Z = -2.16$, $P = 0.08$; Fig. 3.3).

Overall 41.4% ± 0.034% of 215 strawberries were deemed marketable. Plants in the hoverfly-pollinated cages tended to produce the highest proportion of marketable fruit at 58.8% ± 6.11%, compared to 37.1% ± 6.11% for hand-pollinated and 29.0% ± 5.61% for pollinator-excluded fruit ($\chi^2(2) = 10.48$, $P = 0.005$; Fig. 3.2).

Fruit yield per cage differed significantly according to pollination treatment and cage row. Pollination treatment significantly affected fruit yield per cage ($F_{2,10} = 4.84$, $P = 0.034$), with hoverfly-pollinated cages producing a mean of 129.8 ± 12.69 g, compared with 111.5 ± 12.69 g for hand-pollination and 75.0 ± 12.69 g for pollinator-excluded (Fig. 3.2). Cage row also affected the yield of strawberries per cage ($F_{5,10} = 4.74$, $P = 0.018$). Across rows, mean yields per cage ranged from 59.5 ± 17.95 g (row 4) to 171.9 ± 17.95 g (row 1).

The mean fruit firmness (64 strawberries) was 6.0 ± 0.20 Newtons (N) but varied among cage rows ($\chi^2(5) = 12.48$, $P = 0.029$), with means ranging from 5.3 ± 0.30 N (row 1) to 6.6 ± 0.47 N (row 3). Pollination method had no effect on fruit firmness ($\chi^2(2) = 2.57$, $P = 0.28$). Lastly, none of the fixed

effects affected percent dry matter. However, the random effect of cage significantly influenced fruit percent dry matter ($\chi^2(1) = 11.73$, $P < 0.001$).
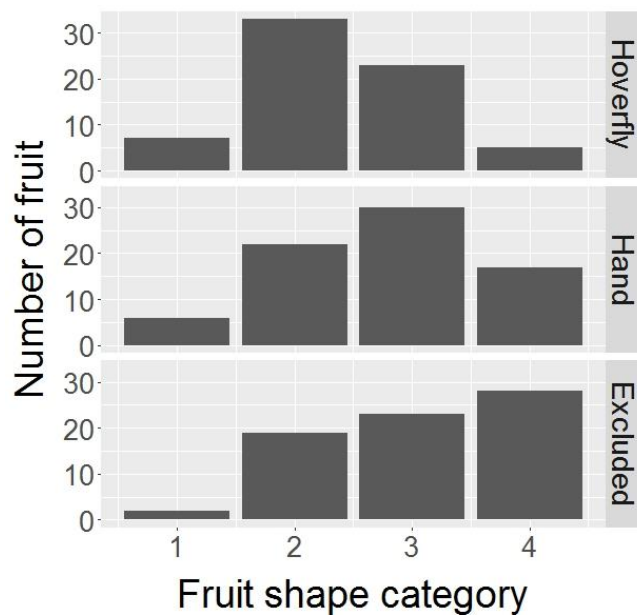
Figure 3.3 Fruit shape category frequency distributions by pollination treatment (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations). Fruit that fell into category 3 or 4 were deemed unmarketable.

Effect of varying brush pollination frequency on fruit quality

The frequency of brush pollinations had significant effects on berry weight, Brix and number of fertilised achenes. Mean fruit weight was influenced by the number of pollination events ($\chi^2(3) = 13.82$, $P = 0.003$). Fruit from flowers brushed twice were the heaviest (8.0 ± 0.49 g), compared to flowers brushed once (7.4 ± 0.61 g), unbrushed control strawberries (5.5 ± 0.71 g) or flowers brushed three times (5.0 ± 0.90 g; Fig. 3.4), suggesting that two hand pollination events with a paintbrush gave optimal pollination.

Pollination method also had a significant effect on fruit Brix ($\chi^2(3) = 19.92$, $P < 0.001$), with fruit brushed three times having the highest Brix levels (8.4% ± 0.43%) compared to fruit brushed once (7.0% ± 0.29%), fruit brushed twice (6.8% ± 0.24%) and unbrushed control fruit (6.0% ± 0.36%; Fig. 3.4). The fixed factor of cage had a significant effect on Brix ($\chi^2(3) = 24.71$, $P < 0.001$).

Pollination success, as measured by number of fertilised seeds, was significantly affected by the frequency of brushes used to hand-pollinate strawberry flowers (χ^2(3) = 14.27, *P* = 0.003). Fruit brushed twice had more seeds (32.5 ± 2.99) than fruit brushed once (30.7 ± 3.54), unbrushed control fruit (18.8 ± 3.24) and fruit brushed three times (17.7 ± 4.09; Fig. 3.4). Thus, two brushes achieved the highest pollination success.

In contrast to the effects described above, the number of hand pollination events did not have a significant effect on fruit diameter (mean = 22.1 ± 0.43 mm, *N* = 82; χ^2(3) = 5.95, *P* = 0.11), percent dry matter of strawberries (mean = 8.1% ± 0.23%, *N* = 82; χ^2(3) = 4.26, *P* = 0.24), the frequency distribution of shape scores (mean shape score = 2.74 ± 0.11, *N* = 82; χ^2(3) = 1.62, *P* = 0.7) or the proportion of marketable fruit (mean = 46.3% ± 0.055%, *N* = 82; χ^2(3) = 3.07, *P* = 0.4).
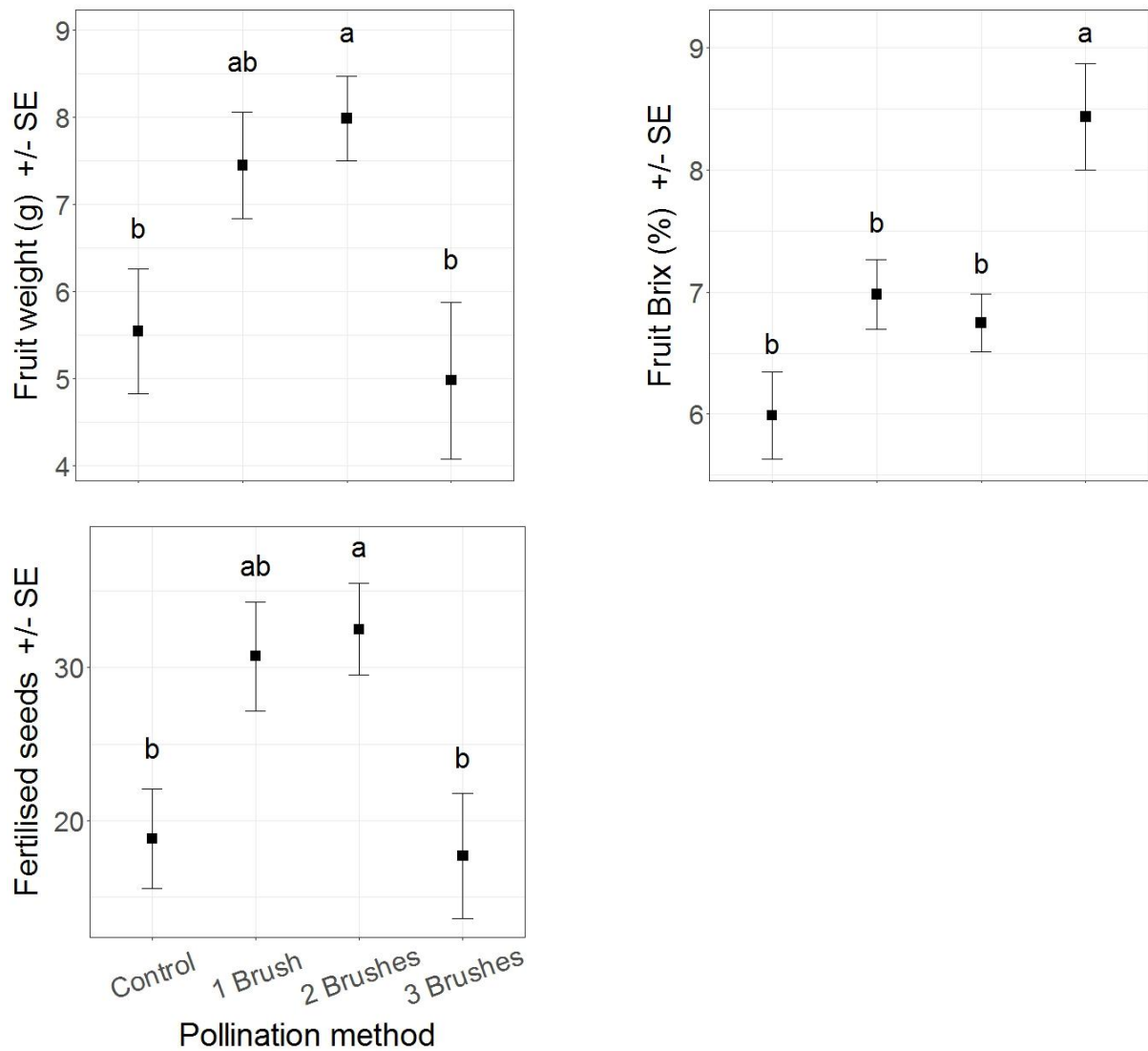
Figure 3.4 Mean fruit weight, Brix and number of fertilised seeds per fruit half by pollination method. Boxes indicate least square means ± standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

Effect of hoverfly species flower visits on fruit quality

When compared to pollinator-excluded controls, pollination by *Episyrphus balteatus* and *Eupeodes latifasciatus* significantly improved strawberry yields and fruit shape score distributions, but only visits from *Eupeodes latifasciatus* enhanced additional fruit quality measures. Pollination treatment significantly influenced fruit weight (χ^2(3) = 9.52, *P* = 0.023). Hand-pollinated fruit were the heaviest (4.5 ± 0.21 g), followed by fruit pollinated by *Eupeodes latifasciatus* (4.4 ± 0.22 g), *Episyrphus balteatus*-pollinated fruit (4.2 ± 0.21 g) and finally insect-excluded fruit (3.6 ± 0.21 g; Fig.

3.5). Growth position also had a significant effect on fruit weight (N = 1083; $\chi^2(2)$ = 231.67, P < 0.001), with primary fruit larger (5.6 ± 0.19 g) than secondary fruit (4.4 ± 0.13 g) and tertiary fruit (2.8 ± 0.12 g). The random effect of cage also significantly influenced fruit weight ($\chi^2(1)$ = 6.95, P = 0.008).

Pollination method also significantly affected Brix ($\chi^2(3)$ = 12.58, P = 0.006). Pollinator-excluded fruit had higher Brix (5.3% ± 0.17%), compared with fruit pollinated by *Episyrphus balteatus* (5.1% ± 0.16%), *Eupeodes latifasciatus* (4.9% ± 0.14%) or hand-pollinated fruit (4.6% ± 0.13%; Fig. 3.5). Cage column affected fruit Brix (N = 1076; $\chi^2(2)$ = 7.70, P = 0.021), with fruit from the central column of cages possessing the highest mean Brix (5.2% ± 0.13%), followed by fruit from the column nearest the irrigation source (4.9% ± 0.13%) and the column farthest from the irrigation source (4.8% ± 0.13%). Primary fruit tended to have higher sugar concentrations (5.3% ± 0.11%) than secondary (5.0% ± 0.083%) or tertiary fruit (4.7% ± 0.088%; $\chi^2(2)$ = 34.03, P < 0.001). Lastly, the random effect of cage also significantly influenced fruit Brix ($\chi^2(1)$ = 9.52, P = 0.002).

Pollinator-excluded fruit had the highest percent dry weight (6.6% ± 0.15%), followed by fruit pollinated by *Episyrphus balteatus* (6.4% ± 0.15%), *Eupeodes latifasciatus* (5.7% ± 0.11%) and hand-pollinated fruit (5.7% ± 0.10%; $\chi^2(3)$ = 16.68, P < 0.001; Fig. 3.5). Fruit from the central column had the highest percent dry matter (6.3% ± 0.097%), followed by the column nearest the irrigation source (6.0% ± 0.091%) and the column farthest from the irrigation source (5.9% ± 0.11%; N = 1075; $\chi^2(2)$ = 7.72, P = 0.021). Analysis of the influence of cage row revealed that percent dry weight generally decreased as distance from the irrigation source increased ($\chi^2(6)$ = 16.72, P = 0.010).

Hand-pollinated fruit had the highest mean seed count (47.7 ± 2.70 seeds) compared with fruit pollinated by *Eupeodes latifasciatus* (44.6 ± 2.68 seeds), *Episyrphus balteatus* (41.9 ± 2.58 seeds) and pollinator-excluded fruit (32.5 ± 2.45 seeds; $\chi^2(3)$ = 15.90, P = 0.0012; Fig. 3.5). Primary fruit had greatest number of fertilised seeds (51.5 ± 2.38), followed by secondary (44.7 ± 1.91) and tertiary fruit (29.8 ± 1.68; N = 1141; $\chi^2(2)$ = 45.74, P < 0.001). However, the random effect of growth position nested within cage also significantly influenced fertilised seeds counts ($\chi^2(2)$ = 21.66, P < 0.001).

Strawberries pollinated by *Eupeodes latifasciatus* had the best mean shape score (2.43 ± 0.054), compared to hand-pollinated fruit (2.46 ± 0.046), fruit pollinated by *Episyrphus balteatus* (2.63 ± 0.048) and pollinator-excluded fruit (2.99 ± 0.057). Moreover, the frequency distribution of shape scores for *Eupeodes latifasciatus*-pollinated fruit significantly differed from that of *Episyrphus balteatus*-pollinated fruit (Z = 3.42, *P* = 0.004). In contrast, the shape score distribution for hand-pollinated fruit was not significantly different from either of the hoverfly-pollinated treatments (hand-*Episyrphus balteatus* comparison: Z = 2.06, *P* = 0.17; hand-*Eupeodes latifasciatus*: Z = -1.75, *P* = 0.30). However, the shape score distribution for pollinator-excluded fruit differed significantly from all other treatments (excluded-*Episyrphus balteatus*: Z = -4.25, *P* < 0.001; excluded-*Eupeodes latifasciatus*: Z = -8.30, *P* < 0.001; excluded-hand: Z = -7.31, *P* < 0.001; Fig. 3.6). Cage row significantly influenced shape score ($\chi^2(6)$ = 24.69, *P* < 0.001), with mean scores ranging from 2.47 – 2.80 across cage rows;. Primary fruit had the highest mean shape score (2.81 ± 0.060), followed by secondary (2.55 ± 0.037) and tertiary fruit (2.54 ± 0.045; $\chi^2(2)$ = 18.14, *P* < 0.001).

Pollination method significantly affected the proportion of marketable fruit (*N* = 1071; $\chi^2(3)$ = 26.11, *P* < 0.001). Plants in the *Eupeodes latifasciatus*-pollinated cages produced the highest proportion of marketable fruit (54.0% ± 3.42%), compared to hand-pollinated (43.8% ± 3.21%), *Episyrphus balteatus*-pollinated (29.7% ± 3.44%) and pollinator-excluded fruit (23.4% ± 3.31%; Fig. 3.5). Proportions of marketable fruit across cage rows varied from 19.1% - 48.7% ($\chi^2(6)$ = 20.65, *P* = 0.002). Finally, secondary fruit possessed the highest proportion of marketable fruit (45.4% ± 2.37%), followed by primary (36.7% ± 3.28%) and tertiary fruit (29.8% ± 2.67%; $\chi^2(2)$ = 20.29, *P* < 0.001).
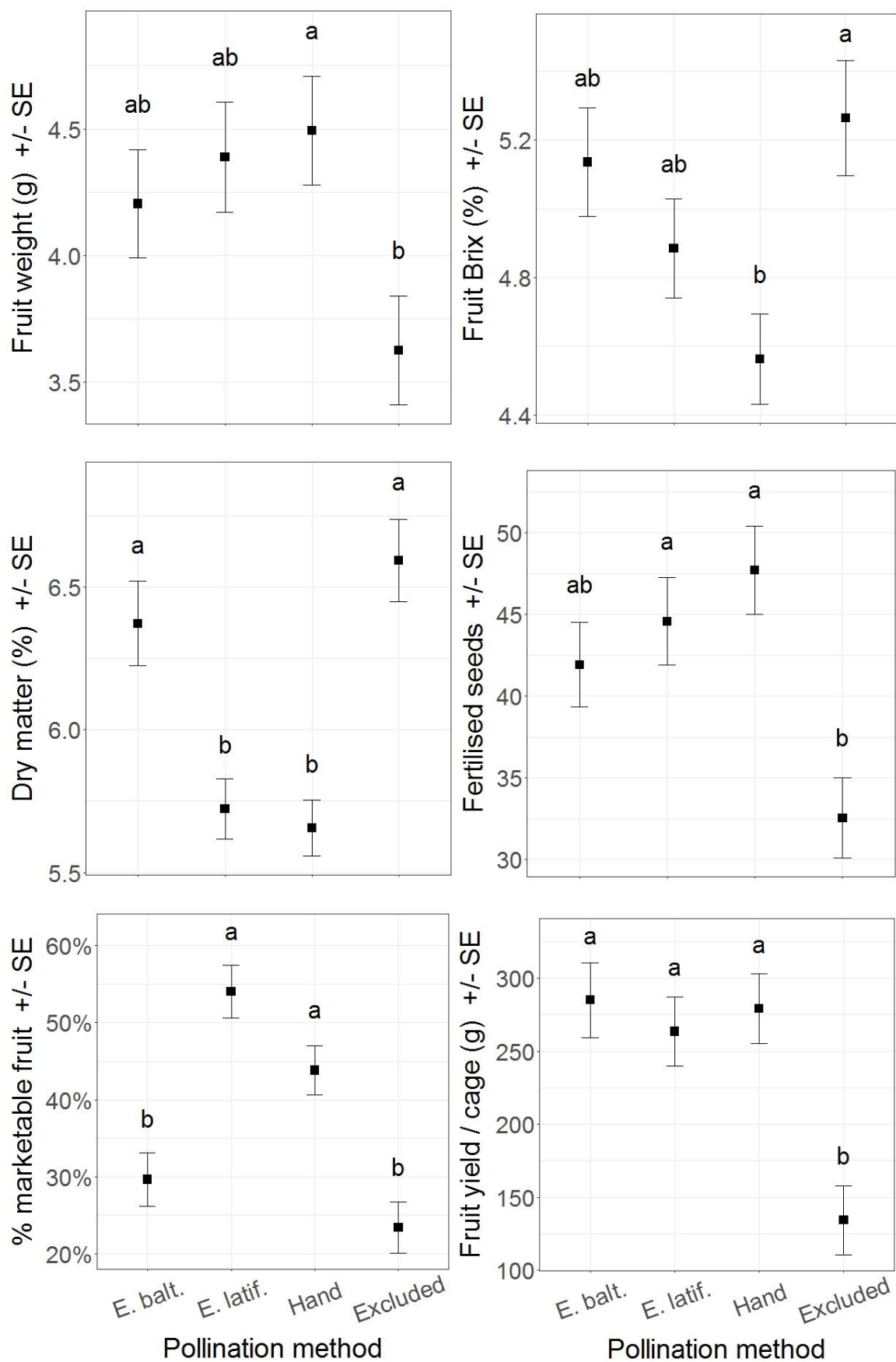
Figure 3.5 Mean fruit weight, Brix, percent dry weight, fertilised seeds per fruit half, proportion of marketable fruit and yield per cage by pollination method. "E. balt." is an abbreviation of *Episyrphus balteatus*. "E. latif." is an abbreviation of *Eupeodes latifasciatus*. Boxes indicate least square means ± standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

Pollination treatment also significantly influenced fruit yield per cage ($F_{3,10}$ = 9.26, *P* = 0.003), with *Episyrphus balteatus*-pollinated cages producing the highest yields (285.0 ± 25.73 g), compared with hand-pollinated (279.1 ± 23.76 g), *Eupeodes latifasciatus*-pollinated (263.4 ± 23.53 g) and pollinator-excluded cages (134.2 ± 23.58 g; Fig. 3.4). In addition, cage row had a significant effect on the yield of strawberries per cage ($F_{6,10}$ = 6.83, *P* = 0.004).

Finally, growth position was the only fixed factor to have a significant effect on fruit diameter (*N* = 1082 strawberries; $\chi^2(2)$ = 252.53, *P* < 0.001). Primary fruit were larger (22.9 ± 0.26 mm) compared to secondary fruit (20.7 ± 0.20 mm) and tertiary fruit (17.9 ± 0.24 mm). Pollination method did not affect fruit diameter ($\chi^2(3)$ = 5.90, *P* = 0.12). In addition to growth position, the random effect of cage also influenced fruit diameter ($\chi^2(1)$ = 6.52, *P* = 0.011).
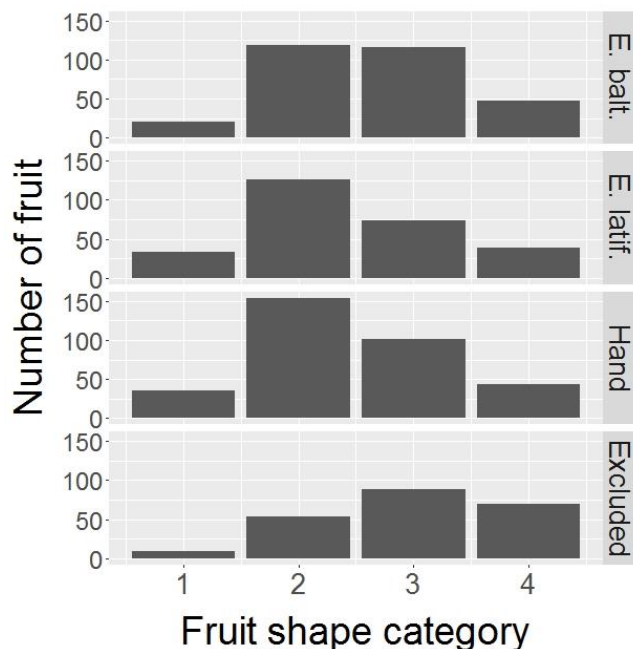
Figure 3.6 Fruit shape category frequency distributions by pollination treatment (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations).

Fruit that fell into category 3 or 4 were deemed unmarketable. "E. balt." is an abbreviation of *Episyrphus balteatus*. "E. latif." is an abbreviation of *Eupeodes latifasciatus*.

Discussion

This study compared the effects of aphidophagous hoverfly flower visits on strawberry fruit quality and yield. Hoverfly pollination enhanced fruit quality and yield when compared to strawberry flowers that received no insect visits. Strawberry flowers visited by a mix of aphidophagous hoverfly species produced fruit with a greater diameter, weight, number of fertilised achenes and fewer malformations. These characteristics, in turn, meant that proportions of fruit that were marketable doubled from 29.0% in insect-excluded cages to 58.8% in hoverfly pollination cages. In addition to improving fruit quality, yields of strawberries increased by 73.1% when hoverflies were added to cages.

These improvements in fruit quality may be explained in part by the use of a mix of hoverfly species as flower visitors. Previous research has demonstrated that a diverse pollinator assemblage will more effectively pollinate crops (Blitzer et al. 2016), with several studies showing that diversity, rather than pollinator abundance *per se*, enhances seed set (Klein et al. 2003; Hoehn et al. 2008; Mallinger and Gratton 2015; Martins et al. 2015). These authors promote the concept of niche complementarity as an explanation for the positive relationship between pollinator diversity and crop quality. Different pollinator taxa tend to visit flowers at different heights and times of day. Furthermore, taxa with different body sizes carry varying pollen loads and behave differently on flower heads (Chagnon et al. 1993; Hoehn et al. 2008). All of these factors suggest that each pollinator functional group will deliver pollen grains in a unique manner. Moreover, when acting in concert, diverse pollinator guilds complement one another resulting in the provision of more complete pollination (Chagnon et al. 1993; Hoehn et al. 2008; Blitzer et al. 2016). In this study, hoverfly species varied in their average body size and typical behaviours on the strawberry flower receptacle, with larger species tending to feed while standing on the receptacle and smaller species touching the edge of the receptacle while standing on petals (personal obs.). Therefore, some

degree of niche complementarity could have contributed to the improved pollination success and fruit quality observed in hoverfly-pollinated strawberries, and quantifying this should be the focus of future studies.

Despite these findings, fruit Brix, firmness and percent dry matter did not benefit from the introduction of a mix of hoverfly species. In each case, mean values for the hoverfly pollination treatment did not differ significantly from those of the insect-excluded treatment. One possible explanation is that any benefit from hoverfly pollination was mitigated by a subsequent increase in water concentration during the rapid cell expansion that occurs as a result of an influx of auxin and gibberellic acid when strawberries mature (Csukasi et al. 2011). This swelling of the fruit tissue may have lowered Brix, firmness and percent dry matter.

Although intended to serve as a positive control, the hand pollination treatment in the mixed-species experiment did not produce more marketable fruit. For most fruit quality measures, strawberries from the hand pollination treatment scored either significantly lower than hoverfly-pollinated fruit, or else not significantly different from either hoverfly-pollinated or insect-excluded berries. Overly vigorous brushing of the receptacle can result in poor pollination success (A. B. Whitehouse, pers. comm. 2017). Because all open strawberry flowers were brushed with pollen twice a week as long as they remained open, receptacles may have become damaged, thereby lowering the pollination success rate and causing the observed reductions in fruit quality.

The subsequent hand pollination experiment revealed that brush pollinating strawberry flowers twice only yielded better-quality fruit than either no brushing or three-brush treatments, both in terms of fruit weight and number of pollinated achenes. As with the hoverfly pollination experiment, better-pollinated fruit tended to have lower Brix, most likely due to the increased water content. The decrease in fruit quality observed in the three-brush treatment may represent the threshold at which the receptacles began to suffer damage from being brushed too often. This phenomenon may be analogous to the effect of having too many visits from insect pollinators, which has previously been shown to cause reduced pollination success (Gómez et al. 2007; Albrecht et al. 2012).

In the trial comparing the pollination effectiveness of two hoverfly species, strawberries visited by *Eupeodes latifasciatus* and hand-pollinated flowers yielded better-quality fruit than the insect-excluded treatment as evidenced by the 37.2% and 46.8% increases, respectively, in number of pollinated achenes, and the 130.8% and 87.2% increases in proportion of marketable fruit. Allowing *Episyrphus balteatus* to visit the strawberry flowers did not significantly improve fruit weight, pollination success or marketability. However, berries from both hoverfly pollination treatments and hand-pollinated fruit had lower frequencies of malformations than insect-excluded strawberries. Interestingly, the shape score distribution for *Eupeodes latifasciatus* differed significantly from that of *Episyrphus balteatus*, which possessed a smaller proportion of berries in the marketable fruit shape categories (45.9%) than the former species (58.6%). In both hoverfly species treatments and hand pollination cages, fruit yields per cage were enhanced by more than 90% when compared to pollinator-excluded cages. Thus, pollination by both hoverfly species would benefit strawberry growers by increasing yields and reducing rates of malformed fruit. However, based on its impacts on pollination success, fruit weight and marketability, *Eupeodes latifasciatus* appears to be a more effective pollinator of strawberry flowers than *Episyrphus balteatus*.

As in previous cage trials, Brix was higher for treatments that tended to have a lower pollination success rate. In this case, percent dry matter also followed Brix in having higher values for treatments with poorly-pollinated berries. In both instances, the smaller cells of poorly-pollinated fruit likely explain the observed differences in Brix and percent dry matter.

When the pollination efficacy of single species of hoverfly is compared against the results from the mixed-species experiment, several similarities emerge in the effect that the insects have on fruit quality parameters. Most notably, fruit yields were significantly augmented by both mixed-species assemblages of hoverflies and visits from only *Episyrphus balteatus* or *Eupeodes latifasciatus*. In the mixed-species experiment, fruit yields grew by 73.1% in hoverfly-pollinated cages when compared to controls, while the difference was even more pronounced in the single-species experiment. In that trial, introducing *Episyrphus balteatus* and *Eupeodes latifasciatus* to cages resulted in yield increases

of 112.4% and 96.3%, respectively. The mean proportion of marketable fruit in mixed-species and in

Eupeodes latifasciatus cages was over double that of pollinator-excluded cages in both experiments:

mixed species of hoverflies increased proportions of marketable fruit by 102.8%, and *Eupeodes*

latifasciatus enhanced rates of marketable fruit by 130.8%. By contrast, *Episyrphus balteatus* did not

significantly improve fruit marketability when compared to the pollinator-excluded controls. In

terms of pollination success rates, visitation from a mixed of hoverfly species led to a 108.4%

increase in the number of fertilised seeds, while visits from *Eupeodes latifasciatus* improved

pollination success rates by 37.2% over pollinator-excluded controls. Research by Klatt et al. (2014)

documented a 61.7% rise in the number of fertilised achenes when bee-pollinated fruit were

compared against self-pollinated controls using different strawberry cultivars; therefore, syrphine

hoverflies may be as effective strawberry pollinators as bees.

Moreover, though *Eupeodes latifasciatus* outperformed mixed-species assemblages of

hoverflies in enhancing yields and fruit marketability, visits from a group of hoverfly species resulted

in a larger increase in numbers of fertilised achenes, when compared against fruit from control

cages. Although these results seem to indicate slight differences in the pollination efficacy of

Eupeodes latifasciatus as compared to a mixed group of hoverfly species, in order to uncover true

differences, future research should compare single- and multiple-species assemblages in the same

experiment.

The findings of this study provide the first evidence to suggest that hoverflies with

aphidophagous larvae are effective pollinators of strawberry. Given that aphids are the primary prey

of syrphine larvae (Rotheray and Gilbert 2011), these hoverflies may be capable of delivering both

pollination and pest control ecosystem services for strawberry growers. Syrphine hoverflies have

been shown to pollinate other crops, such as oilseed rape (Jauker and Wolters 2008; Jauker et al.

2012; Garratt et al. 2014) and apple (Garratt et al. 2016). Though these studies found that

aphidophagous hoverflies were less effective pollinators than bees, syrphines may nonetheless

supplement bee pollination and provide pest control services in these and other crops.

The main limitation of this study is that, as a cage trial, these results provide evidence that syrphines are capable of pollinating strawberry flowers; however, whether hoverflies pollinate strawberries effectively in the field remains to be demonstrated. Hoverflies may not visit strawberry flowers as frequently in the field and therefore their potential value as pollinators may not be as high as our findings imply (Albano et al. 2009b). Furthermore, although syrphine hoverflies are able to improve fruit quality and yields in cages, other flower-visiting taxa may prove to be even more effective pollinators of strawberry. Previous research has shown that honeybees, bumblebees, halictid bees and eristaline hoverflies are also effective strawberry pollinators (Albano et al. 2009b; Gibson 2012). In order to assess the pollination efficacy of syrphines in relation to other taxa, one method that may prove useful is comparing the pollination success and fruit quality after a single visit from flower visitors (King et al. 2013). Such single visit deposition rates can then be coupled with flower visitation rates in the field to obtain a more complete picture of the pollination effectiveness of different species groups, as was done by Albano et al. (2009b) using honeybees, halictid bees and eristaline hoverflies as focal taxa.

To conclude, our findings demonstrate that aphidophagous syrphine hoverflies are effective pollinators of strawberry, boosting yields by over 70% and doubling proportions of marketable fruit. Moreover, even when strawberry flowers were only visited by a single species, both *Eupeodes latifasciatus* and *Episyrphus balteatus* were able to improve fruit yields by over 96% when compared to pollinator-excluded plants. These results suggest that syrphine hoverflies may provide the dual benefits of more complete pollination and aphid biocontrol in strawberry fields. Future studies could compare the pollination effectiveness of syrphine hoverflies with that of *Eristalis* hoverflies, the common strawberry-visiting hoverfly *Syritta pipiens* and bees in a field setting. Though our results suggest that syrphines are effective strawberry pollinators in cages, gaining a better understanding of how well these hoverflies pollinate in the field and how they perform relative to other flower visitors would improve our knowledge of their relative importance as strawberry pollinators.

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Appendix 1 – Hoverfly taxa used in mixed-species efficiency experiment

Category 1: large-bodied species in the *Syrphus* and *Eupeodes* genera

Eupeodes corollae
Eupeodes latifasciatus
Eupeodes luniger
Syrphus ribesii

Category 2: large-bodied *Episyrphus balteatus*

Episyrphus balteatus

Category 3: smaller-bodied *Sphaerophoria* hoverflies

Sphaerophoria scripta
Sphaerophoria sp. (females cannot be identified to species based on morphological characters)

Category 4: smaller-bodied *Melanostoma* and *Platycheirus* hoverflies

Melanostoma mellinum
Platycheirus albimanus
Platycheirus clypeatus
Platycheirus nielseni
Platycheirus peltatus
Platycherius scutatus

Chapter 4: The effect of within-crop floral resources on pollination, aphid control and fruit quality in commercial strawberry

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Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are entirely my own. Where I have consulted the work of others, this is clearly stated.

Signed: _____

Date: _____

Abstract

In the absence of pollinating insects commercial strawberry (*Fragaria x ananassa*) yields would fall by approximately 45%, while damage from aphids is estimated to cost growers at least 1% of annual yields in the UK alone. This combined effect could result in losses of over £100 million per year for the UK economy. We investigated whether incorporating floral resources within commercial polythene-clad tunnels could improve pollination services and aphid control and, hence, strawberry fruit quality. In a randomised block experiment (using 3 x 6 m plots), coriander (*Coriandrum sativum)* field forget-me-not (*Myosotis arvensis*) and corn mint (*Mentha arvensis*) plants were inserted in rows of commercially-grown strawberries. Their effect on numbers of naturally occurring flower visitors, aphids and aphid predators was compared. Counts of flower visitors, including visitors to sown flower species, were higher in coriander and mint plots. However strawberry flowers received more insect visits in control plots. Fruit from forget-me-not plots were lower in sugar than fruit from controls, while fruit from mint plots had fewer fertilised seeds than fruit from control plots. Strawberries from coriander plots did not differ from control fruit on any fruit quality measures. Crucially, proportions of marketable fruit did not differ across treatments. Aphid numbers did not differ between treatments even though coriander significantly increased the numbers of lacewing eggs laid on aphid-infested strawberry plants in coriander plots. Hence, although there were no negative effects on the proportions of marketable fruits with intercropping, the benefits received were limited. It might be that in a different growing season the effect would be more pronounced but this would need to be weighed up against the cost of implementing such interventions.

Introduction

Though the process of agricultural intensification has produced larger harvests, huge areas of farmland are now at greater risk of pest outbreaks due to the loss of both genetic diversity within the crops and biodiversity in the wider landscape (Matson et al. 1997; Crowder and Harwood 2014). Equally concerning are recent declines in wild and managed pollinating insects (Biesmeijer et al. 2006; Potts et al. 2010; Carvalheiro et al. 2013) that are necessary for the pollination of 84% of crop species in Europe (Klein et al. 2007). Consequently, ecological intensification, or conserving species involved in delivering vital ecosystem services, such as biological control of pests and animal-mediated crop pollination, is paramount to sustaining agricultural productivity without causing irreparable damage to the environment (Bommarco et al. 2013; Garibaldi et al. 2016). Creating within-farm semi-natural habitat elements such as sown wildflower strips is a direct and widely-applicable strategy for bolstering populations of biological control agents and pollinating insects (Haaland et al. 2011; Campbell et al. 2012).

Crop field margins can provide essential pollen and nectar resources (Garratt et al. 2014) when crops are not in flower, for pollinating insects and pest natural enemies. Moreover, field edges have become a focus of insect conservation efforts in agroecosystems because crop margins are the least productive part of the field, are more prone to drought and shading, and typically possess the greatest floral diversity within the field (Kells et al. 2001; Pywell et al. 2005). Sowing wildflower strips along field boundaries can increase the abundance of pollinators in fields (Marshall et al. 2006; Kohler et al. 2008; Blaauw and Isaacs 2014; Feltham et al. 2015; Campbell et al. 2017), impact populations of aphid predators (Bowie, 1999; van Rijn et al., 2006; Prasad et al., 2009; Hogg et al., 2011; Walton and Isaacs, 2011; Gontijo et al., 2013; Ramsden et al., 2014) and reduce aphid numbers within crops (Bowie, 1999; Jankowska, 2007; Hogg et al., 2011; Skirvin et al., 2011; Gontijo et al., 2013). However, the impact of field-edge interventions does not always impact the centre of the crop (Bowie 1999; Marshall et al. 2006; van Rijn et al. 2006; Kohler et al. 2008; Skirvin et al. 2011; Walton and Isaacs 2011). For example, flower visiting hoverfly abundance, along a gradient of distance from a wildflower margin, declined

significantly in samples farther than 50 m from patches of floral resources (Kohler et al. 2008), and Skirvin et al. (2011) observed that improvements in pest control did not extend beyond 10 m into lettuce crops. Likewise, van Rijn et al. (2006) documented twice as many aphid-eating, or aphidophagous, hoverflies within 4 m of wildflower strips compared to other parts of the crop.

Other studies have investigated introducing additional floral resources within crops (Patt et al. 1997; Jankowska 2007; Wang et al. 2009; Gillespie et al., 2011). Pest species abundances were reduced in aubergine intercropped with dill flowers (Patt et al. 1997) and wheat intercropped with oilseed rape flowers (Wang et al. 2009). However, introducing rows of alyssum flowers into lettuce fields did not improve aphid suppression or hoverfly fecundity (Gillespie et al. 2011).

As an aggregate flower strawberries (*Fragaria x ananassa* Duch.) require that all carpels be evenly distributed and fully pollinated in order to produce high-quality fruit (Carew et al. 2003). Studies have shown that in the absence of insect pollinators, the pollination rate for strawberry flowers rarely exceeds 60%, with malformations present on around 47% of fruit, demonstrating a significant reliance on animal-mediated pollen transport for optimal fruit development (Chagnon et al. 1993). Indeed, research for the UK National Ecosystem Assessment has revealed that in 2007, British strawberry growers relied on insect pollination for 45% of crop yields, representing around £72 million of the market value in the UK that year (Smith et al. 2011).

Few studies have looked at both pest control and pollination benefits simultaneously (Haaland et al. 2011; Campbell et al. 2012; Wratten et al. 2012; Pywell et al. 2015). Moreover, to our knowledge, no study has measured flower visitation rates, natural enemy and resultant pest abundances, and crop quality with and without additional floral resources. In a previous study aphidophagous hoverflies were shown to be effective pollinators of strawberry, increasing marketable yields (Hodgkiss et al. 2018). In this study we hypothesized that integrating additional nectar and pollen sources within crops would enhance aphidophagous hoverflies, providing both an additional pollination service and aphid suppression, resulting in higher commercial strawberry fruit quality.

Methods

The study was done between April–August 2016 in a commercial strawberry (*Fragaria x ananassa* Duch. cv. 'Jubilee'), plantation in Kent, UK (51.25038° N, 0.34955° E; elevation: 104.9 m at northern end, and 94.0 m at southern end of field). Strawberry plants were in 1 litre pots on table-tops under polythene-clad Spanish tunnels. The 1.88 ha field contained twelve contiguous tunnels, of which only the central ten (each 7 m x 165 m, with 150 micron polythene cladding) were used for the study to mitigate edge effects (Chacoff and Aizen, 2006).

Coriander (*Coriandrum sativum* L.), field forget-me-not (*Myosotis arvensis* (L.) Hill) and field mint (*Mentha arvensis* L.) were selected as focal flower species using the following criteria: (1) attractive to hoverflies as a source of nectar and/or pollen (Colley and Luna 2000; Morris and Li 2000; Haenke et al. 2009; Hassan et al. 2017), (2) produce flowers in the first year after sowing, (3) grow to a maximum height of less than one metre, (4) not harmful or poisonous to humans, (5) not known to be attractive to major strawberry pests, and (6) neither invasive nor considered pernicious arable weeds.

A randomised block design was used with ten replicates of each of four treatments (coriander, forget-me-not, field mint, or untreated – control). Each replicate was in a separate tunnel, hence a tunnel was a block. The control treatment had no additional floral resources. Each plot was 3 x 6 m and separated by 28 m. Within each treated plot, plug plants of one of the three species were inserted, in pots, between the strawberry plants, which were also in pots. Every third plant pot contained the additional plug plant species (one plug plant per metre; 18 additional flower containers per plot). Pots were drip-irrigated on the same line as the strawberries. Coriander and mint seedlings were planted on 8-11 April, and forget-me-not plants on 10-11 May. In the untreated control, empty flower pots were introduced at the same density as the plug plants.

The heights of 18 strawberry plants in control plots and 18 sown flower plants in treated plots were recorded in two randomly-selected tunnels on 1 September. Finally, on 11 August, after the coriander plants had finished flowering, they were cut to 50 cm to avoid any contact with farm machinery passing through the tunnel.

Flower visitor surveys

All plots were surveyed six times during the flowering period of the strawberries: May – August 2016. A seventh survey was carried out in the last two weeks of August during which only field mint and control plots were visited, as coriander and forget-me-not plants had ceased flowering. Ten minute timed observations were done in each plot and strawberry flower and/or sown flower insect visitors recorded. Insects that were observed visiting both strawberry and sown flower species were counted as 'strawberry flower visitors' to avoid double-counting these insects in the calculation of total flower visitors per plot. Flower visitors were identified to functional group (FG). FGs were categorised as follows: (1) bumblebees: *Bombus spp.*; (2) honeybees: *Apis mellifera*; (3) hoverflies: all flies in the Syrphidae family; and (4) other flies: all dipterans excluding hoverflies. During each round of surveys, tunnels and plots were surveyed in a random sequence to account for any time of day or day of the year bias (Rotheray and Gilbert 2011).

Flower visitor surveys were conducted between 09:00 and 17:00 on dry days > 10°C if cloud cover was less than 5 oktas, and > 14°C on cloudier days. Wind speeds were less than 29 km/h (Beaufort scale 5; Pywell et al. 2005).

At each survey, in each tunnel, ambient temperature, relative humidity, wind speed and cloud cover were recorded. Temperature (°C) and humidity (% RH) were measured using a data logger (Omega EL-USB-2, Manchester, UK). Wind speed (km/h) was recorded using a handheld anemometer (Mastech MS6252B, Hong Kong). Cloud cover was assessed in oktas using a circular convex mirror divided equally into eighths.

Finally, counts of open strawberry flowers on each of the ten strawberry plants were made to give an index of floral units available to potential pollinators in each plot. A "floral unit" was defined as one flower head, which would result in a single strawberry fruit.

Aphid and aphid natural enemy surveys

Once the timed observation of flower visitors was completed, ten strawberry plants in the middle row of each plot were hand-searched for aphids and their natural enemies (Hogg et al., 2011). All sides of leaves, stems and flowers were thoroughly examined, and numbers of aphids and natural enemies were recorded. Natural enemies, including immature hoverflies and lacewings, were identified to family level in the field. In the final two surveys (13 July – 29 August), only five plants were surveyed per plot because high numbers of aphids were present. Lastly, in the final survey round (18 – 29 August), only mint and control plots were surveyed because coriander and forget-me-not plants had finished flowering.

Fruit quality assessments

To compare fruit quality ten strawberry flowers were tagged when the introduced plants were also in flower and then collected (100 berries per treatment). Where possible, one flower was tagged and picked from each of the ten plants in the central row of the plot. Strawberry flowers in coriander, forget-me-not and control plots were tagged on 4 July and fruit collected on 27-28 July. Mint and control plot flowers were tagged on 9 August and fruit picked on 1 September. Strawberries were picked when 25-75% of the fruit surface area was pinkish-red. Each berry was then assessed for: fruit shape class, diameter, fresh weight, dry weight, soluble solids content (an index of Brix), number of fertilised achenes and marketability (Klatt et al. 2014).

Shape was assessed as follows: 1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations or 4 = fruit with major malformations. Diameter was measured to the nearest tenth of a millimetre using callipers and mass (Sartorius, Göttingen, Germany) to the nearest tenth of a gram. Each berry was evenly sliced in half. One half was weighed and dried overnight in an oven at 60°C, then re-weighed and the dry weight recorded. The other half of the berry was used for digital refractometer Brix measurement (Palette, Atago, Tokyo, Japan) and counts of fertilised achenes. Each

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berry was placed in a blender (Minipro, Tefal, Rumilly, France) with 200 ml of water and blended for 20 seconds. The contents were then transferred to a 500 ml beaker and allowed to settle. Sunken fertilised seeds were counted by lifting the glass beaker and counting the achenes on the bottom. Lastly, marketability was assessed by classing strawberries with a minimum diameter of 18 mm and a shape score of 1 or 2 as marketable. Berries that failed to meet both criteria were deemed unmarketable (Conti et al. 2014; Klatt et al. 2014).

Flower visitors data analysis

All analyses were done in R version 3.4.1 (R Core Team 2017). Unless otherwise stated, all averages are presented as mean values ± standard error of the mean. As flower visitor survey data often contained many zeros, zero-inflated generalised linear mixed models (GLMMs) were fitted using the 'glmmADMB' package (Fournier et al. 2012; Skaug et al. 2016). A zero-inflated model for each response variable was compared against a GLMM without zero-inflation using Akaike's information criterion, corrected for small sample size (AICc; Thomas 2017). As suggested by Warton (2005), zero-inflated models did not improve model fit. GLMMs were fitted with a Poisson distribution unless over-dispersion was detected, then a negative binomial distribution was used (Zuur et al. 2009).

For the flower visitor surveys, differences between treated and control plots were compared separately, as the plants flowered during different periods. For each treatment, data were only compared against controls from the survey rounds when the sown species was in flower. Flower visitor data from each plot were analysed twice: once with only insect visitors recorded on strawberry flowers, and then with strawberry flower visitors and flower visitors pooled together. Response variables included counts of flower visitors, functional group (FG) richness and FG diversity (inverse Simpson's diversity index). In each analysis, response variables were weighted by counts of open strawberry flowers, following Reitan and Nielsen (2016).

To reduce the number of predictor variables included in our models, a principal components analysis (PCA) was performed on temperature, humidity, cloud cover, wind speed and time of day

(expressed as a proportion of a whole with 0 = 00 hrs 00 mins and 1 = 23 hrs 59 mins). All factors included in the PCA were centred and scaled to have unit variance prior to the analysis because variances differed substantially among factors (Crawley 2007). The number of principal components to include as fixed effects in subsequent analyses was determined by examining scree plots and applying Kaiser's criterion (Yong and Pearce 2013). After principal components were added to the model, the remaining fixed effects included, plot position within the tunnel, tunnel number and the interaction between survey round and treatment (coriander, forget-me-not, mint or control).

The optimal model was chosen starting with a full model and running the 'drop1' function in R to identify and then remove the least significant fixed effect. This was repeated until only significant fixed effects remained.

Counts of open strawberry flowers were analysed using zero-inflated GLMMs with a negative binomial distribution due to over-dispersion. Plot position, tunnel number, survey round and treatment were included in the full model as fixed effects, and the interaction between plot number and survey round was the random effect. The full model was then reduced following the procedure described above for flower visitor data until only significant fixed effects remained.

Aphid and aphid natural enemies data analysis

Aphid, hoverfly larva and egg, lacewing egg and mummified aphid count data contained overabundances of zeros (50.7%, 89.6%, 83.2%, 92.7%, 79.1% of all values, respectively). As a result, these response variables were first pooled across plants within a plot and then analysed using a zero-inflated GLMM and a standard GLMM. The two models were compared using AICc, and the zero-inflated model was only chosen when it significantly improved the model fit (Warton 2005). Aphid and aphid predator counts were modelled using either a Poisson distribution or a negative binomial distribution when data were over-dispersed. In each case, plot totals of aphids and their predators were weighted by the number of plants surveyed in the plot, as this number was changed from ten to five in the last two survey rounds. Fixed effects included plot position within the tunnel, tunnel

number, survey round and treatment (coriander, forget-me-not, mint or control). The interaction between plot number (1-40) and survey round was included as a random effect. The optimal model was chosen following the same procedure used for flower visitor survey data.

Numbers of aphids, hoverfly larvae, hoverfly eggs, lacewing eggs and mummified aphids per plot were also analysed separately for each survey round. GLMs were employed, using either a Poisson distribution or a negative binomial distribution when data were over-dispersed. No weights were used on the count data as within each survey round, the same number of plants was surveyed in every plot. The full model was compared against an otherwise-identical zero-inflated version of the GLM. The zero-inflated model was preferred only when it improved goodness of fit over original full model according to AICc (Warton 2005). Fixed effects included plot position within the tunnel, tunnel number and treatment. The optimal model was chosen following the same procedure used for flower visitor survey data.

Lastly, Spearman's rank correlation test was used to check for correlations between aphid numbers and counts of the four groups of predators and parasitoids (Gillespie et al. 2011).

Fruit quality data analysis

For fertilised seed counts, the mass of the fruit half divided by the mass of the whole fruit was calculated and then used to calculate the mean number of seeds per fruit half. LMMs were used on all normally-distributed fruit quality measurements. Response variables were transformed where necessary. Normality was confirmed by inspecting quantile-quantile plots and the Shapiro-Wilk test, and homoscedasticity was confirmed using Bartlett's test. When transformations failed to produce normally-distributed data, GLMMs were employed. For continuous variables, a gamma distribution was used, and for marketability, a binomial distribution was employed. Fruit shape score frequency distributions were analysed using cumulative link mixed models with a probit link function, as this was a latent continuous variable (Christensen 2015).

Plot position within the tunnel, tunnel number and treatment were selected as fixed effects for the full model of each analyses and optimal model as for flower visitor survey data. As ten berries were collected from each plot, plot number (1-40) was used as the random effect for each model. The significance of the random effect was tested by comparing the optimal model against an identical model that only contained fixed effects using the likelihood ratio test.

Results

The presence or absence of sown flowers in plots did not affect numbers of open strawberry floral units per plant ($\chi^2(3) = 0.55$, $P = 0.91$). Counts of open strawberry flowers only varied significantly according to survey round ($\chi^2(4) = 190.51$, $P < 0.001$; Fig. 4.1). Coriander (*C. sativum*) grew to an average height of 93.4 ± 1.2 cm, over three times as tall as the strawberry plants (mean = 28.9 ± 0.4 cm). Coriander flowered from 27 May to 25 July (Fig. 4.1). Field forget-me-not (*M. arvensis*) grew to approximately half the height (mean = 46.0 ± 2.19 cm) of coriander and flowered from 28 June to 25 July (Fig. 4.1). Field mint (*M. arvensis*) were slightly taller (mean = 33.8 ± 1.3 cm) than strawberry plants and flowered in the final two survey rounds: from 13 July to 29 August (Fig. 4.1).
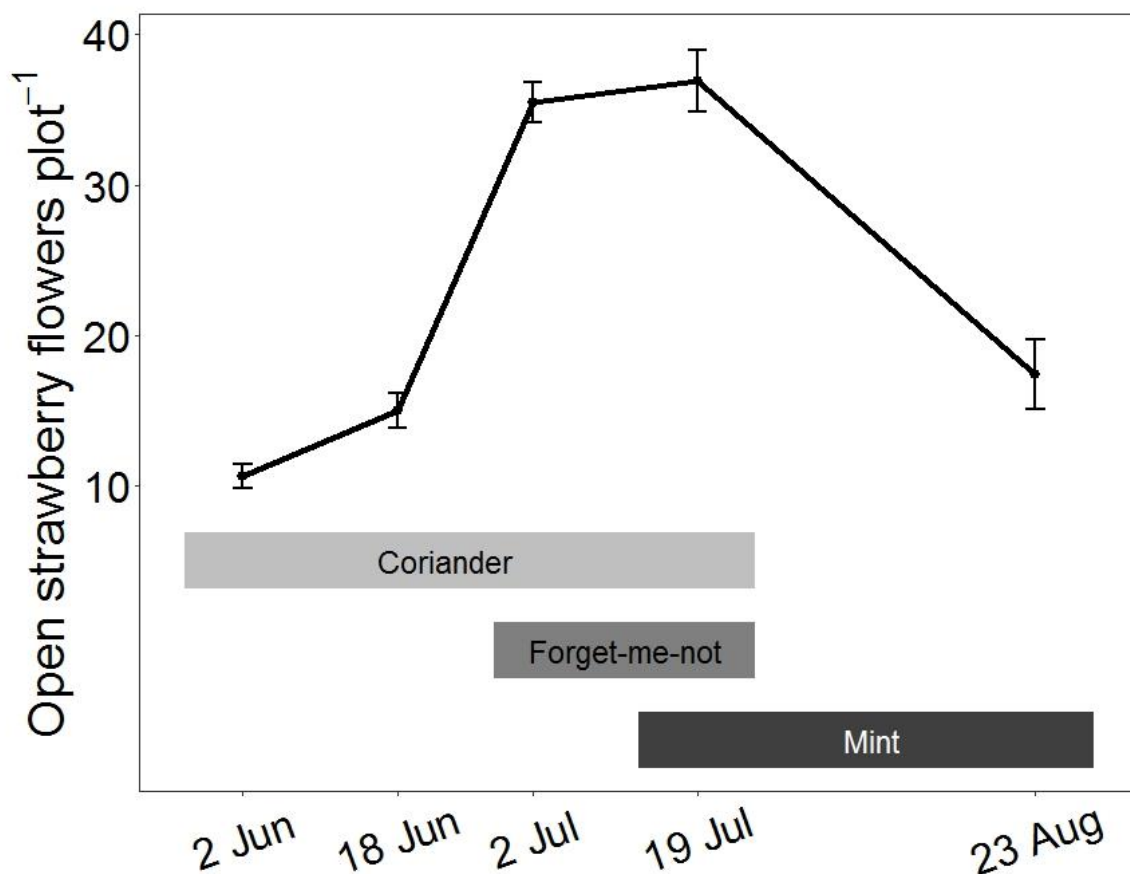
Figure 4.1 Counts of open strawberry flowers during each survey round when sown species were also in flower. Points represent mean counts ± SE per plot. Dates indicated on the x axis show the midpoint of each survey round. Flowering periods of the three sown flower species are represented by bars below the line graph.

Flower visitor surveys

Coriander

Strawberry flower visitors were more numerous and from a richer and more diverse range of functional groups (FGs) in the control plots compared to the coriander plots (abundance: $Z = -3.52$, $P < 0.001$; FG richness: $Z = -2.23$, $P = 0.026$; FG diversity: $Z = 3.56$, $P < 0.001$; Fig. 2; Table 4.1). By contrast, when insects visiting coriander flowers only were added to counts of strawberry flower visitors, FG richness and diversity were both greater in coriander plots than in controls ($Z = 3.71$, $P < 0.001$; $Z = -4.83$, $P < 0.001$, respectively; Table 4.1). Total flower visitor abundance was influenced by the

interaction between survey round and treatment ($\chi^2(3)$ = 18.70, P < 0.001). During the two survey rounds at peak flowering of the coriander plants (covering 13 June – 6 July), flower visitor abundance was significantly greater in coriander plots than controls (Z = 5.16, P < 0.001; Z = 5.93, P < 0.001, respectively). However, in the survey rounds at the start and end of the coriander flowering period, there was no difference between counts of all flower visitors in coriander or control plots (Z = 0.58, P = 1.0; Z = 0.39, P = 1.0, respectively).

Apart from strawberry flower visitor FG diversity, all other flower visitor analyses in coriander and control plots varied according to survey round. Surveys conducted between 13 – 23 June recorded the highest number (strawberry visitors: least-square (LS) mean = 1.32 ± 0.19; total visitors: LS mean = 15.06 ± 2.12) and diversity of flower visitors (strawberry visitor FG richness: LS mean = 1.01 ± 0.12; total visitor FG richness: LS mean = 3.40 ± 0.52; total visitor FG diversity: LS mean = 1.91 ± 0.22) compared to other survey rounds.

Finally, the first principal component derived from weather-related and time-of-day variables was negatively correlated to strawberry flower visitor abundance ($\chi^2(1)$ = 4.60, P = 0.032). Relative humidity and amount of cloud cover both had high positive loadings (0.598 and 0.534, respectively) on the first principal component, while temperature had a high negative loading (-0.567). Therefore, numbers of strawberry flower visitors tended to increase as humidity and amount of cloud cover decreased and temperature increased.

Field forget-me-not
Only strawberry flower visitor FG diversity was affected by the addition of forget-me-not plants to plots ($\chi^2(1)$ = 5.33, P = 0.021). Control plots attracted a higher diversity of FGs to strawberry flowers than forget-me-not plots (Z = 2.25, P = 0.024; Table 4.1).

Table 4.1 Comparisons of insect flower visitor abundance, FG richness and inverse Simpson's diversity (1/D). "Cor" = "Coriander", "Ctrl" = "Control", "strawb" = "strawberry", "Fmn" = "Field Forget-me-not", "Mint" = "Field Mint". Significant differences are in bold with the degree of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Tukey-adjusted comparisons). Where the interaction between survey round and treatment was significant, a letter was used in place of asterisks: X) all flower visitors in coriander plots more abundant than in controls at peak coriander flowering; Y) all flower visitors more abundant in mint plots than controls - both survey rounds; and Z) strawberry flower visitor FG diversity higher in control plots than mint in the second survey.

Analysis	Abundance	FG Richness	FG Diversity
Coriander vs. control (all visitors)	**Cor > Ctrl1^X**	**Cor > Ctrl1*****	**Cor > Ctrl1*****
Coriander vs. control (strawb visitors only)	**Cor < Ctrl1*****	**Cor < Ctrl1***	**Cor < Ctrl1*****
Forget-me-not vs. control (all visitors)	Fmn = Ctrl2	Fmn = Ctrl2	Fmn = Ctrl2
Forget-me-not vs. control (strawb visitors only)	Fmn = Ctrl2	Fmn = Ctrl2	**Fmn < Ctrl2***
Mint vs. control (all visitors)	**Mint > Ctrl3^Y**	**Mint > Ctrl3***	Mint = Ctrl3
Mint vs. control (strawb visitors only)	**Mint < Ctrl3****	Mint = Ctrl3	**Mint < Ctrl3^Z**

Field mint

Strawberry flower visitors were more numerous in control plots than in mint plots, and strawberry visitor FG diversity was influenced by the interaction between survey round and treatment ($\chi^2(1) = 7.65$, $P = 0.0057$; $\chi^2(1) = 9.12$, $P = 0.0025$, respectively). No difference was found in FG diversities between mint and control plots in surveys carried out between 13 – 25 July ($Z = 0.23$, $P = 1.0$); but during the second survey round (18 – 29 August), strawberry visitor FG diversity was higher in control plots ($Z = 2.98$, $P = 0.016$). Strawberry visitor FG richness did not vary according to treatment ($\chi^2(1) = 1.63$, $P = 0.20$). When insects visiting mint flowers were added to strawberry visitors, total flower visitor counts were found to be significantly influenced by the interaction between survey round and treatment ($\chi^2(1) = 8.45$, $P = 0.0037$). Numbers of flower visitors increased significantly in both treatments from the first mint survey round (13 – 25 July) to the second (18 – 29 August; mint: Z

= -10.52, *P* < 0.001; control: Z = -3.23, *P* = 0.0068), but insect counts in mint plots were significantly higher than controls in both rounds (13 − 25 July: Z = -2.77, *P* = 0.028; 18 − 29 August: Z = 4.98, *P* < 0.001; Fig. 4.2; Table 4.1). FG richness of all flower visitors was also significantly higher in mint plots than controls, but total FG diversity did not differ between plot types (FG richness: Z = 2.23, *P* = 0.026; FG diversity: $\chi^2(1)$ = 0. 18, *P* = 0.67; Table 4.1).
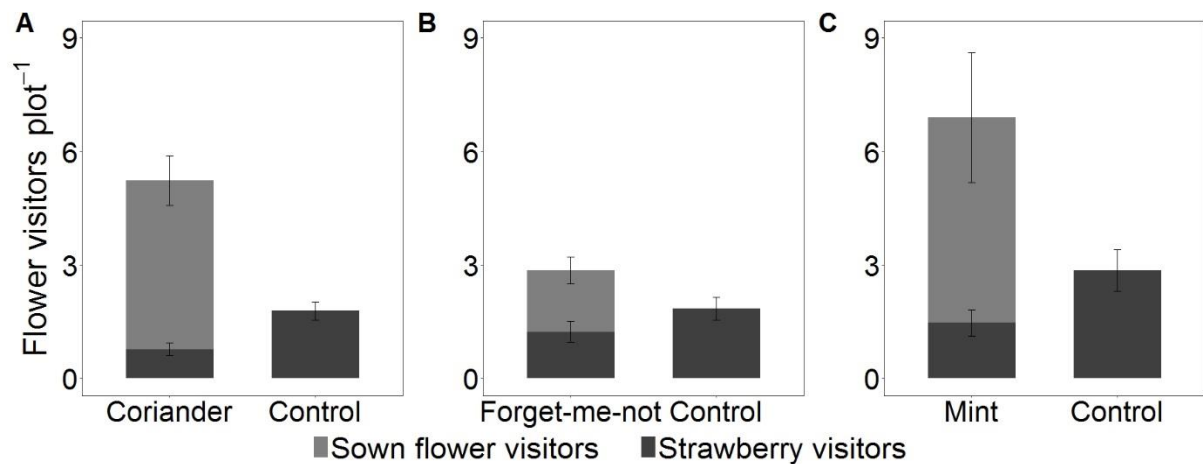
Figure 4.2 Mean ± SE flower visitor counts, weighted by counts of open strawberry flowers per plot by treatment for the three sown flower species. Only flower visitors observed during the flowering period of the sown species are included in each graph: A) coriander (27 May − 25 July); B) field forget-me-not (28 June − 25 July); C) field mint (13 July − 29 August).

In addition to affecting counts of total flower visitors, survey round also had a significant impact on abundances of strawberry flower visitors, strawberry visitor FG richness and total FG richness. In each case, the response variable increased significantly from the first (13 − 25 July) to the second mint survey round (18 − 29 August; strawberry visitor abundance: Z = -3.90, *P* < 0.001; strawberry visitor FG richness: Z = -1.97, *P* = 0.049; total visitor FG richness: Z = -4.61, *P* < 0.001).

Abundances of strawberry flower visitors were negatively correlated to the first principal component of weather-related and time-of-day variables ($\chi^2(1)$ = 7.90, *P* = 0.0049). Humidity and cloud cover had high positive loadings on this principal component (0.589 and 0.533, respectively), while temperature had a high negative loading (-0.577). Therefore, strawberry visitor numbers increased with decreasing humidity and cloud cover, and increasing temperature.

Fruit quality assessments

The presence of introduced flowering plants within strawberry tables had a significant effect on some fruit quality measures. However, proportions of marketable fruit did not differ among any of the treatments (Fig. 4.3). When fruit from mint plots was compared against controls, only the number of fertilised achenes per fruit half was significantly affected by treatment ($\chi^2(1) = 5.10$, *P* = 0.024). Fruit from control plots had significantly more fertilised seeds than berries from mint plots (Z = -2.41, *P* = 0.016; Fig. 4.3). In the analysis of fruit from coriander, forget-me-not and control plots, Brix and percent dry weight were both significantly influenced by treatment (Brix: $\chi^2(2) = 14.35$, *P* < 0.001; percent dry weight: $\chi^2(2) = 6.23$, *P* = 0.044). Berries from control plots scored the highest, followed by fruit from coriander and then forget-me-not plots. However, only the difference in Brix between control and forget-me-not plots was statistically significant (Z = -2.79, *P* = 0.014; Fig. 4.5). For all other fruit quality measurements, treatment had no effect on mean scores (Fig. 4.3 – 4.5).
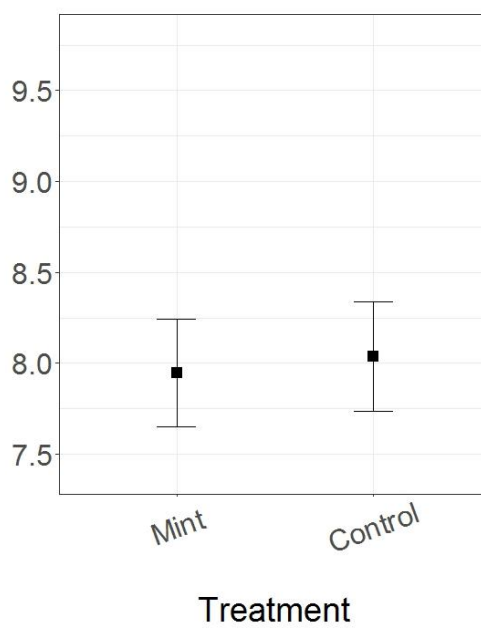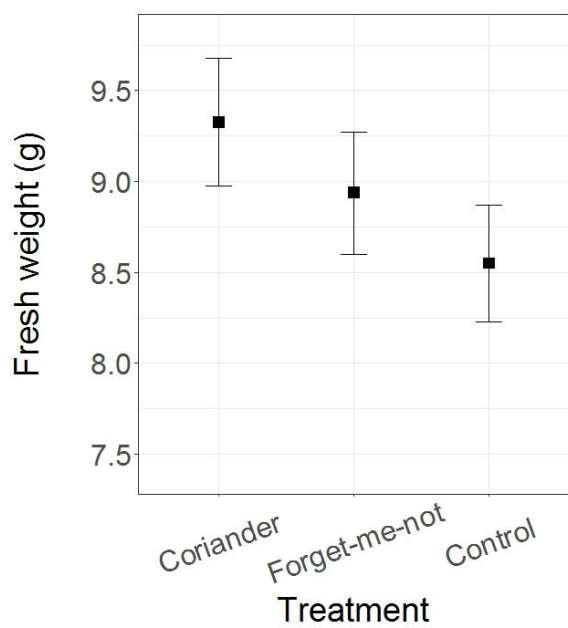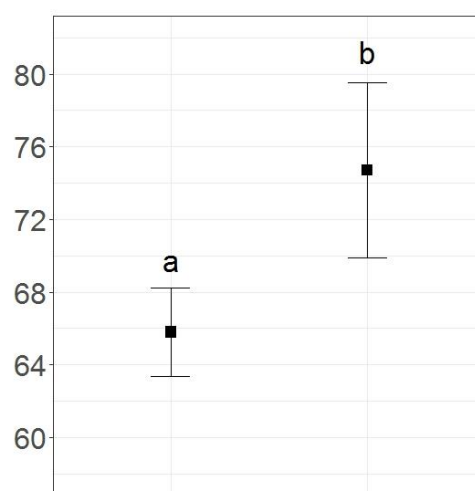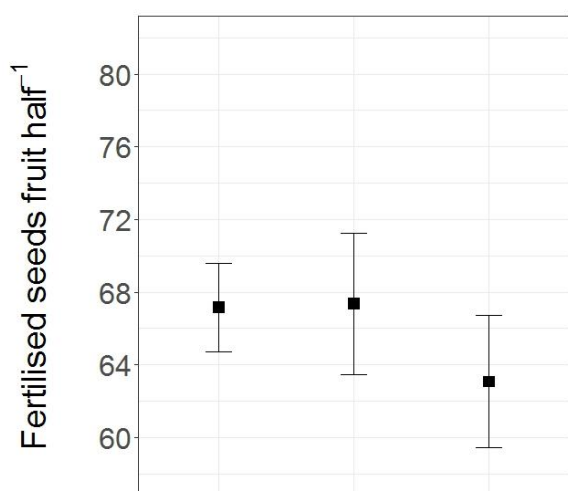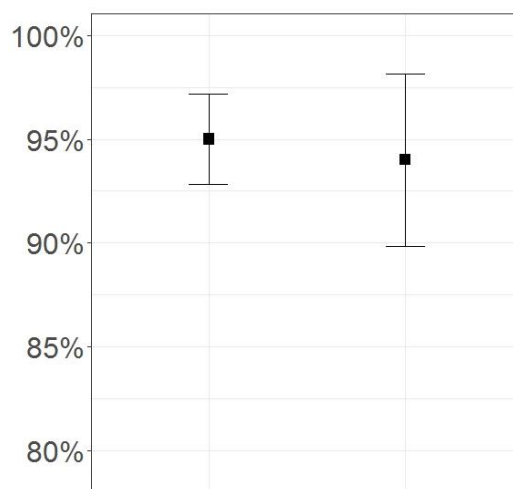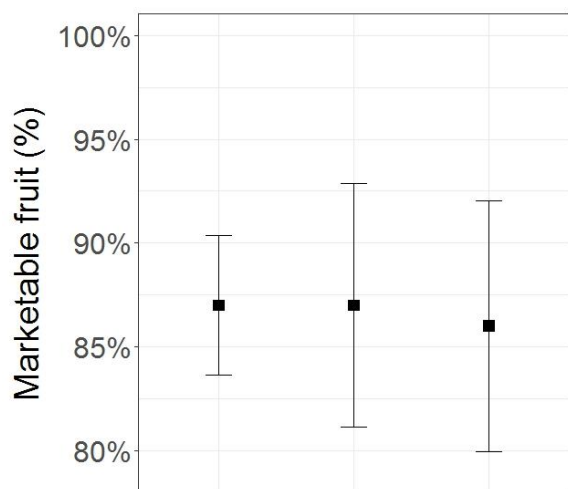
Figure 4.3 Proportion of marketable fruit, fertilised strawberry seeds per fruit half and berry weight by treatment. Points and error bars represent least-square means ± SE. Coriander, forget-me-not and control berries were collected on 27–28 July. Mint and control berries were collected on 1 September. Where present, significant differences ($\alpha = 0.05$; Tukey-adjusted comparisons) are labelled with different letters.
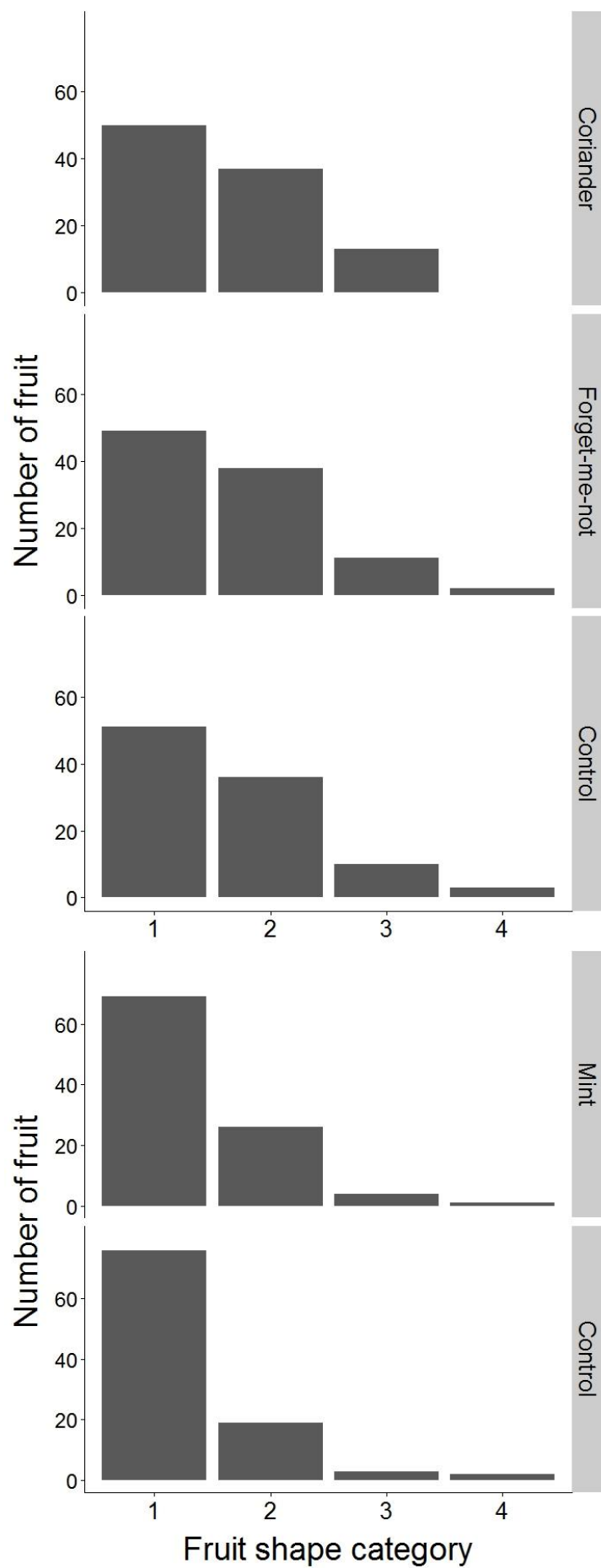
Figure 4.4 Fruit shape category frequency distributions by treatment (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations). Fruit that fell into category 3 or 4 were deemed unmarketable. The top graph shows fruit totals per shape category for all strawberries collected on 27 – 28 July, while the lower chart displays all berries collected on 1 September.

Aphid and aphid natural enemy surveys

Aphids

Across all treatments and survey rounds, an average of 113.7 ± 10.98 aphids (per 10 plants) were recorded in each plot. The addition of introduced flowering plants did not significantly affect aphid abundance ($\chi^2(3) = 5.70$, $P = 0.13$; Fig. 4.6). Finally, aphid counts reached a peak in the second survey round (19 – 26 May: LS mean = 181.9 ± 58.5 aphids per plot). Mean aphid abundance in the second survey round was higher than mean counts recorded in survey rounds 1, 4, 6 and 7 ($Z = -3.22$, $P = 0.022$; $Z = 4.36$, $P < 0.001$; $Z = 3.07$, $P = 0.035$; and $Z = 3.38$, $P = 0.013$, respectively). Mean counts in the remaining survey rounds ranged from 49.9 – 89.5 aphids per plot and did not differ significantly from each other.

Lacewing eggs

Numbers of lacewing eggs per plot were significantly affected by treatment and survey round ($\chi^2(3) = 52.99$, $P < 0.001$; $\chi^2(6) = 212.35$, $P < 0.001$, respectively). Lacewings were found to oviposit on strawberry plants in coriander plots over four times more often than in any other treatment.
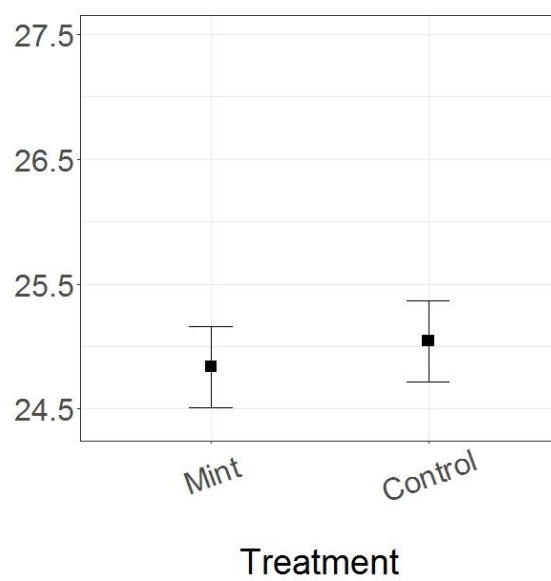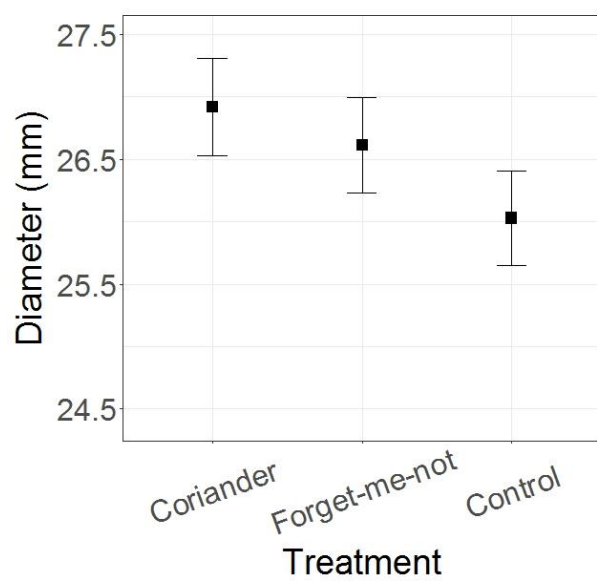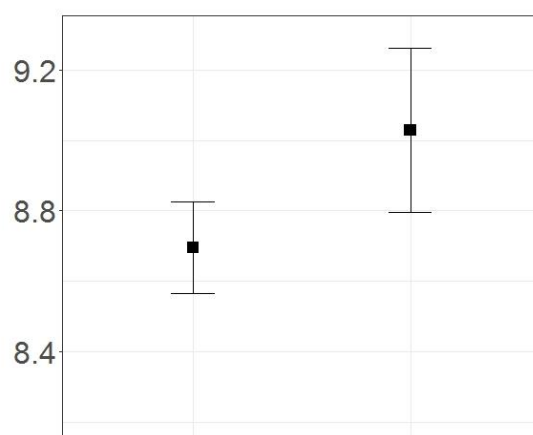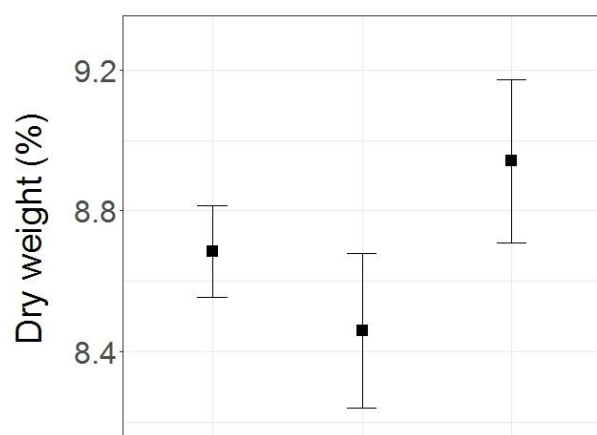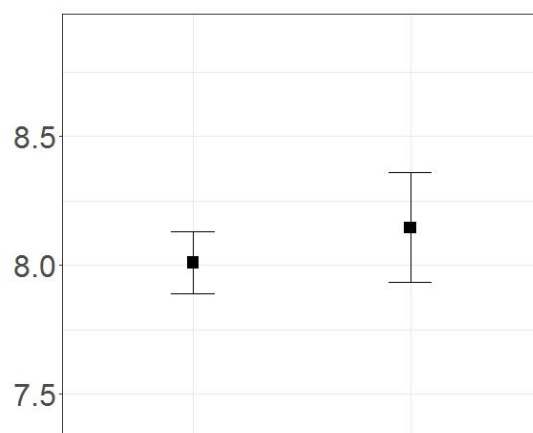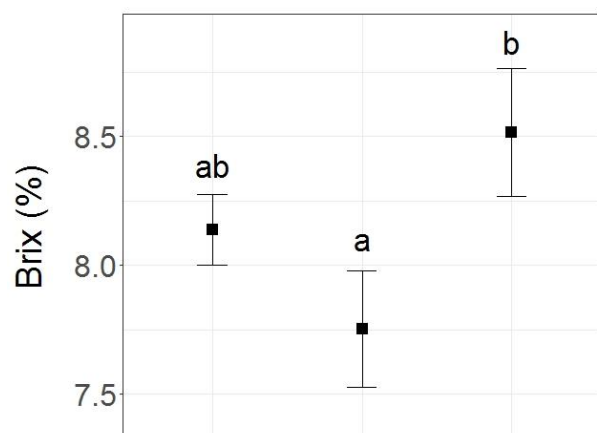
Figure 4.5 Brix, percent dry weight and fruit diameter by treatment. Points represent least-square means ± standard error. Means sharing the same letter do not differ significantly (α = 0.05; Tukey-adjusted comparisons). Coriander, forget-me-not and control berries were collected on 27 – 28 July. Mint and control berries (again) were collected on 1 September. Where present, significant differences (α = 0.05; Tukey-adjusted comparisons) are labelled with different lowercase letters.

Hoverfly eggs

Counts of hoverfly eggs on strawberry plants were also influenced by treatment ($\chi^2(3)$ = 8.31, *P* = 0.040), though differences among treatment means were not significant once *P*-values were adjusted by the Tukey method for multiple comparisons. Trends in counts of hoverfly eggs by survey round mirrored those of aphid counts. Hoverfly egg abundances peaked in survey round 2 (19 – 26 May: LS mean = 16.7 ± 4.6 eggs per plot). Egg counts in all other survey rounds were significantly lower and ranged from 1.9 – 3.0 eggs per plot.
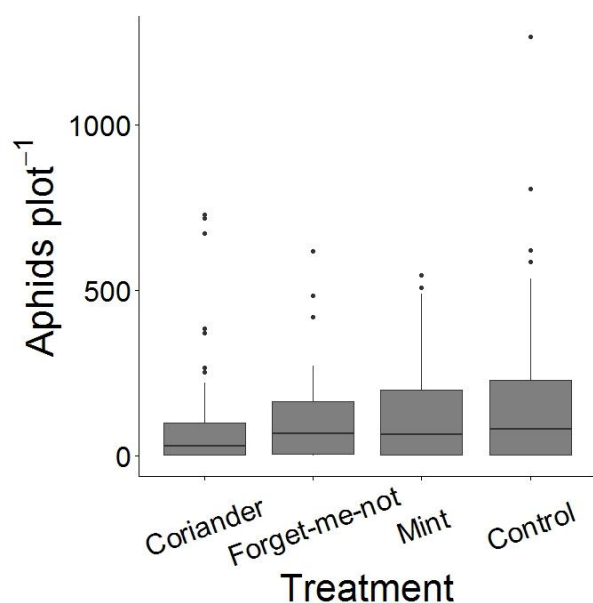
Figure 4.6 Box plots of aphid counts in the coriander, forget-me-not, mint and control treatments over the entire sampling period (13 May – 23 August). There were no significant differences between treatments.

Hoverfly larvae

An average of 1.4 ± 0.17 hoverfly larvae were recorded in each plot over the course of the entire sampling period. However, numbers of hoverfly larvae did not respond positively or negatively to

additional floral resources (χ^2(3) = 6.15, P = 0.10; Fig. 4.7). Lastly, hoverfly larvae peaked in survey round 2 (19 – 26 May) and were significantly lower in survey rounds 3, 4, 5 and 7 (27 May – 6 July; and 18 – 29 August, respectively).

Mummified aphids

On average, 4.4 ± 0.47 mummified aphids were recorded in each plot over the course of the entire sampling period. Aphids parasitized by parasitoid wasps did not respond to additional floral resources (χ^2(3) = 5.81, P = 0.12; Fig. 4.5). As with hoverfly eggs and larvae, mummified aphids fluctuated according to aphid densities: counts of mummified aphids peaked in survey round 2 (19 – 26 May: LS mean = 115.0 ± 31.6 per plot) and were significantly lower in all other survey rounds (range = 1.9 – 4.2 per plot).

Aphid and natural enemy counts by survey round

When aphid and aphid predator abundances were analysed for each round separately, treatment had a significant effect only on lacewing egg abundances between 28 June – 25 July (survey rounds 5 and 6; round 5: χ^2(3) = 43.86, P < 0.001; round 6: χ^2(3) = 26.96, P < 0.001). In both survey rounds, lacewing egg counts in plots provisioned with coriander were significantly higher than counts in all other treatments. For aphids and all other aphid natural enemies, treatment means did not differ significantly during any survey round.
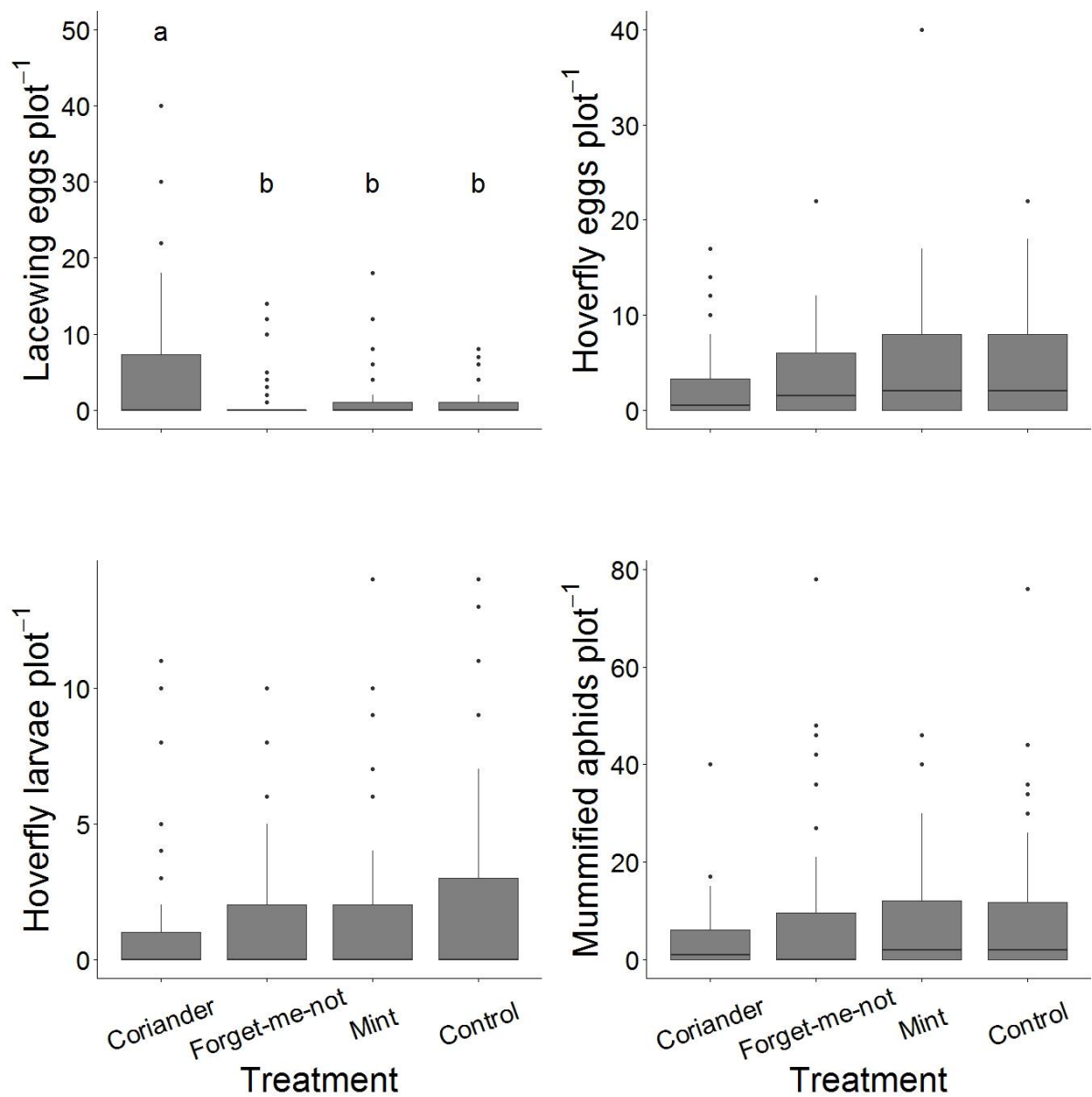
Figure 4.7 Box plots of aphid natural enemy counts per plot by treatment over the entire sampling period (13 May – 23 August). Where present, significant differences between treatments (α = 0.05; Tukey-adjusted comparisons) are labelled with different letters.

Natural enemy responses to aphid abundance

Counts of all aphid predator and parasitoid groups were positively correlated to aphid abundance (lacewing eggs: ρ = 0.14, P < 0.001; hoverfly eggs: ρ = 0.46, P < 0.001; hoverfly larvae: ρ = 0.40, P < 0.001; mummified aphids: ρ = 0.47, P < 0.001).

Discussion

This study assessed the impact of intercropping flowering plants within a commercial strawberry crop on flower insect visitor abundance and diversity, aphid predator abundances, aphids and strawberry fruit quality. For most response variables, inter-planting coriander, forget-me-not or mint plants in strawberry rows had no impact. However, counts of total flower visitors were significantly enhanced in plots sown with coriander or mint flowers. Moreover, aphidophagous lacewings oviposited over four times more eggs in coriander plots than in any other treatment. Despite these differences in insect communities within the plots, adding sown flowers to strawberry rows had no impact on proportions of marketable fruit.

Flower visitors

Coriander and mint treatments attracted more flower visitors than control plots, and visitors in these plots had a greater FG (bumblebees, honeybees, hoverflies, and other flies) richness than controls. However, the enhanced number and diversity of all flower visitors did not translate to greater numbers or diversities of strawberry flower visitors. In fact, in coriander and mint plots, fewer insects were recorded visiting strawberry flowers than in control plots. In forget-me-not plots, insects were observed visiting strawberry flowers as often as in the controls, but the FG diversity of strawberry flower visitors was higher in control plots.

The fact that more flower visitors were present in two of the three treatments with additional floral resources corroborates previous research where flower visitor abundance was positively correlated to counts of floral units (Bates et al. 2011; Feltham et al. 2015). However, we found that forget-me-not did not enhance numbers of flower visitors suggesting that the attractiveness of the additional sown species also plays a role in determining whether flower visitor abundance is enhanced. Thus, our results indicate that while forget-me-not failed to entice more insect flower visitors into strawberry rows, coriander and mint were effective at boosting abundances of flower visitors within strawberry crops under polytunnels.

Furthermore, in coriander and mint plots, flower visitors appeared to find the introduced flower species more attractive than strawberry flowers. Insects in coriander and mint plots were observed visiting strawberry flowers less frequently than in control plots, even though more flower visitors were present. Seifan et al. (2014) documented a similar trend wherein regularly-spaced, highly-attractive flowers were found to have a negative impact on insect visitation rates to less attractive neighbouring flower species, even though the highly-attractive flowers increased the total number of flower visitors observed in the plots.

While the mechanisms behind pollinator preference to the sown flower species were not a focus of this study, previous research suggests that flower volatile compounds are likely to play a role in attracting flower visitors to floral resources (Pichersky and Gershenzon 2002). Specifically, linalool has been identified as one of the organic compounds that may be especially attractive to insect flower visitors (Raguso and Pichersky 1999). Unlike field forget-me-not, coriander and field mint both produce linalool and the presence of this compound could partly explain the observed discrepancy in attractiveness between forget-me-not and the other two sown species (Gracindo et al. 2006; Shahwar et al. 2012; Znajdek-Awizen et al. 2014).

Finally, abundances of strawberry flower visitors were affected by temperature, relative humidity and cloud cover in the comparisons of coriander and mint plots against controls. In both instances, more insects tended to visit strawberry flowers when temperatures were higher (> 20.5°C), and humidity and cloud cover were lower (< 64.6% RH; and < 5 oktas). This pattern of increased insect activity at higher temperatures and lower humidity and cloud cover is in line with prior research on pollinator visitation rates in relation to weather conditions carried out in temperate woodland-meadow, alpine tundra and Mediterranean scrub habitats (McCall and Primack 1992).

Fruit quality

Despite the observed reductions in strawberry flower visitation rates, pollination success rates, as measured by counts of fertilised seeds, were only significantly lower in mint plots than controls; for

all other sown flower treatments, pollination success rates did not differ from control plots. Likewise, mean values of fruit fresh weight, dry weight, diameter, proportions of marketable fruit and fruit shape scores did not differ significantly from controls across any of the sown flower treatments. Therefore, although the addition of floral resources often reduced visitation rates to strawberry flowers, we found no evidence to suggest that quantities of marketable fruit were affected in any of the treatments.

Brix was lower in berries from forget-me-not plots than controls. Previous research on strawberry pollination has documented a reduction in Brix in berries that were better-pollinated (Hodgkiss et al. 2018). While the difference in pollination success rates between forget-me-not and control plots was not great enough to be statistically significant, the fact that Brix was significantly lower in forget-me-not plots suggests that a larger sample size may have revealed significantly higher pollination success rates in forget-me-not plots. However, further research is required to properly explore this hypothesis.

Aphids and their natural enemies

Mean counts of aphids did not differ according to the presence or absence of additional floral resources. Similarly, hoverfly larva, hoverfly egg and mummified aphid numbers were positively correlated to aphid abundance, but did not respond differently according to treatment. Gillespie et al. (2011) found a similar effect in lettuce fields intercropped with alyssum flowers. In their study system, larval aphidophagous hoverflies responded positively to aphid numbers but were unaffected by the presence of additional floral resources.

In contrast, lacewings were the only aphid natural enemy to respond significantly to treatment. Lacewing eggs were found on strawberry plants in coriander plots four times more often than in any other treatment. Moreover, while lacewing eggs were significantly more common in coriander plots, lacewing abundance was also strongly correlated to aphid numbers across all treatments. Lacewings almost always laid eggs in aphid-infested plots: 96.9% (508 / 524) of lacewing eggs were laid in plots

with aphids. These results support the view that lacewings oviposit in response to the presence of aphids and preferred aphid-infested strawberry plants in coriander plots over all other treatments.

Previous studies on lacewing oviposition rates in response to aphid density in pecan and maize crops have generally found positive relationships between lacewing oviposition rates and aphid densities (Petersen and Hunter, 2002; Kunkel and Cottrell, 2007; Keulder and Van den Berg, 2013). However, Coderre et al. (1987) found that the North American lacewing *Chrysopa oculata* Say usually laid eggs on maize plants without aphids. Bickerton and Hamilton (2012) investigated the impact of intercropping coriander, dill, *Anethum graveolens* L., and buckwheat, *Fagopyrum esculentum* Moench, on biocontrol of the lepidopteran European corn borer, *Ostrinia nubilalis* (Hübner), in bell pepper crops. These researchers found that predation rates by *Chrysoperla* sp. lacewings were equally low irrespective of the presence or absence of additional floral resources. The authors cited the possibility that lacewings were ovipositing primarily in response to aphid honeydew, which may have diluted any attraction to the sown flower species as aphids were present in both intercropped and control plots (Bickerton and Hamilton 2012).

When lacewing egg data were analysed as counts per survey round, the increase in lacewing numbers in coriander plots appeared only from late June onwards. Thus, while there was a noticeable increase in abundance in coriander plots from late June to the end of the growing season, lacewings were scarce in all plots early in the season; only 4.0% (21 / 524) of observed lacewing eggs were recorded before 28 June. This late appearance of lacewings calls into question their effectiveness as aphid biocontrol agents in the early stage of the growing season. Similarly, research from the U.S. on potential biocontrol agents for soybean aphids (*Aphis glycines* Matsumura) found that naturally-occurring lacewing larvae appeared too late in the season to be effective at preventing pest outbreaks in soybean crops (Rutledge et al. 2004). In Europe, most lacewing species commonly found in agroecosystems overwinter as either pre-pupae (e.g., *Chrysopa* spp.) or adults (e.g., *Chrysoperla* spp.; Canard 2005). Consequently, given that the larval stage is the period when lacewings consume the

most number of aphids, there is a delay in resumption of significant aphidophagous activity in spring while adults emerge, reproduce and lay eggs of the next generation (Canard 2005).

The difference in strength of the density-dependent responses to aphid abundance between lacewings and the remaining natural enemies was mirrored in the strength of the correlation between predator and aphid abundances. When Spearman's rank correlation test was performed, correlations between aphid density and hoverfly eggs, hoverfly larvae and mummified aphids reached or exceeded a value of $\rho = 0.40$. On the other hand, though still significant, the correlation between aphid and lacewing egg density was much weaker: $\rho = 0.14$. This difference in the strength of the attraction to aphid abundance may in part be explained by the fact that aphidophagous hoverflies and aphid parasitoids are specialist aphid-eaters. In contrast, lacewings are considered generalist predators of soft-bodied insects, and as such, are less reliant on aphids to undergo larval development (Bickerton and Hamilton 2012).

Management implications and future research

During the course of the experiment, coriander flowers grew over three times as tall as strawberry plants. Consequently, around 25% of plants required manual repositioning after getting knocked over by tractor-mounted pesticide applicators. In contrast, forget-me-not and mint plants grew less than twice the height of strawberry plants and were unaffected by farm machinery. All three wildflower species grew vigorously in the fertilised coir substrate and required pruning to remove approximately 15 cm of lateral growth twice during the growing season to ensure that strawberry rows were easily accessible for farm workers. Therefore, planting sown flowers within strawberry tables incurred additional labour costs on top of the cost of seed, irrigation, plant pots and substrate.

In return, introducing coriander within the crop may allow farmers to reduce pesticide costs to control aphids late in the season. Though our field surveys did not extend late enough in the season to document declines in aphids following the rise in lacewing egg numbers, future work may reveal that lacewings are able to suppress aphid outbreaks below economically damaging levels late in the

year. Further research could also examine whether releasing commercially-reared lacewings early on in the season in combination with coriander may represent a viable method of suppressing aphids before natural lacewing populations become established in the field (Pappas et al. 2011). Although ladybirds (Coleoptera: Coccinellidae) were scarce in our study system, research on intercropping coriander within tomato fields found that the ladybird *Cycloneda sanguinea* L. responded in a similar fashion to lacewings in the present study (Togni et al. 2016). This finding offers the possibility that coriander may be effective at attracting additional aphid predator taxa at locations where they are more numerous.

We found no evidence of enhanced pollination services as a result of sown floral resources. This may have been because pollination services already provided in the crop were adequate and did not need supplementing with additional flower visitors. Conversely, this one-year experiment may not have allowed pollinator populations to grow sufficiently to have a noticeable impact on fruit quality measures. A four-year study on the effect of wildflower strips on blueberry production revealed that yields only grew in the third and fourth years after the establishment of flower margins (Blaauw and Isaacs 2014). The authors explained that pollinator populations did not respond immediately but rather grew gradually in the years after additional pollen and nectar sources were established.

Our experimental plots were only separated by a distance of 28 m. Studies on the effects of floral resources on pollinator abundance into crops have shown that flower visitor numbers can be increased up to 100 m away from sown pollen and nectar sources (Campbell et al. 2017). Therefore, flower visitation rates in control plots in our study may have benefited from the presence of additional sown flowers in plots 28 m away, thereby diluting any treatment effect on flower visitor abundance.

Several previous studies have also shown that adding floral resources to agricultural areas can enhance abundances of flower-visiting insects (Kohler et al. 2008; Haenke et al. 2009; Blaauw and Isaacs 2014; Jönsson et al. 2015; Wood et al. 2015; Campbell et al. 2017). Given recent evidence of drastic declines in flying insects over the last three decades (Hallmann et al. 2017), sowing coriander and mint within strawberry crops may help sustain wild insect populations in horticultural ecosystems.

Research on the population-level impact of agri-environment schemes designed to enhance floral resources for bumblebees found that nest densities were higher at farms with sown flower strips, demonstrating that additional floral resources can augment populations of pollinating insects (Wood et al. 2015). However, more research is required to determine whether added floral resources translate to population-level benefits for flower visitors in our study system, or whether the coriander and mint flowers merely attract pollinators away from other areas, resulting in no net growth in insect populations in the local area (Scheper et al. 2015; Williams et al. 2015).

Conclusions

In light of recent declines in wild pollinators and a growing need for viable alternatives to chemical pesticides (Bommarco et al. 2013), planting floral resources within crops has the potential to enhance numbers of beneficial insects that pollinate crops and prey on pest species. Our research has found that intercropping coriander within strawberry rows increases oviposition rates by aphidophagous lacewings four-fold. Proportions of marketable fruit were not affected by the additional of sown flower species, but benefits to pollinator assemblages, and by extension pollination services, were not seen in the single season in which observations were made. Further work spanning multiple growing seasons is required to confirm these benefits for strawberry growers.

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Chapter 5: Detection rates of aphid DNA in the guts of larval hoverflies (Diptera, Syrphidae, Syrphinae) vary according to the addition of floral resources in a commercial strawberry field

Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are the result of a collaboration between Dr Elizabeth L. Clare and myself. I was responsible for the field study, the dissection of larval digestive tracts, DNA extraction from hoverfly gut specimens, the statistical analyses and the writing of the chapter. Dr Clare carried out the DNA sequencing and bioinformatics and helped to edit the chapter. Where I have consulted the work of others, this is clearly stated.

Signed: _____

Date: _____

Abstract

Aphidophagous hoverflies (Diptera, Syrphidae, Syrphinae) are commonly observed flower visitors and aphid predators in fruit crops. Though the larvae of several species of hoverfly are known to consume aphids (Hemiptera: Aphidoidea), relatively little is known about the predation rates or diet preferences of individual species of aphidophagous hoverfly. Here, we investigate whether aphid prey DNA can be detected in the dissected gut contents of hoverfly larvae found in a commercial strawberry field with experimental intercropping of hoverfly attractive wildflowers. We use high-throughput sequencing targeting the cytochrome c oxidase I (COI) DNA barcode region and a bespoke reference database containing verified aphid and hoverfly DNA sequences taken from public repositories. Hoverfly DNA was recovered from 145 out of 149 (97%) hoverfly larval gut contents, while aphid DNA was found in 55 of these specimens (37%). Aphid prey DNA detection rates did not differ between the two most commonly collected hoverfly species, *Episyrphus balteatus* (De Geer) and *Eupeodes corollae* (Fabricius). Interestingly, prey detection rates were higher near plots with intercropped field forget-me-not, *Myosotis arvensis* (L.) Hill, than plots with sown coriander, *Coriandrum sativum* L. These findings suggest that aphids constitute at least a part of the diet of syrphine hoverflies in UK strawberry fields, and that high-throughput sequencing represents a viable method of identifying aphid DNA in hoverfly gut contents. Furthermore, intercropping strawberry crops with specific floral resources may affect aphid consumption in hoverfly larvae, with potential implications for pest control, though further studies are required to confirm and determine the mechanism of this interaction.

Introduction

Documenting trophic interactions in agroecosystems is a necessary first step in identifying naturally-occurring predators that may be capable of providing effective biological control of pest species. Aphids (Hemiptera: Aphidoidea) are one of the most important crop pests in temperate regions. Aphid colonies damage crops by limiting plant growth, transmitting plant viruses, and encouraging the growth of sooty moulds through the deposition of aphid honeydew on plant tissues (Solomon et al. 2001; Dedryver et al. 2010).

Among the diverse array of predatory arthropods that consume aphids, the insectivorous larvae of syrphine hoverflies (Diptera, Syrphidae, Syrphinae) have been cited as potential biological control agents of aphid pests in a variety of crop systems: apple (Dib et al. 2016), broccoli (Prasad et al. 2009), cabbage (van Rijn et al. 2006; Prasad et al. 2009), lettuce (Smith et al. 2008; Hogg et al. 2011), sorghum (Colares et al. 2015), strawberry (Kovanci et al. 2007), and wheat (Wang et al. 2009). Laboratory experiments have shown that syrphine hoverfly larvae are voracious aphid predators, consuming up to 168 aphids per day and 1,140 aphids during larval development (Tenhumberg and Poehling 1995; Hopper et al. 2011). Moreover, habitat manipulation experiments have demonstrated that sowing additional floral resources can boost populations of aphid natural enemies and lower pest abundances within crops (Bowie et al. 1999; van Rijn et al. 2006; Hogg et al. 2011).

Nevertheless, establishing trophic linkages between species in a field setting is complicated by the fact that visual observation is time-consuming and can disrupt normal predator foraging decisions (Gomez-Polo et al. 2015). Microscopic gut contents analysis is useful when insect diets contain solid food fragments, but hoverfly larvae are exclusively fluid feeders. On the other hand, molecular analysis of gut contents represents an effective tool for investigating the diet of fluid-feeding invertebrate predators (Piñol et al. 2014).

The development of high-throughput sequencing technologies has allowed researchers to examine the dietary breadth of insect predators without the need to predict and develop primers for

target prey species, as is the case in polymerase chain reaction (PCR)-based techniques (Pompanon et al. 2012). Additionally, the shorter (157 bp) amplicon used allows for the detection of smaller DNA fragments, typical of the contents of insect predator guts (Gomez-Polo et al. 2015) where DNA is thought to rapidly degrade. Shorter fragments are more likely to be recovered but may impose a limit on taxonomic resolution thus imposing a trade-off (Clare 2014).

Using commercial strawberry as a focal crop, we explored the impact of intercropping three flower species on trophic interactions between aphids and larval hoverflies. The aims of the study were to determine the following: (1) how frequently aphid DNA could be detected in hoverfly gut contents; (2) whether individual species of hoverfly differed in the proportion of specimens that contained fragments of aphid DNA; and (3) whether prey DNA detection rates varied according to the presence or absence of sown flower species. Based on the results of Gomez-Polo et al. (2015), I hypothesised that aphid DNA would be detected in approximately one third of hoverfly specimens and that this proportion would not vary significantly among hoverfly species. Finally, I predicted there would be higher prey DNA detection rates in control plots due to there being more aphids and fewer hoverfly larvae in the control plots.

Methods

Field study

The field study was carried out between April – August 2016 in a commercial strawberry, *Fragaria x ananassa* Duch. cv. 'Jubilee', plantation at a farm in Kent, UK (51.25038° N, 0.34955° E; elevation: 104.9 m at northern end, and 94.0 m at southern end of field). Strawberry plants were grown in 1-litre plant pots on table tops under polythene-clad Spanish tunnels. The 1.88-ha field contained twelve contiguous tunnels, of which only the central ten were used for the study to mitigate any edge effects (Chacoff and Aizen 2006). Immediately adjacent to the northern field edge was an arable field, and additional fields of protected horticultural crops bordered the remaining three sides. Wind breaks of Italian alder, *Alnus cordata* (Loisel.) Duby, were present along the

eastern and southern edges of the field. Six wildflower species grew naturally at the base of the alder wind break on the eastern edge of the field: bramble, *Rubus fruticosus* agg., cow parsley, *Anthriscus sylvestris* (L.) Hoffm., creeping thistle, *Cirsum arvense* (L.) Scop., hogweed, *Heracleum sphondylium* L., honeysuckle, *Lonicera* sp. L., and scentless mayweed, *Tripleurospermum inodorum* (L.) Sch.Bip. Hemlock, *Conium maculatum* L., grew in three isolated patches along the southern windbreak. Apart from these wildflowers, no additional sources of pollen and/or nectar were present along the field boundaries.

Coriander, *Coriandrum sativum* L., field forget-me-not, *Myosotis arvensis* (L.) Hill and field mint, *Mentha arvensis* L. were selected as focal flower species using the following criteria: (1) attractive to hoverflies as a source of nectar and/or pollen, (2) produce flowers in the first year after sowing, (3) grow to a maximum height of less than one metre, (4) not harmful or poisonous to humans, (5) not known to be attractive to major strawberry pests, and (6) neither invasive nor considered pernicious arable weeds (Morris 1998; Atakan and Uygur 2005; Paini et al. 2007; Streeter 2009).

A randomised block design was used with four treatments in ten adjoining polytunnels (each 7 m wide x 165 m long) covered with cladding (150-micron polythene). Along the strawberry rows, plots with one of the three introduced flower species formed the first three treatments, and these were compared to a control treatment in which no additional floral resources were sown. In each tunnel, four 3 x 6 m plots, separated by a distance of 28 m, were randomly assigned to one of the four treatments. Within each treated plot, plug plants of one of the three sown species were inserted along the central three rows of table-top strawberries so that every third plant pot contained the sown flower species. This pattern resulted in a planting density of one sown flower container per metre along the row and 18 additional flower containers per plot. Rather than removing or replacing pots of strawberries, sown flower pots were placed in gaps between pots of strawberries. Each pot was drip-irrigated on the same line as the strawberries. Coriander and mint seedlings were planted on 8-11 April, and forget-me-not plants were potted on 10-11 May. In the

control treatment, empty flower pots were introduced at the same density as sown flower pots to account for bias arising from the presence of additional pots between strawberry plants.

All plots were surveyed six times during the flowering period of the 'Jubilee' strawberries: May – August 2016. During each round of surveys, tunnels were visited between 09:00 and 17:00 in a random sequence and the plots within each tunnel were then surveyed in random order to mitigate bias due to time of day or day of the year (Rotheray and Gilbert 2011). A seventh round of surveys was carried out in the last two weeks of August during which only field mint and control plots were visited, as coriander and forget-me-not plants had ceased flowering.

During each survey, aphid-infested strawberry plants within six metres of each plot were hand-searched for hoverfly larvae for five minutes (Hogg et al. 2011). All sides of leaves, stems and flowers were thoroughly examined, and hoverfly larvae were collected in 1.5 ml Eppendorf tubes, labelled with the plot and tunnel number and date. All specimens were then stored at -80°C prior to dissection.

Dissection of hoverfly larvae and DNA extraction

Hoverfly larvae were dissected on sterile petri dishes under a dissecting microscope. A scalpel and forceps were used to slice open the larvae immediately behind the mouthparts. The gut contents were then squeezed out of the larval integument, or outer skin, by holding onto the posterior end of the larvae with forceps and applying pressure with a scalpel starting at the posterior end and moving forwards to the head. The extracted contents of each larva were then collected into a new 1.5 ml Eppendorf tube, labelled with the same original identifying information. Forceps and scalpel were sterilised between dissections by first passing the utensils through an open flame and then washing them in ethanol. A new petri dish was used for each specimen to reduce the likelihood of cross-contamination of specimens.

Prior to DNA extraction using the DNeasy Tissue Kit (Qiagen, Hilden, Germany; protocol for animal tissues), larval gut contents were placed in a 1.2 ml tube with 180 µl of ATL buffer, 20 µl of

proteinase K and a metal ball bearing. A negative control sample containing all buffers but no larval

gut content was included to test for cross-contamination during the DNA extraction procedure.

Tubes were sealed tightly and placed in racks, which were shaken at 30 oscillations per second for 2

minutes in a tissue lyser (Qiagen Tissue Lyser II, Hilden, Germany). The lysed contents in solution

were then transferred to a new 1.5 ml Eppendorf tube labelled with the sample number, and the

DNeasy extraction protocol was resumed at the incubation step. Extracted DNA was eluted in 100 µl

AE buffer provided by the manufacturer and stored at -80°C in a new 1.5 ml Eppendorf tube, labelled

with the sample number and extraction date.

DNA sequencing

PCR and sequencing were performed by the Genome Centre, Queen Mary University of London.

In brief, amplification of a 157bp fragment of the mitochondrial cytochrome c oxidase subunit 1 was

performed using primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al. 2011) adapted to include Fluidigm

tags CS1 and CS2. Each 10 µL PCR reaction contained 5 µL of Qiagen multiplex PCR (Qiagen, CA)

master mix, 3 µL of water, 0.5 µL of each 10 µM primer and 1 µL of eluted DNA. PCR amplification

was as follows: 95°C for 15 min; 50 cycles of 95°C for 30 s; 52°C for 30 s; 72°C for 30 s and 72°C for 10

min. Amplicon QC was performed using a DNA D1000 TapeStation (Agilent Technologies) and

quantification was performed using a QuBit dsDNA HS Assay Kit (Invitrogen, Life Tecnologies).

Sequencing was performed bi-directionally with 10bp Fluidigm indexes following manufacturer's

protocols and sequencing was run on the MiSeqv2 Chemistry using a 2x150bp run with 300 cycle run

(Illumina).

Reads were merged in Mothur (Schloss et al. 2009) and then processed using the Galaxy

platform (Giardine et al. 2005; Goecks et al. 2010; Afgan et al. 2018). Primer sequences and adaptors

were removed using CLIP from the FASTX-toolkit (Assaf 2010) and all sequences that were longer or

shorter than the target amplicon length of 157bp were filtered out. Sequences were collapsed into

unique haplotypes using COLLAPSE from the FASTX-toolkit (Assaf 2010). All haplotypes were

screened using a BLAST search against a reference database of >600,000 sequences extracted from Genbank. Because initial results from Genbank returned species identifications that included taxa apart from aphidophagous hoverflies and aphids, a bespoke reference database was created using sequences belonging only to UK species of aphids and syrphine hoverfly, which were cherry-picked from the results of our initial screening of our sequences against the full BOLD sequence library. We parsed this output using custom python scripts and filtered out any matches lower than 98% identical to a sequence in the reference database. Only 98-100% matches were retained for the analysis because of the generally-accepted consensus that DNA sequence matches of 3% and above represent poor matches at the species level (Herbert et al. 2003).

Statistical analysis

All analyses were carried out in R version 3.4.1 (R Core Team 2017). Prey DNA detection rates were modelled using generalised linear mixed models (GLMMs) with a binomial distribution. GLMMs were fitted using the 'lme4' package (Bates et al. 2015). Fixed effects included plot position within the tunnel (1-4, from north to south), tunnel number (1-10 from west to east) and the interaction between survey round and treatment (coriander, forget-me-not, mint or control). The interaction between plot number and survey round was included as a random effect to assess the effect of any differences in trends within individual plots from one survey week to the next.

The optimal model was chosen by starting with a full model containing all possible fixed effects and running the 'drop1' function in R to identify and then remove the least significant fixed effect from the full model. This process was then repeated, continually removing the least significant explanatory variable, until only significant fixed effects remained. The significance of the random effect was tested by removing the random effect and comparing the optimal model against the corresponding GLM using the likelihood ratio test.

Results

A total of 149 hoverfly larvae were collected from all plots over the course of the field study. Of these, 145 (97.3%) returned sequences that matched at least one hoverfly species in the bespoke sequence library. Approximately half (72/145; 49.7%) of all larval specimens returned a 100% match to just one of the following hoverfly taxa: *Episyrphus balteatus* (35/72; 48.6%), *Eupeodes corollae* (22/72; 30.6%), *Platycheirus scutatus* (9/72; 12.5%), *Sphaerophoria philanthus/scripta* (4/72; 5.6%), *Eupeodes luniger* (2/72; 2.8%; Fig 5.1.). A further 18 specimens contained 100% matches to more than one hoverfly species (Table 5.1; Appendix 2). Finally, 55 samples contained only 98-99% matches to hoverfly species (Appendix 2). Of these, 27/55 (49.1%) returned a 98-99% match to a single species of hoverfly (Appendix 2).

Of the 145 samples containing hoverfly DNA, 55 (37.9%; bootstrapped 95% confidence interval: 29.7% - 45.5%) also contained DNA fragments that matched at least one aphid species (Table 5.1). Thus, approximately one in three hoverfly larva specimens tested positive for aphid DNA.

Table 5.1 Summary of hoverfly and prey DNA detection frequencies from hoverfly larva gut contents.

Category	Tally
Total number of hoverfly larvae collected	149
Specimens with hoverfly DNA detected	145
Number of hoverfly samples that could be identified to species	72
Specimens with hoverfly and aphid DNA detected	55
Number of hoverfly samples that contained multiple 100% matches to hoverfly species	18

Prey DNA detection rates by hoverfly species

Detection rates of aphid DNA fragments in hoverfly larva specimens did not differ between the two commonest hoverfly species, *Episyrphus balteatus* (9/26; 34.6%) and *Eupeodes corollae* (5/17; 29.4%; $\chi^2(1) = 1.22$, *P* = 0.27; Fig. 5.1). A similar ratio of *Sphaerophoria philanthus/scripta* specimens contained aphid DNA (1/4; 25%), but no prey DNA was detected in either *Platycheirus scutatus* (0/9) or *Eupeodes luniger* (0/2) specimens (Fig. 5.1).
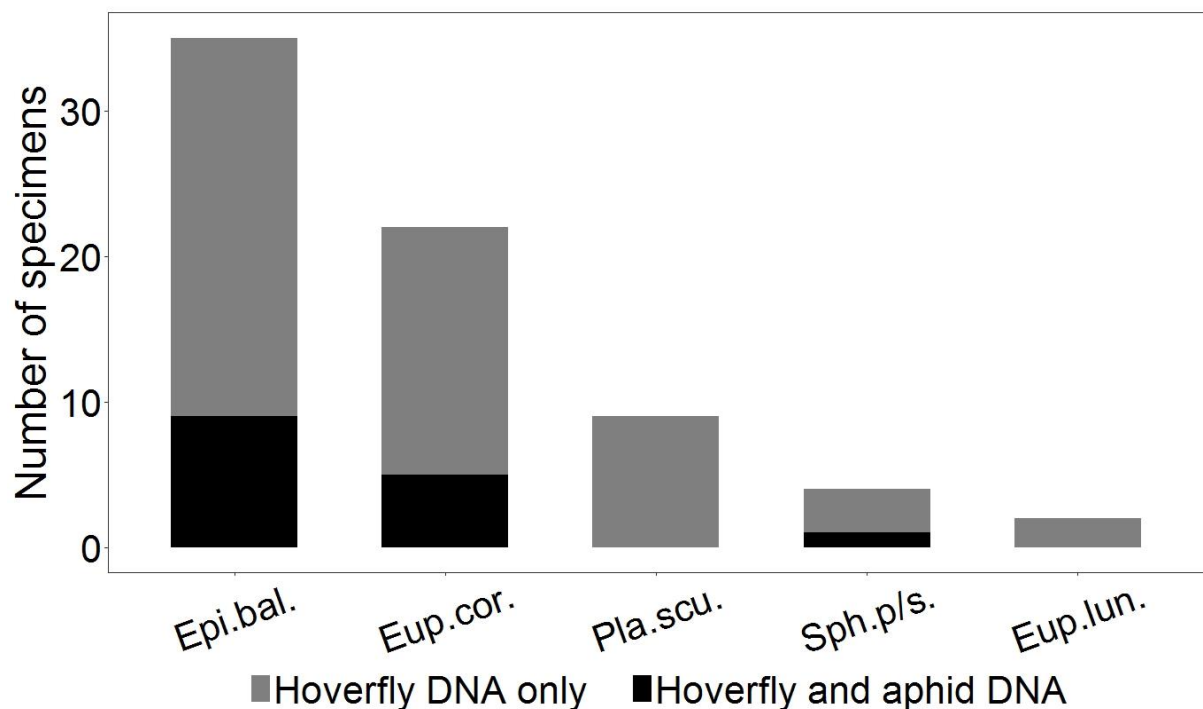
Figure 5.1 Prey DNA detection frequencies among samples with 100% matches for one hoverfly taxon. 'Epi.bal.' = *Episyrphus balteatus*; 'Eup.cor.' = *Eupeodes corollae*; 'Pla.scu.' = *Platycheirus scutatus*; 'Sph.p/s.' = *Sphaerophoria philanthus/scripta*; 'Eup.lun.' = *Eupeodes luniger*.

Aphid DNA species identifications

Out of 55 specimens that returned matches to aphid species in the reference database, only two specimens did not contain at least a 98% match to the aphids *Ericaphis scammelli* and *Wahlgreniella nervata*, two species which were inseparable using our barcoding protocol. The two hoverfly larva specimens containing other aphid species included single 100% matches to *Aphis gossypii* and *Myzus persicae* (Appendix 2).

Prey DNA detection rates by sown flower treatment

The presence of additional sown floral resources had a significant impact on aphid DNA detection rates in hoverfly larval gut contents (χ^2(3) = 12.79, *P* = 0.0051). Over half of hoverfly specimens (17/30; 56.67%) collected within 6 metres of forget-me-not plots contained aphid DNA, compared to under 10% of specimens (2/22; 9.09%) collected near coriander plots (Z = -3.11, *P* = 0.010; Fig. 5.2). Prey DNA detection rates in specimens from mint (15/38; 39.47%) and control plots (21/55; 38.18%) were intermediate between rates from coriander and forget-me-not plots (Fig. 5.2).
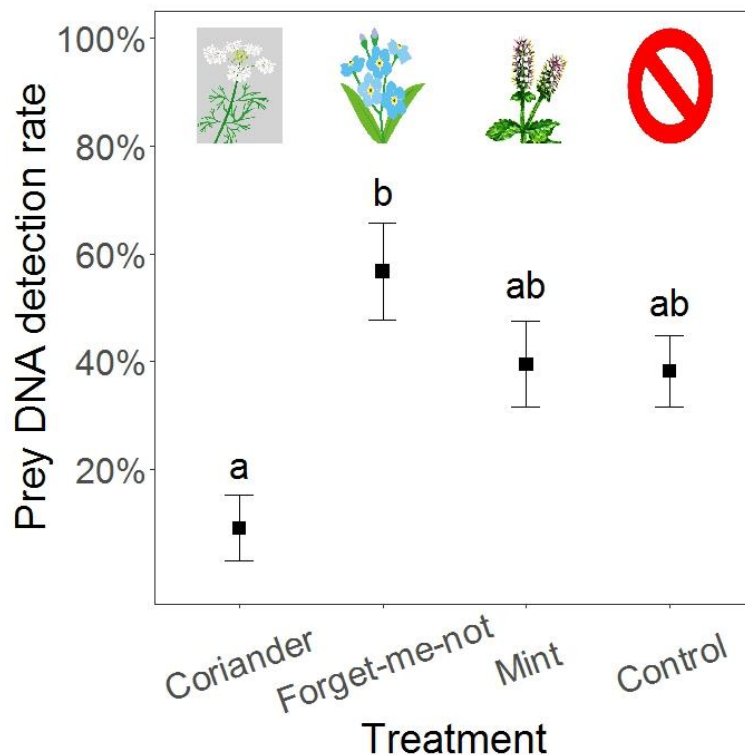
Figure 5.2 Mean detection rates of aphid prey DNA recovered from hoverfly larvae collected in each of the four treatments. Points represent least-square means from the binomial GLMM ± standard error. Means sharing the same letter do not differ significantly (α = 0.05).

Discussion

Using high-throughput sequencing technology, we demonstrated that aphid DNA was present in approximately one in three hoverfly larvae recovered from strawberry plants. Aphid DNA detection rates did not differ between the two commonest species of hoverfly: *Episyrphus balteatus* and

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Eupeodes corollae. Lastly, aphid DNA detection rates were significantly higher in larvae collected near forget-me-not plots as compared to larvae from coriander plots.

The aphid DNA detection rate in our study (55/145 hoverfly specimens; 37.9%) was almost identical to the 36% prey DNA detection rate documented among *Episyrphus balteatus* larvae reared in Mediterranean lettuce crops (Gomez-Polo et al. 2015). While this detection rate indicates that hoverfly larvae were consuming aphid prey within the strawberry crop, it is likely to be a conservative estimate. Although it is most parsimonious to take this estimate at face-value, in fact the 62.1% of hoverfly specimens which did not return segments of aphid DNA may represent cases where aphid DNA had become degraded through digestion so that no 157-bp fragments were detected in the sequencing process (Symondson 2002). Additionally, a proportion of the older, third-instar larvae may have stopped feeding prior to pupating at the time of collection (Rotheray 1993). Finally, as we cannot detect false negative rates and our taxonomic assignment method (>98% match) is likely conservative, we suspect this is an underestimate of the impact of predation on aphids.

In a review of invertebrate gut contents studies, Symondson (2002) documented that prey DNA detection rates were often below 100%, even in predators that had been fed prey less than 24 hours prior to the gut contents analysis. As hoverfly larvae are primarily active at night (Rotheray and Gilbert 2011), the fact that specimens were collected during the day may have meant that for many samples prey DNA had already become undetectable in the guts of the hoverfly larvae. Moreover, previous researchers have documented an array of other potential sources of variation in the detectability of prey DNA including temperature, mass of predator, quantity of prey consumed, number of DNA sequences present in aphid prey, and the preservation of the sample (Weber and Lundgren 2009).

Previous research has shown that prey DNA detection times can vary considerably according to predator species (Symondson 2002). However, in the case of the most abundant hoverfly species in the present study, *Episyrphus balteatus* and *Eupeodes corollae*, aphid DNA detection rates were

similar. The fact that these two species shared similar prey DNA recovery rates may indicate that their aphid consumption rates are also comparable, though further work is required to confirm this hypothesis. Three other hoverfly species were also identified from the larval specimens, but for these species sample sizes were too small to include in the analysis. The observed frequencies of larval hoverfly species corresponded closely to relative frequencies of adult hoverfly species recorded during flower visitor surveys conducted at the same study site (Hodgkiss et al. 2019). Moreover, all larval hoverflies found in the strawberry crop belonged to common, widely-distributed UK species (Ball and Morris 2015).

Aphid DNA detection rates varied according to the provision of additional floral resources. Hoverfly larvae collected on strawberry plants near forget-me-not plots were more likely to contain aphid DNA fragments than larvae found near plots intercropped with coriander. Prey DNA detection rates in mint and control plots did not differ significantly from either coriander or forget-me-not plots. Significantly more lacewing eggs were recorded on strawberry plants near coriander plots (Hodgkiss et al. 2019). There is potential for additional aphid predators to reduce numbers of aphids on strawberry plants near coriander plots, thereby reducing the available numbers of aphids present for hoverfly larvae to consume in these plots.

The fact that 18 samples contained multiple 100% matches to hoverfly species could indicate that intraguild predation occurred in these instances, as was assumed in the same circumstances among larvae collected in lettuce crops (Appendix 2; Gomez-Polo et al. 2015). An alternative explanation is these examples represent instances of contamination rather than intraguild predation. That said, dissection equipment was sterilised after each use to minimise the likelihood of contamination. Furthermore, a negative control sample containing only DNA extraction reagents was tested to check for contamination. Therefore, while still a possibility, contamination appears unlikely. Nonetheless, the true explanation for these results cannot be reliably determined from our experimental design. An alternative explanation might be shared barcodes in some hoverfly species. While the sequences used in our reference set clearly differentiated the species, the available

number of references was minimal and additional sampling of the hoverflies could yield barcode convergence. This cannot be ruled out except with the expansion of reference collections.

Comparing prey DNA against the reference database revealed that 96% of specimens with aphid DNA contained at least a 98% match to either *Ericaphis scammelli* or *Wahlgreniella nervata*, two species which could not be separated using the target region of COI (the regions are identical). These species are not known to use commercial strawberry as a host plant, preferring blueberry and rose or ericaceous plants, respectively (Blackman 2010). DNA sequences from aphid species that are commonly known to feed on commercial strawberry were present in the reference database. Thus if these matches represent misidentifications, then these sequences may belong to additional aphid species that are uncommon pests of strawberry and whose sequences were not present in the BOLD sequence library. This also assumes that the reference collection is correctly identified. Since it has been drawn from a public database the possibility of a misidentification contaminating the reference set remains. Individual specimens with DNA matching two common strawberry-feeding aphids, *Aphis gossypii* and *Myzus persicae*, were also recorded. Furthermore, although aphid colonies were not systematically identified to species in the field, we know that at least 20% of colonies were *Macrosiphum euphorbiae*, a species which was not detected in the digestive tracts of hoverfly larvae. Thus our results may indicate that hoverfly larvae avoid predating *M. euphorbiae*, but further research is required to test this hypothesis.

This study provides new evidence to suggest that hoverflies may be capable of providing strawberry growers with aphid pest control services. Prior research has already indicated that adult hoverflies may play an important role as strawberry pollinators (Albano et al. 2009; Hodgkiss et al. 2018); therefore, hoverflies with aphidophagous larvae could provide strawberry growers with both pollination and pest control ecosystem services. Aphid DNA was found in more than one in three hoverfly larvae. Moreover, the two commonest hoverfly species, *Episyrphus balteatus* and *Eupeodes corolla*e, did not differ in their prey DNA detection rates, which suggests that these two hoverfly species may be equally voracious aphid predators in this strawberry crop. Further research in other

strawberry fields is necessary to determine whether these results are site-specific or whether they hold true across multiple sites in the UK and Europe.

Future work could explore dietary breadth of hoverfly larvae found in strawberry crops by developing primers to identify individual species of aphid using PCR-based methods (Weber and Lundgren 2009). Such information would go some way to providing strawberry growers with useful information on which species of aphid are predated by hoverfly larvae. Then, by determining the dietary breadth of different hoverfly species, those that consume the widest array of aphid species could be targeted as particularly important biological control agents. Within-crop habitat manipulations could then be developed to entice the most effective aphid predators into strawberry rows, thereby reducing the need for chemical pesticides.

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Appendix 2 – Table of species identifications from DNA barcoding

Table 1. Hoverfly and aphid species identifications for each hoverfly larva specimen. Identifications highlighted in bold matched reference sequences 100%. Remaining identifications represent 98-99% matches to reference sequences. Treatment abbreviations are as follows: "C" = coriander; "F" = field forget-me-not; "M" = field mint; "X" = control. Hoverfly species abbrevations are as follows: "Epi.bal." = *Episyrphus balteatus*; "Eup.cor." = *Eupeodes corollae*; "Eup.lat." = *Eupeodes latifasciatus*; "Eup.lun." = *Eupeodes luniger*; "Mel.aur." = *Meliscaeva auricollis*; "Pla.scu." = *Platycheirus scutatus*; "Sph.p/s." = *Sphaerophoria philanthus/scripta*; "Sph.tae." = *Sphaerophoria taeniata*. Aphid species abbreviations are as follows: "Aph.gos." = *Aphis gossypii*; "Eri.s./Wah.n." = *Ericaphis scammelli/Wahlgreniella nervata*; "Myz.per." = *Myzus persicae*; "Wah.vac." = *Wahlgreniella vaccinii*.

No.	Date collected	Trt	Hoverfly identifications					Aphid identifications	
1	16/05/2016	X	**Epi.bal.**	Eup.cor.				-	
2	16/05/2016	X	Epi.bal.	Eup.cor.				-	
3	17/05/2016	X	Eup.cor.					-	
4	17/05/2016	X	Epi.bal.	Eup.cor.	Sph.p/s.	Sph.tae.		-	
5	17/05/2016	X	**Epi.bal.**	**Eup.cor.**				-	
6	17/05/2016	X	Eup.cor.					Eri.s./Wah.n.	Wah.vac.
7	17/05/2016	X	**Eup.lun.**	**Sph.p/s.**	Sph.tae.	Eup.lat.		Eri.s./Wah.n.	Wah.vac.
8	17/05/2016	X	Epi.bal.					Eri.s./Wah.n.	Wah.vac.
9	17/05/2016	X	**Epi.bal.**					Eri.s./Wah.n.	Wah.vac.
10	17/05/2016	X	**Epi.bal.**	**Eup.cor.**	Pla.scu.			**Aph.gos.**	
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14	20/05/2016	C	Eup.cor.					-	
15	20/05/2016	F	Epi.bal.					-	
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18	20/05/2016	X	**Eup.cor.**	Sph.p/s.				-	
19	20/05/2016	C	**Pla.scu.**	Eup.cor.	Epi.bal.			-	
20	20/05/2016	F	**Eup.cor.**					**Eri.s./Wah.n.**	Wah.vac.
21	20/05/2016	C	**Eup.cor.**	Epi.bal.	Pla.scu.			-	
22	20/05/2016	F	**Eup.cor.**	Epi.bal.	Pla.scu.	Sph.p/s.	Sph.tae.	-	
23	20/05/2016	X	**Eup.cor.**	Sph.p/s.	Sph.tae.			-	
24	20/05/2016	F	**Eup.cor.**	**Pla.scu.**	**Sph.p/s.**	Sph.tae.	Epi.bal.	Eri.s./Wah.n.	Wah.vac.
25	20/05/2016	C	Eup.cor.					-	
26	20/05/2016	M	**Epi.bal.**	**Eup.cor.**				-	
27	20/05/2016	C	Epi.bal.	Eup.cor.	Eup.lun.	Sph.p/s.	Sph.tae.	-	
28	20/05/2016	C	**Eup.cor.**					-	
29	23/05/2016	X	**Pla.scu.**	Eup.cor.	Epi.bal.			-	
30	23/05/2016	M	**Sph.p/s.**	Sph.tae.	Epi.bal.	Eup.cor.	Eup.lun.	-	
31	23/05/2016	X	**Epi.bal.**	**Pla.scu.**	Eup.cor.			-	

No.	Date								
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34	24/05/2016	M	**Epi.bal.**	Eup.cor.				**Eri.s./Wah.n.**	Wah.vac.
35	24/05/2016	M	**Epi.bal.**					**Eri.s./Wah.n.**	Wah.vac.
36	24/05/2016	C	Eup.cor.	Eup.lun.				-	
37	24/05/2016	M	**Epi.bal.**					Eri.s./Wah.n.	Wah.vac.
38	24/05/2016	M	**Eup.cor.**					**Eri.s./Wah.n.**	Wah.vac.
39	24/05/2016	M	**Epi.bal.**					**Eri.s./Wah.n.**	Wah.vac.
40	24/05/2016	M	**Eup.cor.**	**Sph.p/s.**	Sph.tae.	Eup.lun.		-	
41	24/05/2016	M	**Eup.cor.**	Sph.p/s.	Sph.tae.	Pla.scu.		-	
42	24/05/2016	M	**Epi.bal.**	Eup.cor.				**Eri.s./Wah.n.**	Wah.vac.
43	24/05/2016	M	Eup.cor.					-	
44	24/05/2016	C	**Sph.p/s.**	Sph.tae.	Eup.cor.			-	
45	24/05/2016	X	**Eup.cor.**	**Pla.scu.**	Epi.bal.			-	
46	24/05/2016	M	Eup.cor.					-	
47	24/05/2016	M	Epi.bal.	Eup.cor.	Pla.scu.			-	
48	24/05/2016	M	**Eup.cor.**					-	
49	26/05/2016	X	**Eup.cor.**	Epi.bal.				-	
50	26/05/2016	X	Eup.cor.					-	
51	26/05/2016	M	**Eup.cor.**					-	
52	26/05/2016	X	**Eup.cor.**					-	
53	26/05/2016	F	Eup.cor.	Eup.lun.	Pla.scu.			Eri.s./Wah.n.	Wah.vac.
54	26/05/2016	M	**Epi.bal.**					-	
55	26/05/2016	M	Eup.cor.	Eup.lun.	Pla.scu.			-	
56	26/05/2016	X	Eup.cor.					Eri.s./Wah.n.	Wah.vac.
57	26/05/2016	M	**Eup.cor.**	Epi.bal.				-	
58	26/05/2016	X	**Eup.cor.**	Epi.bal.	Pla.scu.	Eup.lun.		-	
59	26/05/2016	X	Eup.cor.					Eri.s./Wah.n.	Wah.vac.
60	26/05/2016	F	Epi.bal.	Eup.cor.	Eup.lun.			-	
61	26/05/2016	X	Epi.bal.	Eup.cor.				Eri.s./Wah.n.	Wah.vac.
62	26/05/2016	X	**Epi.bal.**	Eup.cor.	Sph.p/s.			-	
63	26/05/2016	X	**Epi.bal.**	**Eup.cor.**				**Eri.s./Wah.n.**	Wah.vac.
64	26/05/2016	X	Eup.cor.	Eup.lun.				-	
65	26/05/2016	X	Eup.cor.					-	
66	26/05/2016	X	**Eup.lun.**	Eup.lat.				-	
67	26/05/2016	X	Epi.bal.	Eup.cor.				Eri.s./Wah.n.	Wah.vac.
68	02/06/2016	F	**Eup.cor.**					-	
69	02/06/2016	X	**Eup.cor.**	Pla.scu.				**Eri.s./Wah.n.**	Wah.vac.
70	02/06/2016	X	**Epi.bal.**	**Sph.p/s.**	Sph.tae.			-	
71	03/06/2016	X	**Eup.cor.**	Pla.scu.				-	
72	03/06/2016	X	Eup.cor.					-	
73	03/06/2016	X	Eup.cor.	Eup.lun.				Eri.s./Wah.n.	Wah.vac.
74	03/06/2016	M	**Pla.scu.**	Epi.bal.				Eri.s./Wah.n.	
75	06/06/2016	X	Epi.bal.	Pla.scu.				Eri.s./Wah.n.	Wah.vac.
76	07/06/2016	M	-					-	
77	07/06/2016	C	**Pla.scu.**					-	

78	09/06/2016	M	Epi.bal.				Eri.s./Wah.n.	Wah.vac.
79	09/06/2016	F	**Epi.bal.**				-	
80	09/06/2016	M	Eup.cor.	Eup.lun.			-	
81	09/06/2016	F	**Epi.bal.**	**Sph.p/s.**	Sph.tae.		**Eri.s./Wah.n.**	Wah.vac.
82	09/06/2016	X	Eup.cor.	Pla.scu.			Eri.s./Wah.n.	Wah.vac.
83	09/06/2016	M	Eup.cor.	Sph.p/s.	Sph.tae.		Eri.s./Wah.n.	Wah.vac.
84	09/06/2016	F	Epi.bal.	Pla.scu.			Eri.s./Wah.n.	Wah.vac.
85	09/06/2016	F	Epi.bal.	Eup.cor.			Eri.s./Wah.n.	Wah.vac.
86	09/06/2016	M	Epi.bal.	Eup.cor.	Eup.lun.		-	
87	09/06/2016	F	**Epi.bal.**				**Eri.s./Wah.n.**	Wah.vac.
88	09/06/2016	M	**Epi.bal.**	Pla.scu.			Eri.s./Wah.n.	Wah.vac.
89	13/06/2016	M	**Epi.bal.**				-	
90	14/06/2016	F	**Eup.lun.**	Eup.lat.	Epi.bal.		Eri.s./Wah.n.	Wah.vac.
91	14/06/2016	F	Epi.bal.	Eup.cor.	Eup.lun.		-	
92	14/06/2016	F	**Eup.cor.**	**Pla.scu.**			**Eri.s./Wah.n.**	Wah.vac.
93	14/06/2016	F	Epi.bal.				-	
94	15/06/2016	M	**Eup.cor.**	Sph.p/s.	Sph.tae.		**Eri.s./Wah.n.**	Wah.vac.
95	15/06/2016	M	Epi.bal.				Eri.s./Wah.n.	Wah.vac.
96	15/06/2016	X	Eup.cor.	Eup.lun.			-	
97	16/06/2016	F	**Epi.bal.**				**Myz.per.**	
98	16/06/2016	F	**Epi.bal.**				**Eri.s./Wah.n.**	Wah.vac.
99	17/06/2016	X	Epi.bal.	Eup.cor.	Sph.p/s.	Sph.tae.	-	
100	17/06/2016	F	**Epi.bal.**	**Pla.scu.**	Eup.cor.	Eup.lun.	Eri.s./Wah.n.	Wah.vac.
101	17/06/2016	F	Eup.cor.				Eri.s./Wah.n.	Wah.vac.
102	17/06/2016	C	**Pla.scu.**	Eup.cor.	Eup.lun.		-	
103	17/06/2016	F	**Epi.bal.**	Pla.scu.			-	
104	17/06/2016	F	**Epi.bal.**	Eup.cor.			Eri.s./Wah.n.	Wah.vac.
105	17/06/2016	X	**Eup.cor.**	Eup.lun.			Eri.s./Wah.n.	Wah.vac.
106	22/06/2016	X	**Epi.bal.**				-	
107	22/06/2016	M	-				-	
108	22/06/2016	X	**Epi.bal.**				-	
109	22/06/2016	M	-				-	
110	22/06/2016	M	**Pla.scu.**	Epi.bal.			-	
111	23/06/2016	M	Epi.bal.				-	
112	23/06/2016	C	Epi.bal.				Eri.s./Wah.n.	Wah.vac.
113	23/06/2016	C	Epi.bal.	Eup.cor.	Pla.scu.		-	
114	23/06/2016	M	**Epi.bal.**	**Sph.p/s.**	Sph.tae.		**Eri.s./Wah.n.**	Wah.vac.
115	23/06/2016	F	**Epi.bal.**				**Eri.s./Wah.n.**	Wah.vac.
116	23/06/2016	M	**Epi.bal.**	Eup.cor.			-	
117	29/06/2016	X	**Epi.bal.**				-	
118	29/06/2016	C	**Epi.bal.**				-	
119	29/06/2016	F	Epi.bal.	Eup.cor.			-	
120	29/06/2016	X	Epi.bal.	Pla.scu.			-	
121	29/06/2016	M	**Epi.bal.**				-	
122	29/06/2016	C	**Epi.bal.**	Sph.p/s.	Sph.tae.		Eri.s./Wah.n.	Wah.vac.
123	30/06/2016	M	**Eup.cor.**	**Mel.aur.**			-	

124	30/06/2016	F	**Epi.bal.**	Eup.cor.	Sph.p/s.	Sph.tae.			Eri.s./Wah.n.	Wah.vac.	
125	01/07/2016	F	**Pla.scu.**	Eup.cor.					-		
126	01/07/2016	C	**Pla.scu.**						-		
127	01/07/2016	X	**Epi.bal.**	**Pla.scu.**	Sph.p/s.	Sph.tae.	Pla.scu.		Eri.s./Wah.n.		
128	04/07/2016	X	**Epi.bal.**	**Eup.cor.**					-		
129	04/07/2016	C	**Sph.p/s.**	**Sph.phi.**	Sph.tae.				-		
130	06/07/2016	M	**Epi.bal.**	Sph.p/s.	Sph.tae.				Eri.s./Wah.n.	Wah.vac.	
131	07/07/2016	X	Epi.bal.						Eri.s./Wah.n.	Wah.vac.	
132	07/07/2016	X	Epi.bal.						Eri.s./Wah.n.	Wah.vac.	
133	08/07/2016	F	**Epi.bal.**	**Sph.p/s.**	Sph.tae.				-		
134	11/07/2016	X	**Epi.bal.**						-		
135	19/07/2016	M	**Epi.bal.**	Eup.cor.					-		
136	19/07/2016	M	**Epi.bal.**	Sph.p/s.	Sph.tae.				-		
137	19/07/2016	F	Epi.bal.						-		
138	19/07/2016	C	Sph.p/s.	Sph.tae.					-		
139	20/07/2016	F	Epi.bal.						-		
140	20/07/2016	C	**Pla.scu.**						-		
141	20/07/2016	X	**Epi.bal.**	Pla.scu.					**Eri.s./Wah.n.**	Wah.vac.	
142	20/07/2016	X	Pla.scu.						-		
143	20/07/2016	M	-						-		
144	21/07/2016	X	Epi.bal.						-		
145	21/07/2016	C	**Epi.bal.**						-		
146	26/07/2016	X	**Epi.bal.**						-		
147	23/08/2016	X	**Epi.bal.**	Eup.cor.	Sph.p/s.	Sph.tae.			-		
148	25/08/2016	X	**Eup.cor.**	Epi.bal.	Eup.lun.				-		
149	25/08/2016	M	Epi.bal.	Eup.cor.					Eri.s./Wah.n.	Wah.vac.	Myz.per.

Chapter 6: General Discussion

Declaration of Authorship:

I, Dylan Hodgkiss, hereby declare that this thesis chapter and the work presented herein are entirely my own. Where I have consulted the work of others, this is clearly stated.

Signed: _____

Date: _____

The aim of this thesis was to investigate the importance of hoverflies (Diptera: Syrphidae) in commercial strawberry. The project investigated the contribution that hoverflies make to strawberry pollination and attempted to investigate the importance of aphidophagous hoverfly larvae for aphid biocontrol. At the onset of the study there was very little published evidence on the role of hoverflies in delivering these two important ecosystem services in modern commercial strawberry fields. Nonetheless, there was evidence to suggest that hoverflies, especially *Eristalis* spp., may be effective strawberry pollinators (Albano et al. 2009). In addition, aphidophagous hoverflies had been identified as the most abundant aphid predator in organic strawberry fields in Turkey (Kovanci et al. 2007). Thus hoverflies appear to have the potential to provide both pollination and aphid biocontrol services. In this thesis, the following hypotheses were proposed:

- Strawberry flowers are visited by a range of insects, amongst which a variety of hoverfly species is included. The abundance, species richness and diversity differ between commercial strawberry plantations.

- Aphidophagous hoverflies are crucial to strawberry production because visits to flowers improve pollen transfer and resultant yield and fruit quality. In addition, pollination efficacy can vary according to the species of hoverfly.

- The provision of additional floral resources within commercial strawberry crops can improve fruit quality and reduce the incidence of aphids by promoting hoverfly abundance.

- It is possible to determine the predation rate of aphids by hoverflies in crops through the detection of aphid genetic material in the digestive tract of hoverfly larvae.

In Chapter 2, field surveys of strawberry flower-visiting insects at eight fruit farms in the southeast of Britain revealed management and environmental factors that impacted flower visitor assemblages. The effectiveness of aphidophagous hoverflies at pollinating strawberry was tested in small cages in multiple replicated experiments (Chapter 3). Then, a field experiment at a commercial fruit farm addressed the possibility of introducing additional floral resources into strawberry rows in

an effort to improve pollination (through insect visitation frequency) and aphid biocontrol services (Chapter 4). Lastly, hoverfly larvae were collected at the same commercial strawberry farm to determine whether aphid DNA could be detected in the digestive tracts and whether prey DNA detection rates differed according to the provision of additional floral resources (Chapter 5). In this final chapter key findings from preceding chapters are summarised and directions for future research are explored, as well as management recommendations based on the results of these experiments and those published in the literature.

Key findings

Abundance and diversity of strawberry flower visitors

Strawberry growers are reliant on insect pollinators for approximately 45% of annual strawberry yields (Smith et al. 2011). Previous research established that different types of flower-visiting insect vary in their behaviours on the receptacle of the strawberry flower, thereby pollinating different parts of the flower head (Chagnon et al. 1993). Therefore, maintaining diverse assemblages of flower visitors is key to the sustained provision of pollination ecosystem services.

Surveys of eight fruit farms revealed that assemblages of flower visitors in strawberry fields varied from site to site and from week to week, with bumblebees, most likely from managed colonies, predominating early on in the flowering period, and hoverflies and honeybees becoming the most abundant functional groups in June and July (Chapters 2 and 4). These results corroborate previous work, which also highlighted seasonal changes in the relative importance of strawberry flower visitor taxa across a single growing season (Albano et al. 2009; Gibson 2012; Ellis et al. 2017). Across all farms, the most common hoverfly species recorded visiting strawberry flowers were *Episyrphus balteatus*, and hoverflies in the genera *Eupeodes*, *Sphaerophoria*, *Eristalis* and *Platycheirus*. Of these, all but the *Eristalis* spp. have aphidophagous larvae (Ball and Morris 2015).

Flower visitor species compositions varied by both growing method (covered vs. open) and farm management type (retail vs. PYO), with contrasting sites having more dissimilar flower visitor assemblages than sites belonging to the same category. To put these results into context, an estimated 85-90% of UK strawberry fields are grown under polytunnels (Lovelidge 2012). Moreover, approximately 8% of the UK land area devoted to strawberry production is grown for PYO, compared to 90% grown for retail (Garthwaite et al. 2016). Given that different growing methods appear to rely on dissimilar groups of pollinators, these findings support the notion that diverse flower visitor assemblages are required to ensure that optimal pollination services are delivered to all site types.

Though abundance and species richness of flower visitors were not affected by the presence of protective coverings over the crops (polytunnels), species diversity was higher at sites which did not have plastic coverings (open), suggesting that polytunnels may limit the range of species able to reach the strawberry crops at covered sites. As 85-90% of UK strawberry crops are grown under tunnels, the reduced insect diversity, which is potentially due to the use of polytunnels, could leave the vast majority of strawberry growers at risk should key pollinator species become scarce in the future. That said, most open sites grew 'Malwina' strawberries and sites with polytunnels grew 'Elsanta'. Hence it cannot be ruled out that the differences in species assemblages of flower visitors may be the result of relative attractiveness of the two strawberry cultivars (Chapter 2). Since the studies outlined here, the main June-bearer and everbearer strawberry varieties grown for retail have changed again. Further research is necessary to determine whether strawberry variety or the use of polytunnels plays a more significant role in shaping the diversity of flower visitors at different sites.

When breeding strawberry varieties little thought is given to the attractiveness (floral scent or nectar and pollen) or accessibility of flowers to insect pollinators and this could be an area for future consideration. For instance there is a move towards breeding strawberries for robotic picking because of labour shortages (De Preter et al. 2018). These newer varieties have longer trusses which

are more freely presented to insect flower visitors. It is not known if this improved accessibility to insect pollinators has an impact on resultant fruit quality.

Pollination effectiveness of aphidophagous hoverflies

Previous research reported that hoverflies, particularly honeybee-mimicking drone flies with saprophagous larvae (*Eristalis* spp.), are effective pollinators of commercial strawberry (Nye and Anderson 1974; Albano et al. 2009; Gibson 2012). However, this study was the first to demonstrate that aphidophagous hoverflies are also effective strawberry pollinators. Fruit from flowers visited by syrphine hoverflies had almost twice as many fertilised achenes as fruit from flowers excluded from insect visitation. Moreover, only 29% of strawberries in insect-excluded cages developed into marketable fruit, compared to 59% of berries in cages provisioned with hoverflies. Fruit yields were also enhanced by 73% in cages with mixed assemblages of aphidophagous hoverflies when compared to insect-excluded cages (Chapter 3). Previous research found that insect pollination accounts for approximately 45% of strawberry yields (Smith et al. 2011). My results broadly corroborate this view, as the proportion of strawberry yields due to hoverfly pollination in Chapter 3 were between 42-53%. These values equate to a £105 - £132.5 million contribution to the UK economy per year from pollination services alone.

Cage trials further revealed that different species of syrphine hoverfly varied in their pollination efficiency. While both *Episyrphus balteatus* and *Eupeodes latifasciatus* significantly improved yields of strawberries compared to insect-excluded controls, *E. latifasciatus* enhanced pollination success and proportions of marketable fruit, whereas fruit visited by *E. balteatus* did not achieve higher rates of pollination success than insect-excluded controls. Therefore, the identity of flower visitors played an important role in determining fruit quality and marketability (Chapter 3).

Consequently, aphidophagous hoverflies are frequent visitors to strawberry flowers in commercial strawberry crops and effectively pollinate strawberry flowers (Chapters 2, 3 and 4).

These findings lend further support to the growing consensus in the scientific community that bees are not the only insects capable of providing economically-important pollination services (Ssymank et al. 2008; Rader et al. 2011, 2016; Orford et al. 2015). Moreover, diverse pollinator assemblages provide insurance against natural fluctuations in populations of individual pollinator taxa (Ollerton 2017). By gaining a broader understanding of the full suite of insects that effectively pollinate important crop species, growers become better equipped to safeguard the future delivery of crop pollination services. Future research should continue to examine the roles that less-studied flower-visiting insects play in delivering pollination services, including other families of Diptera and Coleoptera, two groups commonly observed visiting strawberry flowers in the field (Fig. 6.1; Chapters 2 and 4).

Figure 6.1 Strawberry flower visitors. Clockwise from top left: *Bombus terrestris/lucorum*; *Episyrphus balteatus*; *Cantharis rustica*; *Coccinella septempunctata*; *Eupeodes corollae*; *Apis mellifera*.

Additional floral resources to boost pollination and aphid biocontrol

Intercropping coriander, field forget-me-not and field mint plants within strawberry rows generated mixed results. Additional floral resources boosted the abundance of flower-visiting insects within the crop, but this observed increased abundance did not translate to improved fruit quality; neither strawberry fruit quality nor pollination success was improved by the intervention (Fig. 6.2; Chapter 4). The high rates of pollination success across all treatments could indicate that pollination services were already adequately provided by wild pollinators at this site. Alternatively, the additional floral resources may have boosted abundances of flower visitors throughout the crop so that even control plots benefited from increased flower visitation thanks to spill-over from adjacent plots with added floral resources. Previous research in arable crops suggests that additional floral resources can boost flower visitor numbers as far as 100 m away from sown pollen and nectar

sources (Campbell et al. 2017). Therefore, future studies should aim to separate experimental plots by distances greater than 100 m.

Field Forget-me-not

Coriander

Control

Field Mint
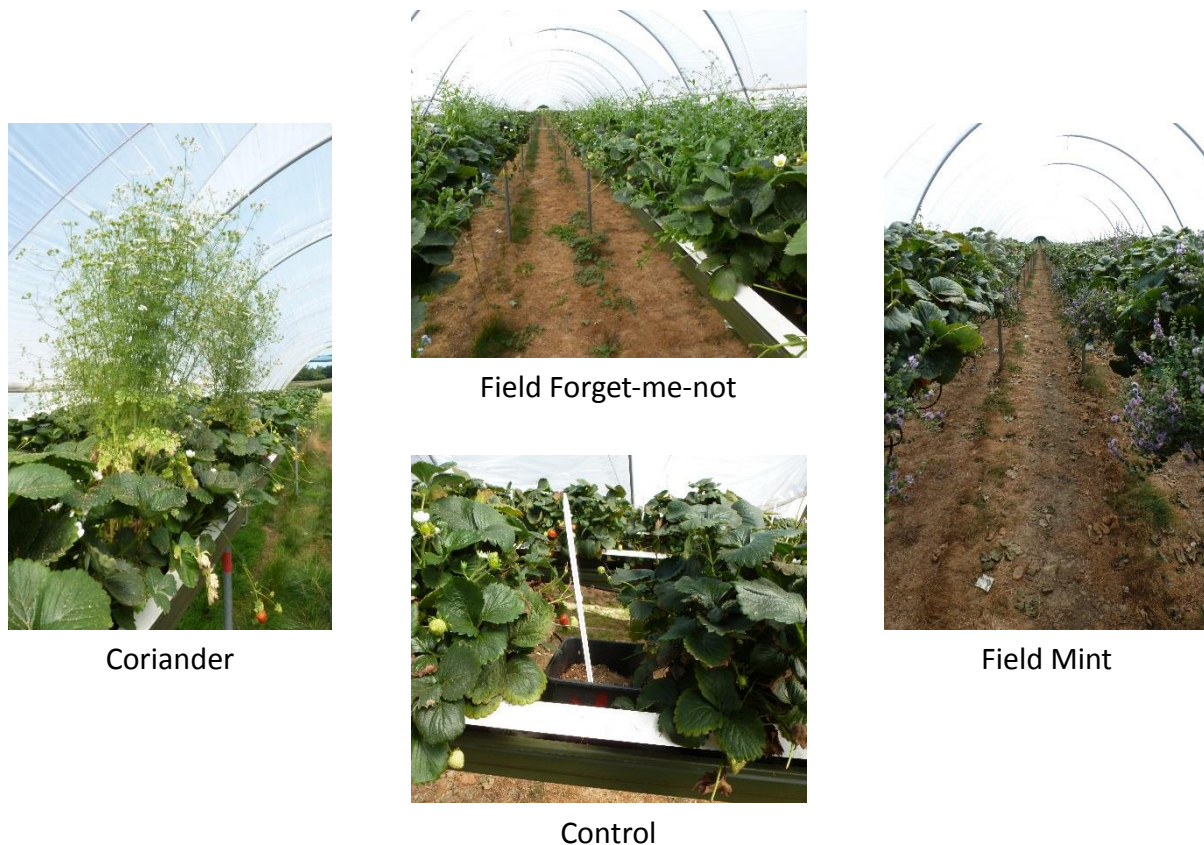
Figure 6.2 Intercropped wildflowers and untreated controls.

Among the three species of wildflowers tested in this trial, only field forget-me-not does not produce the volatile compound linalool (Gracindo et al. 2006; Shahwar et al. 2012; Znajdek-Awiżeń et al. 2014). Thus, the presence of linalool in coriander and field mint may be part of the explanation as to why more flower visitors were attracted to plots provisioned with these two flower species. Moreover, Klatt et al. (2013) analysed commercial strawberry volatiles and found that, like field forget-me-not, linalool was not among the volatiles produced by strawberry flowers. Assuming linalool is a highly attractive volatile for flower visitors, the observed trend of sown flower species

attracting more flower visitors than strawberry flowers may again be partly explained by the presence of linalool in coriander and field mint but not in strawberry flowers.

However, linalool is just one of several volatile compounds that likely work in concert, along with visual cues, to attract flower visitors (Willmer 2011; Nordström et al. 2017). Furthermore, recent research suggests that the same visual and olfactory attractants that are effective in one environmental context may fail to attract insects in another geographical region (Nordström et al. 2017). In addition to attracting pollinators, volatile compounds have also been shown to attract natural enemies into crops (Klatt et al. 2013; Orre Gordon et al. 2013). Whether floral scent and/or visual cues played a role in attracting aphid natural enemies to strawberry plants that were intercropped with coriander could be the focus of future research. Thus, future work is required to determine the relative importance of linalool in attracting beneficial insects into crops.

A key finding in this study was that more aphidophagous lacewing (Neuroptera: Chrysopidae) eggs were recorded on strawberry plants in plots provisioned with interplantings of coriander than in any other treatment. However, aphid abundance remained unaffected by the presence of additional floral resources. Lacewing eggs appeared late in the growing season and thus were probably not present in the crop early enough to have an impact on aphid numbers. As was the case for flower visitors, plots in the field centre recorded as many aphid natural enemies as plots at the field edge (Chapter 4).

Although lacewings appeared too late to effectively suppress aphid outbreaks early on in the year, intercropping coriander to attract lacewings into strawberry fields may allow growers to reduce insecticide applications later on in the growing season. As increased demand for strawberries throughout the year has prolonged the growing season in the UK (Alford 2011), late-season aphid biocontrol using naturally-occurring lacewings could represent a cost-effective and sustainable method of controlling aphid infestations. Further research is required to confirm the effectiveness of lacewings as aphid biocontrol agents in strawberry.

Hoverfly larva gut contents analyses

Aphid DNA was recovered from the digestive tracts of 37% of hoverfly larva specimens collected from strawberry plants in a commercial field. This finding mirrored previous work which found prey DNA in 36% of *Episyrphus balteatus* hoverflies reared in Mediterranean lettuce crops (Gomez-Polo et al. 2015). In the present study significantly more samples with aphid DNA were found near plots with field forget-me-not compared to coriander plots. Prey DNA detection rates in field mint and control plots were intermediate and did not differ from either forget-me-not or coriander plots (Chapter 5).

The two commonest predatory hoverfly species from the study site in the southeast of England were *Episyrphus balteatus* and *Eupeodes corollae*. In contrast to previous research indicating that prey DNA detection rates vary between species (Symondson 2002), aphid DNA detection rates among samples of *E. balteatus* and *E. corollae* did not differ significantly (Chapter 5). These findings suggest that syrphine hoverflies not only provide pollination to commercial strawberry, but also a degree of aphid control. *Eupeodes* spp. hoverflies were the most effective pollinators of strawberry flowers and, along with *Episyrphus balteatus*, were the most frequently-encountered species of predatory larval hoverfly found in the strawberry crop (Chapters 2, 4 and 5). Thus, efforts to boost numbers of *Eupeodes* spp. hoverflies may enhance both pollination and aphid biocontrol in commercial strawberry.

Recommendations

Sown wildflowers

Syrphine hoverflies are common strawberry flower visitors and effective pollinators (Chapters 2 and 3). Consequently, improving farmland habitats by planting additional floral resources tailored to hoverflies will provide valuable food sources when crops are not in flower and encourage hoverflies into the crop (Chapter 4). In particular, planting wildflowers belonging to the families Apiaceae,

Asteraceae, Boraginaceae, Lamiaceae, Ranunculaceae and Rosaceae can attract a broad range of beneficial insects, including hoverflies with aphid-eating larvae. My research found that field forget-me-not, field mint and especially coriander were attractive to aphidophagous hoverflies. Indeed, sown species may prove to be more attractive than strawberry flowers. However, in the present study this phenomenon had no effect on subsequent fruit quality, and pollinator abundances were higher in plots with wildflowers (Chapter 4).

While field margins are often the most suitable habitats to establish wildflowers, planting additional floral resources within the crop represents an alternative strategy that may enhance the ability of beneficial insects to reach central areas of crop fields. The sown flowers may also encourage pollinating insects to remain in the middle of fields and visit crop flowers there. Intercropping with sown wildflowers offers the added benefit of making plant establishment much easier given that the wildflowers can be supplied with the same irrigation and fertilisers as the crop plants. In addition, cultivating wildflowers in the same growing medium as the crop plants avoids the risk of weed species out-competing the sown flower species.

Aphid predators

Intercropping coriander among strawberry rows may represent an effective means of attracting aphidophagous lacewings into the crop. Though the beneficial lacewings arrived too late in the season to reduce aphid numbers, the observed increases in lacewing abundance could carry over to successive years, potentially providing effective aphid control in subsequent growing seasons. In sum, multifunctional wildflower plantings that attract both pollinators and aphid predators are likely to be the most cost-effective method of facilitating the delivery of these two key ecosystem services.

Future Research

Pollination effectiveness

While Chapter 3 provided useful insights on pollination effectiveness of syrphine hoverflies, future studies could examine the relative pollination efficiency of aphidophagous hoverflies in comparison with other taxa known to be effective strawberry pollinators (e.g. bumblebees, honeybees and other hoverflies). Given that previous research has already established the pollination effectiveness of *Eristalis* spp. hoverflies (Nye and Anderson 1974; Albano et al. 2009; Gibson 2012), further cage trial studies could compare directly the effectiveness of *Eupeodes* spp. with *Eristalis* spp. hoverflies. Moreover, research comparing the pollination effectiveness of mixed assemblages of hoverflies versus single-species assemblages would reveal whether diverse groups of flower visitors optimise the delivery pollination services in commercial strawberry. Finally, while bees, butterflies and hoverflies are commonly studied as pollinators in agricultural and conservation contexts, non-syrphid flies and beetles may yet prove to be important pollinators of commercial strawberry based on field surveys of strawberry flower visitors in eight fruit farms (Orford et al. 2015; Rader et al. 2016). In addition no direct comparisons of the effectiveness of pollination by commercial bumblebees versus hoverflies have been done. Given current concerns with release of commercial bumblebees and their long foraging ranges it might be more prudent to release hoverflies for strawberry pollination.

Intercropping coriander to boost aphid natural enemies

The observed positive correlation between coriander plots and counts of lacewing eggs provides initial evidence to suggest that aphid biocontrol by naturally-occurring predators may be enhanced by the provision of coriander within the strawberry crop (Chapter 4). Future work could examine whether aphid infestations can be controlled late in the growing season by naturally-occurring populations of lacewings attracted into the crop by intercropped coriander plants.

Alternatively, inter-planting coriander could be coupled with controlled releases of commercially-reared lacewings early on in the growing season to determine whether coriander promotes lacewing numbers enough to control aphid populations throughout the growing season.

Additionally, determining the relative effectiveness of different aphidophagous predators would improve our ability to optimise aphid biocontrol options in strawberry. In a review of 108 biological control projects using single-species or multiple-species predator assemblages, Denoth et al. (2002) found that in the majority of cases a single species was responsible for the effective suppression of the insect pest species. Releasing multiple predator species did not improve success rates. Similar results were reported in studies of predators of soybean aphids (*Aphis glycines*; Costamagna et al. 2008) and green peach aphids (*Myzus persicae*; Straub and Snyder 2006). In these cases ladybirds (Coleoptera: Coccinellidae) were found to be the most effective at controlling aphid populations. Therefore, identifying the predator species that is most effective at suppressing strawberry pests is a necessary first step in developing successful biological control programmes.

Finally, future studies could test the possibility of growing companion plants, like coriander, beneath strawberry tables as an alternative method of establishing additional floral resources within strawberry crops. This method was trialled in an established covered crop of table-top strawberries at NIAB EMR in 2015; however, sown plants were repeatedly overwhelmed by established perennial weeds, such as broad-leaved dock (*Rumex obtusifolius*) and stinging nettle (*Urtica dioica*), which were not attractive to hoverflies. As a consequence, all three sown species (cow parsley, *Anthriscus sylvestris*; cat's-ear, *Hypochaeris radicata*; and bulbous buttercup, *Ranunculus bulbosus*) failed to establish. A better approach might be to establish additional floral resources in horticultural plantings before installing strawberry tables and polytunnels above the sown flowers.

Digestive tract contents analyses to establish trophic linkages

Having established that hoverfly larvae are consuming aphids within commercial strawberry crops (Chapter 5), future work could develop species-specific primers for aphids to reliably identify aphids in the guts of hoverfly larvae. Knowledge of the dietary breadth of different hoverfly species would reveal whether biocontrol could be achievable for all common strawberry-feeding aphid species, or only a subset of pest species. Furthermore, gut content analyses could also be performed using other species of aphid predator such as lacewings, ladybirds and *Aphidoletes* midges (Diptera: Cecidomyiidae). By gaining a broader understanding of the dietary breadths of these predators, researchers could better assess the relative potential of these groups as aphid biocontrol agents in strawberry.

Another phenomenon that deserves further examination is the role of intraguild predation in the effective suppression of strawberry aphid pests. Prior work investigating the trophic relationships among the hoverfly *Episyrphus balteatus*, the lacewing *Chrysoperla carnea*, the ladybird *Coccinella septempunctata* and the midge *Aphidoletes aphidimyza* revealed that each species was capable of playing the role of intraguild predator or intraguild prey, depending on the relative body size of the individuals involved (Hindayana et al. 2001). Intraguild predation occurred most commonly when aphid prey was scarce. High-throughput sequencing techniques could be used in future studies to determine intraguild predation rates among aphidophagous predators in strawberry crops.

Conclusion

In order to sustain production of insect-pollinated crops for future generations, society must address competing challenges arising from pest outbreaks and pollinator declines among other threats. A necessary first step in achieving sustainable solutions to these obstacles is developing a better picture of the beneficial insects involved in predating pest species and pollinating valuable fruit and vegetable crops. This project has afforded new evidence to suggest that hoverflies with

aphid-eating larvae are capable of both pollinating strawberry crops and consuming aphids that harm strawberry plants. Moreover, planting coriander within strawberry rows has been identified as a potential tool to increase numbers of aphidophagous lacewings within the crop. Finally, common predatory hoverfly species have been identified within commercial strawberry and their ability to consume important pest aphids has been analysed using molecular methods. Gut contents analyses revealed that aphid DNA was the main form of prey DNA, present in over a third of hoverfly specimens.

Future research is necessary to elucidate the relative pollination effectiveness of aphidophagous hoverflies in comparison to other taxa; to confirm the potential of coriander to boost aphid biocontrol by predatory lacewings; and to identify the potential of other aphid natural enemies to serve as effective biocontrol agents in strawberry. Ultimately, harnessing sustainable techniques for safeguarding pollination and pest control services represents the most effective means of ensuring that future generations are able to benefit from stable food production.

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