The pollination ecology of cultivated raspberry (*Rubus idaeus*) in the UK: comparing pollinator supply and demand at field scale

Imogen Ryan

1002517

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University of East Anglia, UK
School of Biological Sciences

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Abstract

Pollinators play a crucial role in the production of food crops worldwide. Managed honey bees are often used to ensure the productivity of crops that are highly dependent on pollination, however they have been found to have negative impacts on wild pollinator communities due to competition for floral resources and the transmission of pests and pathogens. To reduce these potential impacts, the use of managed pollinators should be informed by crop-specific estimates of the pollination requirements to yield along with site-specific monitoring of pollination service provision. Implementing managed pollinators according to their requirements will prevent overstocking and reduce the associated risks of high densities of managed pollinators to wild pollinator communities.

This thesis provides a comprehensive overview of the pollination ecology of two commercial raspberry *Rubus ideaus* L. cultivars studied at a commercial soft fruit farm in south England, assessing their pollinator dependence and the relative importance of managed honey bee *Apis mellifera* and wild bumble bees *Bombus terrestris* for marketable fruit production. A three-year pollinator exclusion study found a 64% loss in marketable fruit weight when pollinators were excluded, showing that the raspberry cultivars studied have a high pollinator dependence. Single visit pollinator efficiency studies revealed that *Apis mellifera and Bombus terrestris* were equally effective per visit at pollinating raspberry flowers in this system and that two visits from either species were sufficient to produce marketable quality fruit. Pollinator visitation rates were measured using timed flower counts and day-long video observations. We found that visitation rates were significantly higher on the first day after flower opening and at the corners of the fields (<20m from both perpendicular edges) compared to locations that were <20m from a field edge. Crop flowers received an average of ~364 insect visits across the fields in 2020 and ~71 in 2021 over the flowers' receptive period of 2.5 days. Average visitation rates did not differ between the short, timed flower counts and day-long video observations.

Monitoring raspberry crop pollinators using short, timed flower counts in the centre of fields could therefore provide valuable information on the minimum pollination service being provided to crop

flowers by wild pollinators along with the need for managed pollinators. Where visitation rates meet or exceed the two visits required to produce marketable fruit, growers should maintain or reduce the stocking densities of managed pollinators to minimize the risks to wild pollinators, while visitation rates that fall short of the required two visits per flower indicate the need for additional managed pollinators to be introduced or wild pollinator conservation to be improved to maximise crop yields.

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1.0 General introduction to pollinators and their importance for agriculture and raspberry pollination

1.1 Pollinators and their conservation concern

Pollinators are any animal that assists in the transfer of pollen from one flower's anther to the stigma of the same or another flower to facilitate sexual reproduction. Up to 350,000 animal species worldwide are thought to carry out this process, with many more yet to be identified (Ollerton, 2017). While vertebrates such as birds and bats provide pollination in some geographical regions (Regan *et al.*, 2015; Tremlett *et al.*, 2019: Ratto *et al.*, 2018), they account for less than 0.5% of pollinators, with invertebrates making up the rest (Ollerton, 2017). The majority of these belong to the four largest insect orders: Lepidoptera (butterflies and moths), Hymenoptera (bees, wasps, sawflies, and ants), Diptera (true flies) and Coleoptera (beetles) (Ollerton, 2017; Wardhaugh, 2015). This thesis solely considered insect pollinators as vertebrate pollinators were not economically important in the study system. The main objective of this thesis is to offer raspberry growers guidance on monitoring and managing crop pollination to optimize crop yield while decreasing dependence on managed pollinators. This introduction provides an overview of current research on the value of pollinators to crops, emphasising key findings and identifying gaps in our understanding. I aim to outline the importance of insect pollinators to agricultural production, with a particular emphasis on soft fruit production, specifically raspberries. I also highlight the existing threats to wild pollinator populations, as they deliver important pollination services to crops, and their perceived and actual declines demonstrate the rationale and necessity of managed pollinators in cropping systems. The crucial role of pollinators in facilitating the successful fertilization of crops has significant implications for agriculture and food security in the face of biodiversity declines.

Insects have been widely reported to be experiencing dramatic declines in abundance. This picture of insect declines is largely due to sensationalized media coverage. This coverage has been fuelled in part by inflated claims made in institutional press releases for three studies (Hallman *et al.,* 2017, Lister and Garcia, 2018 and Sanchez-Bayo and Wyckhuys, 2019) that have become highly cited

(Didham *et al.,* 2020; Saunders *et al.,* 2019). Hallman *et al.* (2017) documented long term local declines in flying insect biomass in protected areas in Germany and Lister and Garcia (2018) found reductions in arthropod counts in a Puerto Rican rainforest over the course of approximated two decades. While these studies do document insect declines, these were measured at a local level rather than the global or national level reported by the press releases (Saunders *et al.*, 2019). A heavily criticised (Mupepele *et al.,* 2019; Simmons *et al.,* 2019) review by Sanchez-Bayo and Wyckhuys (2019) stated that there have been global declines in insect populations, while recent reviews of the empirical evidence for insect declines have revealed a more nuanced and varied picture (Klink *et al.,* 2020; Wagner, 2020). While some insect groups have decreased in abundance, there are others that have increased or remained stable. The criticism for the review by Sanchez-Bayo and Wyckhuys (2019) lead to calls for more robust data and analysis (Didham *et al.,* 2020; Thomas *et al.,* 2019). Powney *et al.* (2019) documented the decline in occupancy of bees and hoverflies in the UK of 25% and 24% respectively between 1980 and 2013 using long term monitoring data, although this varied considerably between species. Known dominant crop pollinators (Kleijn *et al.,* 2015) such as *Bombus* and *Andrena* increased on average in occupancy from 1980 to 2013 and for eusocial species, most of which were *Bombus* sp., average occupancy increased by 38% while solitary bees experienced a decline of 32%. Declines also varied in severity between upland and southern species. Mupepele *et al.* (2019) calculated the effect size of the relationship between time and Hymenoptera (mainly bees) abundance taken from the review by Sanchez-Bayo and Wyckhuys (2019) and found that bees and wasps have declined over time though the strength of this decline was not able to be calculated. Moths and butterflies have also been documented to be in decline in the UK (Conrad *et al.,* 2004, 2006; Fox *et al.,* 2011; 2015; Thomas *et al.,* 2004), Belgium (Maes and Van Dyck, 2001), Germany (Habel *et al.,* 2019) and Sweden (Franzén and Johannesson, 2007). It appears that declines are species and geographically specific, with some groups increasing in abundance. However, there is evidence of declines in certain groups, such as bees and butterflies, which play important roles in pollination. It seems that dominant pollinators are not declining, but diversity probably is, which may have negative effects on pollination service provision.

Pollinator declines are of concern for crop pollination however, as they make up <0.1% of flowering plant species it is important to consider the effects on wild plant communities (Ollerton *et al.,* 2011). 78% of temperate-zone flowering plants and 94% of flowering plants in the tropics are animal pollinated (Ollerton *et al.*, 2011). The absence of pollinators could lead to a decline in fertility of over 79% for half of all flowering plants, and a third would not produce seeds at all (Rodger *et al.,* 2021). I'm not considering the causes of these declines here but recent reviews have identified the main drivers of pollinator declines as habitat loss, climate change, pesticide use and the spread of parasites and diseases (Dicks *et al.,* 2021; Goulson *et al.*, 2015; Potts *et al.* 2016). The potential additive interaction between these drivers is also being explored (Oliver *et al.,* 2010; Goulson *et al.,* 2015; Outhwaite *et al.,* 2022) but there is still much to learn, and more research still needs to be done to determine the relative importance of these drivers on insect declines (Weisser *et al.,* 2023).

1.2 Importance of pollinators for agriculture

According to Klein *et al.* (2007), insect pollinated plants make up at least 35% of total crop production by volume, and up to 75% of global food crops rely on this pollination service to enhance yield. The economic value of crop pollination services is often used to convey the importance of pollinators for agriculture. Lautenbach *et al.* (2012) used Klein *et al.*'s (2007) data on crop yield losses in the absence of pollinators, in combination with 2009 market prices and production, to estimate the annual economic value of pollinators to global crop output. This figure, when adjusted for inflation to 2015 US dollars, ranges between US$235 and 577 billion (Potts et al., 2016). For England alone, Breeze *et al.* (2011) estimated the value to be over GBP 918 million in 2007. While there is ongoing debate regarding the accuracy of economic valuations of pollination services and the methods used to calculate them (Breeze *et al,* 2016), it is apparent that pollinators have significant globally economic importance, and these monetary figures serve as a crucial means of highlighting their ecological importance to agriculture. These economic valuations of pollinators are important due to the growing concern about the decline of pollinators worldwide and the potential consequences of this decline for crop production and food security. Climate change is expected to exacerbate the already challenging situation for pollinators, with potentially severe consequences for agricultural systems that depend on them.

Although around half of calories produced for human consumption are from wind-pollinated crops such as wheat, rice, maize, and barley (D'Odorico *et al.*, 2014), animal-pollinated crops contribute significantly to our daily intake of essential micronutrients such as vitamins, lipids and folic acid (Eilers *et al.*, 2011). Up to 70% of Vitamin A is produced by insect pollinated plants (Eilers *et al.*, 2011). Threats to insect pollinators therefore also threaten human health, especially in countries that heavily rely on insect pollinated crops such as beans for calories and nutrients (Smith *et al.*, 2022).

There is evidence to suggest that the value of pollinators to crop production, in terms of the proportion of food produced that is due to pollinators, could increase with rising global temperatures and more frequent heat waves. Bishop *et al.* (2016) found that bumble bee pollination mitigated against yield losses associated with heat stress in faba bean *Vicia faba*. Bumble bee pollination reduced yield loss from 15% to 2.5% after exposure to a temperature of 30°C. The crop flowers were also found to be more pollinator dependent under the 30°C-temperature treatment compared to the control (which grouped 18, 22 and 26°C treatments), but there was no difference at 34°C. The increase in outcrossing with insect pollination, which increases the probability of pollen transfer from non-heat stressed to heat stressed plants, is thought to be the mechanism behind this (Bishop *et al.*, 2017). Thus, insect pollination may help to mitigate against yield losses under increased heat wave probability, although not at extreme temperatures.

Insect biodiversity can further enhance the resilience and long-term stability of crop production (Senapathi *et al.*, 2021; Winfree *et al.*, 2009) and in some cases can even improve crop yield such as for coffee *Coffee arabica* (Klein, *et al.*, 2003) and pumpkin *Cucurbita moschata* (Hoehn *et al.*, 2008). However, it has also been argued that it is the abundance of common dominant species that drives pollination service provision (Winfree *et al.*, 2015).

Within the four main pollinating groups, bees are the most effective crop pollinator in most cases due to the high frequency of their visits as well as the long distances travelled between visits, driven by the reliance of both adults and larvae on pollen and nectar for survival (Willmer, 2011). However, non-bee flower visitors such as butterflies and flies have been shown to provide pollination services to those flowers that bees have not visited (Cusser *et al.,* 2021). Bee pollination has been shown to increase the yield of multiple crops (Klein *et al*., 2007) and is essential to produce marketable strawberries (MacInnis and Forrest, 2019). They are therefore often the subject of pollination management efforts for the purpose of crop pollination.

1.3 Synergies between honey bees and wild pollinators for crop pollination

Managed pollinators are pollinating species that can be farmed, such as honey bees, or bred in lab settings, such as some species of bumble bee and solitary bee, for the purpose of crop pollination. They can provide pollination services at much higher densities than wild pollinators and are seen as more reliable than wild pollinator communities. Managed honey bees are often used for crop pollination, especially short term, due to their ease of management and can supplement wild insect pollination services to ensure crop yields are maximised. However, most studies that compare pollination services from honey bees to wild bees find that wild bees are more effective on a single visit basis (Page *et al.,* 2021). A meta-analysis of 168 studies by Page *et al.* (2021) found that although honey bee single visit pollination effectiveness did not differ from the average single visit pollination effectiveness of all other pollinators, they were less effective than other bee species across crop and non-crop plants. Garibaldi *et al.* (2013) found that across a range of animal-pollinated crops, the benefit to fruit set of frequent wild bee visits was twice that of an equivalent number of visits by honey bees and Norfolk *et al.* (2016) found that for almonds there was no benefit of the presence of honey bee hives on fruit set and that fruit set was correlated with wild pollinator visitation but not honey bee visitation. For fruit crops honey bees have also been shown to be less effective than wild bees. MacInnis and Forrest (2019) found that strawberries were heavier when their flowers were pollinated by wild bees compared to when they were pollinated by the same number of honey bee visitors while Ahmad *et al.* (2021) found that wild pollinators yielded loquats (*Eriobotrya japonica)* that were heavier, larger, had more seeds and greater sugar, antioxidant, moisture, and fibre content compared to those that were pollinated by *Apis mellifera* only. Sun *et al.* (2021) found that blueberry flowers visited by bumble bees yielded more Grade 1 fruit than those visited by two different species of honey bee. This greater pollination service may be due to a greater degree of outcrossing by wild pollinators, which has been found to improve yields (Cusser *et al*., 2016). However, Andrikopoulos and Cane (2018a) found that flowers that received a single *Apis mellifera* or a single bumble bee visit did not differ in the drupelet set of the raspberries they produced. The best pollinator for raspberry pollination is therefore debateable.

Interestingly, at least four studies have shown that the effectiveness of honey bees as crop pollinators varies, according to whether there are wild bees foraging alongside them, or not. This facilitation of pollination services is thought to be a result of induced changes to honey bee behaviour. According to Greenleaf and Kremen's (2006) study on sunflower pollination, female *S. obliqua expurgata, Anthophora urbana, Diadasia* spp. and both male and female *Melissodes* spp. were more effective than *A. mellifera* on a single visit basis. However, the number of seeds produced after a single *A. mellifera* visit increased as the abundance and species richness of wild bees increased, making them the second most effective species after *S. obliqua expurgate*. This may be because wild bees foraging on pollen-rich male flowers caused *Apis mellifera* they interacted with on a flower to move from male to female sunflowers. Honey bees specialize in either pollen or nectar collection on a foraging bout, and in this case, they focus on either male (pollen) or female (nectar) flowers, making them less effective pollinators since pollen transfer between male and female flowers is limited. Thus, the presence of wild bees enhanced the pollination effectiveness of *Apis mellifera* in this study. Brittain *et al.* (2013) found that honey bees foraging on almond flowers, changed crop rows more often when *Osmia lignaria* were present and that this resulted in more pollen tubes reaching the base of the style and greater fruit set despite lower overall visitation rates per flower. This was likely due to greater cross pollination between almond varieties with increased movement between rows (of different varieties). Eeraerts *et al.* (2020) found that honey bee movements between rows in sweet cherry fields were increased with bumble bee abundance while Carvalheiro *et al* (2011) showed that honey bee movement between sunflower heads was enhanced when they interacted with other bee species as well as butterflies and moths. However, Rogers *et al.* (2013) found no increase in honey bee movement away from the current flower after interacting with either a honey bee or a bumble bee within a flight arena. Although there is some discrepancy in these findings, these studies demonstrate how wild and managed pollinators can complement each other and how promoting wild pollinators can improve pollination services to the crops, even when they are not as effective as honey bees for pollinating crop flowers.

1.4 Competition between honey bees and wild pollinators

While many studies have found that wild pollinators are superior to honey bees in terms of their per-visit pollination services and that wild pollinators can help improve honey bee pollination services, there are other risks associated with relying on a single pollinator species for crop pollination. Increasing pollinator species diversity can enhance the stability and resilience of pollination services, leading to greater crop yields and quality. Having a more diverse pollinator community also increases its resilience to anthropogenic threats such as climate and land-use change due to interspecific differences in response and tolerance to change (Brittain *et al*., 2013). Cusser *et al.* (2016) found that pollen limitation of cotton decreased with pollinator abundance and richness while Mallinger and Gratton (2014) found that fruit set of apples significantly increased with species richness but not with honey bee presence or the number of hives. Other studies have documented the importance of functional group rather than species richness. Functional groups are groups of species that share morphological features or occupy the same niche, therefore functional diversity is the range of those niches or morphological characteristics that are represented. Albrecht *et al.* (2012) compared the importance of species richness and functional diversity for the pollination of radishes *R. sativus* spp. *oleiformes.* Functional groups were social bees (eusocial, large bees), solitary bees (solitary and primitively eusocial, smaller bees) and hoverflies. The study found that functional diversity increased fruit and seed set of radishes more than species diversity in caged plots. Three species across three functional groups lead to greater yields than three species in one functional group. Species richness increased fruit and seed set when only one functional group (social bees) was present but did not increase fruit or seed set when three functional groups were present. One species in each of three functional groups yielded slightly greater fruit and seed set than nine species across three functional groups. Blitzer *et al.* (2016) found that seeds per apple increased, and pollen limitation decreased with the number of bee species, wild bee abundance and the number of functional guilds but not with honey bee abundance. However, functional group diversity explained more variation in apple seed set than species richness. Other studies have also shown that it is functional diversity rather than species diversity that is important for crop pollination. Bluthgen and Klein (2011) explored how a variety of functional traits within wild pollinator communities can lead to foraging complementarity such as phenological complementarity where pollinators forage at different times of day and architectural complementarity where pollinators forage at different flower heights as shown by Hoehn *et al.* (2008). This can improve the quality of pollination services to crops.

Maintaining and improving wild pollinator species and functional diversity could therefore improve crop pollination through complementary flower use, enhanced pollination of honey bees or improved per flower pollination service by multiple species or functional groups of flower visitors. It is therefore important to identify the wild pollinators providing pollination services to crops and study the ways that their populations can be conserved within agricultural landscapes.

Increasing the flowering plant diversity within crop fields may provide improved pollination services by increasing flower visitor diversity. Pereira *et al.* (2015) found that intercropping bell peppers with flowering basil increased the richness and abundance of visiting bees. *Paratrigona lineata, Apis mellifera* and *Tetragonisca angustula* were all attracted to the basil and peppers that were intercropped were wider, longer and heavier and had more seeds than those in the field without basil. This benefit of non-crop flowers within crop fields has also been shown for almonds; Cusser *et al.* (2016) found that abundance and species richness of pollinators was positively correlated with natural land cover. Norfolk *et al.* (2015) also found that high species richness of wild and cultivated flowering ground vegetation was positively correlated with both pollinator abundance and fruit set but did not investigate the effect of the floral diversity on pollinator diversity. Carvalheiro *et al.* (2011) also found that 'weed' diversity increased flower visitor diversity for sunflowers. Intercropping with commercially valuable understory crops or leaving wildflowers within crop fields are compatible with most commercial agricultural systems and so could be feasible wild pollinator management options for growers.

Despite evidence of synergistic benefits of honey bees and wild pollinators on crop yields, high densities of honey bee hives have been linked to reductions in wild bee visits to flowering crops. Though research examining the effects of honey bees on wild bees has conflicting results (Mallinger *et al.,* 2017), in some cases, honey bees can alter native bee communities because of their relatively high level of pathogen loads, degree of resource (pollen and nectar) removal, and their foraging interactions with native bees. 53% of studies looking at the effects of managed pollinators on competition for floral and nesting resources found negative effects on wild bees, 28% found no effect and 19% found mixed effects (Mallinger *et al.*, 2017). Most of these studies lacked controls or experimental manipulations or did not measure critical parameters such as wild bee fitness or population level responses. However, studies that did, largely agreed with the overall findings (55% negative, 33% no effect, 11% mixed).

Nielsen *et al.* (2017) observed that wild bumble bees foraged on wildflower areas at higher abundances than on crop flowers, which honey bees showed the opposite trend. The authors propose that this was due to flower constancy as well as exploitative competition from the honey bees. Roger *et al.* (2013) found the bumble bees stopped foraging at the foraging platform after interacting with a honey bee compared to a small, but significant, reduction in foraging after interacting with a conspecific. In oilseed rape fields, the densities of bumble bees, solitary bees, hoverflies and other pollinating insects were found to decrease in the presence of honey bee hives, according to Lindström *et al* (2016). When the same areas were surveyed after the oilseed rape harvest, there were fewer bumble bees observed within field margins and road verges when honey bees were present and this effect was exacerbated in more homogenous landscapes with low levels of semi-natural grassland (Herbertsson *et al,* 2016). Hudewenz and Klein (2013) also found a reduction of wild bee foraging in uncropped areas when honey bee hives were present. The abundance of stem-nesting bee species was also reduced within the nature reserve in the areas surrounding honey bee hives. Angelella *et al.* (2021) found that the presence of honey bee hives at farms growing pollinator dependent crops was correlated with a 48% decrease in wild bee abundance and a 20% decrease in species richness at field edges compared to those on farms that didn't have hives on site. Strawberry fruit set was 18% lower on farms with honey bees in this study, suggesting that on average honey bee hives did not benefit strawberry yields. In contrast, Mallinger and Gratton (2014) found no negative effect of honey bee hive presence on wild bee abundance and species richness in apple orchards. The effect of honey bee presence on wild pollinator abundance and species richness is therefore variable between crops and locations. Two known studies have tested the effect of honey bee stocking density on the abundance of wild pollinators on crop flowers. Arrington and DeVetter (2018) found no effect of stocking density on non-*Apis* visitation rates between blueberry farms stocked with honey bees at 10 hives/ha and 20 hives/ha. Walther-Hellwig *et al.* (2006) found that *Bombus terrestris* showed spatial avoidance when foraging on *Phacelia* when honey bee hives were at 10 hives/ha. They showed a preference for foraging off-crop on floral resources that were less dense and situated farther away from the honey bee hives. However, this effect was not seen at 1 hive/ha indicating that there may be a density dependent effect of honey bee hives on bumble bee abundance on crop flowers. The stocking density between 1 and 10 hives/ha at which the effect of honey bee presence is reduced was not tested. The effect of reducing honey bee stocking densities rather than removing honey bees from agricultural systems therefore still needs to be further explored.

This competition between honey bees and wild pollinators can have negative impacts on the health of wild pollinators if they are displaced from pollen and nectar resources. This is highlighted in a study by Cane and Tepedino (2016), who calculated the amount of pollen collected by a single honey bee colony compared to the pollen requirements of solitary bee progeny. They found that between June and August, a honey bee colony collects enough pollen per month to rear 33,000 alfalfa leafcutter bee

(*M. rotundata)* progeny. However, the effects of honey bee presence on the health of wild pollinators have been found to be mixed. Hudewenz and Klein (2013) found no effect of honey bee hive presence or proximity on the reproductive success of ground or stem nesting solitary bees in a nature reserve, while Paini *et al.* (2005) found no effect of low honey bee hive numbers on the reproductive success of a native Australian *Mechachile* species, despite a resource overlap. However, the effect of honey bee hive presence on bumble bee worker size (Goulson and Sparrow, 2008), colony growth and reproductive success (Elbgami *et al.*, 2014; Thomson, 2004) has been found to be negative. A reduction in nectar availability when honey bees are present could prompt bumble bee colonies to divert pollen collecting workers to nectar thus reducing larval production (Thomson, 2004). This could also impact bee size. Goulson *et al.* (2002) found that larger bumble bees were more likely to be foragers and mass of both pollen and nectar collected per bee was positively correlated with thorax width. There is therefore likely to be a negative feedback mechanism when honey bee presence reduced floral resource availability or causes displacement of bumble bees to lower quality floral resources. The difference in the effect of honey bee hives on the health of solitary and social bees may be due to their inherent differences in foraging strategy and number of offspring, or to the fact that bumble bee colony health is more easily observed.

Wild pollinator colony health can also be detrimentally affected by honey bee hive presence through the transmission of pathogens and parasites (Manley *et al*. (2015). This can occur through shared flower use (Figueroa et al 2019; Graystock, Goulson and Hughes, 2015; Graystock et al 2020). The presence of Deformed Wing Virus, a viral pathogen previously only found in *Apis mellifera*, in other bee and arthropod species suggests interspecific transmission (Levitt *et al*, 2013 and Singh *et al*, 2010). The disease has been found to cause crumpled wings, discolouration, decreased longevity and mortality in bumble bees (Cilia *et al*, 2021; *Fürst et al*, 2014; Genersch *et al*, 2006; Gusachenko *et al* 2020) and is thus a growing concern for wild bumble bee populations despite the pathway of infection not being known (Gusachenko *et al* 2020). The pathway could be through shared flower foraging as found for *Nosema ceranae*, a parasite of honey bees (Graystock *et al.*, 2013; Graystock, Goulson and Hughes, 2015; Graystock *et al*, 2020). The parasite was found in 20-47% of wild-caught bumble bees across the UK (Graystock *et al,* 2013). When infected, this fungal micro parasite absorbs nutrients from the gut and body fat and has been shown to cause both lethal and sublethal effects in bumble bees (Graystock *et al,* 2013). Mallinger *et al.* (2017) found that 70% of studies on pathogen transmission between managed and wild pollinators found reported potential negative effects of managed pollinators on wild bees though the majority did not measure direct effects of transmission on wild pollinator populations. However, caution should be exercised when looking to introduce managed pollinators to areas that support pollinator species of conservation concern.

Both perceived and actual decline in wild pollinators can lead to an increased use of or reliance on managed pollinators such as bumble bees, solitary bees and, most commonly, honey bees. The use of which can further exacerbate any declines in wild pollinators. This creates a self-reinforcing cycle where the use of managed pollinators becomes more necessary with population decline of wild pollinators, but the increased use of managed pollinators can also negatively impact the survival and health of wild pollinators, perpetuating the need for further management intervention. To mitigate any adverse effects of honey bee colonies on wild pollinator populations and crop pollination, it is essential to monitor crop pollination services. This approach will provide information on the need and stocking densities of honey bees for crop pollination. This will help ensure that the use of honey bees does not conflict with the conservation of wild pollinators and have a negative impact on crop yields.

1.5 Raspberry production

UK soft fruit production was valued at £575.3 million in 2021 (Defra, 2021). Raspberries and strawberries make up £147.5 and £352.4 of this respectively. While raspberry production and area harvested have remained stable over the last ten years, the yield per hectare increased dramatically between 1997 and 2011 (FAOSTAT, 2021). Figure 1.1 shows that UK area under raspberry cultivation decreased dramatically between 1990 and 2002, but that tonnes of raspberries produced stopped following the pattern of area under cultivation after 1997. This shows that raspberry production has become more efficient through agricultural intensification. The use of pesticides and synthetic fertilisers has improved the yields of many crops over the last few decades, enabling the production of more food from less land. For many horticultural crops the increase in the use and technology around protected cropping such as greenhouses has improved yields by enabling temperature and humidity control as well as protection from frost and other adverse weather conditions. For raspberries, protected cropping normally comes in the form of plastic high tunnels or poly tunnels. Spanish polytunnels were introduced into the UK in the mid-1990s (Calleja and Mills, 2012) which coincides with the increase in yield per hectare for both raspberries and strawberries (FAOSTAT, 2021). Polytunnels increase and maintain higher temperatures and increase humidity enabling many crops to be grown ‘out of season’ (Johnson, Young and Karley, 2012; Singh *et al*,

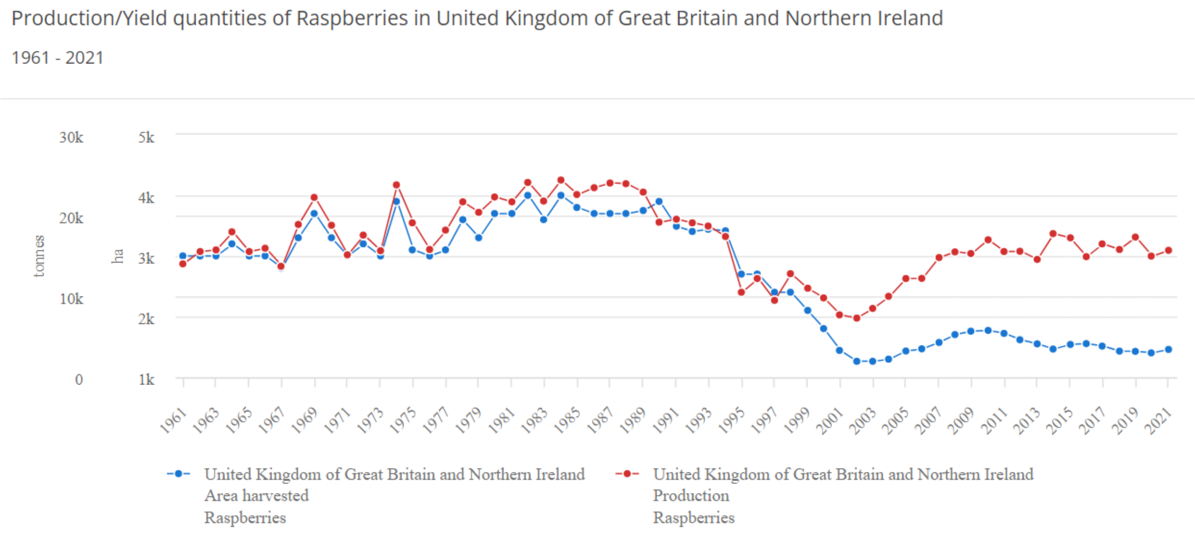

Figure 1.1 Area of raspberry production and tonnes harvested between 1961 and 2021 in the UK (FAOSTAT, 2021)

2012). Growers capitalise on this, often concentrating their production on early and late season varieties when prices are high (Kempler, et al 2002). They also provide shelter from wind for fragile crops such as raspberries (Wells and Loy, 1993; Lamont, 2009), cold protection (McIntosh and Klingaman, 1993) and enable the humidity and temperature of the growing environment to be monitored and controlled by manual ventilation. For insect pollinated crops such as raspberry (Free, 1993; McGregor, 1976) producing higher yields from less land not only relies on access to traditional agricultural inputs such as fertilizer and pesticides, but pollination services also need to be met to enable yields to be maximised. Pollination of mass flowering crops can prove difficult as although they can often provide a valuable short-term resource for wild pollinators and managed honey bees in agricultural landscapes, these high levels of floral resources are not available consistently throughout the spring, summer and early autumn and so cannot maintain high abundances of pollinators. The short-term need for high numbers of pollinators that cannot be supported outside of crop flowering periods, means that managed pollinated such as the European honey bee *Apis mellifera* are often introduced. As previously discussed, introducing high volumes of managed pollinators could be detrimental to wild pollinator communities. Therefore, by introducing managed pollinators to cropped systems, growers may be reducing the diversity of pollinators that visit the crop with potential negative effects on crop yield.

Although commercial raspberries are self-fertile, due to their flower morphology they rely on insect pollinators to reach complete pollination. The stamens of a raspberry flower are arranged in concentric circles around the edge of a central receptacle with the pistils arising spirally from the receptacle (Fig. 1.2). This structure means that the inner-most stigmas are morphologically isolated from the anthers of the same flower and therefore require an external pollinating agent to pollinate all the stigmas of the flower (Free, 1993). Raspberries are termed aggregate fruits as each fertilised ovary develops into its own fruiting body called a drupelet of which there are 20-150 per berry. Each ovary has two ovules and thus the potential for two seeds to be produced per ovule. However, in most cases only one ovule is fertilised resulting in single seeded drupelets (Funt and Hall, 2013). Due to the structure of raspberry fruits, the number of fertilised ovules is directly correlated to the fruit yield; the more ovules that are fertilised, the more drupelets the raspberry has. If too few ovules are fertilised the cohesion of the drupelets is reduced resulting in crumbly unmarketable fruits (Andrikopoulos and Cane, 2018a), often with a terminal tuft of dried unpollinated pistils (Cane, 2005; Free, 1993). Insect pollination is therefore essential for achieving high fruit yield and quality of commercial crops (Chagnon et al, 1991; Cane, 2005; Prodorutti and Frilli, 2008; Ellis et al, 2017; Andrikopoulos and Cane, 2018a and Strelin and Aizen, 2018). Pollination dependency makes commercial raspberry crop yields vulnerable to pollinator declines and fluctuations in the abundance of wild bees. This vulnerability means that the majority of soft fruit growers purchase or rent managed pollinators such as honey bees and bumble bees in order to subsidise the systems' wild pollinator community and ensure maximum pollination (Free, 1993; BerryWorld and Hall hunter pers. comms). High densities of *Apis mellifera* and *Bombus terrestris* have been associated with raspberry stigma damage and resulting reductions in fruit yields (Saez *et al.*, 2014), demonstrating that there is an important balance between achieving maximum pollination and avoiding negative impacts of high visitation rates on crop yields.

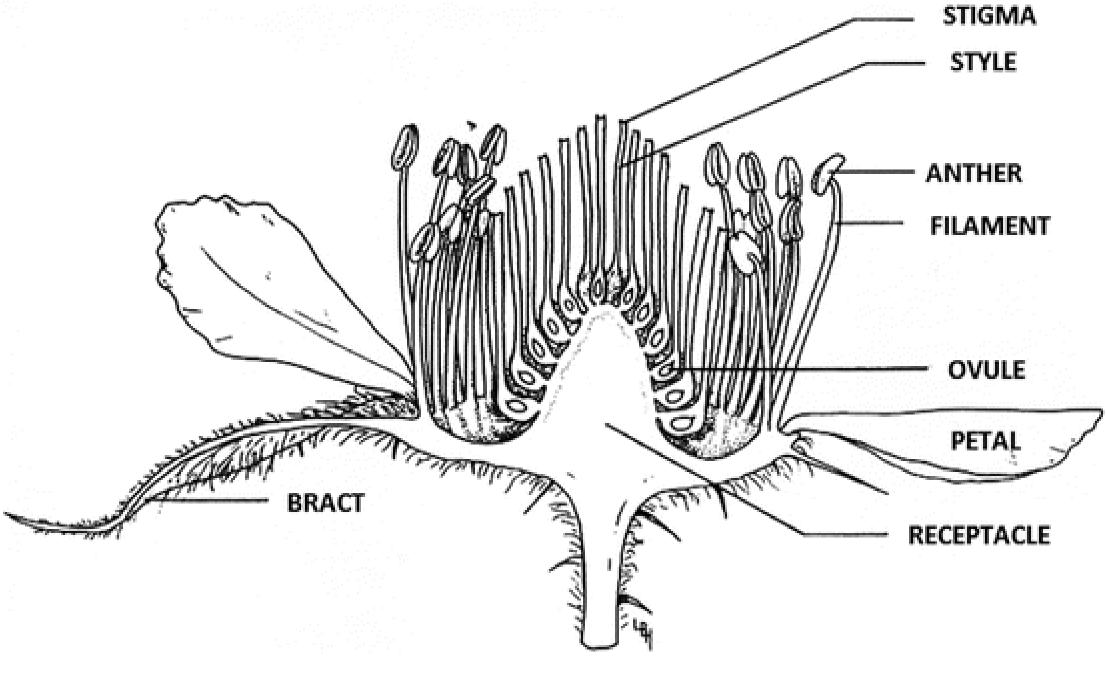

Figure 1.2 Longitudinal section of 'Willamett' raspberry flower (McGregor, 1976).

1.6 Raspberry pollinator dependence meta-analysis

Pollinator exclusion studies are often used to determine the pollinator dependence of crop plants (Klein *et al.,* 2007). ‘Pollinator dependence’ is the percentage of a plants reproductive output or yield that is directly attributable to animal pollination. Flowers are excluded from pollinators using mesh and the yield from pollinator excluded and animal pollinated flowers are compared. For fruit crops fruit set, size, weight, or seed set is usually compared. I performed a meta-analysis to identify the pollinator dependence of raspberry commercial crops.

1.6.1 Methods

Web of Science was used to perform an extensive literature search, last updated December 2021. The search string: [raspberr* OR "red raspberr*" OR "Rubus idaeus"] AND [pollinat* OR "pollinator dependence*"] AND [exclusion* OR "crop productivity*" OR "crop yield*" OR "yield gain*" OR "fruit set*" OR "fruit size*" OR "seed set*"] was used and returned 40 results. The titles and abstracts of the articles identified using this search were then screened to determine their relevance using the predefined exclusion criteria detailed below. For those articles where the inclusion criteria could not be assessed from the title and abstract alone, the full article was viewed before inclusion or exclusion. Studies were not excluded based on location however only articles published in English were included and so research from some geographic regions are likely to have been excluded. The inclusion criteria were as follows:

1. The study must empirically measure the effect of insect pollination on raspberry fruit production
2. The study must contain original results
3. The study must measure at least one of the following variables: fruit set, seed set, fruit weight or fruit size
4. The study must compare fruit production between pollinator excluded and insect pollinated flowers

The title, abstract and in some cases the full article was read to identify whether the study included original empirical data, included raspberry as a study crop, measured fruit set, seed set, fruit weight or fruit size of fruit produced by flowers that were insect pollinated and by flowers that had been excluded from insect visitors. The key words of fruit set, seed set, fruit weight and size along with pollinator and exclusion were scanned for. Reviews and meta-analyses were excluded from this analysis. Studies quantifying the insect visitors to raspberry crop flowers were not included in this review unless they included yield values from an exclusion experiment. The reviewer excluded studies that had no pollination treatments or exclusion studies, did not include raspberries in their study, were not a study on pollination or had no abstract or full text available. This led to six studies being selected for the analyses. I then extracted the mean, standard deviation and sample size from the text, supplementary material, or figures for both the control (insect pollinated) and experimental (insect excluded) treatments. Web plot digitiser was used to extract these values from plots where they were not stated in the text. One study was excluded at this point as no sample sizes were given, resulting in five studies being included in the analysis. One of the studies was carried out across two years and measured two of the desired yield metrics and so was represented four times in the analysis. Another also measured two yield metrics and two of the remaining studies measured the same yield metric but for multiple cultivars. This resulted in 12 replicates being included in the analysis. The *metafor* package in R (Viechtbauer, 2010) was used to calculate Hedge's *g* effect sizes for each replicate and fit a random-effects meta-analysis model to the effect sizes with a restricted maximum likelihood tau^2 estimator.

1.6.2 Results

There was an overall significantly negative effect of exclusion of pollinators on raspberry fruit yield (Est=-1.9278, SE = 0.4546, z=-4.2404, p<0.0001) (Figure 1.3). The test for heterogeneity was significant (Q (df = 11) =338.08, p<0.0001), indicating that the effect sizes across the replicates were not consistent.

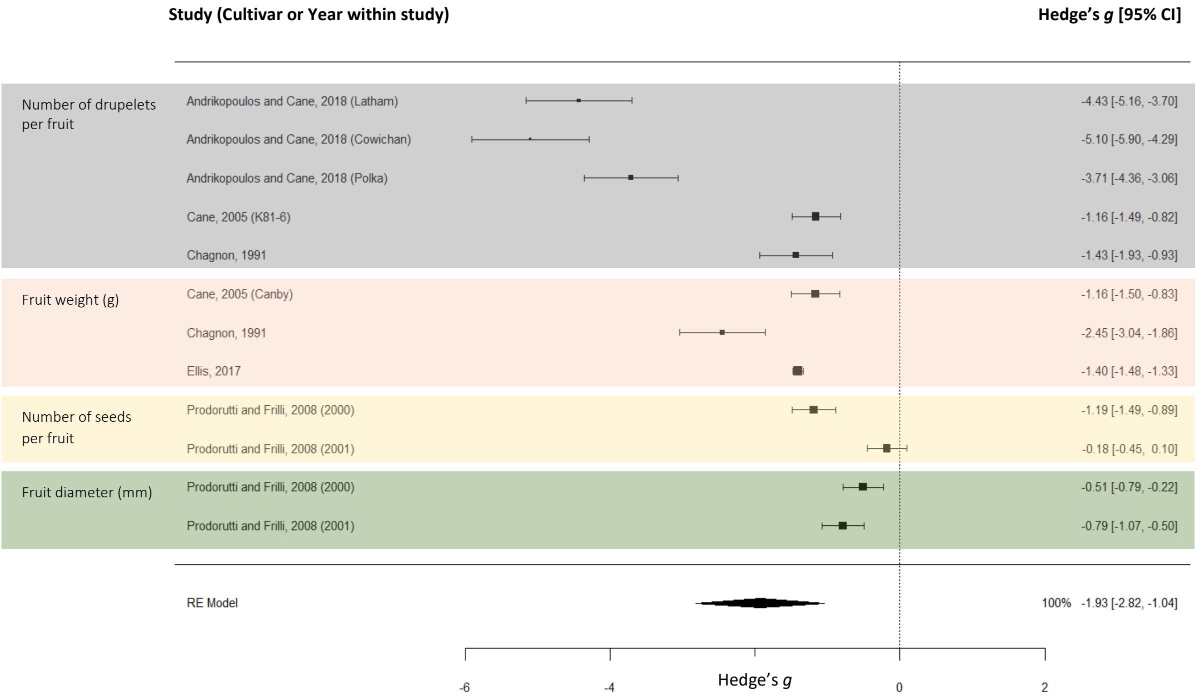

Figure 1.3 Forest plot of Hedge's *g* (black square) and 95% confidence intervals (whiskers) and the pooled Hedge's *g* effect size and its standard error (black diamond). The size of the square indicates the relative weight of the study on the overall Hedge's g

The mean pollinator dependence ($\bar{D}$) was calculated using the following formula:

$$D_i = \frac{\bar{x}_{IP}{}^{i} - \bar{x}_{IE}{}^{i}}{\bar{x}_{IP}{}^{i}}$$

D: pollinator dependence ratio

$\bar{x}_{IP}$: mean yield for insect pollinated flowers

$\bar{x}_{IE}$: mean yield for insect excluded flowers

i: replicate

Overall, the pollinator dependence was found to be 0.30, meaning that 30% of the yield of insect pollinated flowers was directly attributable to insect pollinators. There was, however, considerable variation observed between replicates, ranging from 0.06-0.68 in pollinator dependence.

1.6.3 Discussion

These findings indicate that the reliance of raspberries on pollinators for their yield is influenced by factors such as the yield metric, year, cultivar, and specific study conditions. Differences in pollination service provision may result in varying degrees of dependence, with the extent of reliance on pollinators fluctuating across different scenarios and studies. Some of the study sites may have experienced pollination deficits, but the assessment of such deficits requires the supplementation of hand pollen to open-pollinated flowers. Without this intervention, it remains challenging to determine whether observed differences between studies are attributable to varying pollination service provisions. Moreover, the study conducted by Prodorutti and Frilli (2008) suggests that pollinator service provision may also vary from year to year. To ensure accurate measurements of pollinator dependence it is crucial to incorporate pollen supplementation in pollinator dependence studies, thereby addressing and quantifying any potential deficits. Andrikopoulos and Cane (2018) found a between cultivar difference in pollinator dependence, thus assessing the pollinator dependence for multiple cultivars is important for determining crop level pollinator dependence estimates. The variance within and between studies could also be due to environmental factors that affect yield, and differ between sites, such as weather, soil nutrients and agricultural inputs and their interactions with pollinator service provision.

Soil fertility, fertilizer application, water availability and pest control have all been shown to affect pollinator dependence (Tamburini *et al.,* 2019). For example, low fertilizer availability can reduce the pollinator dependence of plants by limiting crop yield (Chen *et al.,* 2021; Garratt *et al.,* 2018; Tamburini *et al.,* 2017). Sites with suboptimal nutrient conditions are therefore likely to have low pollinator dependence and not achieve maximum yield levels despite high levels of insect pollination.

High nutrient availability has mixed effects on pollinator dependence. The relationship between fertilizer input and pollinator dependence has been found to be positive for Oilseed Rape (Garratt *et al.,* 2018) and unimodal for Sunflowers due to the compensatory effect of high fertilizer levels on pollinator limitation (Tamburini *et al.,* 2017). Variability in pollinator dependence between sites is therefore likely to be highly influenced by crop and soil management.

In highly managed crop systems like soft fruit cultivation, within-farm variation in soil fertility and water and fertilizer availability is mitigated through a combination of potted plants, drip fertigation and irrigation. However, exposure to weather, temperature and pollination service provision can vary along polytunnels and across fields and result in inconsistent yields (Hall *et al.,* 2020; Nielsen *et al.,* 2017). The limitation on yields that these environmental variables create could thus affect pollinator dependence at a plant level.

Based on the pollinator dependence values calculated for these studies, it can be concluded that raspberries fall into the categories of little (>0-<10% yield reduction), modest (10-<40% yield reduction) and great (40- <90% yield reduction) need for animal-mediated pollination, as defined by Klein *et al.* (2007). The variability observed between and within these studies prompted the exclusion study carried out in Chapter 2 and the exploration of the potential causes of pollination service variability (Chapters 3, 4 and 5) that may be determine the observed pollinator dependence. The meta-analysis comprises studies conducted in Utah, Quebec, north-eastern Italy and Scotland. In contrast our pollinator exclusion study, presented in Chapter 2, investigated pollinator dependence in southern England thereby expanding the scope of geographic regions examined and enhancing our understanding of pollination in the UK. Most of these studies use continuous measurements of fruit yield without imposing marketability thresholds such as aesthetic qualities such as drupelet evenness or minimum required size or weight. The absence of these marketable metrics may mean that the true value of pollinators to commercial raspberry crop yields has been underestimated.

1.7 Measuring the pollinator effectiveness of raspberry pollinators

Pollinator effectiveness studies can be incorporated into pollinator exclusion studies to assess the use of different pollinator species for the pollination of a specific crop. Pollinator effectiveness (PE) is the proportion of the yield attributable to animal pollinators that can be achieved from a single visit (SV) from a particular pollinator species:

$$PE = \frac{SV - IE}{IP - IE}$$

Andrikopoulos and Cane (2018a), a study included in the meta- analysis, evaluated the pollinator effectiveness of five species of insect pollinators that are current or potential managed pollinators of raspberry in the US. The effectiveness of *Apis mellifera, Bombus* sp. (consisting of *Bombus huntii, Bombus nevadensis, Bombus griseocollis* and *Bombus fervidus*), *Osmia lignaria, Osmia bruneri* and *Osmia aglaia* were compared across three commercial cultivars (‘Royalty’, ‘Cowichan’ and ‘Latham’). Of the pollinator species tested, only *Apis mellifera* is native to the UK. *O. aglaia* yielded more drupelets per raspberry than *A. mellifera* for ‘Royalty’, while *Osmia bruneri* was not as effective as the other species tested for Latham. *O. bruneri, O. lignaria*, *Bombus* sp. and *A. mellifera* did not differ in their pollinator effectiveness for ‘Cowichan’. In a separate study, Cane (2005) also determined the pollinator effectiveness of *Apis mellifera* and *Osmia aglaia* for raspberry pollination and found that the two species were equal pollinators of raspberry. Saez *et al.* (2014) compared the deposition of raspberry pollen per visit between *Apis mellifera* and *Bombus terrestris,* reporting that pollen deposition increased significantly with *Apis mellifera* visits but not with *Bombus terrestris* visits. The study found no significant correlation of pollen grains per stigma with drupelet set. The variation between pollinator species and crop cultivar could be due to different floral characteristics such as availability of pollen or nectar or the foraging behaviour and duration. The degree of self-compatibility could also vary between cultivars as previously documented by Pinczinger *et al* (2021) for red raspberry. Thus, it is crucial to assess the effectiveness of pollinator species native to the UK to compare them to honey bees and to help inform pollination management on farms, especially in light of the negative impacts honey bees can have on native pollinator communities and the common use of honey bees for raspberry pollination in the UK (Ellis *et al.*, 2017; BerryWorld pers. comms.) and globally (Andrikopoulos and Cane, 2018a and Prodorutti and Frilli, 2001).

1.8 Quantifying raspberry pollinator requirements

While pollinator effectiveness studies are valuable for comparing different pollinator species, controlled visitation experiments that allow multiple visits to crop flowers are more accurate in estimating the number of visits required for each pollinator species to produce a marketable fruit (Kendall *et al*, 2020). This can be studied by permitting different numbers of visits to crop flowers and comparing their resulting yields. The optimal number of pollinator visits required to maximize raspberry crop yield is uncertain, with carrying estimates reported in the literature. Simulation modelling by Saez *et al*. (2018) suggested that drupelet set reached its maximum at 99% success for flowers receiving around 15-35 visits from *Apis mellifera* or 10-20 visits from *Bombus terrestris.* Similarly, Saez *et al.* (2014) observed that flowers that received approximately 10 pollinator visits per day were not pollen limited, based on visitation rates to open-pollinated flowers. Nevertheless, as lower visitation rates were not tested, it remains possible that fewer insect visits could still result in full fruit set. The findings of Chagnon *et al.* (1991) support this idea, as they determined that pollination and fruit set reached its maximum after 5-6 visits or ~150 accumulative seconds of visit time by *Apis mellifera*. Similarly, Andrikopoulos and Cane (2018b) found that approximately 127 seconds of visitation over two days, from either a honey bee or a native bumble bee was sufficient for maximal fruit set in the US, with no difference in drupelet set of the resulting berries compared to open pollinated flowers. The visits recorded were the first visit to a flower on two consecutive days. Thus, raspberry flowers are likely to require at least 5-6 visits in a single day or a single visit on at least two days during their receptive period to achieve maximum pollination. Garibaldi *et al.* (2020) proposed that these estimates be used in combination with site-specific visitation rates to inform pollinator management for crop pollination but depending on which estimate of the require number of visits to maximise raspberry yield is used, required visitation rates could range from 17-195 visits to

100 flowers per hour. Using the incorrect estimate could result in overstocking of managed bees, which could have negative impacts on both the crop and wild pollinator communities. To ensure that managed pollinators are stocked appropriately, more pollinator effectiveness studies are needed to help quantify the number of visits required more accurately for optimal pollination of raspberries and other crops. This would also aid in the understanding of why these estimates vary so greatly between studies.

1.9 Exploring spatial and temporal variation in pollinator visitation rate for informing pollinator monitoring methods

Understanding how visitation rates to crop flowers vary within and between days as well as across crop fields is important for predicting whether crops are limited by pollination or if managed pollinators are required. Nielsen *et al.* (2017) found that honey bee visitation rate to raspberry flowers was higher in areas that were not under polytunnels than those that were covered. The study also found that visitation rates were higher in the tunnel openings compared to in the middle of the tunnels. This effect was also found for raspberry and blueberry crops under tunnels in Australia (Hall *et al.,* 2020). Varying visitation rates to crop flowers along the length of polytunnels could lead to uneven pollination service provision and is important to account for if using target visit numbers as proposed by Garibaldi *et al.* (2020).

1.10 Thesis outline and aims

As discussed throughout sections 1.5-1.7 of this general introduction, although there are some studies that explore the pollinator dependence and requirements of commercial raspberry crops, the results vary considerably between studies and there remains to be any clear guidance on pollination management for raspberry crops or other soft fruit crops. I collected empirical field data with the aim of addressing main aims:

1.10.1 Quantify the pollinator dependence of raspberry using commercially important metrics

Although pollinator dependence has been measured for raspberry (Andrikopoulos and Cane, 2018a; Cane, 2005; Chagnon *et al.,* 1991; Ellis *et al.,* 2017; Prodorutti and Frilli, 2008; Chen *et al.*, 2021), only Ellis *et al.* (2017) considers marketability or the commercially important metrics of the resulting fruits. Their study does, however, use strawberry marketing standards to categorise raspberries as first and second class. Pollinator dependence ratios are often used to calculate the value of pollinators for crop production (Klein *et al.,* and references therein) as well as economic valuations of pollination services (Lautenbach *et al.,* 2012; Potts *et al.,* 2016). They can also be important to farmers and growers to determine the vulnerability of their crops to pollinator deficits and declines. Estimates therefore need to be accurate and crop specific and should be determined using commercially relevant metrics and thresholds to accurately inform pollination management and conservation. In Chapter 2, I address this flaw in most pollinator dependence studies and calculate the pollinator dependence of commercial raspberries using two commercially important metrics, marketable fruit set and marketable fruit weight. I also explore how these estimates can vary with study year and cultivar, highlighting the need for more multi-year studies and crop level pollinator dependence to be based on multiple cultivars. The two cultivars used in the following studies are the high-end commercial cultivars ‘Diamond Jubilee’ and ‘Sapphire’ which together make up ~17% of the UK’s raspberry production in tonnes. While not a large proportion of total raspberry production, their high-quality means that they represent a greater proportion of UK raspberry production by total market value and an important cultivar for growers due to their higher price point per unit.

1.10.2 Quantify pollinator requirements of raspberry crops

While the pollinator effectiveness of *Apis mellifera* for raspberry crop pollination has been quantified in other studies (Andrikopoulos and Cane, 2018a; Cane, 2005) other UK pollinator species are yet to be tested. If reliance on honey bees is to be reduced to aid yield stability and wild pollinator conservation, it is important that the ability of wild pollinators to pollinate this economically important crop is assessed. In Chapter 3, I compare the pollinator effectiveness of *Bombus terrestris,* a common UK bumble bee, with honey bees *Apis mellifera* to determine the ability of wild pollinators to provide pollination services to raspberry crop flowers in the absence of honey bee hives.

1.10.3 Explore the drivers behind visitation rate variation to inform pollinator monitoring methods

Determining the visitation rate of managed and wild pollinators to crop flowers is a vital monitoring method for informing pollinator management (Garibaldi *et al.,* 2020) and identifying whether managed pollinators are overstocked. Spatial and temporal variability in visitation rates could therefore hinder accurate estimation of pollination service provision. In Chapter 4, I compare visitation rates to crop flowers in different locations within the field to add to the growing knowledge base of the effect of polytunnels on fruit crop pollination (Hall *et al.,* 2020; Nielsen *et al.,* 2017). In Chapter 5, I compare the visitation rate to crop flowers throughout their receptive period to determine the distribution of insect visits. This is crucial information for informing target visitation rates as a method of pollination service monitoring. I also compare visitation rate data from Raspberry Pi cameras and short timed flower counts to determine the most efficient and accurate method of measuring pollinator visitation. I am also currently helping to develop an AI tool to enable Raspberry Pi cameras to be used to measure day long visitation rate to other crops. My thesis has a heavy focus on the methods we use to measure the importance of pollinators to crops and monitor pollination services as well as the more specific pollinator requirements of commercial raspberries.

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2.0 The importance of multi-year studies and commercial yield metrics in measuring pollinator dependence ratios: a case study in UK raspberries Rubus idaeus L.

2.1 Abstract

1. The benefit of pollinators to crop production is normally calculated using ‘pollinator dependence ratios’, which reflect the proportion of yield lost (here reported as a value between 0 and 1) in the absence of pollinators; these ratios are quantified experimentally using pollinator exclusion experiments. Pollinator dependence ratio estimates can vary considerably for a single crop, creating large, frequently overlooked, uncertainty in economic valuations of pollinators. The source of this variation is usually unclear.
2. We experimentally measured the pollinator dependence ratio of two UK commercial cultivars of raspberry *Rubus idaeus* L., using a range of yield metrics - fruit set, marketable fruit set, fruit weight and marketable fruit weight- over three years (2019-2021), to quantify the effects of yield metric, inter-annual variation, and cultivar on pollinator dependence ratio estimates.
3. We found a difference in pollinator dependence ratio for fruit set of 0.71 between 2019 and 2020, showing the importance of carrying out exclusion studies over multiple years
4. Averaged over multiple years and two cultivars, the dependence ratio was 0.68 measured using marketable fruit set and 0.64 using marketable fruit weight. Imposing a quality threshold (size, shape) below which fruits would not be of commercial value (marketable fruit set/weight) dramatically increased both the pollinator dependence ratio, and subsequent economic valuations of pollination service derived from it.
5. Our study shows that, for raspberry, estimates of the pollinator dependence ratio, and therefore the economic value of insect pollinators, are highly sensitive to the choice of yield metric, and can change between years and cultivars. Many economic decisions about pollinator management, at farm, regional and national scales, rely on estimates of pollinator dependence. We therefore recommend that, for estimating pollinator dependence ratios,

pollinator exclusion studies are conducted over three or more years and use yield metrics that incorporate quality criteria linked to actual market values and commercial thresholds.

2.2 Introduction

It is well-known that pollinators are important for the reproduction of flowering plants. An estimated 79% of angiosperms have improved seed set in the presence of pollinators (Rodger *et al.*, 2021), while 75% of the world's major food crops depend on pollination to some extent, to produce the edible or marketable parts of the plant (Klein et al, 2007). This dependence translates to between 5 and 8% (by volume) of human food produced globally being a direct result of animal pollination (Aizen et al., 2009; Potts et al., 2016). These estimates are all based on empirically derived dependence ratios, which measure the loss of yield in the absence of pollinators (Gallai *et al* 2009), for each type of crop. The dependence ratios from Klein et al (2007), along with 2009 market prices and production, were used by Lautenbach et al. (2012) to provide an annual economic value of pollinators to global crop output. Inflated to 2015 US$, this equates to US$235 - 577 billion per year (Potts et al., 2016).

These estimates of the overall economic value of pollinators for crop production allow us to assess the potential economic consequences of observed pollinator declines (e.g. UK declines shown by Powney *et al.,* 2019). They are used as motives or incentives for pollinator conservation (Kleijn et al 2015), and to evaluate how much should reasonably be invested in crop pollination service management (Allsopp *et al.,* 2008) and pollinator monitoring (Breeze *et al.*, 2021).

Exclusion studies can be used to measure dependence ratios by preventing pollinators from visiting study flowers using mesh bags or cages and comparing the resulting yield to that from flowers which had access to pollinators. Pollination deficits (any loss in yield due to a lack of pollination) can be quantified by providing additional pollen to flowers that have access to pollinators (usually done by hand using a paintbrush) and comparing the resulting yield to flowers that were not pollen supplemented. Pollination deficits can be used to determine whether and how much pollination service management needs to be improved to maximise yields. Pollinator exclusion and pollen supplementation experiments have been used to quantify the pollinator dependence and pollination deficits of over 80 different crops (Klein *et al,* 2007), including apple (Garratt *et al,* 2014), strawberry (Klatt *et al,* 2014) and raspberry (Andrikopoulos and Cane, 2018; Cane, 2005; Chagnon *et al*, 1991; Chen *et al*, 2021; Ellis *et al*, 2017; Prodorutti and Frilli, 2008). Pollinator dependence and pollen limitation has also been assessed for some wild flowering plants (Koch *et al,* 2020; Ratto *et al,* 2018; Rodger *et al,* 2021).

The yield metrics used for comparison between pollination treatments, and thus the basis for dependence and economic valuations, vary between studies. Even within yield quantity parameters e.g. mass per unit or number of units, selecting a yield parameter of 'direct economic benefit' is important as the relationships between yield metrics can be non-linear as shown in field bean by Bishop *et al* (2020). Yield metrics used for fruit crops include the proportion of crop flowers that produce a fruit (% fruit set), and the weight and size of the resulting fruit. These metrics reflect commercial value, as produce is normally paid for by weight or volume. However, in industry there can be minimum thresholds for commercially acceptable sizes or weights, below which fruits cannot be sold or have reduced value. These thresholds are frequently overlooked. Many raspberry pollination studies, for example, do not account for a lower size threshold of marketability in measuring yield attributable to insect pollinators (e.g Chagnon *et al*. (1991), Cane. (2005), Prodorutti and Frilli (2008), Andrikopoulos and Cane (2018), Chen *et al* (2021, 2022)). Species-specific yield metrics used to reveal details of the pollination process, such as drupelet set in raspberries (e.g. Andrikopoulos and Cane, 2018), can also be less relevant to industry.

As well as influencing the *quantity* of fruit, insect pollination can also impact aesthetic fruit qualities such as shape and colour. These metrics, along with size or weight, can affect the price per tonne, for example when produce is given a classification or grading for market, based on both quantity and quality metrics as for apples (Garratt *et al.,* 2014). Accounting for the difference in price per unit between classes is therefore important for economic valuations of pollinators to crops as shown by Garratt *et al.* (2014). These aesthetic qualities such as shape, colour or, in the case of raspberries, uniformity and wholeness can also result in fruit being classified as unmarketable and not entering the market place at all (BerryWorld pers. comm., December 2019). It is therefore important to measure the benefits to crop quality as well as quantity, when calculating the contribution of pollinators to crop production value. This is especially true for those species such as strawberries, dessert apples and raspberries which are self-fertile in their reproduction and able to produce some fruit in the absence of pollinators but require pollinators to produce marketable quality fruit, as measured by Ellis *et al* (2017), Garratt *et al.* (2014) and Klatt *et al* (2014). Yield quality is also important for seed crops as their market value lies in the viability and vigour of the seeds and so including seed quality in pollinator dependence valuations, such as in Fijen, *et al* (2018), is essential for measuring the true value of pollinators to crop production. Including fruit that are too small or light, or otherwise unsuitable for market in calculations of pollinator dependence ratios and resulting economic valuations will underestimate the economic value of pollinators to food crops in cases where fruit quality, or price band, is improved by pollination. Likewise, the benefit of pollinators to yield can be overestimated if quality metrics aren't considered where high densities of pollinators have detrimental impacts on fruit quality (Aizen *et al,* 2014; Monasterolo *et al,* 2022; Sáez *et al,* 2014).

In 2020, UK raspberry (*Rubus idaeus* L.*)* production was valued at £133.3 million, 12.8% of total UK fruit production value for that year and the third largest income for a single type of fruit behind strawberries and dessert apples (Defra, 2021). Despite most commercial cultivars being self-fertile i.e. being able produce seeds with pollen from the same plant, unlike wild varieties, the structure of the reproductive parts of *Rubus idaeus* flowers prevents unaided complete self-pollination (Free, 1970; McGregor, 1976) and thus the production of commercially viable fruit. Each pollinated pistil produces a single fruiting body, called a drupelet, containing a single seed, or in some cases two (Funt and Hall, 2013). The more stigmas that are pollinated, the more drupelets will develop and the larger the fruit. Raspberries are termed aggregate fruit, as each 'fruit', or 'berry', is made up of multiple drupelets. When picked they are separated from their receptacle. This means that to remain whole, there needs to be enough cohesion between drupelets. If too few drupelets develop, the fruit is crumbly and unmarketable (Andrikopoulos and Cane, 2018). The majority of a flower's ovules need to be fertilized to produce commercially marketable fruit (Cane, 2005). The benefit of pollinators to fruit quality can therefore make the difference between marketable and unmarketable fruit.

Various pollinator exclusion studies (Andrikopoulos and Cane, 2018; Cane, 2005; Chagnon *et al*, 1991; Chen *et al*, 2021; Ellis *et al*, 2017; Prodorutti and Frilli, 2008) have enabled pollinator dependence ratios to be calculated for raspberries (Klein *et al.*, 2007). These studies found a reduction in fruit yield of between 10 and 70% when pollinators were excluded, in comparison to open pollinated flowers. This range runs substantially lower than the dependence category of 40-90% yield loss in the absence of pollinators, reported for raspberry by Klein *et al.* (2007), and its central point is lower than the central value of 65% used in economic analyses by Lautenbach et al. (2012) and Potts et al. (2016). This suggests that although pollinators improve raspberry yields, they aren't essential for raspberry plants to produce fruit and their value may previously have been over-estimated. Although most cultivars are considered self-fertile (Keep, 1968), perhaps gaining this ability during their domestication (Jennings, 1988), the number of fruit produced and the number of seeds and drupelets within those fruit when left to self, varies between cultivars (Pinczinger *et al*, 2021).

To some extent, the differences in pollinator dependence estimated by these studies may reflect the true pollinator dependence of different raspberry cultivars. This highlights the need to assess the pollinator dependence in multiple cultivars, also highlighted for apple by Garratt *et al*, (2014), when looking to determine the pollinator dependence of raspberry crops as a whole. However, a number of other factors are known to contribute to variation in measured pollinator dependence ratio, including abiotic and biotic factors such as soil fertility (especially nitrogen availability), temperature, water availability, and the composition of the pollinator community (e.g. see Chen et al 2021) as well as pest levels or control (Lundin, *et al* 2013; Sutter and Albrecht, 2016). The extent to which these factors explain the differences in estimates of pollinator dependence in the literature is largely unknown and requires substantial additional research in each crop type, across multiple systems, to elucidate.

Methodological details, including the selected yield parameters and number of study years, are also likely to be important sources of variation in pollinator dependence ratio, as shown clearly for *Vicia faba* by Bishop et al (2020). Rather surprisingly, to our knowledge, commercial quality and size thresholds below which fruit would not be marketable, have not previously been used to study pollinator dependence in raspberry. Unlike strawberries and apples there is no government class specification for raspberries in the UK, instead this is normally dictated by retailers who differ in their requirements (BerryWorld pers. Comms, December 2019).

Of the raspberry pollinator exclusion studies cited above, only Prodorutti and Frilli (2008) performed exclusion studies for more than one year. Pollinator community composition has been shown to vary markedly between years at the same site (Rader *et al.,* 2012; Russo *et al.,* 2015; Senapathi *et al.,* 2021), and we might expect measured pollinator dependence also to vary as a result. This is because the comparison between bagged and open flowers in standard exclusion experiments measures the pollination service being provided by the pollinator community that happens to be present, in that particular ecological context. Garibaldi *et al.* (2011) reported that interannual stability of pollinator-dependent crops is lower than the stability of pollinator-independent crops, likely due to this variability and the close relationship between pollinator species richness and plant reproductive success and yield (Albrecht *et al.,* 2012). Multi-year analyses are therefore essential, to determine how inter-annual variability affects individual pollinator-dependent crops and their resulting pollinator dependence, especially when estimating the economic value of pollinators.

Using exclusion studies over three years, we experimentally tested the combined effects of pollination treatment, study year and crop cultivar on raspberry yield for two different metrics (fruit set and fruit weight), with and without accounting for marketability. We calculated the different pollinator dependence ratios and determined whether there was a pollination deficit despite high managed pollinator input. We asked whether and how much the pollinator dependence ratio differed between years, cultivars and yield metrics to determine whether these are likely causes of the variation in pollinator dependence found between raspberry pollination studies. We asked whether the mean pollinator dependence taken from Klein *et al.* (2007) and used by Lautenbach *et al*. (2012) and Potts *et al*. (2016) is representative of raspberry pollinator dependence in our study system considering this variation between years, varieties and yield metrics. We also calculated the impact of implementing market thresholds within the yield metrics, on economic valuations of pollination service provision to commercial UK raspberry crops.

2.3 Methods

2.3.1 Study site

The study was carried out on an 81-hectare commercial soft fruit farm near Reading, south England (51°29′32″N, 000°52′28″W) throughout the period of June to September in 2019, 2020 and 2021. Two self-compatible cultivars of red raspberry (*Rubus idaeus*); 'Diamond Jubilee' and 'Sapphire', were included in the study, both developed by BerryWorld and made available for growers in 2013 (BerryWorld pers. comm., December 2022). Both cultivars were grown throughout each study period. Each experimental site was made up of one commercial field of >1.5 ha surrounded by uncropped field margins. There were small areas of semi-natural grassland and patchy woodland on the farm and within the immediate surrounding area. Both raspberry cultivars were grown under Spanish polytunnels in drip fertilized and irrigated pots, each with two canes per pot. Raspberry canes are only harvested for one growing season. 153 and 149 rented honeybee hives were in place at the farm during 2019 and 2020 respectively, equating to ~2 hives/ha of farmland, throughout the raspberry flowering season, for the purpose of crop pollination of both raspberries and strawberries. This dropped to 81 colonies in 2021, due to colony losses and relocation to other sites reducing the stocking rate to 1 hive/ha. No managed bumblebee colonies (*Bombus terrestris*) were active on the farm during the study periods in 2019 and 2020. A few colonies were still active in an adjacent field to Diamond Jubilee in 2021 however, they were at the end of their 10th week *in situ* when the first study flowers opened. It is therefore likely that the bees leaving these colonies were gynes and males.

2.3.2. Exclusion study pollination treatments

Crop plants were randomly selected across each field in each year; 19 in 2019 (Diamond Jubilee: n=9, Sapphire: n=10), 30 in 2020 (Diamond Jubilee: n=10, Sapphire: n=20) and 36 in 2021 (Diamond Jubilee: n=16, Sapphire: n=20). Canes were only used for one growing season at the study site and so new canes were selected each year for the exclusion study. Cultivars of raspberry and strawberry were also rotated between fields and so repeated sampling from the same field in multiple years was not possible. Sample size was increased in later years to provide contingency, since some inflorescences were lost from the experiment in 2019 and 2020, due to accidental picking or disease. For each plant three lateral branches with ≥7 flower buds were selected and randomly assigned to one of three treatments; insect pollination (IP), insect exclusion (IE) and insect exclusion with pollen supplementation (IES) or ‘hand pollination’, for 2020 and 2021 this was exactly 10 buds per treatment on each plant but for 2019 this varied between 7 and 18. Any open flowers were removed at the start of the study. The flowers assigned to the insect exclusion and insect exclusion with pollen supplementation treatments were covered by 27x27cm bags made of 1 mm mesh, tied at the bottom with string to prevent pollination by insects (see Fig. S2.1 for an image of this set up). The tops of the bags were folded and sealed with paperclips to allow them to be easily opened during hand pollination and harvesting without damaging the flowers or developing fruit. Another treatment of insect pollination with pollen supplementation (IPS) was included in 2020 and 2021 to give a maximum potential fruit production value, as this wasn’t provided in 2019 by the insect exclusion with pollen supplementation treatment due to hand pollinated flowers in bags yielding significantly less fruit than open pollinated flowers. The flowers for this treatment were left un-bagged to allow insect pollinators to visit and were hand pollinated with additional pollen. The pollen supplemented flowers were hand-cross pollinated using a soft bristle paintbrush to transfer pollen from non-study to study flowers of the same cultivar. Flowers were not emasculated for any of the treatments and so self-pollination was still possible. By leaving flowers intact, our treatments replicate the current commercial yield (insect pollination), the maximum possible fruit yield (insect pollination with pollen supplementation) and the expected yield if all insect pollinators were lost, with (insect exclusion with pollen supplementation) and without (insect exclusion) human intervention. Hand pollinated flowers were pollinated at least twice during their receptive period (≥2 days), with the first pollination event no more than two days since flower opening (Anrikopoulos and Cane, 2018 and Bekey, 1985). Flowers were only pollinated on dry days as pollen was hard to collect and transfer when wet.

Many Diamond Jubilee fruit were lost in 2019 to commercial harvests and so, for Sapphire, which flowered later in 2019 and for both cultivars in 2020 and 2021, bags were placed over the developing fruit for the insect pollinated treatments once all flowers had dropped all their petals and the tips of their stamens had turned brown and started drying. In 2021, some insect pollination with pollen supplementation fruit were lost to commercial harvests before bags could be added. Fruit picked by the harvesters were included in both fruit set analyses, identified by the presence of a receptacle, as it was assumed that they were marketable when picked.

2.3.3 Fruit collection and measurement of fruit set and quality

Fruit (entire raspberries, comprising multiple drupelets) were harvested when bright red and the fruit could be detached easily from the receptacle and counted. In 2020 and 2021, fruit were also weighed and measured (length and width) at the widest points using callipers. Fruit that were visibly infected with moulds such as *Botrytis* or *Phytopthora* species, which both cause small, hardened unripe fruit, were excluded from the analysis as pollinator dependence could not be assessed. All other fruit were included in the analysis for fruit set, but only those that were classed as 'marketable' by satisfying the criteria for commercial whole fruit sales were included as marketable fruit. To be counted as 'marketable', each fruit had to be whole (i.e., *not* missing drupelets or crumbly), without excessive bubbled drupelets (drupelets of dramatically different sizes, see Fig. S2.2a for examples), have a length of ≥15mm and a weight of ≥3g (the minimum requirement of any BerryWorld affiliated retailer; BerryWorld pers. Comms, December 2019). The pollinator dependence ratio of the crop was defined as the proportion of yield in the insect pollinated (IP) yield treatment that was directly attributable to insect pollinators. We calculated this for each of the following yield metrics; fruit set

(%), marketable fruit set (%), fruit weight (g) of each individual fruit, and marketable fruit weight (g) of each individual fruit (excluding unmarketable fruit).

This was calculated using the following formula:

$$D = \frac{\bar{x}_{IP} - \bar{x}_{IE}}{\bar{x}_{IP}} \tag{1}$$

D: pollinator dependence ratio

$\bar{x}_{IP}$: mean yield metric (e.g. % fruit set) for insect pollinated flowers

$\bar{x}_{IE}$: mean yield metric (e.g. % fruit set) for insect excluded flowers

Fruit set was calculated as the percentage of flowers that resulted in a fruit, so buds that did not result in a flower were excluded from this analysis.

2.3.4 Economic valuation

The economic value of insect pollination to raspberry production was calculated following the bioeconomic approach of Gallai *et al* (2009). This equation uses the pollinator dependence ratio (D), along with the quantity of commercial crop (in tonnes) produced (Q) and the price per tonne received at market (P):

$$Economic\ value\ of\ insect\ pollination = D \times Q \times P \tag{2}$$

For this study we used Defra’s horticultural statistics (Defra, 2021) which gave a total national production economic value ($Q \times P$) for 2020 (2021 data not confirmed at time of submission) multiplied by our dependence ratios for fruit set and marketable fruit set for the two cultivars and three years combined. Due to the differing prices per tonne for each retailer, cultivar specific economic valuations of pollination services to the crop were not calculated. For illustration purposes only we used the pollinator dependence ratios calculated in this study to represent all UK-grown commercial raspberry crops in this equation.

2.3.5 Statistical Analyses

Analyses were conducted using general(ised) linear models (GLMMs) in R statistical software (version 4.1.3)(R Core Team, 2022) using the glmmTMB R package (v. 1.1.5; Brooks *et al,* 2017) and the lmerTest R package (v.3.1.3; Kuznetsova *et al*, 2017). Four responses were analysed: fruit set, marketable fruit set, fruit weight and marketable fruit weight, and each was tested against two major explanatory fixed effects: pollination treatment, and year. Cultivar was also included as a fixed effect for the fruit set models as both cultivars were modelled together. Models testing fruit set and marketable fruit set had a binomial response of the number of successes (flowers that developed into fruit/marketable fruit) and failures (flowers that did not develop a fruit/developed an unmarketable fruit) per treatment branch, accounting for differences in the number of flowers per branch, and a beta-binomial error distribution was used to account for overdispersion. Fruit weight and marketable fruit weight were normally distributed and modelled with a Gaussian error structure. Nested random effects were included in each model to account for the structure of the experimental design, shared growing conditions and pollinator exposure, and avoid pseudoreplication issues, with plant ID within field ID used for fruit set and marketable fruit set models, and branch ID within plant ID for fruit weight and marketable fruit weight models. Branch ID was not necessary as a random effect in the fruit set models as the response was already modelled per branch, and field ID was not included in the fruit weight model due to lack of power and very minimal impact. For per berry marketable fruit weight, the two cultivars Diamond Jubilee and Sapphire were modelled separately as the differences in fruit size and appearance are already known and are the primary reason for the cultivation of both varieties at the study farm. Maximal models were employed without simplification and acceptable model fit was assessed from residual plots. Fruit width and length were not modelled as they were both found to have a significant positive correlation with fruit weight (Corr.coeff = 0.895, $p<0.0001$, and Corr.coeff= 0.855, $p<0.0001$ respectively). Full details of all variables and maximal models are shown in Table 2.1.

Table 2.1. Description of the structure of each response and explanatory variable used, and the GLMM structures constructed from them. The years included in each model are provided separately for the fruit set and fruit weight models as only two years of fruit weight data were used.

Type	**Variable**	**Distribution (link/offset)**	**Definition**
Response	Fruit set	Beta binomial (logit)	Proportion of flowers producing a fruit (fruit/no fruit) accounting for number of study flowers on each lateral branch
	Marketable fruit set	Beta binomial (logit)	Proportion of flowers producing a fruit of a marketable size and quality (marketable/not marketable) accounting for number of study flowers on each lateral branch*
	Fruit weight	Gaussian (identity)	Mass in grams of individual fruit
	Marketable fruit weight	Gaussian (identity)	Mass in grams of individual fruit that were of a marketable size and quality*
Explanatory	Pollination treatment	Four level categorical factor	Insect pollination (IP), Insect exclusion (IE), insect pollination with hand pollen supplementation (IPS) and insect exclusion with hand pollen supplementation (IES)
	Year	Three level categorical factor for fruit set/ marketable fruit set models Two level categorical factor for fruit weight/marketable fruit weight models	2019-2021 2020-2021
	Crop cultivar	Two-level categorical factor	Two varieties of commercial raspberry (Diamond Jubilee and Sapphire), grown in separate fields
Random	Field/Plant_ID	Six level categorical factor/72 level categorical factor	Unique identifier for each study plant within each field (72 plants across 6 fields and 3 years (2019:2021))
	Plant_ID/Branch	60 level categorical factor/four level categorical factor	Unique identifier for each study plant and each branch within study plant (four branches on each of 60 plants across 2020 and 2021)
Response	**Model structure**		
Fruit set	Crop cultivar + Pollination treatment + Year + (1|Field/Plant_ID)		
Marketable fruit set	Crop cultivar + Pollination treatment + Year + (1|Field/Plant_ID)		

Diamond Jubilee weight	Pollination treatment + Year + (1|Plant_ID/Branch)
Sapphire weight	Pollination treatment + Year + (1|Plant_ID/Branch)
Diamond Jubilee marketable weight	Pollination treatment + Year + (1|Plant_ID/Branch)
Sapphire marketable weight	Pollination treatment + Year + (1|Plant_ID/Branch)

*See text, section 2.2.3 for a description of the required size and quality for marketability

2.4 Results

In total 2,456 ripe fruit were harvested from 2,733 study raspberry crop flowers across both varieties, all years and pollination treatments. 110 of these were harvested by commercial pickers so we could not weigh or measure them, though they were included as marketable fruit in the fruit set analyses. 385 fruit were excluded from all analyses as they were infected with *Botrytis sp.* or *Phytopthora sp.* Flowers and fruit that were on lateral branches damaged by humans were also removed from the study. Of the 447 unmarketable berries across all pollination treatments in 2020 and 2021, 60% were deemed unmarketable due to bubbles or crumbliness, 31% was due to both bubbles or crumbliness and being underweight (due to low drupelet numbers) and only 6% was due to being underweight without the presence of bubbles or crumbliness.

2.4.1 Fruit set

The percentage of raspberry flowers that produced marketable fruit was related to crop cultivar, year, and pollination treatment (Fig.2.1a-c; Table S2.1). Hand pollen supplementation of insect pollinated flowers (IPS: n=58 branches, 92.53% ± 2.58) did not significantly increase percentage marketable fruit set compared to insect pollinated branches (IP: n=72, 93.93% ± 2.00) (Fig. 2.1a; z=-2.574, P=0.01), in fact marketable fruit set was significantly lower for pollen supplemented flowers, perhaps due to interference between pollen tubes. These results show that there was no pollination deficit for raspberry crop flowers in this system when producing marketable fruit. Insect excluded branches (IE:

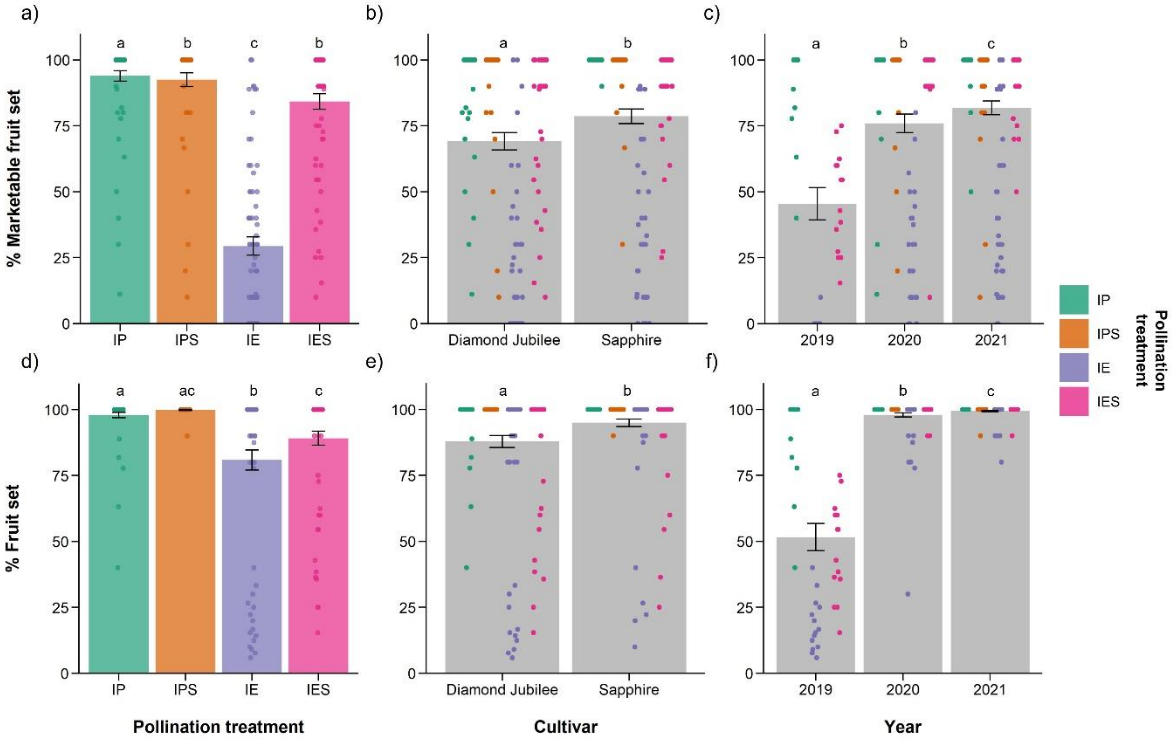

Figure 2.1 The mean percentage of study flowers that produced a marketable fruit (a-c) and a fruit whether marketable or not (d-f) under a, d) four different pollination treatments, for b, e) two commercial cultivars of red raspberry *Rubus idaeus*; ‘Diamond Jubilee’ and ‘Sapphire’, and c, f) three consecutive study years. Pollination treatments were Insect Pollinated (IP), Insect Pollinated with hand pollen Supplementation (IPS), Insect Exclusion (IE), and Insect Excluded with hand pollen Supplementation (IES). Standard errors are shown. Different letters show significant differences between levels of each variable.

n=72, 29.41% ± 3.48) yielded significantly less marketable fruit than insect pollinated (Fig. 2.1a; z=-11.556, P<0.0001), showing that pollinators are important for producing high marketable yields of these cultivars of raspberry. Hand pollinated bagged branches (IES: n=71, 84.23% ± 2.91) yielded significantly less marketable fruit than insect pollinated branches (Fig. 2.1a; z=-4.592, P<0.0001), indicating a negative effect of hand pollination, perhaps due to physical damage or incomplete pollination. Cultivar was a significant predictor of marketable fruit set, with Sapphire (n=142 branches, 78.64% ± 2.80) yielding a significantly higher % marketable fruit set from all study branches across all treatments compared to Diamond Jubilee (n=131 branches, 69.16% ± 3.27) (Fig. 2.1b; z=2.173, p=0.0298). Total marketable fruit set differed between all three years (Fig. 2.1c), highlighting the variability of crop yields between years; fruit set was significantly higher in 2020 (n=100 branches, 75.96% ± 3.54) and 2021 (n=131 branches, 81.84% ± 2.59) than in 2019 (n=42 branches, 45.49% ± 6.11) (z=5.200, P<0.0001 and z=6.764, P<0.0001 respectively) and was also significantly higher in 2021 than in 2020 (z=2.115, P=0.0344) (Fig. 2.1c; Table S2.1).

We found similar results when using total fruit set without the marketability threshold (Fig. 2.1d-f; Table S2.1). Pollen supplementation of insect pollinated flowers (IPS: n=58 branches, 99.83% ± 0.17) did not significantly increase percentage fruit set compared to insect pollinated branches (IP: n=72, 97.93% ± 1.05) (Fig 2.1d; z=-1.059, P=0.2896). Insect excluded branches (IE: n=72, 80.89% ± 3.84) yielded significantly less fruit than insect pollinated (Fig. 2.1d; z=-9.040, P<0.0001). Hand pollinated bagged branches (IES: n=71, 89.13% ± 2.67) yielded significantly less fruit than insect pollinated branches (Fig. 2.1d; z=-6.178, P<0.0001). Cultivar was a significant predictor of fruit set, with Sapphire (n=142 branches, 94.97% ± 1.43) yielding a significantly higher % fruit set from all study branches across all treatments compared to Diamond Jubilee (n=131 branches, 87.85% ± 2.27) (Fig. 1e; z=2.198, p=0.0279). Fruit set was significantly higher in 2020 (n= 100 branches, 97.95% ± 0.90) and 2021 (n=131 branches, 99.47% ± 0.23) than in 2019 (n=42 branches, 51.63% ± 4.71) (z=10.067, P<0.0001 and z=9.365, P<0.0001 respectively) and was also significantly higher in 2021 than in 2020 (z=2.253, P=0.0242) (Fig. 2.1f; Table S2.1). The pollinator dependence ratio for both varieties combined is shown in Table 2.2 and visualised in Figure 2.3, showing the variability in dependence ratios between years and yield criteria.

2.4.2 Fruit weight

Our results for the effects of pollination treatment and year on marketable fruit weight are shown in Figure 2.2 and Table S2.2. Similar results for total fruit weight (including non-marketable fruit) are provided in Figure S3 and Table S2.3. Pollen supplementation of insect pollinated flowers did not significantly increase marketable fruit weight compared to fruit from insect pollinated flowers for either Diamond Jubilee (IP: n=154 fruit from 26 branches, 5.41 ± 0.15, IPS: n=222 fruit from 26 branches, 5.62g ± 0.17) (t=0.610, p=0.544) or Sapphire (IP: n=206 fruit from 32 branches, 7.11g ± 0.10, IPS: n=313 fruit from 32 branches, 6.80g ± 0.11) (t=-1.027, p=0.307) suggesting that marketable fruit weight was not pollen limited in either variety. The pollinator dependence ratio calculated using marketable fruit weight for both cultivars is shown in Table 2.2 in comparison to the other yield metrics measured in this study. We have also shown the pollinator dependence ratio using fruit weight including non-marketable fruit, to show how accounting for marketable quality when measuring the % of crop yield in grams affected the resulting pollinator dependence ratio. There was no significant year effect for either cultivar (Diamond Jubilee: $F = 3.313$, $p = .08$, Sapphire: $F = 1.828$, $p = .187$; Figure 2.2 b,d).

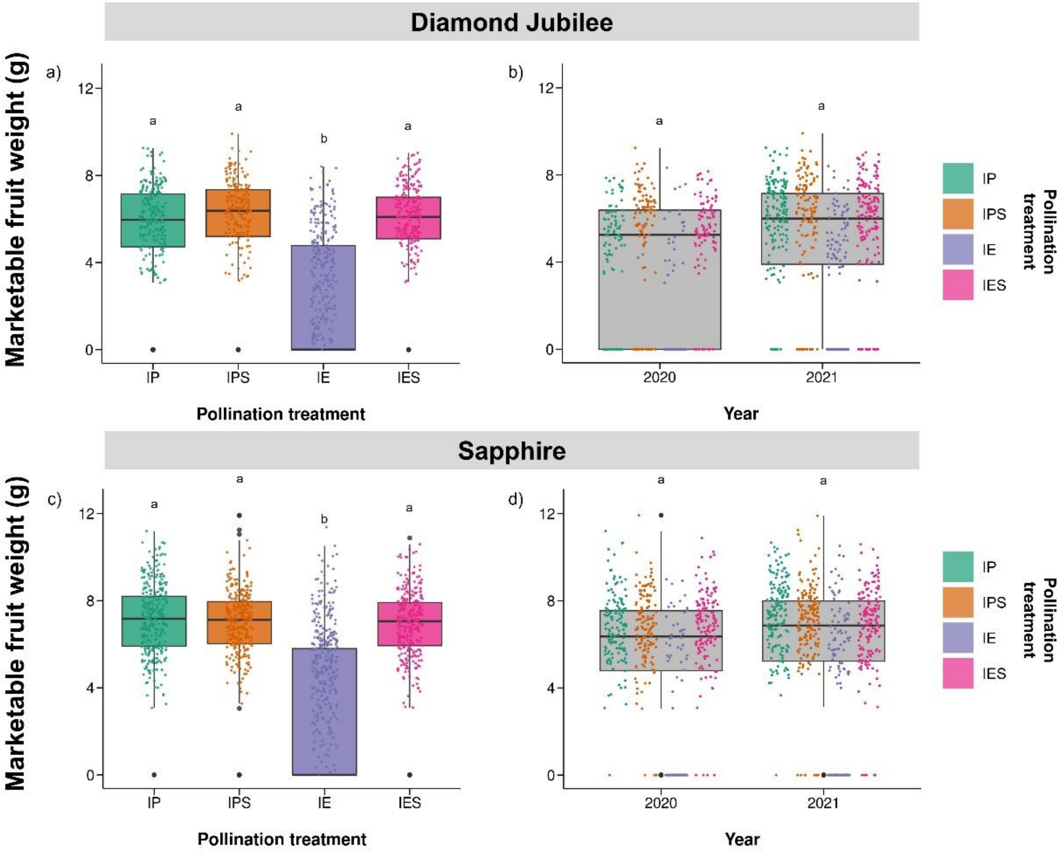

Figure 2.2. The median marketable fruit weight in grams of fruit produced by study flowers under four different pollination treatments (a&c) for two commercial cultivars of red raspberry *Rubus idaeus*; ‘Diamond Jubilee’ (a&b) and ‘Sapphire’ (c&d) for two consecutive years; 2020, 2021. Pollination treatments were Insect Pollinated with hand pollen Supplementation (IPS), Insect Pollinated (IP), Insect Excluded with hand pollen Supplementation (IES) and Insect Exclusion (IE). IQR, minima, maxima and outliers are shown. Outliers were <1.5*IQR from either end of the box. Different letters show significant differences between levels of each variable and each combination of variables within interactions. See text for sample sizes.

Table 2.2. Pollinator dependence ratios for each yield metric for both Diamond Jubilee and Sapphire over three years. Pollinator dependence calculated using equation 1. Values used for economic valuation are shown in bold.

		Pollinator dependence ratios			
		Fruit set (%)		**Fruit weight**	
Cultivar	**Year**	**Total**	**Marketable**	**Total**	**Marketable**
Year totals					
Diamond Jubilee: Sapphire	2019	0.78	0.99	-	-
Diamond Jubilee: Sapphire	2020	0.07	0.72	0.30	0.60
Diamond Jubilee: Sapphire	2021	0.02	0.54	0.30	0.70
Cultivar totals					
Diamond Jubilee	2019:2021	0.23	0.70	-	-
Sapphire	2019:2021	0.10	0.66	-	-
Diamond Jubilee	2020:2021	-	-	0.35	0.62
Sapphire	2020:2021	-	-	0.27	0.65
Metric totals					
Diamond Jubilee: Sapphire	2019: 2021	**0.16**	**0.68**	-	-
Diamond Jubilee: Sapphire	2020: 2021	-	-	**0.30**	**0.64**

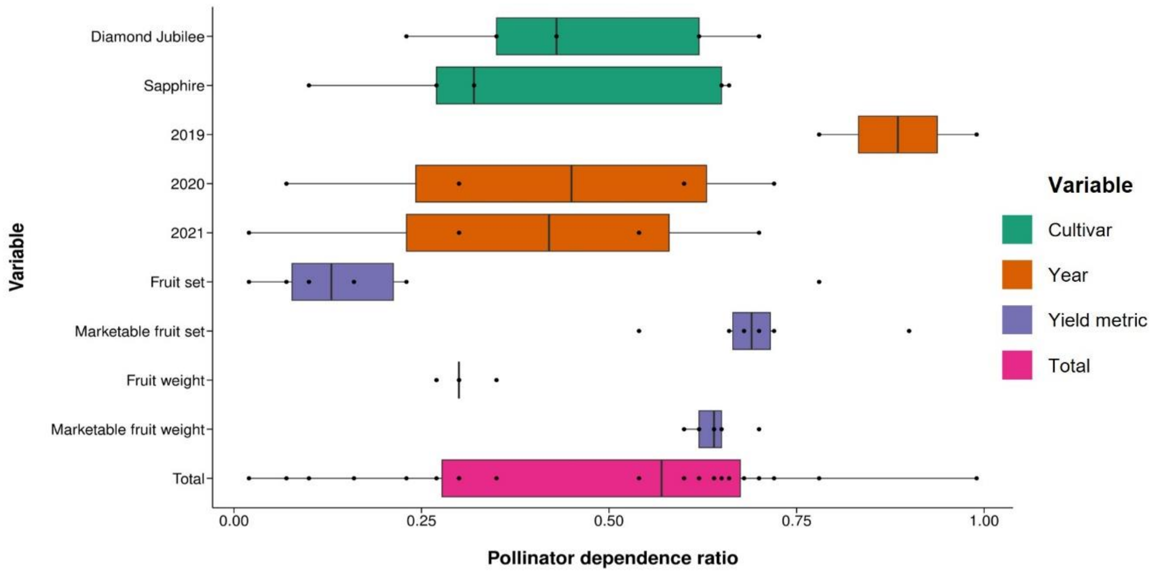

Figure 2.3. The median pollinator dependence of red raspberry *Rubus idaeus* for ‘Diamond Jubilee’ and ‘Sapphire’ cultivars in three consecutive years (2019, 2020, 2021) calculated using Fruit set (%), Marketable fruit set (%), Fruit weight and Marketable fruit weight. This is a visualisation of the pollinator dependence values in Table 2. IQR, minima, maxima and data points are shown.

2.4.3. Economic valuation of pollination to UK raspberry production

The economic value of insect pollination to raspberry production was calculated using equation 2, multiplying the UK total national production economic value for raspberry (Defra, 2021) by the overall pollinator dependence ratios for % fruit set (0.16), % marketable fruit set (0.68), fruit weight (0.30) and marketable fruit weight (0.64) for all years and cultivars combined. This valued the benefit of insect pollinators to UK raspberry production in 2020 at £21.3 million using % fruit set and this value was quadrupled to £90.6 million when market thresholds were taken into account using % marketable fruit set. For fruit weight this was £39.9 million, more than doubling to £85.3 million using marketable fruit weight.

2.5 Discussion

The importance of insect pollination to UK raspberry production is evidenced by the average reduction in marketable fruit set of 68.2% (Diamond Jubilee: 70.4%, Sapphire: 66.4%, $D = 0.68$) across our two varieties and three study years (Table 2.2), when pollinators were excluded. This is similar to the central dependence value for raspberry by Klein *et al* (2007). The benefit provided by pollinators in this system was valued much lower using % fruit set (16.34%, $D = 0.16$), as expected due to the self-compatibility of cultivated *R. idaeus*. This yield metric included low-quality fruit that would not reach market as fresh raspberries, and therefore have no economic value for commercial producers in our system, negating its usefulness in pollinator dependence ratios for commercial crops.

When our pollinator dependence ratios were used to estimate the economic value of insect pollination to UK raspberry production, the value dramatically changed depending on which yield metric was used. The value to UK raspberry production in 2020 using our total marketable fruit set dependence ratio was ~£90 million compared to £21.3 million using fruit set. Smith et al (2011) give the pollinator dependence of UK raspberries as 0.45, quoting a 2007 monetary value of pollinators to UK raspberries as £39 million per annum. Since 2007, the economic value of UK raspberry production has increased by almost 50% from £90.7 million in 2007 to £133.3 million in 2020. Insect pollination of raspberry crops was therefore worth ~£60 million in 2020 using the same 0.45 dependence ratio. The £30 million per annum difference in pollinator value reflects the different estimates of the dependence ratio (0.68 in this study vs 0.45 in Smith *et al*. 2011). The economic value to our study cultivars is also likely to differ due to their differing pollinator dependence values. Calculating cultivar specific pollinator dependence values prevents over or underestimating the value of pollinators to individual cultivars using averaged crop pollinator dependence (Garratt *et al.* 2014) and informs the cultivar specific importance of effective pollination management. Where cultivar specific production quantities and prices are available, these should be used to provide the economic value of pollinators to each cultivar. If economic valuations continue to be used to highlight the risks associated with pollinator declines (Silva *et al*, 2021), and to argue the necessity of pollinator conservation efforts (but see Kleijn *et al*, 2015, more accurate pollinator dependence ratios are required, using commercially relevant yield metrics.

Measuring fruit set alone does not accurately capture the benefits of insect pollinators to crop yield, because quality criteria relating to fruit weight, size and appearance can exclude some fruit from the market (BerryWorld pers. Comms, December 2019). Marketable fruit weight and marketable fruit set together provide a more complete picture of the benefit of insect pollinators to crop yield where yield is paid for by weight rather than units. In this study, bubbles or crumbliness (Fig. S2a) were the main cause of berries being considered unmarketable, rather than berries being underweight. The large disparity between the fruit set and marketable fruit set pollinator dependence ratios in this study is therefore mainly due to the positive effect of pollinators on the uniformity and number of seeds/drupelets and the resulting fruit cohesion rather than their benefit to fruit weight. Not accounting for the benefit pollinators provide to fruit aesthetics in this study therefore substantially underestimates the commercial value of their pollination service provision. Where pollination levels affect crop size, weight or aesthetics, as demonstrated here and also in pollination studies for apples (Garratt *et al.*, 2014), cotton and sesame (Stein *et al.*, 2017), strawberries, oilseed rape and buckwheat (Klatt *et al.*, 2014; Bartomeus *et al.*, 2014), fruit set alone does not capture the true pollinator dependence of commercial crop production and the benefit pollinators have on crop yields.

Hand pollination was included as a treatment in the first year (2019) as this was thought to represent the maximum fruit set as in other crops (Garratt *et al.*, 2014). However, in that year, hand pollination yielded significantly fewer marketable fruit than insect pollinated flowers. The insect pollinated with hand supplementation treatment was therefore added in 2020 to represent maximum potential fruit set to determine whether there was a pollination deficit. Having both treatments helps separate the effect of the bag from the effect of preventing cross pollination. Both bagged treatments yielded significantly fewer marketable fruit in 2019 than in either of the other study years. The extremely low yields for insect excluded flowers in 2019, which lead to the higher dependence ratio values, are therefore not solely due to lack of pollination. The UK experienced a heatwave in July 2019 (max daily temp of ≥35°c for 5 days recorded on the farm, reaching 40°c on one of these days) during the flowering period of the study raspberry crops. The effect of these high temperatures on raspberry yields has not been previously studied, but pollen viability and seed set in flowering plants can be negatively impacted by high temperatures (Descamps *et al.*, 2018; Devasirvatham *et al.*, 2012; Hedhly, 2011) and these effects can be mediated by insect cross pollination (Bishop *et al*, 2016). Mesh, like that used for our exclusion bags, has been shown to increase the temperature underneath or inside it by 0.7°C (Alaphilippe *et al.*, 2016). Our mesh size was even finer than this and so could have increased the temperature inside by a larger margin, potentially enhancing the negative effect of pollinator exclusion on fruit set and inhibiting insect excluded flowers from self-fertilising, as well as causing a reduction in the hand pollinated fruit set compared to 2020 and 2021. The unexpected reduction in fruit set in hand pollinated flowers relative to insect pollinated flowers in 2019 could be explained either by a temperature-related effect of the mesh bag, or by the mediative effects of pollinators on open-pollinated flowers that were also potentially damaged by excessive heat (Bishop *et al*, 2016). This potential interaction between temperature and pollination treatment is rarely accounted for and so we retained the results from this year as the low yields in 2019 represent a genuine source of variation in results between sites, and years, for a given crop. Temperature sensors could be placed inside exclusion bags to monitor temperatures to assess this effect in future studies. Climate variation has been found to explain a third of global crop yield variability for the globally important crops wheat, maize, rice and soybean (Ray *et al*, 2015), while fruit set and fruit weight of strawberries (Menzel, 2019) and tomatoes (Vijayakumar *et al,* 2021) has been found to decrease with temperature increases. Considering the effect of temperature on crop yields and pollinator dependence is thus important, especially in the context of climate change, with increasingly unpredictable weather conditions expected (IPCC, 2022).

The mean pollinator dependence ratio using % fruit set ($D_i = 0.16$) for this study was similar to the 20% mean yield reduction reported by Prodorutti and Frilli (2008), for ripe fruit set measured over two years, despite our study having a much larger range in pollinator dependence using this yield metric (0.02-0.78 compared to 0.14-0.26). This shows the importance of multi-year studies for providing representative mean pollinator dependence ratios despite inter-annual variation in fruit yields. For marketable fruit set our pollinator dependence ratio varied less between years but was still highly variable (0.54-0.99). This inter-annual variation in dependence demonstrates substantial stochastic uncertainty in estimates of pollinator dependence, even for a specific cultivar in a single location, and suggests that multi-year analyses are necessary to establish estimates with realistic uncertainty ranges. Taken as a whole, our dataset provides strong evidence of inter-annual variation in pollinator dependence estimates, which can have dramatic effects on subsequent crop pollination service economic valuations, as shown in this study. The causes of this interannual variation could be due to the differing pollinator community, environmental effects such as weather, temperature, pest levels, or differing fertilizer and water inputs (Chen *et al,* 2022) or limitations or it could be due to plant fertility and genetics. These potential causes of variation in crop yield exist within commercial farms and thus measuring pollinator dependence in multiple years at the same site can help capture the pollinator dependence range of that crop. Future exclusion studies should endeavour to collect data in as many years as possible to allow this uncertainty to be accounted for and, ideally, measured.

The total variation in pollinator dependence ratio, dependent on yield metric, year and cultivar, in this study (0.02-0.99) was greater than the between study variation for previous raspberry pollination studies (10-70% yield reduction in the absence of pollinators). This suggests that between study variation is likely to be at least in part, explained by differences in yield metric, study year and cultivar. Using a multi-year study and a more appropriate yield metric we have validated the *Rubus ideaus* pollinator dependence value used by Lautenbach et al. (2012) and Potts et al. (2016).

2.6 Conclusion

We have shown that two varieties of commercially produced raspberry in the UK (Diamond Jubilee and Sapphire) are pollinator dependent using six different yield metrics over three study years. However, the strength of this dependence is highly sensitive to the cultivar, year, yield metric used and the environmental conditions of the study, as well as the criteria used to decide which fruit are included in 'yield' measurements. Where exclusion studies are used to calculate the economic value of pollination services using dependence ratios, we strongly recommend that studies are conducted over multiple years (three or more) to generate a range of uncertainty, and that commercial quality criteria linked to actual market value are incorporated into the calculations of dependence.

2.7 References

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2.8 Supplementary material

Table S2.1. Results of Generalised Linear Mixed Models (GLMMs) with beta binomial error distributions for the response of **fruit set** for (a) marketable fruit and (b) total fruit.

Variable	Estimate	Std. error	z ratio	P value
a)				
(Intercept)	1.5279	0.3887	3.931	**<0.001**
Cultivar:Sapphire	0.6051	0.2784	2.173	**0.0298**
Year:2020	-2.1501	0.4135	5.200	**<0.001**
Year:2021	2.8115	0.4157	6.764	**<0.001**
Treatment:IE	-5.0481	0.4369	-11.556	**<0.001**
Treatment:IES	-1.7074	0.3718	-4.592	**<0.001**
b)				
(Intercept)	2.1636	0.4031	5.368	**<0.001**
Cultivar:Sapphire	0.7677	0.3492	2.198	**0.0279**
Year:2020	4.3782	0.4349	10.0682	**<0.001**
Year:2021	5.6151	0.5996	9.365	**<0.001**
Treatment:IE	-4.0211	0.4448	-9.040	**<0.001**
Treatment:IES	-2.5462	0.4122	-6.178	**<0.001**
Treatment:IPS	-1.1759	1.1102	-1.059	0.2896

Table S2.2. Results of General Linear Mixed Models (GLMMs) of **marketable fruit weight** with Gaussian errors for (a) Diamond Jubilee and (b) Sapphire

Variable	Estimate	Std. error	t value	P
a)				
(Intercept)	4.8001	0.4793	10.017	**<0.001**
Year:2021	0.9414	0.5172	1.820	0.081
Treatment:IE	-3.3696	0.4183	-8.054	**<0.001**
Treatment:IES	0.2357	0.4164	0.566	0.573
Treatment:IPS	0.2560	0.4197	0.610	0.543
b)				
(Intercept)	6.8261	0.3275	20.843	**<0.001**
Year:2021	0.5129	0.3801	1.352	0.186
Treatment:IE	-4.5700	0.2841	-16.088	**<0.001**
Treatment:IES	-0.3004	0.2863	-1.049	0.297
Treatment:IPS	-0.2911	0.2834	-1.027	0.307

Table S2.3. Results of Generalised Linear Models GLMMs of **fruit weight** with gaussian errors for (a) Diamond Jubilee and (b) Sapphire

Variable	Estimate	Std. error	t value	P
a)				
(Intercept)	5.7783	0.3316	17.423	**<0.001**
Year:2021	0.3078	0.3735	0.824	0.418
Treatment:IE	-2.1234	0.2550	-8.327	**<0.001**
Treatment:IES	0.0978	0.2536	0.386	0.701
Treatment:IPS	0.3459	0.3560	1.351	0.181
b)				
(Intercept)	6.8770	0.2699	25.476	**<0.001**
Year:2021	0.4425	0.3218	1.375	0.179
Treatment:IE	-1.8898	0.2174	-8.691	**<0.001**
Treatment:IES	-0.1561	0.2192	-0.712	0.478
Treatment:IPS	-0.0531	0.2170	-0.245	0.807

Figure S2.1. Example of exclusion study set up with four branches on the same raspberry cane assigned to each of the four pollination treatments and marked with the corresponding colour of tape. Insect exclusion with pollen supplementation (circled in pink) and insect exclusion (circled in lilac) branches have sealed mesh bags to exclude insects from accessing the flowers.

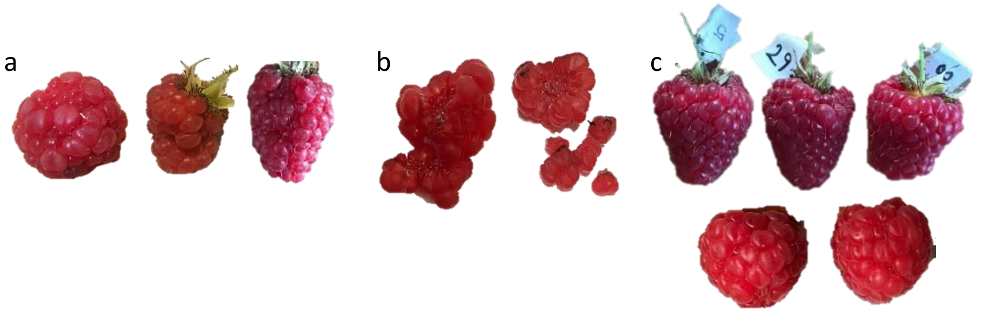


Figure S2.2. Examples of a) unmarketable bubbled fruits, b) unmarketable crumbly fruits and c) marketable whole fruits

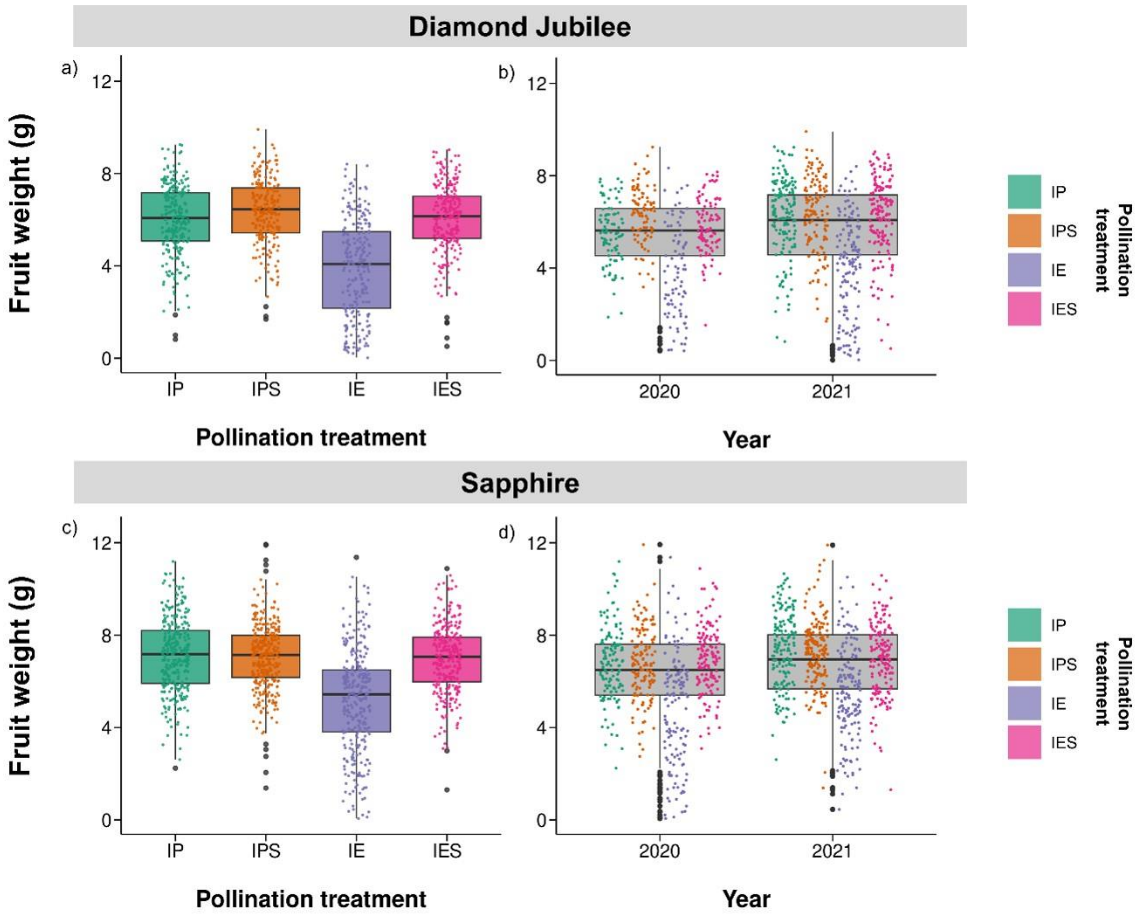


Figure S2.3. The median marketable fruit weight in grams of fruit produced by study flowers under four different pollination treatments (a&c) for two commercial cultivars of red raspberry *Rubus idaeus*; ‘Diamond Jubilee’ (a&b) and ‘Sapphire’ (c&d) for two consecutive years; 2020, 2021. Pollination treatments were Insect Pollinated with hand pollen Supplementation (IPS), Insect Pollinated (IP), Insect Excluded with hand pollen Supplementation (IES) and Insect Exclusion (IE). IQR, minima, maxima and outliers are shown. Outliers were <1.5*IQR from either end of the box. Different letters show significant differences between levels of each variable and each combination of variables within interactions. See text for sample sizes.

3.0 Two visits by a honey bee or bumble bee maximises marketable fruit weight of two cultivars of red raspberry *Rubus idaeus*

3.1 Abstract

Honey bees are an important option for providing consistent pollination services to mass flowering crops, however their high abundance on soft fruit farms can be detrimental to fruit production and wild pollinator conservation. Empirical evidence is therefore needed to compare the pollinator effectiveness of honey bees to native wild pollinators to inform their necessity for raspberry and other soft fruit crop production. This study investigated the effects of honey bee and bumble bee visitation, number, and duration of social bee visits on raspberry yield for two commercial varieties in the UK. We found that honey bees and bumble bees had similar positive effects on fruit weight and marketable fruit set, but a single bumble bee visit resulted in a greater overall marketable yield than a single visit from a honey bee. Two visits from either taxon yielded similar marketable yields to open pollinated flowers suggesting that low visitation rates are sufficient for achieving marketable fruit yields. Additionally, single bumble bee visits produced greater marketable fruit weight per berry than open pollinated flowers, indicating a potential negative effect of high visitation rates to raspberry crop flowers as previously documented by other authors. We therefore recommend reducing reliance on honey bee pollination for raspberries where crop flowers are receiving more than two bumble bees and supporting bumble bee conservation efforts.

Keywords: *Rubus idaeus,* pollination, pollinator effectiveness, pollinator efficiency, soft fruit

3.2 Introduction

Managed pollinators, such as honey bees, are commonly deployed on soft fruit farms to ensure adequate and consistent pollination during the crop flowering period (Breeze *et al*, 2019 and Lye *et al,* 2011). This is largely due to the direct relationship between pollination levels and yield (Chagnon *et al*, 1991) and the low cost of renting honey bee hives (Breeze *et al,* 2017) or buying bumble bee colonies (Agralan growers, 2021). Overestimating the stocking densities required for pollination has also been demonstrated to be detrimental to fruit yield, due to damage to stigma and styles caused by high numbers of visits (Aizen *et al.*, 2014; Saez *et al.*, 2014). Reductions in fruit set can also occur if surplus pollen deposition causes interference between pollen tubes at high densities (Cruzan, 1986). Visitation numbers and fruit set are therefore not linearly related, and so ensuring that the number of visits to each flower reaches but does not exceed their optima is important for maximising fruit production. Saez *et al.* (2014) studied the visitation and damage rates to raspberry crop flowers near National Parks in Argentina. In fields with high *Bombus terrestris* and *Apis mellifera* abundance, crop flowers received ~170 and ~140 daily visits by *Bombus terrestris* and *Apis mellifera* respectively. Pollen deposition increased with the frequency of *A. mellifera* visits but so did the proportion of damaged pistils, although less so than with *B. terrestris.* Drupelet number per fruit decreased with the proportion of damaged pistils and with visitation frequency.

The detriment of overstocking honey bees is not limited to fruit production. High honey bee densities can also spread pathogens and parasites to wild pollinators through shared flower use (Figueroa et al 2019; Graystock, Goulson and Hughes, 2015; Graystock et al 2020). Deformed Wing Virus, a viral pathogen previously only found in *Apis mellifera,* has now been found in other bee and arthropod species, suggesting interspecific transmission perhaps through shared food sources (Bailes *et al.,* 2018; Levitt *et al,* 2013 and Singh *et al,* 2010). The disease has been found to cause crumpled wings, discolouration, decreased longevity and mortality in bumble bees (Cilia *et al,* 2021; *Fürst et al,* 2014; Genersch *et al,* 2006; Gusachenko *et al* 2020) and is thus a growing concern for wild bumble bee populations despite the pathway of infection not being known (Gusachenko *et al* 2020). *Nosema ceranae*, a parasite of honey bees that was found in 20-47% of wild-caught bumble bees sampled at five sites across the UK (Graystock *et al,* 2013) has been shown to be transferred to bumble bees via shared flowers (Graystock, Goulson and Hughes, 2015; Graystock *et al,* 2020). The micro parasite has been shown to cause both lethal and sublethal effects in bumble bees as the fungus absorbs nutrients from the gut and body fat (Graystock *et al,* 2013). When combined with high hive densities and the large foraging range of honey bees, the transmission of parasites and pathogens poses a risk of widespread environmental contamination.

High densities of honey bee hives have also been linked to reductions in wild bee visits to flowering crops. Angelella *et al.* (2021) found a 48% decrease in wild bee abundances and a 20% decrease in species richness when honey bee hives were present at farms growing pollinator dependent crops compared to farms that didn't have hives on site. Strawberry fruit set was also 18% lower on farms with honey bees suggesting there was no net benefit from their pollination. Walther-Hellwig *et al.* (2006) found that *Bombus terrestris* showed spatial avoidance when foraging on a field of *Phacelia,* foraging further away from the honey bee hives despite the flower cover in these areas being lower. Lindström *et al* (2016) found that the presence of honey bee hives depressed the densities of bumble bees, solitary bees, hoverflies and other pollinating insects in within oilseed rape fields

The presence of honey bee hives in agricultural systems can also result in negative impacts on bumble bee colony growth and reproductive success (Elbgami *et al,* 2014; Goulson and Sparrow, 2008; Thomson, 2006). This could be due to reduced nectar availability when honey bees are present, prompting bumble bee colonies to divert pollen collecting workers to nectar thus reducing larval production (Thomson, 2004). Introducing honey bee hives consequently conflicts with wild pollinator conservation, and native pollinator populations could continue to decline if agriculture attempts to rely solely on honey bees instead of providing more semi-natural habitats and floral resources for wild pollinators. Quantitative assessment of the benefit honey bees provide to raspberry crops, and how this relates to managed hive density, is therefore imperative to enable equitable management choices that benefit both growers and wild pollinators.

The recommended honey bee stocking density for raspberry crops is 0.5-2.5 hives per hectare (Delaplane and Mayer, 2000). There is little evidence behind how advised stocking rates are calculated, or how this varies with the surrounding landscape (Gaines-Day and Gratton, 2016) and so they are unlikely to be accurate for all raspberry systems. Honey bees have also been found to have the same pollination effectiveness per single visit as *Bombus* and *Osmia* species (Andrikopoulos and Cane, 2018a and Cane, 2005). Thus, wild pollinators have the potential to provide effective pollination to raspberry crops in the absence of honey bees if they are at sufficient densities.

Discrepancies in the estimated number of visits required to achieve complete pollination and resulting full drupelet set in raspberry contribute to the uncertainty surrounding managed pollinator stocking rate requirements. A model developed by Saez *et al.* (2018) predicted that drupelet set was maximised at 99% success for flowers that received between ~15-35 visits from *Apis mellifera* or ~10-20 visits from *Bombus terrestris*. Saez *et al.* (2014) found that flowers that received ~10 pollinator visits a day were not pollen limited, however this was the lowest visitation rate measured and so a lower number of visits may have been sufficient for full fruit set. The results from Chagnon *et al.* (1991) support this, finding that pollination and fruit set reached its maximum after 5-6 visits or ~150 accumulative seconds of visit time by *Apis mellifera*. Andrikopoulos and Cane (2018b) found similar results, reporting that two prolonged visits, with a mean cumulative visit time of ~127 seconds, from either a honey bee or a native bumble bee to the US were sufficient for maximal fruit set, with no difference in drupelet set of the resulting berries compared to open pollinated flowers. These prolonged visits were the first visit to a flower on two consecutive days. Raspberry flowers are therefore likely to require at least 5-6 visits in a single day or visits on at least two days during their receptive period to reach maximum pollination. Estimates of the required numbers of visits for maximum fruit yields can be used in conjunction with site specific visitation rates to crop flowers to determine whether pollinator communities and managed pollinators are providing sufficient pollination (Garibaldi *et al.,* 2020). Depending on which estimate of raspberry visitation requirements is used, estimated target visitation rates could be anywhere between 17 and 195 visits per hour to 100 flowers. This target number of visits can then be compared to actual visitation rates observed during timed flower observations to determine whether current pollination services are optimal (Garibaldi *et al.*, 2020). Using the wrong estimate of required number of visits to maximise yield could therefore lead to overstocking. More studies that quantify the number of visits needed for complete raspberry pollination, along with many other crops, are therefore needed to help determine a more accurate estimation of the minimum or optimal required number of pollinator visits and ensure that managed pollinators are stocked accordingly.

These studies can be done using single visit studies that measure the single-visit pollination effectiveness and efficiency of different crop pollinators to compare their performance and estimate the number of visits needed from each species or group to maximise per flower yield (Kendall *et al,* 2020; Ne'eman *et al,* 2009). As defined by Kendall *et al* (2020): "single-visit pollination effectiveness refers to a pollinator's contribution to pollen deposition or plant reproductive outcomes (e.g., fruit set or seed set). In contrast, single-visit pollination efficiency refers to the difference between single-visit pollination effectiveness and maximum potential fruit set or seed set, derived from hand-pollination treatments." Single visit studies allow the differences in pollination performance between pollinator taxa to be determined however, to determine the number of visits required by each species to maximize plant reproductive success and the resulting crop yield, controlled repeated visits must be permitted and recorded, as in Andrikopoulos and Cane (2018b). This also allows the contribution per additional visit to be determined, whereas single visit studies assume additive equal contribution to fruit set or weight by each pollinator visit (Kendall *et al,* 2020) and can thus underestimate the number of visits required for maximising yield.

Our study aimed to determine whether honey bees and bumble bees differ in their pollinator effectiveness and provide an estimate for the required numbers of visits and visit duration to achieve marketable fruit set, and fruit weights equivalent to open pollinated flowers. We also aimed to determine whether these two commercially important metrics are correlated with drupelet set, a commonly used metric in raspberry pollination studies, to compare our estimates with pre-existing estimates for other cultivars and geographic regions.

We asked the following research questions:

- Are honey bees more effective at pollinating raspberry crop flowers than bumble bees for two different commercial varieties?
- How many visits are required by honey bees or bumble bees to maximise marketable yield per flower?

- What is the optimal total visit duration for producing marketable raspberries?
- What is the relationship between the number of seeds produced (proxy for drupelet set) and both fruit weight and marketable fruit set?

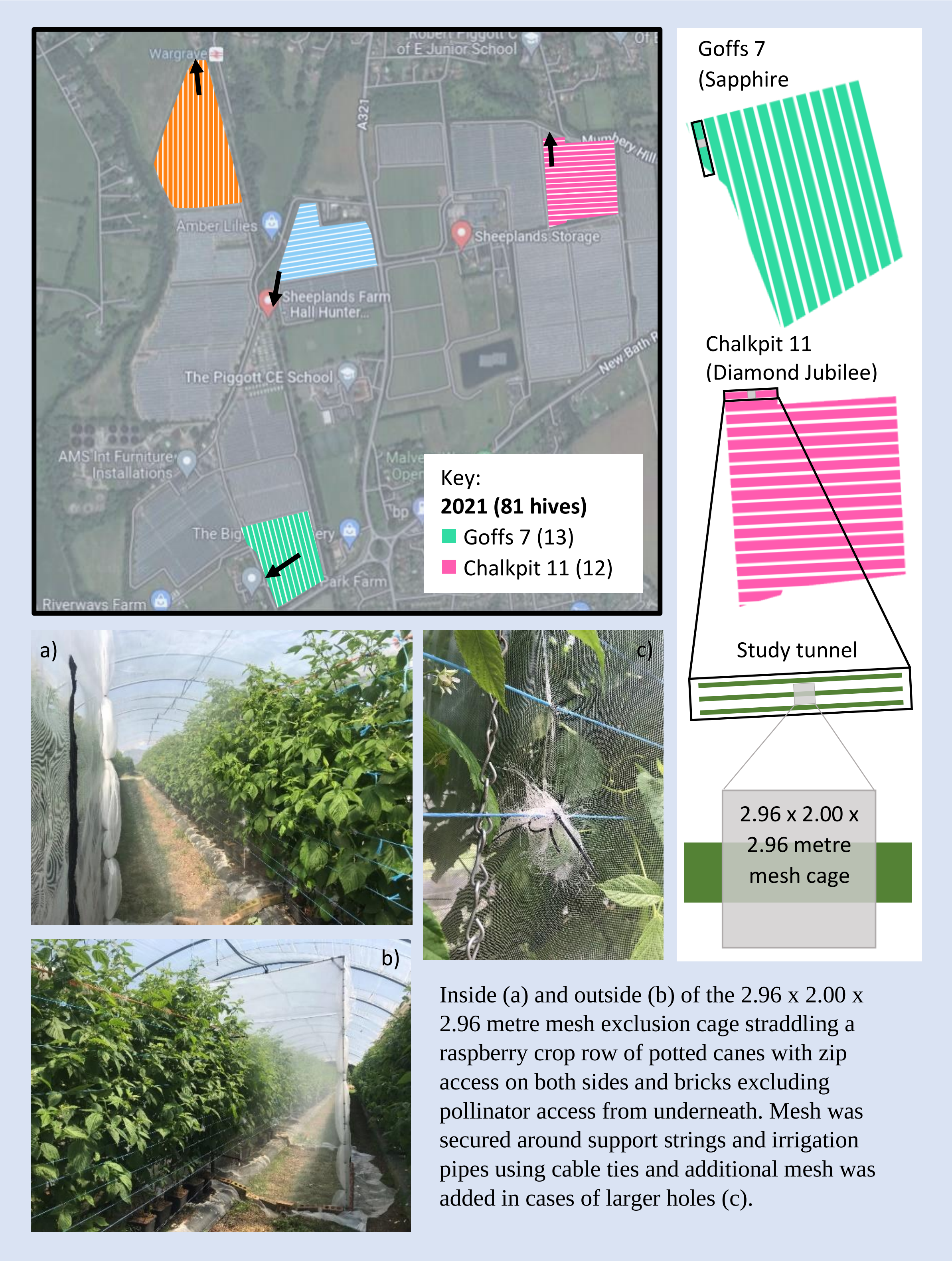
3.3 Methods

3.3.1 Study site

The study was carried out on an 81-hectare commercial soft fruit farm near Reading, south England (51°29′32″N, 000°52′28″W) throughout the period of July to September in 2021. Two cultivars of red raspberry (*Rubus idaeus*); ‘Diamond Jubilee’ and ‘Sapphire’, were included in the study, both developed by BerryWorld and filed for growers in 2013. Each experimental site was made up of one commercial field of >1.5 ha surrounded by uncropped field margins. There were small areas of semi-natural grassland and patchy woodland on the farm and within the immediate surrounding area. Both raspberry cultivars were grown under Spanish polytunnels (7.8 metres wide) with three rows of potted raspberry canes in each tunnel, each row was ~2 metres apart. 81 rented honey bee hives were in place at the farm during 2021, equating to 1 hive/ha of farmland, throughout the raspberry flowering season for the purpose of crop pollination of both raspberries and strawberries. The field and hive locations within the farm are shown in Box1. A few managed bumble bee colonies were still active in an adjacent field to Diamond Jubilee in 2021 however, they were at the end of their 10th week *in situ* when the first study flowers opened. It is therefore likely that the bees leaving the hive were gynes and males and therefore unlikely to have been foraging on the crop flowers.

3.3.2 Single visit pollinator efficiency

To determine the efficiency of honey bee, bumble bee and solitary bee (*Andrena*) individuals, we performed single visit pollination studies. Branches of unopened raspberry flowers in one polytunnel in each of the two crop fields were covered with 27x27cm bags made of 1 mm mesh and tied at the bottom with string to prevent pollination by insects. Each bag was then removed one at a time to allow visits from foraging bee species. Solitary bees were caught and euthanised for later identification to species level under a microscope. A 2.96 x 2.00 x 2.96 metre aluminium frame was constructed and covered in mesh (holes 1.35 mm2 Harrod Horticultural Ltd, Lowestoft, UK) (Figure 3.1). This was used alongside the mesh bags to increase the number of replicates. Individuals from target bee taxa were caught within the crop field whilst foraging on a crop flower and released into the cage. All flowers that were visited were marked with coloured wire and labelled with numbers using white electrical tape to enable identification once fruiting had occurred and the identity of the visitor and length of each visit to that flower was recorded. We did not assess the pollinator effectiveness of hoverflies, other flies or wasps in this study as they were in low abundances in the previous year (see Chapter 4) though some flowers did receive visits from these insects and were excluded from the analyses. 162 virgin flowers were recorded to have been visited by at least one of either *A. mellifera, Bombus* and *Andrena* sp. in this study (86 to Diamond Jubilee and 84 to Sapphire). Single visits from *Bombus* species that were included in this study were from either *B. terrestris/lucorum* (73) or *B.hypnorum* (2). Visits were recorded using a phone camera and the start time and duration of the visit was later recorded using a stopwatch. The identity of the visitor species and the length of that visit was recorded. A subset of flowers also received multiple visits from the same and different pollinator species, and these incidences were used to measure the effect of number of visits on the resulting fruit set. The visited flowers were then recovered with the larger bags and left to develop into berries. Berries were harvested when ripe and their weight, length and width was recorded. For fruit to be counted as ‘marketable’, they had to be whole (i.e., not missing drupelets or crumbly), without excessive bubbled drupelets (drupelets of dramatically different sizes) and have a length of ≥15mm and a weight of ≥3g (Berryworld pers. Comms, December 2019). The berries produced by flowers that had received a controlled number of visits were compared to open pollinated flowers that had been visited by many insects (representing maximum yield) and those that had been excluded from insects and thus received no visits. Each of these two treatments was assigned to a lateral on the same randomly selected plant. The plant was marked, and excess buds were removed so that each study lateral had 10 buds. One of these laterals was left uncovered to be pollinated by insects and the other was covered with a 27x27cm mesh bag to prevent insect visitation. These plants were distributed within the tunnels where the mesh cages were situated. Flowers within the cages that had not been



Inside (a) and outside (b) of the 2.96 x 2.00 x 2.96 metre mesh exclusion cage straddling a raspberry crop row of potted canes with zip access on both sides and bricks excluding pollinator access from underneath. Mesh was secured around support strings and irrigation pipes using cable ties and additional mesh was added in cases of larger holes (c).

Figure 3.1. Map of the study farm with honey bee hive (arrows) and field locations (coloured fields) highlighted. Numbers of hives situated at the farm and in each field are given in parentheses. White lines indicate the orientation of the Spanish polytunnels. Locations of study tunnels and exclusion cages are shown with photos and a description of the mesh cages.

visited by an insect were also randomly sampled to enable the cage exclusion and the bagged exclusion berries to be compared. It was not possible to have openly pollinated flowers within the cage. Means and standard errors are given in parentheses.

3.3.3 Relationship between number of seeds, fruit weight and marketability

We also studied the relationship between seed or drupelet number and marketable yields to enable comparison of our results to the findings of Andrikopoulos and Cane (2018a), the only other study that has compared the yields achieved from single visits by both honey bees and bumble bees, which measured yield benefit using drupelet set. Presenting these findings allows other studies that have measured drupelet or seed set in raspberries under different pollination treatments to be interpreted in terms of marketable yields. Ripe raspberries were harvested from the four pollination treatments (Insect Pollinated, Insect Pollinated with hand pollen Supplementation, Insect Exclusion, and Insect Exclusion with hand pollen Supplementation) on study plants from Chapter 2 that were randomly distributed across two different crop fields, each of a different cultivar, in both 2020 and 2021. All berries were weighed and measured as documented in Chapter 2 and classified as either marketable or unmarketable. The seeds in each fruit were then extracted by pushing each berry individually through a sieve and rinsing any pulp residue away with water. The extracted seeds were then spread onto paper towels to dry, ensuring that seeds were not on top of each other to enable later counting. A random 20% subsample of fruit was then selected from each 1-gram weight range (e.g. 5-5.99g, 6-6.99g berries etc) within each pollination treatment, year and cultivar and their seeds were photographed and counted using the cell counter function in Fiji (Schindelin *et al.*, 2012). Seed number represents a measure of reproductive success and a proxy for number of drupelets where this is time consuming or difficult to count. Each pollinated pistil produces a single drupelet, containing a single seed, or in some cases two (Funt and Hall, 2013).

3.3.4 Data analyses

To compare the observed and expected numbers of successes and failures to produce marketable fruit across the four different pollination treatments (pollinator excluded, single honey bee visit, single bumble bee visit, open pollinated) for Diamond Jubilee and Sapphire separately, Fisher exact tests were performed in the R statistical software (version 4.1.3) (R Core Team, 2022) using the *stats* package (R Core Team, 2022). Successes were flowers that developed into marketable fruit and failures were flowers that did not develop a marketable fruit. The data exhibited quasi-complete separation between pollination treatments, when one group has only successes or failures and so a frequentist binomial generalised linear model produced inflated errors due to having no variance within a category on which to estimate. This made the resultant p values unreliable and so Fisher's exact tests were used. The quasi-complete separation is clear from the zeros in Tables 3.4, 3.5 and 3.6. Pairwise comparisons were generated using the *rstatix* package (v. 0.7.2, Kassambara, 2023) to determine which treatments differed from each other. Bonferroni adjusted residuals and p values were generated for each cell in the contingency table.

Pollinator efficiency scores (PE) for each species of visitor were calculated using the equation for pollinator effectiveness (read efficiency: Kendall *et al*, 2020; Ne'eman *et al*, 2009), from Spears (1983) as:

$$PE_i = \frac{(P_i - Z)}{(U - Z)} \tag{2}$$

where P_i is the mean marketable fruit weight in grams from flowers receiving a single visit by species i, Z is the mean marketable fruit weight from flowers receiving no visitation, and U is the mean marketable fruit weight in grams from flowers receiving unrestrained visitation (open pollination). Flowers that were not marketable had a marketable fruit weight of 0 grams, thereby including both factors influencing marketable fruit weight, the ability to produce a marketable fruit and the weight of that fruit if marketable. The estimated number of visits required for maximum pollination was calculated as 1/PE_i which assumes that all pollination visits equally contributed to the resulting fruit weight.

All other analyses were performed using generalised linear models (GLMs) in the R statistical software (version 4.1.3) (R Core Team, 2022) using the lme4 R package (Bates *et al,* 2015). Maximal models were used without simplification, and acceptable model fit was assessed from residual plots. To determine and compare the pollinator effectiveness of honey bees and bumble bees, marketable fruit weight was compared to that from open pollinated and pollinator excluded flowers using a GLM with a gaussian error distribution. An interaction between pollination treatment and plant location (cage or tunnel) was included to measure any difference in pollinator effectiveness and pollinator dependence between locations. The two cultivars Diamond Jubilee and Sapphire were modelled separately as the differences in fruit size and appearance are already known and are the primary reason for the cultivation of both varieties at the study farm. The effects of number of social bee visits and the duration of visitation on raspberry fruit weight were also compared between plant locations for Diamond Jubilee and Sapphire separately, again using GLMs with gaussian error distributions.

To determine the factors affecting marketable fruit set the effect of the interactions between both the number of social bee visits and the total duration of all observed insect visits to study flowers and flower location (tunnel or cage) GLMs were fitted using a binomial response of success/failure. Successes were flowers that developed into marketable fruit and failures were flowers that did not develop a marketable fruit. Flowers that received controlled insect visits were randomly located within the study tunnels and within the mesh cages according to virgin flower availability and pollinator choice and the identity of the branch or plant was not recorded and so a random effect of plant could not be included in any of the models. Maximal models were employed without simplification and acceptable model fit was assessed from residual plots. Fruit width and length were not modelled as they were both found to have a significant positive correlation with fruit weight (S1: Corr.coeff = 0.895, $p<0.0001$, and Corr.coeff= 0.855, $p<0.0001$ respectively). Full details of all variables and maximal models are shown in Table 3.1

Table 3.1. Description of the structure of GLMs of marketable fruit set and fruit weight of flowers under different pollination treatments, number of visits and total visit durations and all response and explanatory variables. The maximal models are shown and were carried out in R (v.4.1.3).

Type	**Variable**	**Distribution (link/offset)**	**Definition**
Response	Marketable fruit set	Binomial (logit)	Per flower success or failure (1 or 0) in producing a marketable fruit*
	Marketable fruit weight (g)	Gaussian (identity)	Mass in grams of individual fruit that were of a marketable size and quality*
	Fruit weight (g)	Gaussian (identity)	Mass in grams of individual fruit regardless of marketability
	Duration of visit (s)	Gaussian (identity)	Duration of the first insect visit to a virgin flower in seconds
Explanatory	Pollination treatment	Four level categorical factor	None (Pollinator excluded), 1x Honey bee visit, 1x Bumble bee visit, Many visits (Open pollinated)
	Location	Two level categorical factor	Study tunnel and mesh cage
	Number of visits	Discrete	The number of insect visits received (0-4 visits)
	Seconds of visitation	Continuous	The total time in seconds of insect visitation received by a study flower
	Number of seeds per berry	Continuous	The number of seeds per fruit
	Crop cultivar	Two level categorical variable	Two varieties of commercial raspberry (Diamond Jubilee and Sapphire), grown in separate fields
	Visitor group	Two level categorical variable	Honey bee or bumble bee
Response	**Model structure**		
Diamond Jubilee marketable fruit set	Number of visits*Location		
	Seconds of visitation* Location		
	Number of seeds per berry		
Sapphire marketable fruit set	Number of visits*Location		
	Seconds of visitation* Location		
	Number of seeds per berry		

Diamond Jubilee marketable fruit weight	Pollination treatment*Location
Sapphire marketable fruit weight	Pollination treatment*Location
Diamond Jubilee fruit weight	Number of visits*Location
	Seconds of visitation* Location
	Number of seeds per berry
Sapphire fruit weight	Number of visits*Location
	Seconds of visitation* Location
	Number of seeds per berry
Fruit weight	Number of seeds per berry*Cultivar
Diamond Jubilee visit duration	Location*Visitor group
Sapphire visit duration	Location*Visitor group

*See text, section 2.4 for a description of the required size and quality for marketability

3.4 Results

A total of 226 ripe berries were collected in 2021 that had been visited at least once by a target insect pollinator during the single visit pollinator study. This excluded 23 berries that were lost to *Phytopthera* before picking, 17 that were dead or damaged and 9 that were lost as they either could not be relocated or had ripened and fallen off the plant inside the cage before we could harvest them. Two of the 226 berries were overripe when harvested so could not be accurately measured or assessed and thus were excluded from the analysis. Five of the remaining 224 berries were excluded as they were from flowers visited by non-target insects (wasps and hoverflies). 84 had received a single visit from a honey bee, 75 had received a single visit from a bumble bee and 11 had received a single visit from a solitary bee. 19 received two visits from honey bee, 10 received two visits from bumble bee individuals, 8 received a single visit from a honey bee and a bumble bee individual and 8 and 3 received 3 and 4 visits from honey bees respectively. Due to the low sample sizes of solitary bee visits we did not include them in our analysis of single visit pollinator effectiveness.

3.4.1 Single visit pollinator effectiveness

Using Fisher exact tests on the number of marketable and unmarketable Diamond Jubilee fruits produced from flowers across the three pollination treatments in the exclusion cage (pollinator excluded, single bumble bee visit and single honey bee visit) we found that pollination treatment and fruit marketability were not significantly related (Table 3.2) (p=0.0651). For the four pollination treatments in the tunnel, we also found no significant relationship between pollination treatment and marketability (Table 3.3) (p=0.0912). No pollinator treatments significantly differed from the expected marketable fruit set of 60% in the cage and 85% in the tunnel. Therefore, across both the exclusion cage and the study tunnel, pollination treatment did not have a significant effect on Diamond Jubilee marketable fruit set, with all treatments yielding similar proportions of marketable fruit. Pairwise comparisons are shown in Table S3.1.

Table 3.2. Contingency table of the frequency of marketable and unmarketable Diamond Jubilee fruits under three pollination treatments inside a pollinator exclusion cage

Marketable	Pollinator excluded	Single bumble bee visit	Single honey bee visit	Total
Y	8	22	8	38
N	12	8	6	26
Total	20	30	14	64

Table 3.3 Contingency table of the frequency of marketable and unmarketable Diamond Jubilee fruits under four pollination treatments in a polytunnel

Marketable	Pollinator excluded	Single bumble bee visit	Single honey bee visit	Open pollination	Total
Y	42	10	22	36	113
N	15	0	2	6	20
Total	57	10	24	42	133

Using Fisher exact tests on the number of marketable and unmarketable Sapphire fruits produced from flowers across the three pollination treatments in the exclusion cage we found that pollination treatment and fruit marketability were significantly related (Table 3.4) (p<0.0001). Flowers that received a single visit from either a honey bee (63% marketable fruit set) or a bumble bee (79% marketable fruit set) yielded significantly more marketable fruit than those that received no insect visits (0% marketable fruit set) and did not significantly differ from each other. For the four pollination treatments in the tunnel, we found a significant relationship between pollination treatment and marketability (Table 3.5) (p<0.0001). Flowers that received a single visit from a honey bee or a bumble bee did not significantly differ in the proportion of fruits produced that were marketable (HB: 73% marketable fruit set; BB: 100% marketable fruit set). Flowers that received no insect visits (43% marketable fruit set) yielded significantly fewer marketable fruit than those that received a single bumble bee visit but did not significantly differ in marketable fruit set from flowers that received a single honey bee visit. Open pollinated flowers (100% marketable fruit set) yielded a significantly greater marketable fruit set than honey bee (adj. p=0.0029) visited but not bumble bee visited flowers (adj. p=1.000). Pairwise comparisons are shown in Table S3.1.

Table 3.4. Contingency table of the frequency of marketable and unmarketable Sapphire fruits under three pollination treatments inside a pollinator exclusion cage

Marketable	Pollinator excluded	Single bumble bee visit	Single honey bee visit	Total
Y	0	17	19	36
N	20	10	5	35
Total	20	27	24	71

Table 3.5. Contingency table of the frequency of marketable and unmarketable Sapphire fruits under four pollination treatments in a polytunnel

Marketable	Pollinator excluded	Single bumble bee visit	Single honey bee visit	Open pollination	Total
Y	16	8	16	50	90
N	21	0	6	0	27
Total	37	8	22	50	117

Flower location and pollination treatment did not have a significant effect on the fruit weight of marketable fruit for either Diamond Jubilee or Sapphire (Table S3.2). Open pollinated Diamond Jubilee flowers yielded marketable raspberries that weighed 5.67g ± 0.17 on average, flowers that received a single visit from a honey bee or a bumble bee yielded marketable berries that weighed 4.92g ± 0.21 and 5.28g ± 0.23 respectively and pollinator excluded flowers yielded marketable berries that were 5.51g ± 0.14. For Sapphire, Open pollinated flowers yielded marketable fruits that were 5.92g ± 0.13 on average, a single honey bee or bumble bee visit yielded marketable fruit that weighed 6.15g ± 0.25 and 6.61g ± 0.40 respectively and pollinator excluded flowers yielded marketable fruit that were 6.33g ± 0.35 on average. The lack of pollinator dependence and effect of pollination treatment on fruit weight of marketable berries indicates that pollination treatment is not important for fruit weight above the required threshold for marketability.

When non-marketable fruit were included in the analysis of the effect of pollination treatment on fruit weight, we found no significant effect of pollination treatment or location for Diamond Jubilee (Table S3.3). Fruit weight of raspberries produced by flowers that received a single honey bee visit (4.63g ± 0.22), bumble bee visit (4.94g ± 0.25) or open pollination (5.33g ± 0.20) did not significantly differ from those that received no insect visits (4.97g ± 0.15). Pollination treatment and location both had independent significant effects on Sapphire fruit weight. Fruit weight of raspberries produced by flowers that received a single honey bee visit (6.08g ± 0.24), bumble bee visit (5.99g ± 0.40) or open pollination (5.92g ± 0.13) were significantly heavier than those that received no insect visits (4.45g ± 0.24) (Table S3.3). Flowers that received a single bumble bee visit were not significantly heavier than those that had access to unrestrained pollinator visits. However, flowers that received a single honey bee visit were significantly heavier than those that had access to unrestrained pollination. Berries in the study tunnel were significantly heavier than those in the cage averaged across flowers that had received 0 or 1 insect visits.

Table 3.6. Single visit PE scores for bees visiting red raspberry cultivars in the study tunnels

	Diamond Jubilee	Sapphire	Average PE*	Approx. visits†
Honey bee	0.48 (24)	0.57 (22)	0.52	1.92
Bumble bee	1.07 (10)	1.07 (8)	1.07	0.93
Average PE*	0.65	0.70	-	-
Approx. visits†	1.54	1.43	-	-

The numbers of observations are in parentheses.
* Weighted mean PE for each type of bee and each cultivar
† The approximate number of visits required for maximum fruit weight (1/PE).

Only flowers from the tunnel were included in the calculations of pollinator effectiveness because the open pollination treatment (from which *U* is derived) was not possible inside the cage. PE scores and estimated number of visits required for maximum fruit set are shown in Table 3.6.

3.4.2 The effect of number of social bee visits on marketable fruit set and fruit weight

19 of the 84 Diamond Jubilee and Sapphire berries that received a single honey bee visit were malformed, had bubbled drupelets or were too small, which gave a marketable fruit set of 77.4% for flowers under this treatment. 76.0% of flowers that received a single visit from a Bombus individual (n=75) produced a marketable fruit and 90.9% of flowers that received a single visit from a solitary bee (n=11) produced a marketable fruit compared to 93.5% (n=92) for the open pollinated treatment.

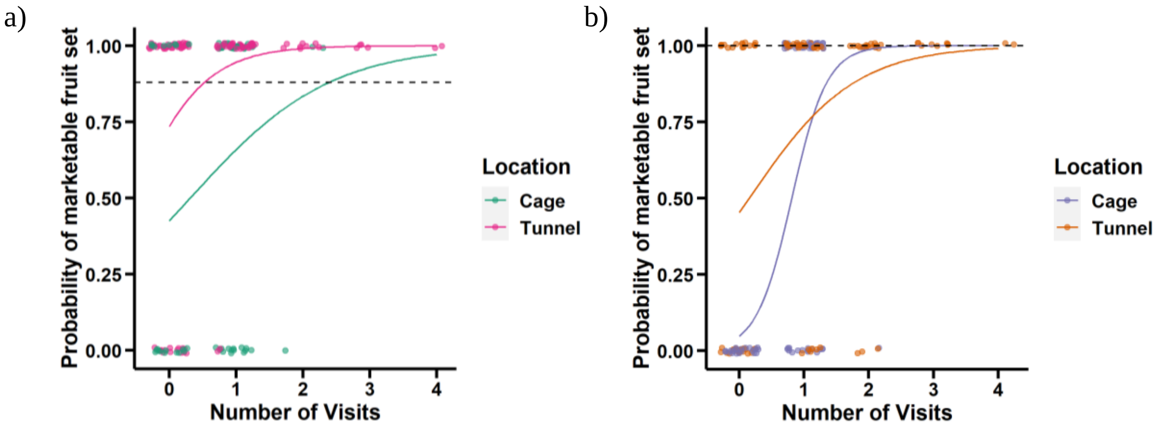


Figure 3.2. The predicted probability of marketable fruit set of study flowers in both the study tunnel and the exclusion cage for a) Diamond Jubilee and b) Sapphire. The bold lines indicate the predicted mean probability of marketable fruit set. The raw data points are shown and jittered for clarity. Dashed lines indicate the open pollination proportion of fruit that were marketable within the study tunnel.

Number of social bee visits was not a significant predictor of marketable fruit set for Diamond Jubilee (Est= 0.960, SE=0.50, z=1.916, p= 0.0553) but flowers in the tunnel yielded almost four times more marketable fruits than those in the exclusion cage (Est=1.323, SE=0.53, z=2.500, p=0.0124) (Figure. 3.2a). The interaction between the number of visits and the location of the flower was significant for Sapphire (Est=-2.458, SE= 1.112, z=-2.209, p=0.0271; Fig 3.2b) but not for Diamond Jubilee (Table S3.4). Flowers in the cage required fewer insect visits to produce a marketable fruit than those in the tunnel. The model outputs are presented in Table S3.4. Marketable fruit set comparable to open pollination was achieved after one social bee visit in the tunnel and three in the exclusion cage for Diamond Jubilee and four social bee visits in the tunnel and two in the exclusion cage for Sapphire.

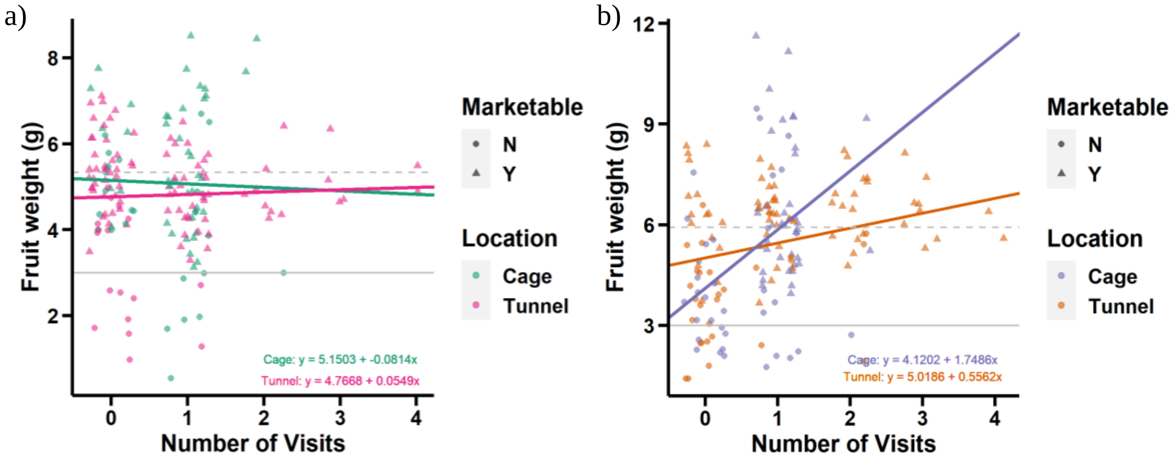


Figure 3.3. Fruit weight of raspberries harvested from study flowers in both the study tunnel and the exclusion cage with increasing numbers of visits for a) Diamond Jubilee and b) Sapphire. The raw data points are shown and jittered horizontally for clarity. Colours indicate the location and shapes indicate whether the fruit was marketable. The grey solid horizontal lines show the minimum marketable fruit weight dictated by the distributer and the grey dashed line shows the mean fruit weight for open pollinated flowers.

Number of social bee visits did not significantly predict fruit weight for Diamond Jubilee (Est=-0.081, SE=0.33, t= -0.249, p=0.803; Fig. 3.3a). There was no significant location or interaction effect (Table S3.4). There was a significant interaction effect of location and number of social bee visits for Sapphire (Est=-1.192, SE=0.46, t=-2.574, p= 0.0109; Fig 3.3b). Fruit weight increased more steeply with number of visits in the tunnel than in the exclusion cage. Fruit weight did not reach open pollination levels for either cage or tunnel flowers for Diamond Jubilee but did for Sapphire. This was reached after one visit in the cage and two in the tunnel. The model outputs are presented in Table S3.5.

3.4.3 The effect of visitation durationon marketable fruit set and fruit weight

Total visit duration was not a significant predictor of marketable fruit set for Diamond Jubilee (Est= 0.002, SE=0.002, z=0.612, p= 0.5403) but there was a significant location effect (Est=0.929, SE=0.44, z=2.132, p=0.0330) (Figure. 3.4a). Flowers in the cage yielded 2.5 times fewer marketable berries across the different visit durations. The interaction between total visit duration and the location of the flower was significant for Sapphire (Est=-0.029, SE=0.01, z=-2.336, p=0.0195; Fig 3.4b).

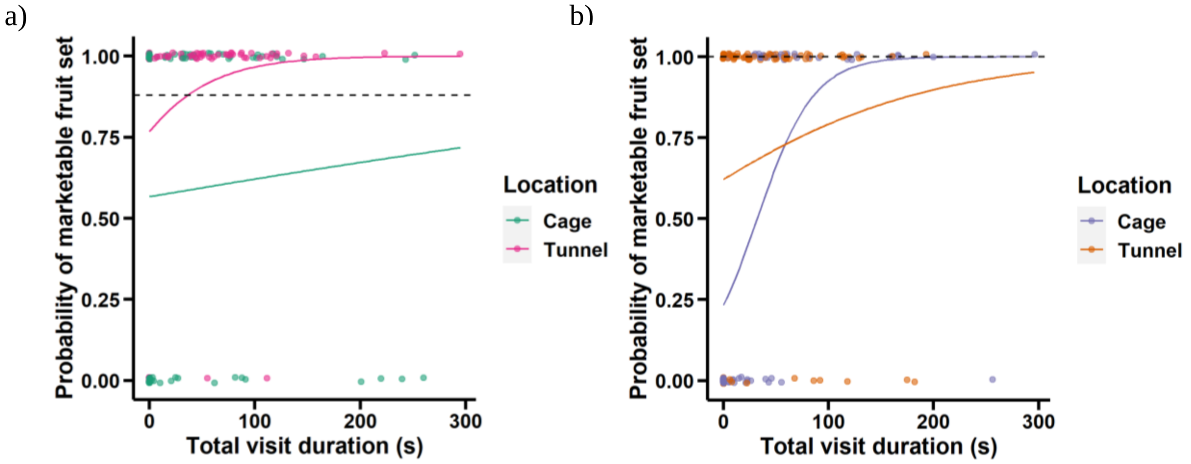


Figure 3.4 The predicted probability of marketable fruit set of study flowers in both the study tunnel and the exclusion cage with total visit duration for a) Diamond Jubilee and b) Sapphire. The bold lines indicate the predicted mean probability of marketable fruit set. The raw data points are shown and jittered for clarity. Dashed lines indicate the open pollination proportion of fruit that were marketable within the study tunnel.

Flowers in the tunnel yielded more marketable fruits when they received low visit durations or no visits, while flowers in the cage increased more steeply in the proportion of marketable fruit produced with increasing total visit duration. The model outputs are presented in Table S3.6. Marketable fruit set comparable to open pollination was achieved after ~30 seconds in the tunnel and was not observed in the exclusion cage for Diamond Jubilee though this could not be compared to open pollination under cage conditions. Marketable fruit set equivalent to open pollination was not achieved in the tunnel for flowers that received controlled insect visitation while ~170 seconds of visitation was required in the cage for Sapphire.

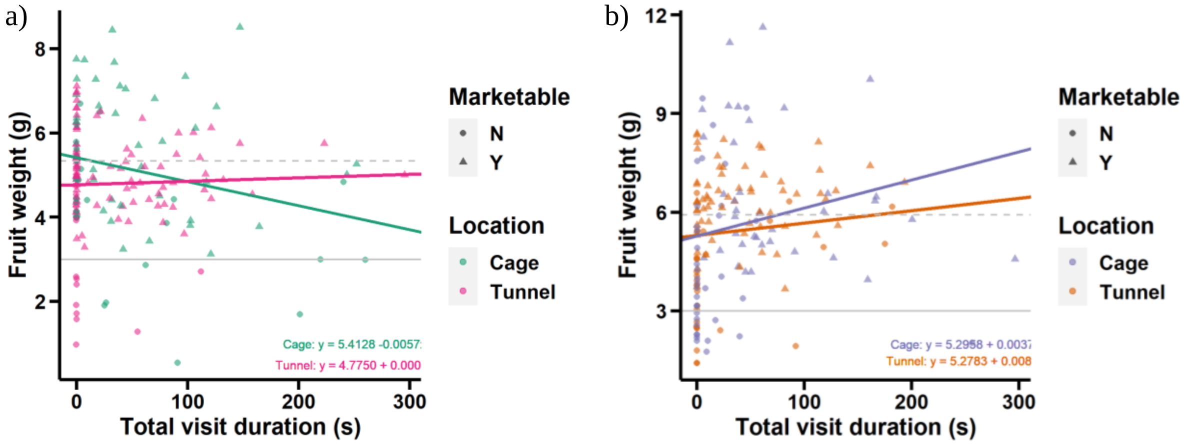


Figure 3.5 Fruit weight of raspberries harvested from study flowers in both the study tunnel and the exclusion cage with increasing total visit duration for a) Diamond Jubilee and b) Sapphire. The raw data points are shown and jittered horizontally for clarity. Colours indicate the location and shapes indicate whether the fruit was marketable. The grey solid horizontal lines show the minimum marketable fruit weight dictated by the distributer and the grey dashed line shows the mean fruit weight for open pollinated flowers.

Total visit duration significantly predicted fruit weight for Diamond Jubilee (Est=-0.006, SE=0.002, t=-2.402, p=0.0174; Fig. 3.5a). Fruit weight decreased slightly with visit duration. There was also a significant location effect (Est=-0.638, SE=0.27, t=-2.358, p=0.0195). Neither location nor total visit duration was a significant predictor for Sapphire fruit weight (Table S3.7; Fig 3.5b).

3.4.4 Visit duration of social bees in the cage vs the tunnel

Visit duration of the first insect visit to a virgin crop flower did not significantly differ between honey bees and bumble bees for either cultivar and were not significantly different between cage and tunnel settings (Table S3.8). Honey bees foraging on Diamond Jubilee flowers had visit durations of 85.9 seconds (±13.2) on average in the tunnel and 104 seconds (±16.6) in the cage. Bumble bees had visit durations of 55.2 seconds (±15.4) in the tunnel and 66.6 (±13.3) in the cage. Honey bee visits to Sapphire flowers were 53.1 seconds (±11.3) in the tunnel and 52.2 (±8.28) in the cage and bumble bee visits were 33.8 seconds (±8.99) and 72.9 seconds (±15.4) in the cage.

3.4.5 The relationship between number of seeds, fruit weight and marketability

There was a significant positive relationship between the number of seeds in a berry and fruit weight for both Diamond Jubilee (Est=0.057, SE=0.008, t=6.964, $p < 0.0001$) and Sapphire independently (Est=0.045, SE=0.01, t=4.410, $p<0.001$) with no significant effect of pollination treatment on this (Table S3.9). The relationship between number of seeds per berry and fruit weight significantly differed between cultivars (Est=-0.010, SE=0.004, t=-2.33, p=0.02). Fruit weight increased by 0.053g for Diamond Jubilee with each additional seed that develops and by 0.043g for Sapphire (Table S3.9; Fig 3.6).

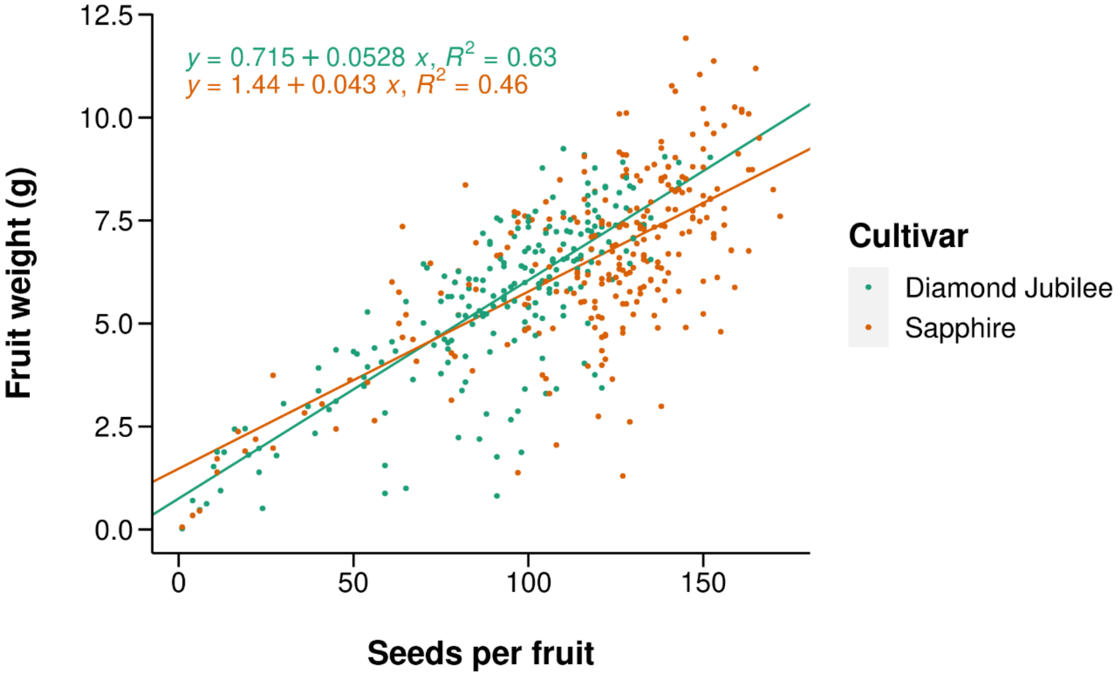


Figure 3.6 The relationship between the number of seeds per fruit and fruit weight with crop cultivar; Diamond Jubilee (n=210) and Sapphire (n=262). The equations of the regression lines along with the R^2 for each cultivar are shown.

Number of seeds per berry was a significant predictor of marketable fruit set for both Diamond Jubilee (Est=0.050, SE=0.01, z=6.517, $p<0.0001$) and Sapphire (Est=0.061, SE=0.01, z=7.029, $p<0.0001$) (Figure. 3.7). The probability of marketable fruit set was over 50% when Diamond Jubilee berries had ~65 seeds and Sapphire berries had ~80 seeds. The model outputs are presented in Table S3.10.

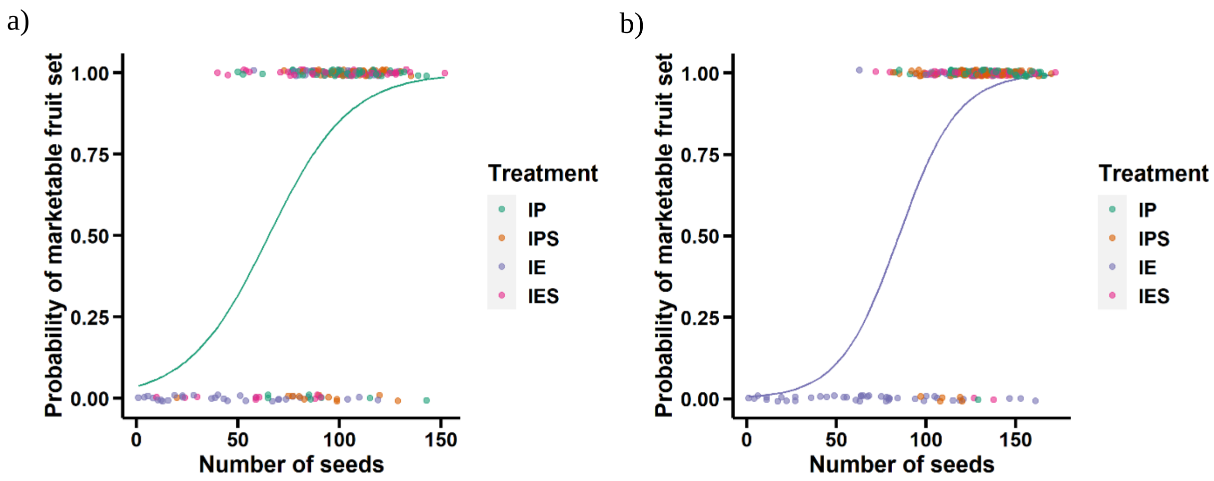


Figure 3.7. The predicted probability of marketable fruit set of study flowers across the study fields for a) Diamond Jubilee and b) Sapphire. The raw data points are shown and jittered for clarity and colours indicate the pollination treatment to show their distribution. Pollination treatments were Insect Pollinated (IP), Insect Pollinated with hand pollen Supplementation (IPS), Insect Exclusion (IE) and Insect Excluded with hand pollen Supplementation (IES).

3.5 Discussion

We found that bumble bees were more effective than honey bees at pollinating raspberry in this system when comparing single visit pollinator effectiveness scores using marketable fruit weight. This metric included both aspects of yield that are important to growers; the ability for the flower to produce a marketable fruit and the resulting weight of that marketable fruit. A single visit from a bumble bee to a crop flower yielded a greater marketable fruit weight than those that received open pollination. This suggests that receiving many pollinator visits may reduce overall fruit yield as found by Aizen *et al.* (2014) and Saez *et al.* (2014) who found that high visitation rates damaged raspberry stigmas and reduced drupelet set.

Two visits from a honey bee were estimated to be sufficient to produce a fruit that did not differ from those produced by open pollinated flowers. However, we found no difference in marketable fruit set or marketable fruit weight between flowers that received a single visit from a bumble bee or a honey bee. This suggests that for achieving marketable fruits and fruit weight beyond the marketability threshold, both social bee groups were equitable, but that bumble bee visits can provide greater yield overall than honey bee visits. Willmer *et al.* (1994) also found that honey bees are not as efficient pollinators of raspberry as bumble bees; carrying less pollen on their bodies and depositing less pollen on raspberry stigmas per visit and foraging at a slower rate than bumble bees. In contrast, Andrikopoulos and Cane (2018a) found no significant difference in the number of drupelets produced after a single visit from a honey bee compared to a bumble bee for two other cultivars of red raspberry (Cowichan and Latham). Our study showed that the number of seeds, a proxy for the number of drupelets, significantly predicted marketable fruit set and so the benefit of honey bee and bumble bee visits to the number of raspberry drupelets produced reported by Andrikopoulos and Cane (2018a) is likely to have also improved marketable fruit set. These results indicate that the effectiveness of different pollinator groups for producing marketable fruit varies between raspberry cultivars, though there are no other studies that compare the pollinator effectiveness between *Bombus* and *Apis* species for raspberry crops. Other studies that examine pollinator effectiveness for raspberry pollination on a single or controlled visit level either don't measure fruit yield (Willmer *et al.* 1994) or focus on the effectiveness of honey bees or *Osmia* (Cane, 2005; Chagnon *et al.*, 1991). The benefit of managed honey bee hives for raspberry pollination is therefore in their high numbers rather than their efficiency.

Other studies have highlighted the risks that honey bees pose to wild pollinators through disease transmission (Figueroa et al 2019; Graystock, Goulson and Hughes, 2015; Graystock et al 2020) and competitive exclusion (Angelella *et al.*, 2021; Lindström *et al.*, 2016; Walther-Hellwig *et al.,* 2006) and the impacts on bumble bee colony growth thought to result from this (Elbgami *et al,* 2014; Goulson and Sparrow, 2008; Thomson, 2004, 2006). Therefore, despite some uncertainty about the impact of managed honey bees on wild pollinator communities (Mallinger *et al.,* 2017), our study highlights that bumble bees can provide equally or more effective per visit pollination services to raspberry crops compared to honey bees. As a result, employing honey bees for raspberry crop pollination may not be worth the associated risks, especially at farms where crop flowers receive at least two visits from bumble bee individuals during their receptive period. In instances where bumble bee populations are currently limited, conservation measures could be implemented to increase their abundance in the surrounding landscape such as pollen and nectar strips with tussocky grasses or banks for providing suitable forage and bumble bee nesting habitat (Carvell *et al.*, 2004, 2007; Kells and Goulson, 2003). This would provide a more sustainable and potentially preferable alternative crop pollinator to honey bees in the future.

The pollinator effectiveness scores in this study showed that less than two visits from a social bee were required to achieve a marketable fruit weight comparable to open pollinated flowers for both cultivars. This agrees with the findings of Andrikopoulos and Cane (2018b), though the visits in our study were not necessarily prolonged. This indicates that it may be the visit number rather than the length of visits that is important for raspberry fruit yields. For Diamond Jubilee we found that marketable yield was limited by fruit weight rather than marketable fruit set. Flowers in the study tunnel that received at least one insect visit exceeded the open pollinated marketable fruit set (Fig 3.2a). However, the flowers that received controlled numbers of visitation did not reach the mean fruit weight for open pollinated flowers (Fig 3.3a) though this difference was not significant. This indicates that there may be an optimal number of visits for Diamond Jubilee not captured in this study that is more than four insect visits but less than the number of visits received by open pollinated flowers. Saez *et al.* (2014) found that the number of drupelets per fruit (which we showed to be significantly positively related to fruit weight) declined with increasing numbers of visits and that number of drupelets was highest at visitation rates of ~10 visits per day. This optimal number of visits for Diamond Jubilee is therefore likely to be between 4 and 25 visits over the 2.5 day receptive period of raspberry flowers. The required number of social bee visits to Sapphire flowers was more limited by marketable fruit set in the study tunnel (Fig. 3.2b) than by fruit weight (Fig.3.3b). An asymptote at the open pollination marketable fruit set level of 100% was reached at ~4 visits (Fig. 3.2b) though this is based on a small number of flowers that received either three or four insect visits. Whereas fruit weight of flowers that received 2 insect visits was equivalent to that of open pollinated flowers (Fig. 3.3b). Therefore, although on average only two visits are required to achieve the same fruit weight as open pollinated flowers, receiving more than two visits increases the proportion of flowers that are achieving marketability and thus overall yield.

For Diamond Jubilee there was no significant effect of increased number of visits on either marketable fruit set or fruit weight. This indicates that receiving more than one or two visits did not lead to significantly better fruit yields. Furthermore, the duration of visits did not affect marketable fruit set but cumulative visit durations resulting in a slight decrease in fruit weight per flower. This suggests that fewer, shorter insect visits are optimal for pollinating Diamond Jubilee, and longer or repeated visits do not increase yield.

The effect of the number and duration of social bee visits on Sapphire fruit weight and marketable fruit set varied depending on the study location. Flowers in the tunnel required more visits and longer cumulative visit durations than those in the exclusion cage. Although not significant, the longer duration of bumble bee visits in the cage compared to the tunnel is likely to have contributed to the reduced number of visits required for Sapphire marketable fruit set and fruit weight in the cage. The differing methods used to facilitate controlled visits between the two survey locations could also have affected the per visit pollination effectiveness. In the tunnel, mesh bags were removed to allow any free flying pollinator to access the virgin flowers. It is possible that visitors to study flowers in the tunnels may not previously visited another crop flower. Whereas, in the mesh cage, bees had to be caught and then released into the cage to forage. Foraging bees were caught while foraging on a crop flower for ease of catching, ensuring that they had raspberry pollen on their bodies before visiting the study flower. This guaranteed that the bee had visited another raspberry crop flower before visiting a study flower, increasing the likelihood of successful pollination.

Although there have been no studies comparing within and between flower pollination effectiveness within a raspberry cultivar, it is likely that flowers visited by bees that have visited another crop flower previously have access to greater pollen availability during these visits. This is due to the high numbers of pollen grains on their bodies (Willmer *et al*., 1994). Studies that compare cross pollination with selfing for fruit yields compare bagged pollinator-excluded or hand-pollinated flowers pollinated with pollen from the same flower, with flowers that have received hand pollination using pollen from a different cultivar (Zurawicz, 2016; Zurawicz *et al.,* 2018). Further research could compare pollen deposition on stigma and pollen tube growth of emasculated vs non-emasculated flowers after no insect visits or a single visit from a pollinator. This would determine the proportion of pollen grains deposited on the stigma that were transported within a flower without insect pollinators and the proportion of pollen deposited by insects that is from the same or a different flower.

3.6 Conclusion

Our study found that honey bees and wild bees had similar benefits to fruit weight and marketable fruit set in raspberry crops. However, a single visit from a bumble bee resulted in greater overall yield in the form of marketable fruit weight than a single visit from a honey bee. Furthermore, our results suggest that high visitation rates may have negative impacts on raspberry yields. Given these findings and the potential risks to wild pollinators posed by honey bee hives, we recommend reducing reliance on honey bee pollination for raspberries and supporting bumble bee conservation efforts. Our study also showed that only two visits from either a honey bee or a bumble bee were needed to achieve marketable yields that did not differ from those of open-pollinated flowers, indicating that very low visitation rates are sufficient for optimal yields. Based on this, we suggest removing or reducing honey bee hives from agricultural systems where raspberries receive at least two bumble bee visits per flower. This can be monitored using timed flower observations.

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3.8 Supplementary material

Table S3.1. Pairwise fisher exact test results for the comparison of **marketable fruit set** between **pollination treatments** for each cultivar and each location. Adjusted p values used a Bonferroni correction for repeated hypothesis tests within the pairwise comparisons. Sample size n is the combined number of fruits collected from flowers of the two treatments being compared.

Cultivar	Location	Group 1	Group 2	n	p	p.adj	p.adj.signif
Diamond Jubilee	Cage	Closed	Single_BB	50	0.0377	0.113	NS
		Closed	Single_HB	34	0.487	0.632	NS
		Single_BB	Single_HB	44	0.316	0.632	NS
	Tunnel	Closed	Open	99	0.214	0.856	NS
		Closed	Single_BB	67	0.101	0.6505	NS
		Closed	Single_HB	81	0.081	0.486	NS
		Open	Single_BB	52	0.582	1.000	NS
		Open	Single_HB	66	0.700	1.000	NS
		Single_BB	Single_HB	34	1	1.000	NS
Sapphire	Cage	Closed	Single_BB	47	<0.0001	<0.0001	****
		Closed	Single_HB	44	<0.0001	<0.0001	****
		Single_BB	Single_HB	51	0.235	0.705	NS
	Tunnel	Closed	Open	87	<0.0001	<0.0001	****
		Closed	Single_BB	45	0.0004	0.0262	*
		Closed	Single_HB	59	0.034	0.204	NS
		Open	Single_BB	58	1.000	1.000	NS
		Open	Single_HB	72	0.0004	0.0029	**
		Single_BB	Single_HB	30	0.155	0.93	NS

Table S3.2. Results of Linear Mixed Models (LM's) for the effect of **pollination treatment** and **location** on the response of **marketable fruit weight** for (a) Diamond Jubilee and (b) Sapphire with a gaussian error distribution

Variable	Estimate	Std. error	t value	p
a)				
(Intercept)	6.0600	0.3779	16.038	<**0.001**
Treatment:Open	0.2621	0.2427	1.080	0.282
Treatment:Single_HB	-0.7917	0.5344	-1.482	0.141
Treatment: Single_BB	-0.6114	0.4412	-1.386	0.168
Location: Tunnel	-0.6538	0.4123	-1.586	0.115
Location: Tunnel x Treatment:Open	NA	NA	NA	NA
Location: Tunnel x Treatment:Single_HB	0.1826	0.6039	0.302	0.763
Location: Tunnel x Treatment:Single_BB	0.1208	0.5797	0.208	0.835
b)				
(Intercept)	7.0006	0.6989	10.016	<**0.001**
Treatment:Open	-0.4109	0.4045	-1.016	0.312
Treatment:Single_HB	-0.9285	0.7700	-1.206	0.230
Treatment: Single_BB	-0.1771	0.6098	0.290	0.772
Location: Tunnel	0.6720	0.6038	1.113	0.268
Location: Tunnel x Treatment:Open	NA	NA	NA	NA
Location: Tunnel x Treatment:Single_HB	-0.8442	0.7700	1.096	0.275
Location: Tunnel x Treatment:Single_BB	NA	NA	NA	NA

Table S3.3. Results of Linear Mixed Models (LM's) for the effect of **pollination treatment** and l**ocation** on the response of **fruit weight** for (a) Diamond Jubilee and (b) Sapphire with a gaussian error distribution

Variable	Estimate	Std. error	t value	p
a)				
(Intercept)	5.3581	0.3107	17.244	**<0.001**
Treatment:Open	0.4968	0.2826	1.758	0.0804
Treatment:Single_HB	-0.6212	0.4842	-1.283	0.2011
Treatment: Single_BB	-0.4114	0.4011	-1.026	0.3064
Location: Tunnel	-0.5283	0.3611	-1.463	0.145
Location: Tunnel x Treatment:Open	NA	NA	NA	NA
Location: Tunnel x Treatment:Single_HB	0.3550	0.5906	0.601	0.5485
Location: Tunnel x Treatment:Single_BB	0.4973	0.6228	0.798	0.4256
b)				
(Intercept)	3.8327	0.3811	10.058	**<0.001**
Treatment:Open	1.1405	0.3696	3.086	**0.002**
Treatment:Single_HB	2.4278	0.5160	4.705	**<0.001**
Treatment: Single_BB	2.1140	0.5028	4.205	**<0.001**
Location: Tunnel	0.9446	0.4730	1.997	**0.047**
Location: Tunnel x Treatment:Open	NA	NA	NA	NA
Location: Tunnel x Treatment:Single_HB	-1.3183	0.6904	-1.909	0.0578
Location: Tunnel x Treatment:Single_BB	-0.7397	0.8332	-0.888	0.3759

Table S3.4. Results of Linear Mixed Models (LM's) for the effect of **number of visits** and **location** on the response of **marketable fruit set** for (a) Diamond Jubilee and (b) Sapphire with a binomial error distribution

Variable	Estimate	Std. error	z value	p
a)				
(Intercept)	-0.3015	0.4367	-0.690	0.490
Number of visits	0.9600	0.5010	1.916	0.055
Location: Tunnel	1.3235	0.5010	2.500	**0.012**
Location: Tunnel x Number of visits	0.8806	0.8806	0.981	0.327
b)				
(Intercept)	-2.984	1.024	-2.913	**0.004**
Number of visits	3.684	1.060	3.476	<**0.001**
Location: Tunnel	2.798	1.071	2.613	**0.009**
Location: Tunnel x Number of visits	-2.458	1.112	-2.209	**0.027**

Table S3.5. Results of Linear Mixed Models (LM's) for the effect of **number of visits** and **location** on the response of **fruit weight** for (a) Diamond Jubilee and (b) Sapphire with a gaussian error distribution

Variable	Estimate	Std. error	t value	p
a)				
(Intercept)	5.1503	0.2983	17.263	<**0.001**
Number of visits	-0.0814	0.3263	-0.249	0.803
Location: Tunnel	-0.3834	0.3436	-1.116	0.266
Location: Tunnel x Number of visits	0.1363	0.3595	0.379	0.705
b)				
(Intercept)	4.1202	0.3931	10.481	<**0.001**
Number of visits	1.7486	0.4260	4.105	<**0.001**
Location: Tunnel	0.8984	0.4756	1.889	**0.061**
Location: Tunnel x Number of visits	-1.1924	0.4632	-2.574	**0.011**

Table S3.6. Results of Linear Mixed Models (LM's) for the effect of **visit duration** and **locatio**n on the response of **marketable fruit set** for (a) Diamond Jubilee and (b) Sapphire with a binomial error distribution

Variable	Estimate	Std. error	z value	p
a)				
(Intercept)	0.2703	0.2170	0.853	0.393
Visit duration	0.0023	0.0037	0.612	0.540
Location: Tunnel	0.9289	0.4357	2.132	**0.033**
Location: Tunnel x Visit duration	0.0195	0.0114	1.702	0.088
b)				
(Intercept)	-1.1907	0.4004	-2.974	**0.003**
Number of visits	0.0372	0.0111	3.363	**<0.001**
Location: Tunnel	1.6870	0.4847	3.481	**<0.001**
Location: Tunnel x Visit duration	-0.0288	0.0123	-2.336	**0.020**

Table S3.7. Results of Linear Mixed Models (LM's) for the effect of **visit duration** and **location** on the response of **fruit weight** for (a) Diamond Jubilee and (b) Sapphire with a gaussian error distribution

Variable	Estimate	Std. error	t value	p
a)				
(Intercept)	5.4128	0.2164	25.008	**<0.001**
Visit duration	-0.0058	0.0024	-2.402	**0.017**
Location: Tunnel	-0.6378	0.2704	-2.358	**0.020**
Location: Tunnel x Visit duration	0.0066	0.0035	1.870	0.063
b)				
(Intercept)	5.2958	0.2876	18.412	**<0.001**
Number of visits	0.0038	0.0038	0.988	0.325
Location: Tunnel	-0.0175	0.3843	-0.046	0.964
Location: Tunnel x Visit duration	0.0048	0.0057	0.843	0.401

Table S3.8. Results of Linear Mixed Models (LM's) for the effect of **pollinator group** and **location** on the response of **visitation duration** for (a) Diamond Jubilee and (b) Sapphire with a gaussian error distribution

Variable	Estimate	Std. error	t value	p
a)				
(Intercept)	66.567	12.050	5.524	<**0.001**
Location: Tunnel	-11.367	24.100	-0.472	0.639
Visitor: Honey bee	37.790	21.362	1.769	**0.081**
Location: Tunnel x Visitor: Honey bee	-7.115	32.764	-0.217	0.829
b)				
(Intercept)	72.89	11.35	6.420	<**0.001**
Location: Tunnel	-39.14	23.75	-1.648	0.103
Visitor: Honey bee	-20.72	16.55	-1.252	0.214
Location: Tunnel x Visitor: Honey bee	40.06	29.45	1.361	0.178

Table S3.9. Results of Linear regressions of **fruit weight** with the interaction of the **number of seeds per fruit** and **pollination treatment** for a) Diamond Jubilee and b) Sapphire and c) a linear regression of **fruit weight** with the interaction of the **number of seeds per fruit** and **cultivar**

Variable	Estimate	Std. error	t value	P
a)				
(Intercept)	-0.0047	0.8491	-0.006	0.996
Seeds per berry	0.0574	0.0082	6.964	**<0.001**
Treatment:IE	0.6856	0.9249	0.741	0.459
Treatment:IES	1.0461	1.0163	1.029	0.305
Treatment:IPS	0.4736	1.3188	0.359	0.720
Seeds per berry x Treatment:IE	-0.0040	0.0095	-0.416	0.678
Seeds per berry x Treatment:IES	-0.0057	0.0101	-0.563	0.574
Seeds per berry x Treatment:IPS	-0.0023	0.0127	-0.185	0.853
b)				
(Intercept)	1.0831	1.3772	0.789	0.431
Seeds per berry	0.0447	0.0101	4.410	**<0.001**
Treatment:IE	-0.0168	1.4393	-0.012	0.991
Treatment:IES	1.1186	1.9751	0.566	0.572
Treatment:IPS	0.7718	1.8576	0.415	0.678
Seeds per berry x Treatment:IE	0.0050	0.0111	0.449	0.654
Seeds per berry x Treatment:IES	-0.0092	0.0149	-0.617	0.538
Seeds per berry x Treatment:IPS	-0.0038	0.0141	-0.270	0.787
c)				
(Intercept)	0.7154	0.3056	2.341	**0.019**
Seeds per berry	0.0528	0.0032	16.552	**<0.001**
Cultivar:Sapphire	0.7247	0.4526	1.601	0.110
Seeds per berry x Cultivar:Sapphire	-0.0097	0.0042	-2.330	**0.020**

Table S3.10. Results of Linear Mixed Models (LM's) for the effect of **number of seeds** on the response of **marketable fruit set** for (a) Diamond Jubilee and (b) Sapphire with a binomial error distribution

Variable	Estimate	Std. error	z value	p
a)				
(Intercept)	-3.2846	0.6721	-4.887	<**0.001**
Number of seeds	0.0503	0.0077	6.517	<**0.001**
b)				
(Intercept)	-5.1339	0.9383	-5.472	<**0.001**
Number of seeds	0.0606	0.0086	7.029	<**0.001**

4.0 Wild pollinators provide more uniform crop visitation under protected cropping than honey bees and at rates sufficient to maximise raspberry marketable fruit set

4.1 Abstract

Polytunnels have enabled the extension of crop growing seasons, stabilized yields and improved the quality of crops produced. However, the impacts of these physical barriers on insect pollinators of insect pollinated crops are poorly understood. The presence of polytunnels could reduce insect pollination services where insects have access issues. Understanding the variation in visitation rate within crop fields can help inform the stocking densities of managed pollinators to ensure all areas of the field are receiving sufficient pollinator visits to maximise yields. We studied the effect of the distance from a tunnel opening and tunnel side on the visitation rate to crop flowers by honey bees and non-honey bee wild insects on a commercial soft fruit farm. 88% of insect visitors to raspberry crop flowers were honey bees though honey bee visitation was significantly reduced in 2021 compared to 2020, with a 46% reduction in hive stocking density. We observed that on average, flowers received more than 60 times the required two pollinator visits at honey bee stocking densities of 1 hive/ha during their ~2.5 day receptive period with some flowers receiving 72 visits in just an hours' worth of observation. In the absence of honey bees, marketable fruit set is still likely to be achieved, as crop flowers received an average of 0.42 non-honey bee pollinator visits per hour equating to ~8 visits in their receptive period, sufficient to achieve optimal fruit yields. We found a higher visitation rate of honey bees at the corners of fields than at distances of >20m from a field edge but found no significant difference in the honey bee visitation rates to flowers at the edges of fields compared to those in the middle. Wild pollinator visits did not significantly differ between field locations. This study highlights that managed pollinator stocking densities don't always reflect pollinator deficits or requirements, and that there is the potential to reduce the advised honey bee stocking densities and rely more heavily on wild pollinators to provide reliable uniform crop pollination services to soft fruits.

4.2 Introduction

Many agricultural and horticultural crops traditionally grown in open field settings are now being produced in covered cropping systems such as greenhouses and plastic tunnels to extend their growing season and permit the production of crops across a wider geographical region (Dalyrymple, 1973; Wittwer, 1993). Plastic crop protection tunnels such as high tunnels or hoop houses (Lamont *et al,* 2002) and polytunnels, have been used in horticulture in Europe since 1945 (Dalrymple, 1973). Spanish polytunnels were introduced into the UK in the mid-1990s (Calleja and Mills, 2012). They are constructed by stretching a clear plastic film or sheeting over hoops of metal or polyvinyl chloride (PVC) and have no electrical services, automated ventilation, or heating systems (Lamont *et al,* 2002), providing passive temperature and humidity control. Crop plants are primarily drip irrigated within the polytunnels for the purpose of watering and fertigation (Lamont *et al,* 2002).

Polytunnels increase and maintain higher winter temperatures, increase humidity and reduce air temperatures in the summer in shaded tunnels, enabling many crops to be grown 'out of season' (Johnson, Young and Karley, 2012; Singh *et al,* 2012). Growers capitalise on this, often concentrating their production on early and late season varieties when prices are high (Kempler, *et al* 2002). In temperate regions the tunnels create a warmer growing environment in early spring or autumn (Gent, 1991; Jiang *et al,* 2004; Wells, 1992, 1996; Wells and Loy, 1993), while in tropical regions they are used to protect against heavy rain during the rainy or monsoon season (Jensen and Malter, 1995) or for providing shade (Balliu and Sallaku, 2008). They provide shelter from wind (Wells and Loy, 1993) for fragile crops such as raspberries (Lamont, 2009), cold protection (McIntosh and Klingaman, 1993) and enable the humidity and temperature of the growing environment to be monitored and controlled by manual ventilation. By protecting crops from extreme weather conditions polytunnels have also expanded the suitable growing regions and reduced the productivity gap between regions making the production of specialised crops more global (Calleja *et al,* 2012; Dalyrymple, 1973; Wittwer, 1993).

While these tunnels provide many benefits to the production of horticultural crops, many of the crops grown under them require insect pollination to achieve optimal yields (Klein *et al,* 2007) and there is little advice on how to manage pollination services while using polytunnels. In a meta-analysis by Kendall *et al.* (2021), 81% of studies measuring *Apis mellifera* abundance in protected cropping systems found population decline and/or reduced colony activity under covers. This was due to reduced reproduction, increased adult mortality, reduced forager activity and increased disease prevalence (Kendall, *et al.,* 2021), which in some cases reduced crop yields (Dag, 2008). Bumblebees may be less affected by crop covers as only 50% of studies found a negative effect on populations and colony activity under covers with the other 50% of studies finding that populations were maintained (Kendall, *et al.,* 2021). The link between polytunnels and these negative impacts on pollinator health are uncertain but have been suggested to be changes to temperature (da Silva *et al.,* 2017) or humidity (Pinzauti, 1994) inside the tunnels, the light quality or quantity and heightened pesticide exposure in the semi-enclosed spaces (Dag, 2008; Kendall *et al*., 2021). Most of these studies look at the effect of protected cropping on managed pollinators put in place inside the tunnels with little consideration for those pollinators entering or attempting to enter polytunnels from outside.

Polytunnels provide a physical barrier to the crop where pollinators are coming from outside of the protected cropping system. Polytunnels are usually closed during early spring and autumn growing seasons to provide the required increase in temperature and humidity to enable the extension of the growing season (Singh *et al*, 2012). Closed polytunnels prevent access to crop flowers by insect pollinators and make it necessary for boxed pollinators such as bumble bees and solitary bees to be introduced inside the tunnels to provide the required crop pollination services. Even when the sides and ends of the tunnels are lifted in the summer to provide ventilation with increasing ambient temperatures, allowing access to the tunnels by insect pollinators from outside the crop field, the tunnels still prevent access to the crop from above ~1m from the ground at the sides of the tunnel and ~2.5m from the ground at either end of the tunnel. Pollinators can also become stuck in folds of the plastic sheeting where the opening flaps or sides are folded back or pushed up (personal observation).

Bumble bees have been found to have an affinity for following linear landscape structures such as pathways, roads and artificial structures and use them during exploratory flights (Brebner *et al*, 2021; Cranmer *et al*, 2012). Honey bees have also been found to use linear landscape features as navigational guides (Collett and Graham, 2015; Degen *et al*, 2016; Menzel *et al*, 2019). Polytunnels could therefore potentially act as guides or funnels for pollinators from outside the field to facilitate penetration into the centre of the field by crop pollinating insects. Although pollinators do enter polytunnels to forage, Hall *et al.* (2020) found that both managed honey bee *Apis mellifera* and stingless bee *Tetragonula carbonaria* visitation rates to raspberry and blueberry crop flowers in Australia declined with increasing distance from the tunnel ends, although no effect on sweat bee *Homalictus urbanus* visitation rate was found. The effect of the distance from tunnel openings on bumble bee and solitary bee visitation rates to crop flowers has not been previously studied.

Raspberries are an important soft fruit crop in the UK, representing 21% of the agricultural land dedicated to soft fruit (Ridley *et al*, 2022) and 20% of all crops grown under tunnels (Evans, 2013). Although 88% of raspberries grown in the UK are grown under tunnels (Ridley *et al*, 2022), the tunnels are usually left open at the ends and sides to facilitate ventilation during the warm summer months of the flowering and fruiting season while still providing shelter from wind and rain. This enables external pollinators to access the tunnels and reduces the need for managed pollinators, especially inside the tunnels. This is especially true for raspberry crops, which are very attractive to bees (Free, 1968). Using these estimates in combination with site specific visitation rates to crop flowers to determine whether current honey bee stocking densities are providing sufficient pollination as proposed by Garibaldi *et al.* (2020), they would vary greatly in their conclusion of between 17-195 visits to 100 flowers per hour. Using the wrong estimate of required number of visits to maximise yield could therefore lead to overstocking. More studies that quantify the number of visits needed for complete raspberry pollination, along with many other crops, are therefore needed to help determine a more accurate estimation of the minimum required number of pollinator visits and ensure that managed pollinators are stocked accordingly.

Understanding the effect of the presence and length of polytunnels on the visitation rate of both managed and wild pollinators to raspberry crop flowers will help inform how pollinators are managed in these protected cropping environments.

Increasing the stocking density of managed pollinators may mitigate against any negative effects of the polytunnels on crop visitation frequency (Kendall *et al.,* 2021). However, this approach might come at the expense of wild pollinator abundance (Angelella *et al.,* 2021; Mallinger *et al.,* 2017; Lindström *et al.,* 2016), leading to a potential trade-off between relying on honey bees or wild pollinators to ensure sufficient and even pollination service provision to crop flowers under polytunnels. Evaluating the relative abundance of managed and wild pollinators in different crop systems under varying managed pollinator stocking densities will provide insights into the effects of managed pollinators on crop pollination service provision and wild pollinator abundance. This assessment is facilitated by the use of diversity indices.

Diversity indices are quantitative measures used to describe the variety and distribution of different species in a community. They enable the assessment and comparison of the diversity of species or groups of species, within specific habitats and enable the comparison and monitoring of ecological communities over time or across different habitats. These indices are valuable tools for assessing the impacts of environmental changes, human activities, and conservation efforts on biodiversity.

In this study, diversity indices allow us to assess the contribution of each pollinator species to the overall pollination service provided to the raspberry crops, enabling comparisons between years. Various diversity indices provide insights into different aspects of community species composition and considered alone don't provide the full picture. We consider the following indices in this study as they capture the main aspects of community composition that are of value in this context.

Species Richness (S) is the simplest measure of diversity. It represents the total number of different species in a given area or sample. However, it does not account for species abundance or evenness, whereas the Shannon Evenness Index (J) quantifies how evenly individuals are distributed among different species without considering the number of species The Shannon-Wiener Index or Shannon Entropy (H' or H) considers both species richness and evenness, incorporating the number of species and their proportional abundance in a community. Higher values indicate greater diversity. Simpson's Diversity or Concentration Index (D) also considers richness and evenness but is more sensitive to changes in the abundance of dominant species. It ranges from 0 to 1, with higher values indicating lower diversity. The Berger-Parker Index (BP), or dominance index, focuses on the abundance of the most dominant species in a community. The index ranges from 0 to 1, with higher values indicating a higher degree of dominance by a single species. However, this index does not account for the presence or abundance of other species in the community. Consequently, it is often used alongside other diversity indices to obtain a more comprehensive understanding of community structure. Assessing all these diversity indices allows us to fully understand the community structure and composition, enabling us to determine how this has changed between years.

Here, we used raspberry as a model crop to investigate the pollinator abundance and species richness of crop flower visitors in a protected cropping system in two different study years and measured the abundance of these visitor groups along the length of polytunnels and their visitation rate to crop flowers at the edges and centre of fields.

The main aims of this study were:

- To determine which insect species or taxon is the main crop pollinator in this protected cropping system in terms of abundance and visitation rate to crop flowers. We expected *Apis mellifera* to play this role, with the highest on-crop abundance and be the most frequent visitors because managed colonies are added by the grower to provide pollination.

- To investigate how pollinator visitation rates vary with distance from the open ends of commercial polytunnels to inform pollination monitoring methods. We expected visitation rates to decline along the polytunnels as found by Hall *et al.,* (2020).
- To determine whether the honey bees and wild pollinators are independently meeting or exceeding the required two visits to achieve marketable fruit set. We expected honey bees to exceed this due to the high density of hives at the farm.

4.3 Methods

4.3.1 Study site

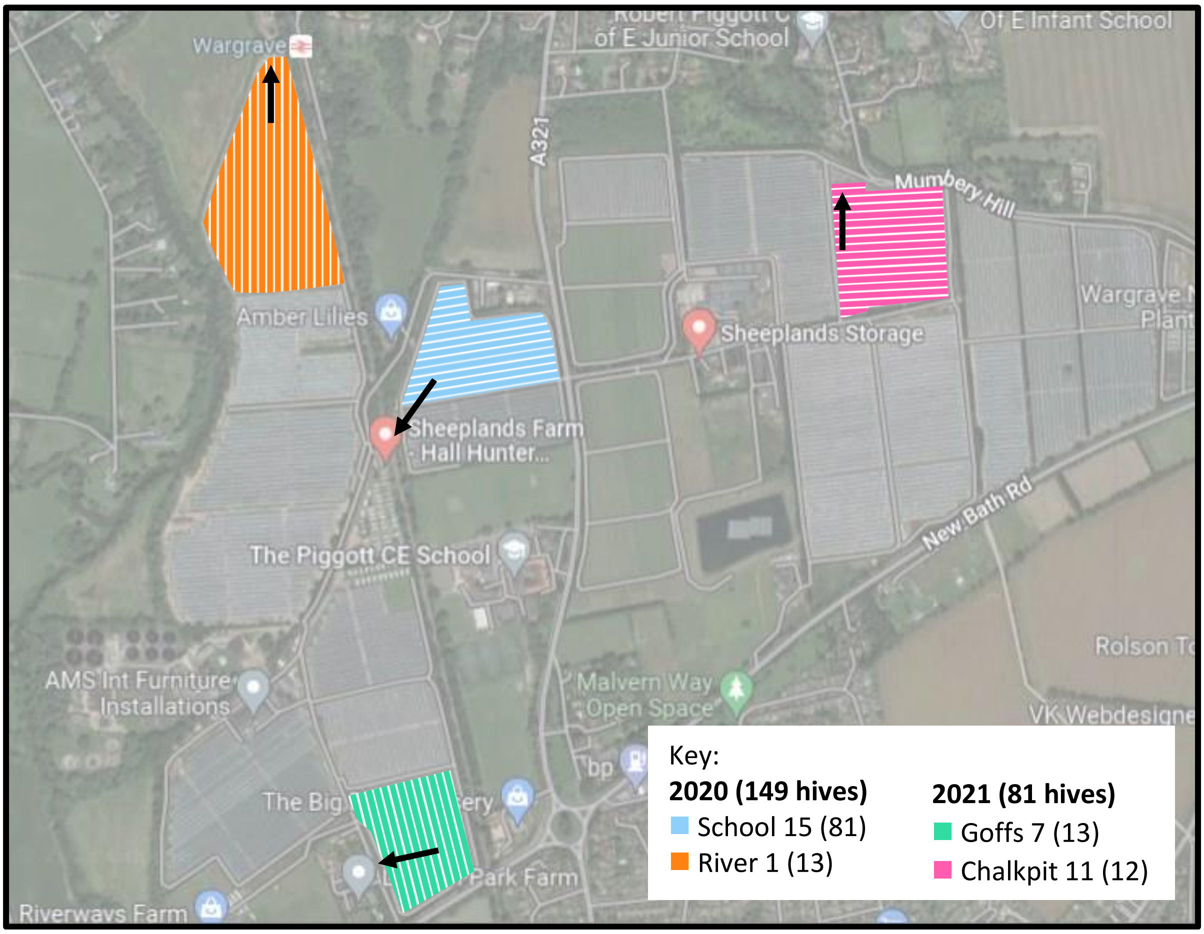

Figure 4.1. Map of the farm showing the locations of the four study fields. The orientation of the white lines on each field indicates the orientation of the Spanish polytunnels. The location of the honey bee hives on the farm are shown by the black arrows and the numbers of hives in each location is given in the key, in parentheses. Fields are labelled with the names given by the farm. The numbers of hives for School 15 and River 1 were from 2020 and the numbers of hives for Goffs 7 and Chalkpit 11 were from 2021.

The study was carried out on an 81-hectare commercial soft fruit farm near Reading, south England (51°29′32″N, 000°52′28″W) throughout the period from July to September in 2020 and 2021. Two cultivars of red raspberry (*Rubus idaeus*); ‘Diamond Jubilee’ and ‘Sapphire’, were included in the studies, both developed by BerryWorld and filed for growers in 2013. Each experimental site was made up of one commercial field of >1.5 ha surrounded by uncropped field margins. There were small areas of semi-natural grassland and patchy woodland on the farm and within the immediate surrounding area (Figure 4.1). Both raspberry cultivars were grown under Spanish polytunnels (7.8

metres wide) with three rows of potted raspberry canes in each tunnel, each row was ~2m apart from each other. 149 rented honey bee hives were in place at the farm during 2020, equating to ~2 hives/ha of farmland, throughout the raspberry flowering season for the purpose of crop pollination of both raspberries and strawberries. This dropped to 81 colonies in 2021 due to colony losses and relocation to other sites reducing the stocking rate to ~1 hive/ha. The field and hive locations within the farm are shown in Figure 4.1. No managed bumble bee colonies were active on the farm during the study period in 2020. A few colonies were still active in an adjacent field to Diamond Jubilee in 2021 however, they were at the end of their 10th week *in situ* when the first study flowers opened. It is therefore likely that the bees leaving the hive were gynes and males.

4.3.2 Pollinator visitation rates to crop flowers at the edge of the field vs the middle

The frequency of visits to crop flowers was assessed by selecting 10 open, receptive flowers at four random locations within a study field. Similar numbers of locations were within 20 m of the edge of the field (n=26) and in the field interior or middle, defined as at least >20 m from any field edge (n = 22). The edge plants were then subdivided based on their closest edge. Tunnel opening was defined as <20 m from a tunnel opening (n = 2), tunnel side was defined as <20 m from the side of a tunnel perpendicular to the tunnel opening (n = 10) and corner <20 m from both the tunnel opening and tunnel side perpendicular to each other (n= 14) (Figure 4.2). These 10 flowers were marked with different coloured wires to enable differentiation between flowers. Each set of 10 flowers was observed for 20 minutes in a single timed flower count. The length of observation time was the minimum needed to obtain accurate estimates of flower visitor rates in this system, as determined by preliminary observations of up to two hours, using the method described by Fijen and Kleijn (2017; see Chapter 5). During timed flower counts, all insect visitors to a flower were recorded, noting the time of the visit, the species or group the insect belonged to (Honey bee, Bumble bee, Solitary bee, Hoverfly, Wasp, Non-syrphid fly, other) and the wire colour of the visited flower. If a pollinator visited a flower, then visited other flowers, in the same or different inflorescences (focal or non-focal flowers), and returned to the first flower, this was considered two visits.

Timed flower counts were repeated three times in a survey day with one replicate in each period of the day (10:00-12:00, 12:00-14:30 and 15:00-17:00) for each set of 10 flowers. This ensured that each location was surveyed across each of the three time periods. In total 48 locations, 4 locations on each of 12 days, were surveyed across the two cultivars. For 'Diamond Jubilee' this was carried out on the 17th and 21st July in 2020 and the 21st of July and 4th August in 2021. For 'Sapphire' this was carried out on the 28th of July and the 1st, 5th, and 10th August in 2020 and the 2nd, 9th, 14th, and 19th July in 2021. A reduced field season in 2020 and adverse weather conditions in 2021 meant that fewer Diamond Jubilee survey days were possible.

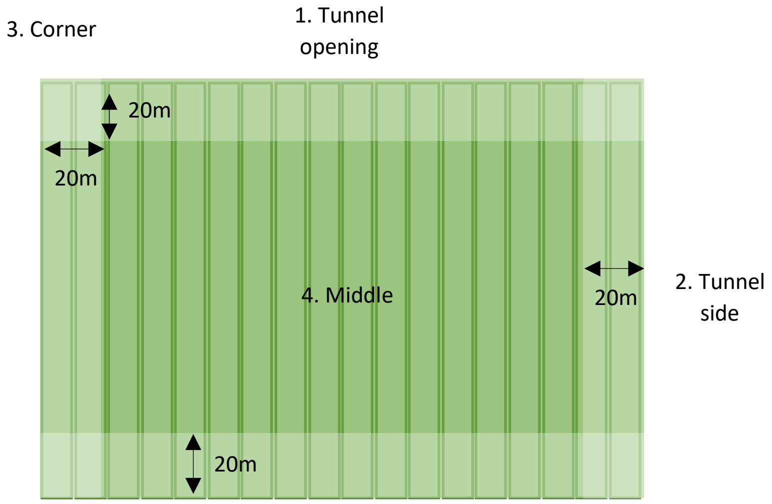

Figure 4.2 Diagram of the 4 location treatments: tunnel opening, tunnel side, corner, and middle

4.3.3 Pollinator abundance on crop flowers

The abundance of crop flower visiting insects was assessed by walking 100m long transects along randomly selected tunnels in each crop field. The tunnels were selected from those that were >20 metres from the parallel sides of the field to enable them to be used to record pollinator abundance at increasing distances from the edge of the field. Each transect was split into five 20 metre sections and the transect was walked at a slow pace over ~30 minutes, scanning all crop flowers on one side of a crop row and recording all insect visitors to crop flowers. Honey bees and bumble bees were identified to species on the wing. Where possible all other insects observed foraging on crop flowers were caught in insect nets and euthanized for later identification under a microscope in the lab. Specimens were identified to genera using taxonomic keys, and to species where this was possible. Each of the three transects were walked three times in a survey day with one replicate in each period of the day as for the timed flower counts. 36 transects, 3 transects on each of 12 days were surveyed across the two cultivars. For 'Diamond Jubilee' this was carried out on the 16th and 20th July in 2020 and the 21st and 29th July in 2021. For 'Sapphire' this was carried out on the 26th and 31st July and the 4th and 9th August in 2020 and the 4th, 9th, 14th and 19th July in 2021. As for the timed flower counts a reduced field season and adverse weather conditions lead to fewer Diamond Jubilee survey days than Sapphire in both years.

4.3.4 Data analyses

To determine the relative abundances of both honey bees and wild pollinators on crop flowers and whether this differed between years, a variety of diversity indices were calculated for both study years (see Table 4.2) using the formulas in Table 4.1. We use the exponent of Shannon's entropy to give the effective number of species, as described by Jost (2006), i.e., the number of species in an equivalent community that has the same value for Shannon's entropy as our community but is composed of equally abundant species. The metric allows comparison between survey years on a linear rather than log scale where a community with twice as many species or genera has an effective number of species/genera of twice as much as that with which it is being compared.

Table 4.1. Descriptions and formula for the diversity indices calculated using our transect data

Metric	Description	Formula
Richness (S)	The number of species or genera recorded	Number of species or genera
Berger-Parker Dominance (BP)	Proportional abundance of only the most abundant species in the population	P_{max}
Shannon's evenness (J)	A ratio of Shannon index calculated from a community, and maximum Shannon index for the community with the same richness (i.e. with S species all having the same relative abundances) The value is 1 for a community with all species at equal abundances	$\frac{H}{ln\, S}$

Simpson's concentration (*D*)	The probability that two randomly selected individuals will be of the same species. Decreases with increasing species richness. Values usually between 1.5-3.5 with the maximum value occurring when all species have the same relative proportion within a community	$\sum P_i^2$
Gini-Simpson (1-*D*)	The inverse of the Simpson's concentration which enables the value to increase with increasing richness	$-\sum P_i^2$
Shannon's entropy (*H*)	The uncertainty with which we can predict which species one randomly selected individual in the community belongs to. Considers both species richness and evenness.	$-\sum P_i \ln(P_i)$
Shannon exponent (effective number of species) (e^H)	Exponent of Shannon's diversity, converts diversity indices to effective number of species or the 'true diversity'	$\exp -\sum P_i \ln(P_i) = e^H$

P_i is the proportion of individuals belonging to species *I*; P_{max} is the proportion of individuals belonging to the most abundant species.
Formulas from McCune and Grace (2002), Shannon (1948), Simpson (1949), Jost (2016) and Zelený (2021)

Analyses were conducted in R statistical software (version 4.1.3) (R Core Team, 2022). We produced species accumulation curves for transects, and individuals, adding samples in a random order, using the *Vegan* package (Oksanen *et al*, 2022). Confidence intervals are the standard deviation of the estimated curve. We conducted three analyses to address how visitation rates and pollinator abundance vary with distance from the edge of field. Negative binomial GLMMs were run with pollinator visitation rate (from timed flower counts) and crop flower visitor abundance (from transect walks) as response variables. Model structures, and details of variables, are shown in Table 4.1. All models included a two-way interaction term between year and pollinator group as an explanatory term, and a nested random factor of field/survey day/location ID or field/survey day/transect ID, to account for resampling the same locations within the same day and field, and across different days within the same field. The other explanatory variables for the timed flower count model were location in the field, interacting with both year and pollinator group independently. The other explanatory variables for the transect walk model were the distance from tunnel opening, interacting with both year and pollinator group independently. Due to the low sample size for tunnel opening using the timed flower count data, we fit a model using the transect data with a pooled pollinator visitor abundance for transect sections that were >20m away from the tunnel opening and compared them to the sections that were 0-20m from the tunnel opening to test whether there was a categorical effect of edge on pollinator abundance. The same interactions were included with distance from tunnel opening as a categorical variable: tunnel opening or middle.

Table 4.2 Description of the structure of GLMMs for abundance (from transect walks) and rate of insect visits to open pollinated flowers (from timed flower counts), with the distance from field edges and all response and explanatory variables. The maximal models are shown and were carried out in R (v.4.1.3).

Type	**Variable**	**Distribution (link/offset)**	**Definition**
Response	Visitor abundance	Negative binomial (log)	The number of visitors observed along 100m of crop
	Visitation rate	Negative binomial (log)	The number of visits observed per flower to marked flowers summed over 60 minutes of observation (3x 20 min observations)
Explanatory	Year	Two level categorical factor	**2020-2021**
	Location	Four level categorical factor	Location of the timed flower count: **Field corner** (≤20m from a tunnel opening *and* the perpendicular edge of the field), **tunnel side** (≤20m from the edge perpendicular to the tunnel openings but >20m from a tunnel opening), **tunnel opening** (≤20m from a tunnel opening but >20m from the perpendicular side of the field) and **middle** (>20m from any field edge)
	Pollinator group	2 level categorical factor	Identity of crop flower visitors; either **honey bees** or **wild pollinators** (all other crop visitors)
	Distance from tunnel opening	Discrete	Metres from the opening of a tunnel along the transect. Split into 5 20 metre sections (0-20m, 20-40m, 40-60m, 60-80m, 80-100m)
	Tunnel opening	2 level categorical factor	**Tunnel opening** (0-20m from tunnel opening) or **middle of tunnel** (>20m from tunnel opening: pooled 20-40m, 40-60m, 60-80m, 80-100m)
Random	Field/Survey day/Survey ID	4 level categorical factor/12 level categorical factor/48 level categorical factor	Unique identifier for each field, survey day within field and timed flower count location within survey day and field (four timed flower counts on each survey day across 2020 and 2021)

	Field/Survey day/Transect ID	4 level categorical factor/12 level categorical factor/36 level categorical factor	Unique identifier for each field, survey day within field and transect within survey day and field (three transects on each survey day across 2020 and 2021)

Response	**Model structure**
Visitation rate	Year*Pollinator group + Year*Location + Pollinator group*Location + (1|Field/Survey day/Survey ID)
Visitor abundance	Year*Pollinator group + Year*Distance from tunnel opening + Distance from tunnel opening*Pollinator group + (1|Field/Survey day/Transect ID)
Visitor abundance	Year*Pollinator group + Year*Tunnel opening + Tunnel opening *Pollinator group + (1|Field/Survey day/Transect ID)

4.4 Results

17,300 insect visits to *R. idaeus* crop flowers were observed over the two years, 12,314 in 2020 and 4,986 in 2021. The main groups of flower visitors are listed in Table 4.3 along with any species that made up at least 0.1% of visits over the two years. The full list of genera and species identified over the two survey seasons is given in Table S4.1. The number of visits to each flower during 1 hour of total observation time (3x20 min timed flower counts) varied between 0 and 72 visits flower^{-1} hour^{-1} (mean 12.01 ± 0.61) for all pollinators combined. Honey bee visitation rate ranged from 0 to 72 visits flower^{-1} hour^{-1} (mean 11.55 ± 0.60), this was almost 30 times as high as that of wild pollinators combined, which ranged from 0 to 4 visits flower^{-1} hour^{-1} (mean 0.42 ± 0.03).

4.4.1 Crop flower visitors

The main insect visitors to the commercial raspberry crops during 2020 and 2021 were *Apis mellifera* (87.9% of total visits) and *Bombus terrestris/lucorum* (9%). Other *Bombus* species (*B.lapidarius, B. pascuorum, B. pratorum* and *B. hypnorum* made up 1.1% of visits. All other insect groups combined made up the remaining 2% of observed visits; hoverflies, solitary bees, social wasps, flies, and other insects all individually represented less than 1% of flower visits observed. 145 visitors (0.8% of all visitors) were not able to be caught for microscope identification to genus. However, 85 of these unkeyed individuals were able to be identified to family level on the wing and so only 0.3% of all insect visitors were not able to be identified to family. Non-syrphid flies and ‘other’ insects were excluded from diversity analyses and the accumulation curve as they made up less than 0.2% of the crop flower visitors and could not be identified to family in the field.

Table 4.3 Abundance of each pollinator group visiting raspberry flowers over two years

Group	Species group	2020 visits	% of 2020 visits	2021 visits	% of 2021 visits
Honey bees	*Apis mellifera*	**11,321**	**92.0**	**3,892**	**78.0**
Bumble bees		**847**	**6.9**	**904**	**18.1**
	Bombus terrestris/lucorum agg.	774	6.3	786	15.8
	Bombus lapidarius	43	0.3	54	1.1
	Bombus pascuorum	27	0.2	61	1.2
Solitary bees		**89**	**0.7**	**23**	**0.5**
	Andrena sp.	54	0.4	11	0.2
	Other identified genera	6	<0.1	2	<0.1
	Not caught/Identified	29	0.2	10	0.2
Hoverflies		**31**	**0.3**	**78**	**1.6**
	Episyrphus balteatus	6	<0.1	16	0.3
	Other identified genera	16	0.1	32	0.6
	Not caught/Identified	9	<0.1	30	0.6
Social wasps		**8**	**<0.1**	**77**	**1.5**
	Vespula vulgaris	2	<0.1	24	0.5
	Other identified genera	1	<0.1	12	0.2
	Not caught/Identified	5	<0.1	41	0.8
Non syrphid flies		**15**	**0.1**	**6**	**0.1**
Other		**3**	**<0.1**	**4**	**<0.1**

The accumulation curve for individuals identified to genus for the transect walks did not exhibit a distinct plateau in either year with number of transects (Fig. 4.3a) or number of individuals (Fig. 4.3b), indicating that we are likely to have not observed all insect genera visiting raspberry crop flowers in this system. 12 genera were observed in both years while there were three genera that were only observed and identified in 2020 and four that were only observed and identified in 2021. The majority of these genera in both years were hoverflies; 8 genera in 2020 and 9 in 2021. However, each of these genera made up less than 0.4% of visits to observed crop flowers in each year. For the full list of genera see Table S4.1.

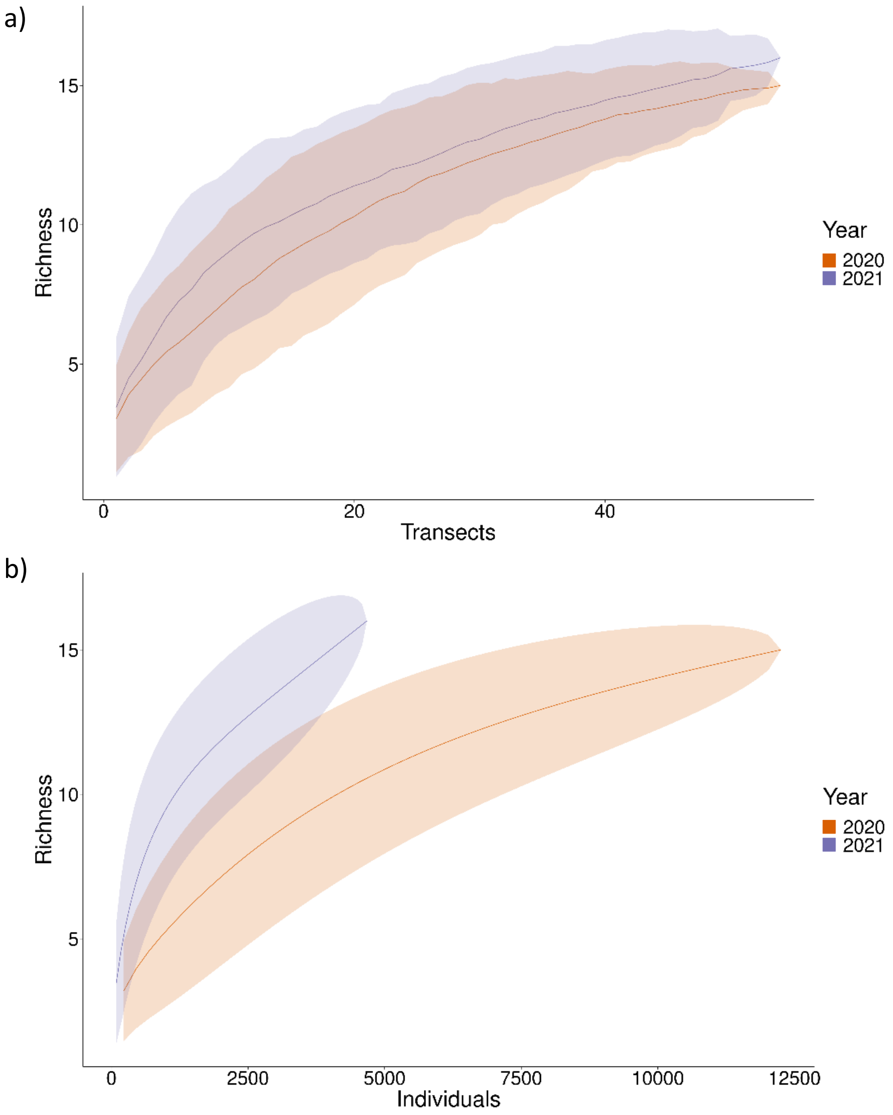

Figure 4.3 The genus accumulation curves a) with sampling effort (number of transects) and b) number of individuals observed. Shaded areas are the standard error of the estimate curve.

Diversity indices were used to compare the insect community visiting the raspberry crop flowers between the two survey years using genus level (Table 4.4). Individuals that were unidentified were assigned a genus based on the known proportions of genera within each insect group. We calculated dominance as the relative abundance of the most abundant genus of visitors. Genera richness was similar between years despite a 60% decrease in visitor abundance in 2021 compared to 2020. We found a 42% increase in the effective number of genera between years, using the exponent of the Shannon diversity, with 1.38 effective genera in 2020 and 1.96 in 2021. For wild pollinators alone there was a ~9% increase in visitor abundance in 2021 compared to 2020. Shannon diversity was greater in 2021 compared to 2020 and when this was converted to effective number of genera to enable the linear comparison between years we found a 15% increase with 1.784 effective wild pollinator genera in 2020 and 2.051 in 2021.

Table 4.4. Genera diversity indices for both the total raspberry pollinator community and the wild pollinator community identified using transect surveys during two study years. Dominance was calculated for honey bees (HB) when all genera were included and for bumble bees (BB) when calculated for wild pollinators only.

Index	**2020 Total**	**2020 Wild**	**2021 Total**	**2021 Wild**
Richness (S)	15	14	16	15
Abundance	12295	974	4950	1058
Berger-Parker Dominance (*BP*)	0.921(HB)	0.870 (BB)	0.786 (HB)	0.854 (BB)
Pielou’s Evenness (*J*)	0.119	0.219	0.243	0.265
Simpson (*D)*	0.853	0.763	0.652	0.733
Gini-Simpson (1-*D)*	0.147	0.237	0.348	0.267
Shannon (H)	0.323	0.579	0.672	0.719
Effective number of genera (e^H)	1.381	1.784	1.959	2.051

4.4.2 The effect of year on visitation rate

Visitation rate to crop flowers, measured in timed flower counts, varied with the interaction between survey year and pollinator group (Chisq = 57.084, $p<0.0001$) and the interaction between pollinator group and location (Chisq = 24.474, $p<0.0001$). The visitation rate of honey bees was significantly lower in 2021 (3.75 visits per flower per hour ± 0.29) than in 2020 (20.3 visits per flower per hour ± 0.93) (Est = 1.625, SE = 1.92, 8.476, $p<0.0001$) but there was no effect of year for wild pollinators (Est = 0.218, SE = 0.244, t=0.895, p=0.8074). Wild pollinators visited raspberry flowers at a rate of 0.49 visits per flower per hour (± 0.05) in 2020 and 0.37 visits per flower per hour (± 0.04) in 2021. Honey bees visited the crop flowers at a significantly higher rate than wild pollinators in both years (2020: Est = 3.066, SE = 0.154, 19.880, $p<0.0001$; 2021: Est = 1.659, SE = 0.166, t=10.014, $p<0.0001$) (Fig. 4.4; Table S4.2).

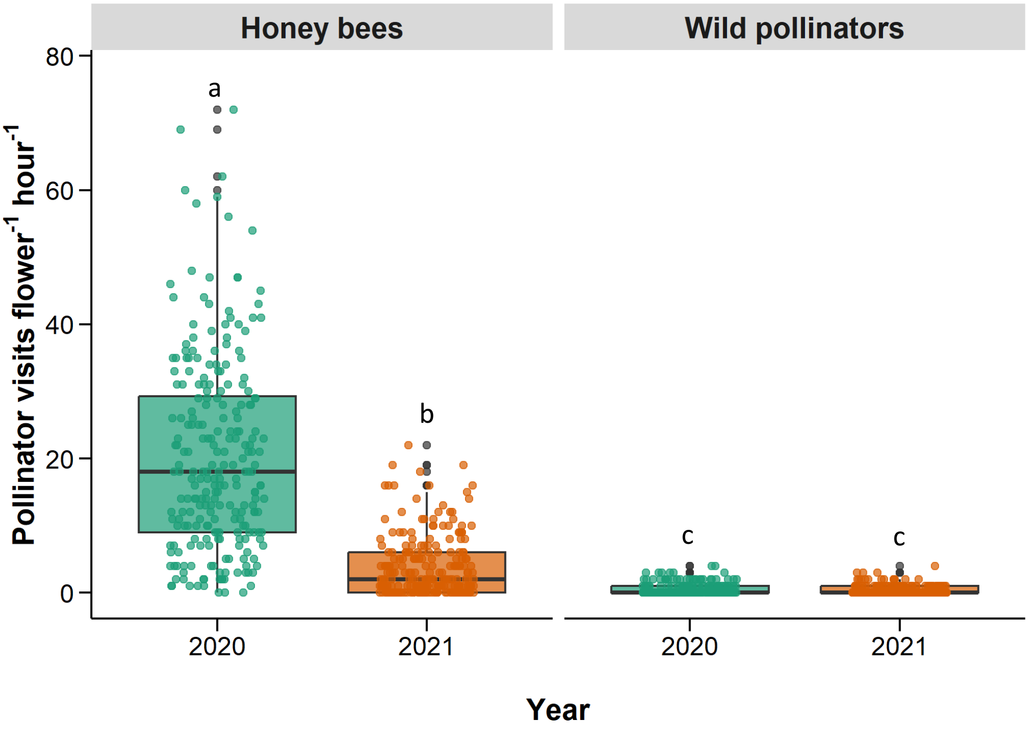

Figure 4.4 Visits per flower per hour for honey bees and wild pollinators. Letters show significant differences. 24 sets of 10 flowers were observed in each year (n=240)

4.4.3 The effect of location on visitation rate

Flowers at the corners of the field (≤20 m from a tunnel opening and ≤20 m from a tunnel side) were visited significantly more times in an hour of observation by honey bees than those in the middle of fields (>20m from any edge) (Est = 0.64, SE = 0.145, t=4.461, p=0.0002) (Figure 4.5a). However, flowers that were within 20m of either a tunnel side (Est = -0.0663, SE = 0.192, t=-0.345, p=1.000) or a tunnel opening (Est = -0.4597, SE =0.317, t=-1.450, p=0.8336) did not significantly differ from those in the middle of the field or those in the corners (Tunnel side: Est = 0.5801, SE=0.211, t=2.749, p=0.1095; Tunnel opening: Est = 0.1867, SE = 211, t=0.576, p=0.9991). This suggests a gradient of honey bee visitation rates with the corners of the fields experiencing the greatest visitation rate and the flowers more than 20 metres away from any edge experiencing the lowest visitation rates. There was no significant locations effect on wild pollinator visitation rates (Figure 4.5b).

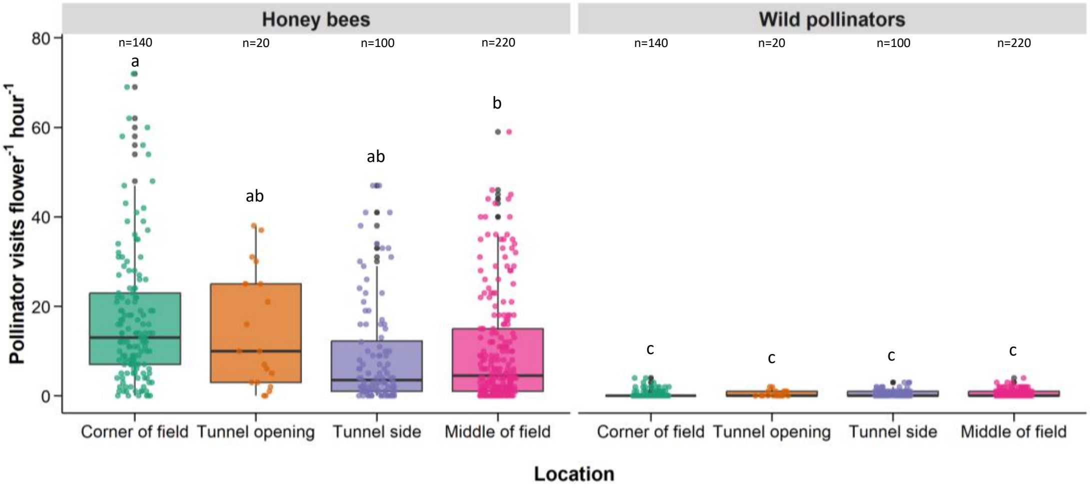

Figure 4.5 Visits per flower per hour for honey bees and wild pollinators at different locations within the field. Letters show significant differences. Flower sample sizes are shown.

4.4.4. The effect of distance from tunnel edge on pollinator abundance

The abundance of insect visitors to crop flowers, measured on transect walks, varied with the interaction between the pollinator group (honey bees or wild pollinators) and the survey year as found with the visitation rate analysis above (Chisq =143.53, p<0.0001). Wild pollinator visitor abundance did not differ between years (Est = -0.212, SE=0.2311, t= -0.919, p=0.7949) but honey bee visitor abundance was significantly higher in 2020 than 2021 (Est = 1.088, SE=0.2121, t=5.131, p<0.0001) (Figure 4.6).

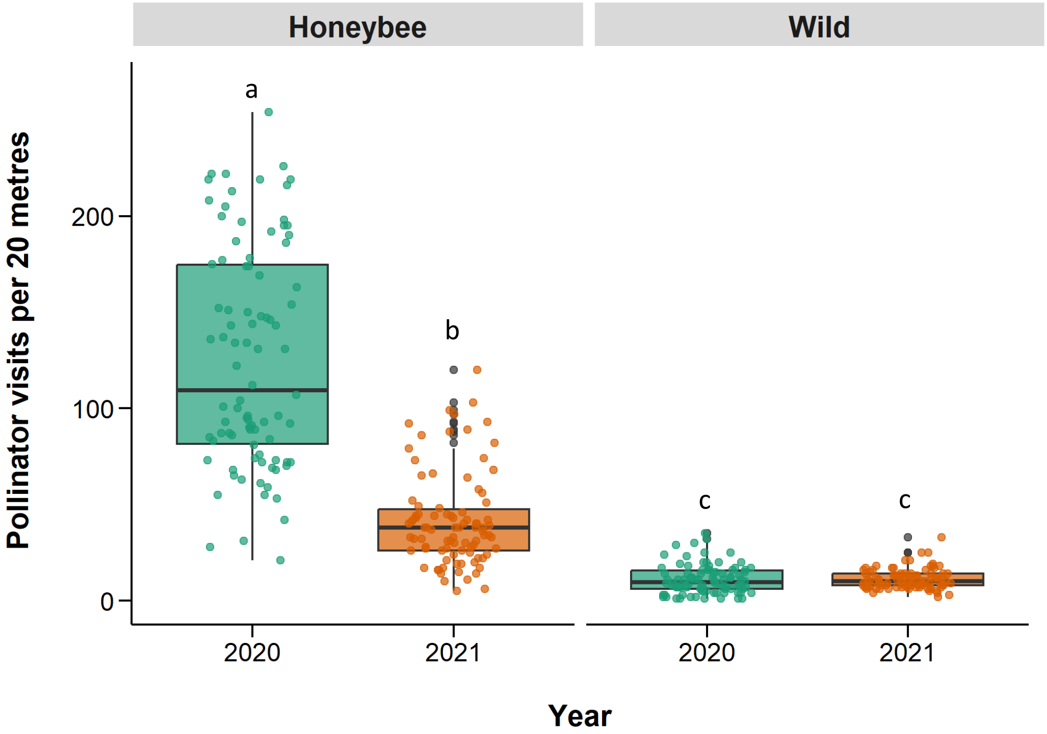

Figure 4.6 Visitor abundance per 20 metres of transect in two years for honey bees and wild pollinator. Letters show significant differences. 18 transects, each with five 20 metre sections were surveyed in each year (n=90)

There was no effect of distance from the tunnel opening on the visitor abundance of either pollinator group (Chisq=0.0506, p=0.8220). When crop visitor counts were grouped into edge (≤20m from the tunnel opening) and middle (>20m from the tunnel opening) as done for the visitation rate analysis we found no significant difference in honey bee visitor abundance at the edge of the field compared to in the middle (Est = 0.115, SE = 0.05, t=2.306, p=0.0987). There was also no effect of edge on wild pollinator abundance on crop flowers (Est = -0.173, SE = 0.126, t=-1.373, p=0.5169) (Figure 4.7).

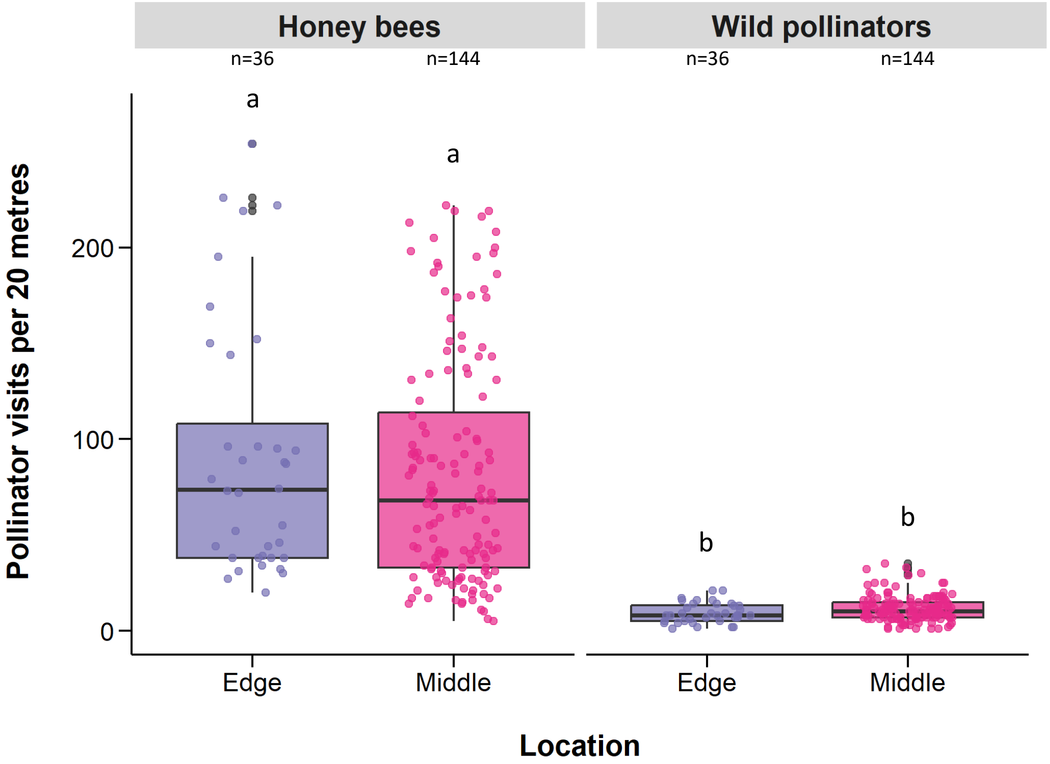

Figure 4.7 Visitor abundance per 20 metres of transect at the edge (≤20m from the tunnel opening) and in the middle (>20m from the tunnel opening) of the field along transect walks for honey bees and wild pollinators. Letters show significant differences. 18 transects, each with five 20 metre sections were surveyed in each year (n=180 sections in total)

4.5 Discussion

As expected, the main crop pollinator in this protected cropping system was honey bees *Apis mellifera*. 88% of crop flower visits across both years were from honey bees, creating a high species dominance, while the richness mainly came from hoverflies. Genera richness of insect visitors to raspberry crop flowers was very similar in 2021 compared to 2020, with 16 and 15 genera visiting the crop in each year respectively. The accumulation curves show that we are likely to have missed some genera present within the community and thus the richness could have been higher. However, this lack of asymptote could, in part, be due to not catching and identifying all observed flower visitors. Those individuals that were not caught were likely to have belonged to the same genera as those that were, increasing the number of transects where these genera were present and thus flattening the curve. The genus accumulation curves were very similar between years, with only 3 genera in 2020 (5 individuals) not identified in 2021 and 4 in 2021 (8 individuals) not identified in 2020. Any genera not represented in our list are likely to present at low numbers in the community due to the dominance of honey bees. Identifying the rare genera not recorded would therefore by very unlikely to change our finding that honey bees are the main crop pollinator in this system. The abundance of honey bees decreased by 66% in 2021 compared to 2020 with a 46% drop in honey bee hive stocking density. Bumble bees were found at similar abundance on crop flowers in both years, while solitary bees were more abundant in 2020 and hoverflies and wasps were more abundant in 2021 (Table 4.3). Insect communities are known to be extremely variable between years (Herrera, 1988), so with only two years of data, we cannot read much into this.

Our single visit study of pollination service (Chapter 3) found all flowers that received at least two visits from either a honey bee or a bumble bee were not significantly different in marketable fruit weight to those that were openly pollinated. In this study, we found that raspberry flowers were visited an average of 20.8 times per hour of observation in 2020 and 4.05 times per hour in 2021. Using the estimated required number of visits from Chapter 3, the average crop flower therefore received enough visits to produce a marketable fruit after under 6 minutes in 2020 when the honey bees were stocked at ~2 hives/ha and in just under 30 minutes in 2021 when honey bees were stocked at 1 hive/ha. Assuming a conservative foraging period of 10am-5pm, as surveyed for this study, crop flowers received 364 insect visits in 2020 and ~71 in 2021 over the flowers' receptive period of ~2.5 days, if visitation rate is averaged across this foraging period. This shows that 1 honey bee hive/ha is more than sufficient to achieve full fruit set. A more conservative estimate of the time required to achieve the maximum fruit weight could be calculated using the values from the pollinator effectiveness studies by Chagnon *et al* (1991) and Andrikopoulos and Cane (2018b). These studies found that full raspberry fruit set was yielded after 5-6 visits during a single day or two prolonged visits one on each of two consecutive days. Using this estimate the crop flowers in this study received, on average, sufficient honey bee visits in less than 18 minutes in 2020 at ~2 hives/ha honey bee stocking density and in 100 minutes in 2021 at 1 hive/ha to produce a marketable fruit. Therefore, visitation rates are more than sufficient to maximise fruit yields in this system and honey bees are overstocked for the purpose of crop pollination.

Over the course of the two survey seasons, the visitation rate to raspberry crop flowers showed significant variability, ranging from no visits to certain flowers during three 20-minute surveys to as many as 72 observed visits (11.55 ± 0.60) to others. The utilization of Fijen and Kleijn’s (2017) method to establish the minimum observation period ensured that this variability was attributable to genuine biological variations in visitation rates to crop flowers. While we selected flowers that appeared to have opened in the past day, we did not record visits to the same flower on consecutive days, hence we cannot ascertain whether the peak attractiveness and visitation to the flower took place during the days and times we observed, nor how visitation rate varied throughout the life of the flower. This variation among flowers may therefore be explained in part by the fact that young flowers are typically visited more frequently than older ones (Bataw, 1996). Bataw (1996) also demonstrated that pollen and nectar availability can differ significantly both within plants and even branches but the mechanisms behind this remain unknown.

The pollinator visitation rates observed in this study would have resulted in raspberry crop flowers receiving two visits from wild pollinators in just over 4 hours in 2020 and less than 6 hours in 2021, most of these visits were from *Bombus terrestris*. Using the more conservative estimate by Chagnon *et al* (1991) of 5-6 pollinator visits, full fruit set could still be reached within the flowers receptive period (2.5 days). Thus, wild pollinators have the potential to provide sufficient fruit set to commercial raspberries in the absence of managed honey bees. Wild pollinators may even be able to provide the required two visits in less time if, as other studies have suggested, their current abundance is currently being depressed by high densities of honey bees in the crop field (Lindström *et al*, 2016; Angelella *et al.*, 2021 and Walther-Hellwig *et al.*, 2006). However, the evidence for this competitive exclusion effect of honey bees on wild pollinators is still uncertain.

Three large scale studies have been conducted to investigate the impact of honey bee hives on wild pollinator abundance and species richness in crop fields. Specifically, Mallinger and Gratton (2014), Lindström *et al.* (2016) and Angelella *et al.* (2021) surveyed the pollinator community at commercial farms growing apples, oilseed rape and mixed crops respectively. They compared the abundance of wild pollinators and honey bees at farms with and without honey bee hives present. Mallinger and Gratton (2014) also measured the visitation rate to apple flowers. Angelella *et al.* (2021) reported a 48% reduction in wild bee abundance and a 20% reduction in species richness, whereas Mallinger and Gratton (2014) observed no effect of the presence of honey bee hives on the abundance or species richness of wild bees. Lindström *et al.* (2016) found a significant negative effect of increasing field size on wild pollinator abundance when honey bee hives were present. These studies demonstrate that honey bees can have detrimental impacts on the abundance of wild pollinators in crop fields in some instances but that this effect is not universal. While these studies have investigated the impact of honey bee hive presence on wild pollinator abundance and species richness in crop fields, it is also important to consider whether the density of honey bee hives has any effect on wild pollinator visitation to crop flowers

Arrington and DeVetter (2018) found no effect of stocking density on non-*Apis* visitation rates between blueberry farms stocked with honey bees at 10 hives/ha and 20 hives/ha. Walther-Hellwig *et al.* (2006) found that *Bombus terrestris* showed spatial avoidance when foraging on *Phacelia* when honey bee hives were stocked at 10 hives/ha compared to fields with no hives present, but not when hives were stocked at 1 hive/ha, indicating that there may be a density dependent effect of honey bee hives on bumble bee abundance on crop flowers. The relatively low stocking densities in comparison for our study may, therefore, not have a detrimental effect on wild pollinator abundances in the field, however, as there was no control without the presence of honey bee hives, their effect on wild pollinators in this system could not be assessed. More studies that measure the effect of honey bee hive presence and their stocking densities need to be done to determine the effect of stocking densities of between 0 and 10 hives/ha on the abundance and diversity of wild pollinators on crop flowers. This could be done by surveying wild pollinator abundance in crop fields across farms with varying hive stocking densities as done by Mallinger and Gratton (2014) and Angelella *et al.* (2021) and testing the correlation between stocking density and wild pollinator abundance. This would allow the reduction of honey bee hives (instead of removal) as a conservation method for wild pollinators to be assessed.

Hall *et al*, (2019) found decreases in honey bee visitation rate to raspberry and blueberry flowers with distance from polytunnel opening when stocked at 8 hives/ha across the farm. This pattern was also seen for wild stingless bee visitation rate to raspberry flowers in the same study. Although we found no significant effect of distance from tunnel openings on the visitation rates to crop flowers, we did

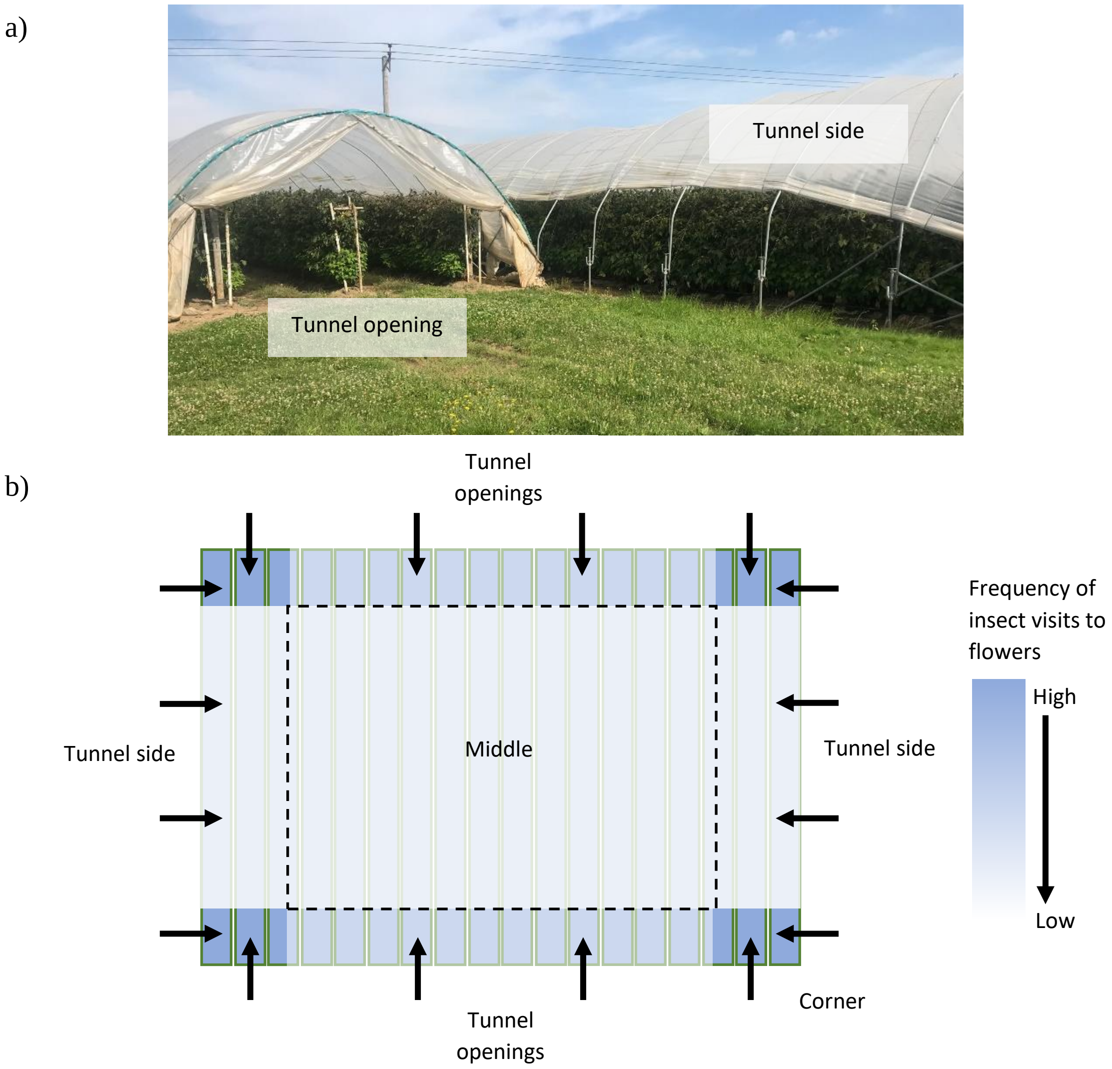

Figure 4.8 a) Open sides and ends of tunnels, b) a heatmap of the observed visitation rates to crop flowers in the polytunnels. The darker the blue the greater the visitation rate. Black arrows indicate potential areas of access to the field for pollinators demonstrating that corners have twice the accessibility of tunnel openings or tunnel sides independently.

find that plants that were >20m from the edge of the field had significantly lower visitation rates from honey bees than plants at the field corners, with an intermediate level at the openings of the tunnel (Figure 4.8). This indicates that visitation rates to crop flowers from honey bees are not uniform throughout the field and that flowers in the centre of the field experienced lower visitation rates as found by Hall *et al.* (2020). It is therefore important to assess the visitation rate to plants in the middle of fields when looking to determine whether the current pollinator community and managed pollinators are providing sufficient visitation to maximise yields. We therefore recommend that growers conduct timed flower counts in the middle of fields or at least 20 metres from any edge of the field to measure the lowest visitation rate occurring in that crop field. The centre of the field still experienced visitation rates of 9.97 visits per flower per hour. Flowers therefore received almost five times the required number of visits in just a single hour. The higher visitation rates in the corners of the fields could be due to an additive effect of the two types of access to the field, though there were greater visitation rates in the tunnel openings (not significant) compared to the sides. Reduced visitation rates in the middle of the field may be due to elevated temperatures further in to the tunnels as found by Hall *et al.* (2020) reducing the ability for honey bees to forage. The high raspberry nectar secretion rates (Willmer *et al.,* 1994), could mean that there is sufficient nectar resources in the tunnel openings for pollinators and thus honey bees don’t need to travel further into the field, creating a bottle neck at the tunnel openings. Wild pollinators provided a more consistent visitation rate across the field of between 0.34-0.50 visits per flower per hour across the different parts of the field. The lack of an effect of distance from field edge for wild pollinators may be due to avoidance of honey bees by wild pollinators (Walther-Hellwig *et al.* (2006), prompting greater dispersion throughout the crop field. The greater foraging rate of bumble bees (Willmer *et al.,* 1994) could also result in dispersion further into the field within a single foraging bout before returning to their colony (Osborne, *et al.,* 2008; Willmer *et al.,* 1994), though the effect of distance from field edge was not able to be assessed on a taxon level for wild pollinators due to low abundances.

The frequency of visits observed in this study is likely to have caused pistil damage, as 40% of raspberry pistils were damaged at similar rates of honey bee visitation during the study by Saez *et al.*

(2014). The high stocking density could therefore have had detrimental effects on crop production given the negative relationship between proportion of pistil damage and the drupelet set shown by Saez *et al.* (2014) however, we did not measure this damage in this study. Given the risks of high honey bee densities to wild pollinators (Nielsen *et al.,* 2017; Lindstrom *et al.*, 2016; Angelella *et al.,* 2021; Goulson and Sparrow, 2008; Elbgami *et al.,* 2014; Manley *et al.,* 2015) and raspberry pollination (Saez *et al.,* 2014) that have been demonstrated in other systems, our study suggests that honey bee hive densities could routinely be reduced to 1 hive/ha for the purpose of crop pollination, 50% of the highest density observed in this study. The results also suggest that even lower hive densities could be tolerated in the system, without any impact on raspberry production. Excess hives should therefore be reduced or removed in the interest of pollinator conservation and local species richness, with any resulting benefits to wild pollinator communities potentially improving the quality and stability of crop pollination services.

4.6 Conclusion

Wild pollinators were found to provide sufficient visitation rates to achieve the minimum required two pollinator visits for optimizing marketable fruit yield. They also visited crop flowers uniformly across the field unlike honey bees which were found at higher densities at the corners of the field. This could be due to corners having two potential entrances to the field-both the end and side of a tunnel. Therefore, wild pollinators have the potential for providing sufficient crop pollination services without the use of honey bee hives. Although honey bees are currently providing the majority of the pollination service to raspberries in this system with the use of managed hives, visitation rates to crop flowers show that honey bees are likely overstocked at 1 hive/ha for the purpose of raspberry pollination and that they provide uneven pollination services across crop fields. We therefore suggest monitoring visitation rates to flowers in the centre of the field and removing or reducing honey bee hives from agricultural systems where raspberries receive at least two bumble bee visits per flower.

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4.8 Supplementary material

Table S4.1. Full species list of pollinators observed during transect walks in 2020 and 2021

Group	Genus	Species	2020 count	2021 count
Honey bees	*Apis*	*mellifera*	11,321	3,892
Bumble bees	*Bombus*	*terrestris/lucorum*	774	786
		lapidarius	43	54
		pascuorum	27	61
		pratorum	3	2
		hypnorum	0	1
Solitary bees	*Andrena*	unidentified sp	54	11
	Hylaeus	*communis*	4	1
	Lassioglossum	unidentified sp	1	0
		malachurum	0	1
	Nomada	*fucata*	1	0
	Not identified		29	10
Hoverflies	*Episyrphus*	*balteatus*	6	16
	Eristalis	*pertinax*	0	1
		tenax	1	5
	Eupeodes	*B*	0	1
		corollae	3	3
		lundbecki	1	0
		luniger	0	6
	Melanostoma sp		1	0
	Myathropa	*florea*	2	1
	Parasyrphus	*nigritarsis*	0	1
	Platycheirus	*albimanus*	3	0
	Sphaerophoria	*interrupta ag*	2	6
		scripta	2	1
	Syritta	*pipiens*	0	1
	Syrphus	*ribesii*	0	1
		vitripennis rectus	0	4
	Volucella	*pellucens*	0	1
		inanis	1	0
	Not identified		9	30
Social wasps	*Vespula*	*vulgaris*	2	24
		germanica	1	10
		rufa	0	1
	Dolichovespula	*media*	0	1
	Not identified		5	41
Non-syrphid flies	Not identified		15	6
Other	*Chrysididae* sp		0	1
	Coccinella	*septempunctata*	1	1
	Coenonympha	*pamphilus*	0	1
	Deraeocoris	*ruber*	1	0
	Harmonia	*axridis*	0	2
	Ichneumonoidea sp		0	1
	Rhagonycha	*fulva*	1	0

Table S4.2. Results of Generalised Linear Mixed Models (GLMMs) with negative binomial error distributions for the response of **visitation rate**.

Variable	Estimate	Std. error	z ratio	P value
a)				
(Intercept)	3.1098	0.1459	21.313	**<0.001**
Year:2021	-1.1927	0.2285	-5.221	**<0.001**
Pollinator group: Wild pollinators	-3.8061	0.2175	-17.503	**<0.001**
Location: Middle	-1.7074	0.3718	-4.592	**<0.001**
Location: Tunnel opening	0.1424	0.4148	0.343	0.7314
Location: Tunnel side	-0.2865	0.2743	-1.044	0.2963
Year2021: PollinatorgroupWildpollinators	1.4067	0.1862	7.555	**<0.001**
Year2021: LocationMiddle	-1.4828	0.2869	-1.682	0.0925
Year2021: LocationTunnelopening	-0.6581	0.6380	-1.032	0.3023
Year2021: LocationTunnelside	-0.5872	0.3586	-1.638	0.1015
PollinatorgroupWildpollinators: LocationMiddle	1.0592	0.2346	4.515	**<0.0001**
PollinatorgroupWildpollinators: LocationTunnelopening	0.7411	0.4504	1.645	0.0999
PollinatorgroupWildpollinators: LocationTunnelside	1.1607	0.2697	4.304	**<0.0001**

5.0 Raspberry Pi's for raspberry pollinators: the use of video setups for measuring visitation rates and visit duration to crop flowers

5.1 Abstract

Measuring pollinator visitation rates to crop flowers helps determine the pollination service being provided and assess the need for managed pollinators to maximise yield. This is usually done using timed flower counts or focal flower observations undertaken by trained observers. However, these human observations in the field are time consuming and require teams of surveyors to allow simultaneous sampling of multiple survey sites or day-long observations. The use of automated camera monitoring has been tested in recent years as a way to streamline these surveys and collect more data. Here, we use both short in-person timed flower counts and manual coding of day-long Raspberry Pi video footage to estimate pollinator visitation rates to raspberry crop flowers, the total number of daily visits per flower, and the duration of, and intervals between, these visits. We compared these estimates with previously determined pollinator requirements for raspberry to establish whether pollinator requirements were being met at our study site. No significant difference in pollinator visitation rate was found between short, timed flower counts and day-long video footage. Raspberry flowers received an average of 94.6 visits over 7 hours on the first day after opening, more than 45 times the number of visits required to maximise fruit yield. Video monitoring made it possible to calculate visit duration and intervals between visits alongside visit number, but it was not always possible to identify pollinator species or group from the video data. In-person counts were more time efficient due to the 1:1 ratio of observation time to data collection time, but videos allowed multiple survey locations to be monitored simultaneously. We propose the use of multiple low-cost Raspberry Pi camera setups to simultaneously record crop flower visitation rates at multiple locations, allowing

analysis of short segments of video instead of in-person timed flower counts. Where simultaneous sampling is not of interest, in-person timed flower counts are sufficient to estimate day-long visitation rates to crop flowers to inform pollination management. For day-long observations, artificial intelligence or machine learning methods would be required to analyse large amounts of video data in a time-efficient manner.

5.2 Introduction

Ensuring that crop flowers receive sufficient visits to maximise their yield, while avoiding the negative effects associated with too many visits or inflated numbers of managed pollinators in the landscape, should be the main objective of crop pollination management. However, crop pollinator management guidelines mainly focus on suggested stocking densities of managed pollinators such as honey bees or laboratory reared bumble bees (Free, 1993; Delaplane and Mayer, 2000) as these are easy to understand and do not require monitoring or farm-level assessments of pre-existing pollinator communities. Despite the widespread use of managed honey bees, there is little empirical evidence to support the suggested hive stocking densities reported in the literature. To accurately predict the number of honey bee hives required, the relationship between hive number and honey bee abundance on crop flowers needs to be determined. The relationship has been shown to vary between studies, with some finding linear relationships at both landscape (Eeraerts *et al.,* 2023) and field level (Benjamin and Winfree, 2014) and others finding that stocking densities do not predict honey bee abundance on crop flowers (Mallinger *et al.,* 2021; Benjamin and Winfree, 2014). A study by Gaines-Day and Gratton (2016) also found a linear increase in cranberry yield with the density of honey bee hives per hectare in some landscapes, but there was no benefit to yield of adding honey bees in heavily wooded landscapes. This variation within and between scales could be due to the attractiveness and availability of non-crop floral resources in the surrounding landscape (Blitzer *et al.,* 2012), the distance of the hives from crops and the variation in colony size and quality of honey bee hives (Garibaldi *et al,* 2020; Geslin *et al.,* 2017; Crane *et al,* 1999; Russell *et al*, 2013). Attempting to predict the required honey bee stocking density from crop pollinator requirements may not be possible. Using generic stocking densities for each crop is therefore likely to over- or underestimate the additional pollination service required at farm level. Monitoring farm-level visitation rates to crops with known hive densities may help inform the increase or decrease in hive density required for yield optimization for that site.

Measuring the number of legitimate visits to crop flowers in a specific context and relating it to the pollination service per visit provided by each flower-visiting species for each crop (often available in the literature) provides an accurate measure of the pollination service currently provided and whether this meets or exceeds the number of visits required to maximise yield. The total duration of visits can also be compared with the number of cumulative seconds of visitation required to maximise pollination and yield. Timed flower counts have been suggested by Garibaldi *et al.* (2020) as the best way to measure this pollinator visitation rate to crop flowers, by observing a set number of flowers over a set period of time and recording all visits to these flowers, repeated over the course of a single day. This can be repeated at different locations within a field or a farm to determine how visitation rates vary across the field, as was done in Chapter 4. Visitation rates can then be compared with estimates of the number of visits each flower requires to maximise yield through complete ovule fertilisation using field studies (Chapter 4) or literature estimates (Garibaldi *et al*, 2020). The target visitation rate for raspberry given by Garibaldi *et al.* (2020) used the required number of visits for raspberry flowers calculated by Saez *et al.* (2018), which estimated the required visitation rate using simulation modelling based on pollen deposition per bee. Garibaldi *et al.* (2020) assumed a pollinator activity period of 6 hours per day and a receptive period of 3 days (Hiregoudar Manju and Bundela, 2019) when calculating the target visitation rate for raspberry. This target visitation rate is given as 55 visits per 100 flowers per hour to allow for complete pollination (Garibaldi *et al.,* 2020). The ability to translate site-level visitation rates into pollination service provision in this way has the potential to improve pollination management and crop yields, by enabling farmers and growers to monitor and manage the pollination service provided to their crops. It can prevent overstocking of pollinators by allowing the farmer to see the visits their crop flowers are receiving before introducing more managed pollinators, as well as the number of excess visits that occur when managed pollinators are overstocked. This could prevent negative impacts on fruit yields due to flower damage through excessive visits (Saez *et al.,* 2014) as well as any potential detrimental impacts of honey bees on wild pollinator communities (e.g. Nielsen *et al.,* 2017; Goulson and Sparrow, 2008; Angelella *et al.,* 2021; Elbgami *et al.,* 2014).

Timed flower observations can provide information on the frequency of visits to crop flowers at the current stocking density of managed pollinators and the current abundance of wild pollinators, but they can be time consuming and, unless there is a team of observers, simultaneous observations at different locations within the field are not possible, reducing the accuracy of spatial variation estimates. Repeated observations within the same day to multiple locations can also prove difficult if many replicates are required. Most studies that observe insect visitors to crop flowers do so for short periods of time (e.g. 5 minutes) (Garibaldi *et al.* 2020; Saez *et al.* 2014). However, the appropriate length of time observing flowers required to ensure that the true visitation rate is being recorded varies depending on the visitation rate. Fijen and Kleijn (2017) present a method for determining the minimum observation time required based on preliminary studies over several days to determine how long subsequent timed flower counts should be for. This results in more accurate estimates of the visitation rate as the minimum observation time captures the variation in rate within and between flowers. However, if this results in long timed flower counts where visitation rates are low or highly variable, it may mean that repeat visits to multiple locations are not possible and therefore the visitation rate across fields cannot be accurately determined.

Pollinator visitation rates to crops often vary throughout the day due to changes in temperature, humidity, and floral reward (Schmidt *et al,* 2015; Willmer *et al,* 1994). Raspberry nectar availability has been found to decrease significantly one day after opening and throughout the day as the nectar secreted during the previous day and overnight is depleted due to day-time secretion rates not fully replenishing nectar stocks between foraging visits (Schmidt *et al,* 2015; Willmer *et al,* 1994). By recording the frequency of visits at different times of the day or continuously throughout the day, the constancy of pollinator visitation rates can be determined, and more accurate estimates of visitation rates and total number of visits can be calculated. Measuring pollinator visitation rates to crop flowers throughout the day is therefore important for pollinator management.

An alternative method for collecting this important day-long visitation rate data is the use of video cameras or motion detectors to record pollinator activity, which can provide high-resolution data on variations in visitation rates over whole days and multiple days, as well as over multiple sites using multiple camera setups. The use of cameras can also make it easier to record both the frequency of visits and their duration. However, processing these large amounts of data can be equally labour intensive and managing large video files can lead to storage problems. In this study, we used short repeated timed flower counts, equivalent to those undertaken in Chapter 4 and day-long observations from video analysis to measure insect pollinator visitation rates and used video analysis to measure visit duration to raspberry crop flowers throughout the day. We compare the accuracy, efficiency, and suitability of the two methods for estimating crop flower visitation rates for the purpose of adaptive pollinator management on farms and compare the estimates of the number of visits flowers receive between the in-person observations of Chapter 4 and the video observation methods presented here.

Our study aimed to determine how visitation rates and durations to raspberry crop flowers vary within and between days to inform when pollinator monitoring should be carried out. We also aimed to determine whether visitation duration differed between *Apis mellifera* and *Bombus* sp. to understand the mechanisms behind interspecific variation in pollinator effectiveness observed in Chapter 2. We also wanted to determine whether honey bees avoid recently visited flowers as seen for other species (ref). Finally, we wanted to compare the efficiency and accuracy of short timed flower counts and day long observations using Raspberry Pi cameras for the true day long mean number of visits per flower.

We asked the following research questions:

- Does visitation rate and duration change with time since flower opening?
- Do honey bees and bumble bees differ in their visit durations on raspberry flowers?
- Do honey bees avoid recently visited flowers?

- Can short, timed flower counts accurately estimate the day-long mean visitation rate?

5.3 Methods

5.3.1 Study site

The study was carried out on an 81-hectare commercial soft fruit farm near Reading, south England (51°29′32″N, 000°52′28″W) throughout the period from July to September in 2020 and 2021. Two cultivars of red raspberry (*Rubus idaeus*); ‘Diamond Jubilee’ and ‘Sapphire’, were included in the studies, both developed by BerryWorld and filed for growers in 2013. Each experimental site was made up of one commercial field of >1.5 ha surrounded by uncropped field margins. There were small areas of semi-natural grassland and patchy woodland on the farm and within the immediate surrounding area. Both raspberry cultivars were grown under Spanish polytunnels (7.8 metres wide) with three rows of potted raspberry canes in each tunnel, each row was ~2m apart from each other. 149 rented honey bee hives were in place at the farm during 2020, equating to ~2 hives/ha of farmland, throughout the raspberry flowering season for the purpose of crop pollination of both raspberries and strawberries. This dropped to 81 colonies in 2021 due to colony losses and relocation to other sites reducing the stocking rate to ~1 hive/ha. No managed bumble bee colonies were active on the farm during the study period in 2020. A few colonies were still active in an adjacent field to Diamond Jubilee in 2021 however, they were at the end of their 10th week *in situ* when the first study flowers opened. It is therefore likely that the bees leaving the hive were gynes and males.

5.3.2 Minimum observation period for timed flower observations

In July 2020 we observed flower visitation by insect pollinators to 10 marked flowers on a single raspberry cane for two one-hour periods between 13:30 and 16:30. We recorded each pollinator that foraged on a marked flower, noting the landing time on the flower to the minute. We identified the species in the field where possible to the lowest possible taxonomic level. All honey bees were recorded as *Apis mellifera* as they are the only honey bee species in the UK. Bumble bees were identified to species or morphospecies for *Bombus terrestris/lucorum.* Individuals not able to be identified to species in the field were not caught so as not to prevent potential subsequent visits to the same flower or other marked flowers. All other visitors were therefore categorised into one of the following groups: social wasps, hoverflies, other flies and solitary bees. To determine the length of our visitation rate surveys we used the minimum observation time method by Fijen and Kleijn (2017) to determine the duration when observing for longer does not significantly improve the accuracy of the visitation rate estimate. This enabled us to maximise our sample size while ensuring we accurately measured visitation rate to crop flowers.

This data was analysed in MS Excel using a simplified version of the method described in Fijen and Kleijn (2017). We divided the pollinator visit observations into time intervals of durations ranging from 1 to 20 min. We then calculated the standard deviation (SD) of the number of visits to all 10 flowers based on each time interval (i.e., 120 one-minute intervals, 60 two-minute intervals). A scatter plot of these SDs was then eyeballed to find the point at which the SD reached an asymptote (18-20 minutes) to determine how long the timed flower counts should be. 20-minute timed flower counts were then carried out, the methods for this are presented in Chapter 4. This observation duration was then later validated using the R code for the minimum observation time method developed by Fijen and Kleijn (2017). As before, the pollinator visit observations was divided into time intervals of lengths ranging from 1 to 20 min and then the standard deviation (SD) of the number of visits to all 10 flowers based on each time interval was calculated. The two observations, each of one hour long, were analysed separately. For each survey, we then calculated the standard deviation (SD) of the number of visits to all 10 flowers based on each time interval (i.e., 60 one-minute intervals, 30 two-minute intervals). The SD of the survey-long observations was calculated as the mean SD from the time intervals from 16-20min, as at those intervals the SD had always reached an asymptote. We then analysed at which observation duration the SD of the estimated visitation rate no longer differed significantly from the SD of the actual visitation rate based on the hour-long observation to determine the minimum observation duration. To do this we calculated bootstrapped 95% confidence intervals (CI) for the SD of the estimated visitation rate for each observation interval, following Anderson and

Santana-Garcon (2015) in the R-package ‘boot’ (Canty & Ripley 2015) with 10,000 bootstrap replicates. Confidence intervals that overlapped with this survey-long mean standard deviation were not significantly different. The first instance that this occurred i.e. the shortest observation duration that had a standard deviation of the visitation rate that did not significantly differ from the standard deviation of the visitation rate over the entire hour survey, was identified as the minimum observation period required for the timed flower observations. This was also repeated using day long Raspberry Pi data to determine the minimum observation time for 2021 as abundance of honey bees and visitation rates were lower in 2021. Due to this data being over a longer period of time we divided into time intervals of lengths ranging from 1 to 80 min. The SD of the day-long observations was calculated as the mean SD from the time intervals from 60-80min, as at those intervals the SD had always reached an asymptote.

5.3.3 Raspberry Pi camera set up

Raspberry Pi cameras were set up in August 2021 at the end of the 2021 field season for 9 days in total to record visits to equivalent sets of 10 flowers over day-long observation periods. The PICT (plant-insect interactions camera trap) system consisted of a Raspberry Pi Camera Module V2.1 connected to a Raspberry Pi Zero W board and powered by a portable power bank using the code and camera setup developed by Droissart *et al*, (2021). Videos were recorded at a resolution of 1296 × 972 at 15 or 24 frames per second (fps) between 10:00am and 7:00pm. Videos were recorded using the H264 codec and later converted to MP4 format for analysis. Camera locations were randomly selected within the survey fields in both edge and non-edge areas of the field and 10 flowers that had opened within the last day were selected and marked. Cameras were placed on tripods and positioned so that all marked flowers were within the frame. The flowers were placed as close together as possible, ensuring that all flowers had opened recently. Once set up, the cameras recorded continuously until they were switched off at the end of the day. Each set of flowers was recorded for at least 2.5 days to capture their receptive period. Data from two separate sets of 10 flowers in different fields and on different days (Flower Set 1:10-12th August, and Flower Set 2: 13-15th August) were analysed in this study.

5.3.4 BORIS behavioural observation coding

Behavioral Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016) was used to analyse two sets of 2 days of Raspberry Pi footage, each on a different set of 10 flowers in two different fields to determine whether the 20-minute timed flower counts are accurate in predicting day-long visitation rates, and whether using BORIS in this way to analyse Raspberry Pi footage is an effective way to measure visitation rates to crop flowers. The footage was manually coded in BORIS. The software enables an ethogram of ‘behaviours’ to be imported and then coded to ‘subjects’ for each ‘event’ throughout the video. For our analysis the ‘subjects’ were the individual flowers (1-10) and the ‘behaviours’ were the insect group: honey bee, bumble bee, solitary bee, hoverfly, fly, wasp, unknown insect and the ‘event’ were foraging visits to the marked raspberry flowers. This provided data on the number, frequency, and duration of these visits, as well as the intervals between visits. Means and standard errors are given in parentheses.

5.3.5 Statistical analyses

Analyses were performed using generalised linear (mixed) models (GLM(M)s) in the R statistical software (version 4.1.3) (R Core Team, 2022) using the lme4 R package (Bates *et al,* 2015). Maximal models were used without simplification, and acceptable model fit was assessed from residual plots. To determine whether visitation rates to the same flowers differed between and within observation days, we compared the number of observed visits to all 10 flowers using the Raspberry Pi video footage between 10:30 and 5:50 in 20-min blocks on both days. To do this, we fitted two separate general linear models using a Poisson distribution for Flower Set 1 and a negative binomial distribution for Flower Set 2 due to overdispersion, with observation day and observation time as fixed effects. The interaction between these fixed effects was also included to determine whether the time-of-day effect varied with day, and a random effect of flower identity was included to avoid pseudoreplication.

To determine whether the duration of honey bee visits, recorded by the Raspberry Pi cameras and observed and coded using BORIS, differed between and within observation days we compared the duration in seconds (to three decimal places) of the visits observed to all 10 flowers between 10:30 and 5:50 in 20-minute blocks over both days as above. We fitted two separate GLMs, one for each set of flowers, using a log-transformed normal distribution because the data was right skewed. The interaction between observation day and observation time was included to determine whether the time-of-day effect varied with day. A random effect of flower identity was included to avoid pseudoreplication. The estimates given in the text and the predicted values shown in the plots are back-transformed exponents of the log coefficients. The original log estimates are given in Table S2. To determine whether the duration of honey bee and bumble bee visits differed between each other and between observation days, we compared the duration in seconds of the honey bee and bumble bee visits observed to all 10 flowers between 10:30 and 18:00 between two observation days for both sets of flowers. Solitary bees, hoverflies, flies, and wasps were not included due to small sample sizes. We fitted a generalised linear model with a Gamma error distribution as the continuous response variable was right-skewed, with observation day and pollinator group as fixed effects. The interaction between these fixed effects was also included to determine whether the difference in visit duration between the two pollinator groups varied with day.

To compare the time between each honey bee visit and the previous visit as a function of the interaction between the identity of the previous visitor and the observation day we calculated the time in seconds between the start of each visit and the end of the previous visit. We then fitted a generalised linear model using a log transformed normal distribution due to the right skew of the data. The R package emmeans (v.1.8.4-1; Lenth, 2023) was used to generate pairwise comparisons for Figure 7 and 9.

To compare the mean number of pollinator visits in 20 minutes calculated from three 20-minute counts, one in each period of the day (10:00am-12:00pm, 12:00pm-2:30pm and 3:00-5:00pm), as done for the in-person time flower counts presented in Chapter 4, with the daylong mean number of visits per 20 minutes using a full day of Raspberry Pi footage, we performed a subsampling t-test. We compared the mean of a subset of the video data (3 x 20-minute counts, one in each period of the day) to the mean of the remaining data using a Welch's t-test in R. We randomly subsampled the pollinator visit count from three sets of 20-minute observations, one from each of the three time periods and compared the mean of the subsampled values to the mean of the remaining values in the data frame. This process was repeated 1000 times using bootstrapping, and the resulting p-values were used to assess the significance of the difference in means.

5.4 Results

5.4.1 Minimum observation period for timed flower observations

During the two 1 hour long timed floral count observations of 10 crop flowers in 2020, 450 insect visitors were observed foraging on the marked flowers, 210 in the first hour and 240 in the second. 447 of these visits were from *Apis mellifera,* a single visit from each of *Bombus terrestris*, *Bombus pratorum* and *Andrena sp* were observed in the first hour. In both surveys, there was a steady increase in cumulative abundance throughout the 60-minute observation (Fig. 5.1).

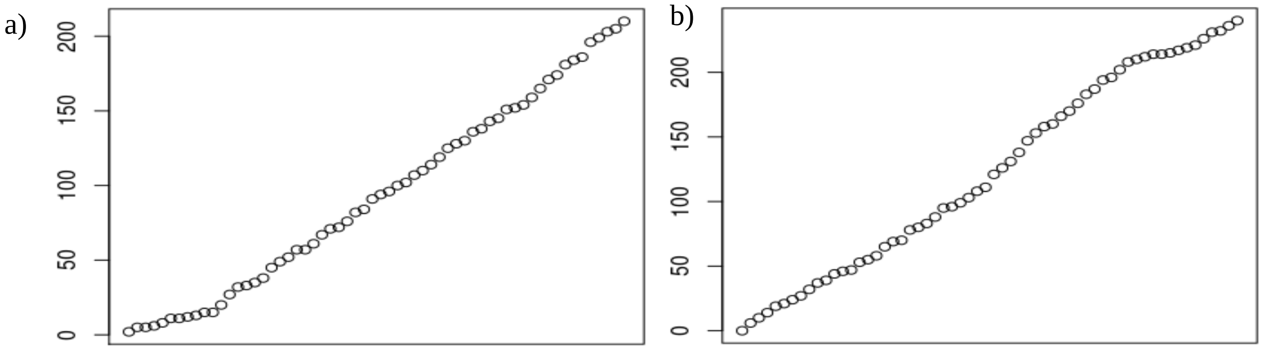

Figure. 5.1 The cumulative abundance of pollinators visiting the 10 focal raspberry flowers over 60 minutes for two surveys. Each visitor that landed on a focal flower was recorded.

Using the R code for the minimum observation time method developed by Fijen and Kleijn (2017) we identified the first observation duration for which the bootstrapped CI overlapped with the SD of the survey-long visitation rate. For the first observation period this was 3 minutes and for the second this was 7 minutes. Figure 5.2 shows the calculated standard deviations of each observation period compared to the survey long visitation rate standard deviation (mean of the 16–20-minute

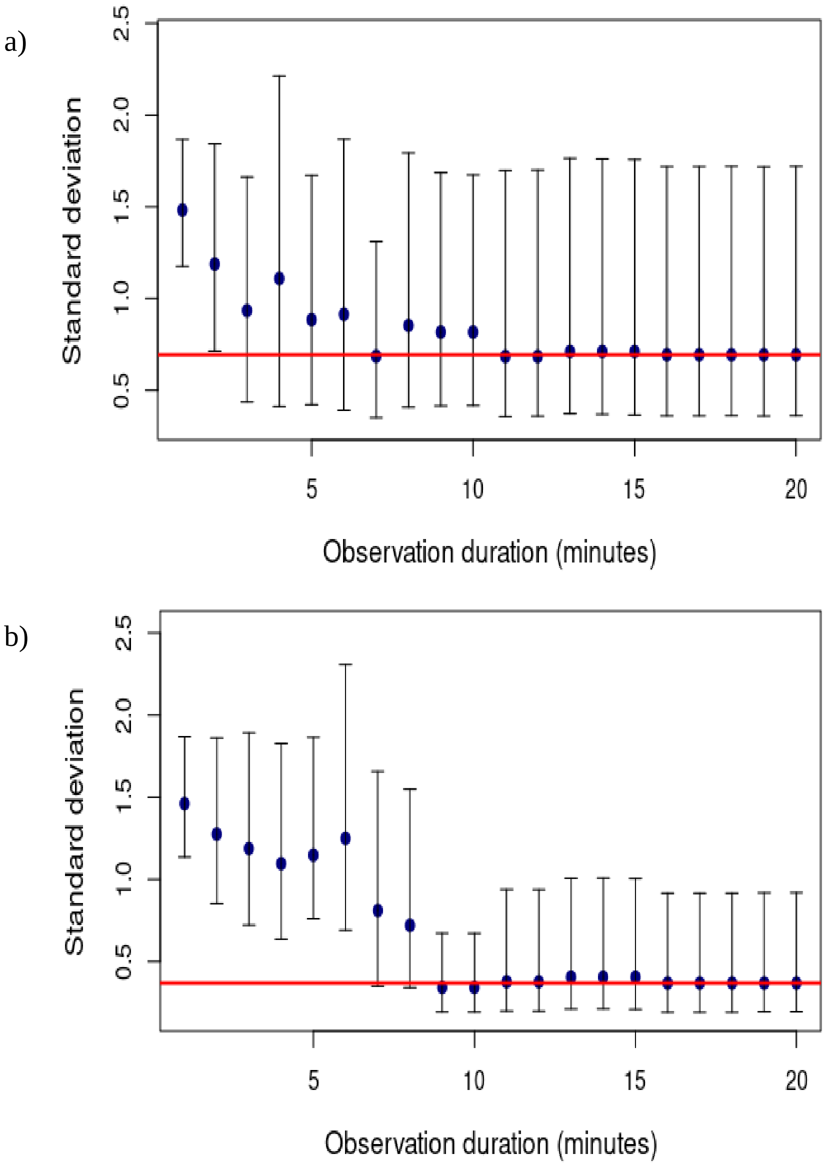

Figure 5.2 Decrease in standard deviation (SD) of visitation rate (pollinators/minute) with increasing observation durations. Points show bootstrapped SDs with 95% confidence interval. The mean SD was calculated for observation interval 16-20 min and is indicated by the solid red line. a) shows the minimum observation time between 13:30 and 14:29 and b) shows the minimum observation time between 15:02 and 16:01.

observations). For 2021, eight hours of Raspberry Pi footage was used to determine the minimum observation time required. Intervals of 1-80 minutes were used due to the much longer observation period. 17 minutes was identified as the minimum required observation time. Figure 5.3 shows the calculated standard deviations of each observation period compared to the survey long visitation rate standard deviation (mean of the 60-80-minute observations). Data collected during the 20-minute point counts were therefore sufficiently accurate for both years and shorter timed floral counts could have been used in 2020.

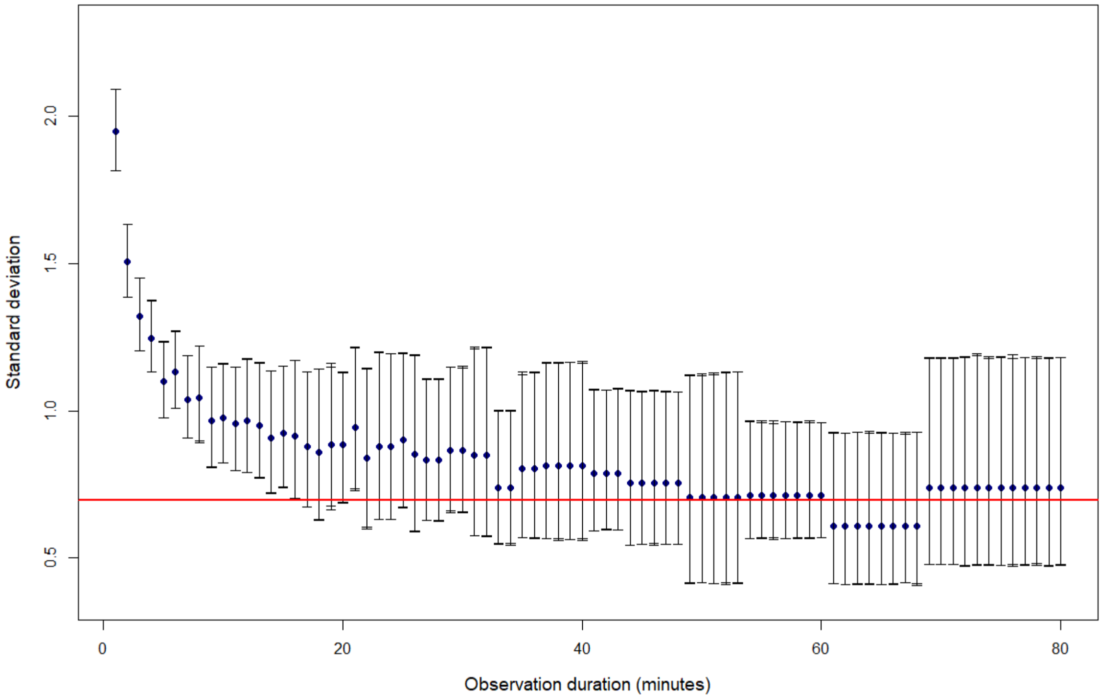

Figure 5.3 Decrease in standard deviation (SD) of visitation rate (pollinators/minute) with increasing observation durations. Points show bootstrapped SDs with 95% confidence interval. The mean SD was calculated for observation interval 60-80 min and is indicated by the solid red line.

5.4.2 In person timed flower counts

The timed flower counts presented in Chapter 4 found a mean honey bee visitation rate of 20.3 visits per flower per hour in 2020 and 3.75 visits per flower per hour in 2021. For wild pollinators this was 0.49 visits per flower per hour in 2020 and 0.37 visits per flower per hour in 2021. Using these mean visitation estimates, assuming six hours of daily pollinator activity as used by Garibaldi *et al* (2020), the observed flowers are therefore likely to have received ~125 pollinator visits during their first day since opening in 2020 and ~25 pollinator visits in 2021. For wild pollinators alone this would have been ~3 visits their first day since opening in 2020 and ~2 in 2021.

5.4.3 Raspberry Pi video analysis

Flower Set 1 was visited a total of 996 times over ~14 hours of camera observations over two consecutive days between the 9:30 and 6pm (Day 1: 12:06-17:56 and Day 2: 09:51-17:56) with each individual flower receiving between 38 and 187 visits over the two observation days (99.6 visits ± 15.8). The second set of ten flowers (Flower Set 2) was visited a total of 1390 times over 15 hours of camera observations over two consecutive days between the 9:30am and 6pm (Day 1: 10:43-18:00 and Day 2: 10:16-18:00) with each individual flower receiving between 68 and 279 visits over the two observation days (139 visits ± 24.2). The cumulative number of visits to each flower over the two days is presented in Figure 5.4.

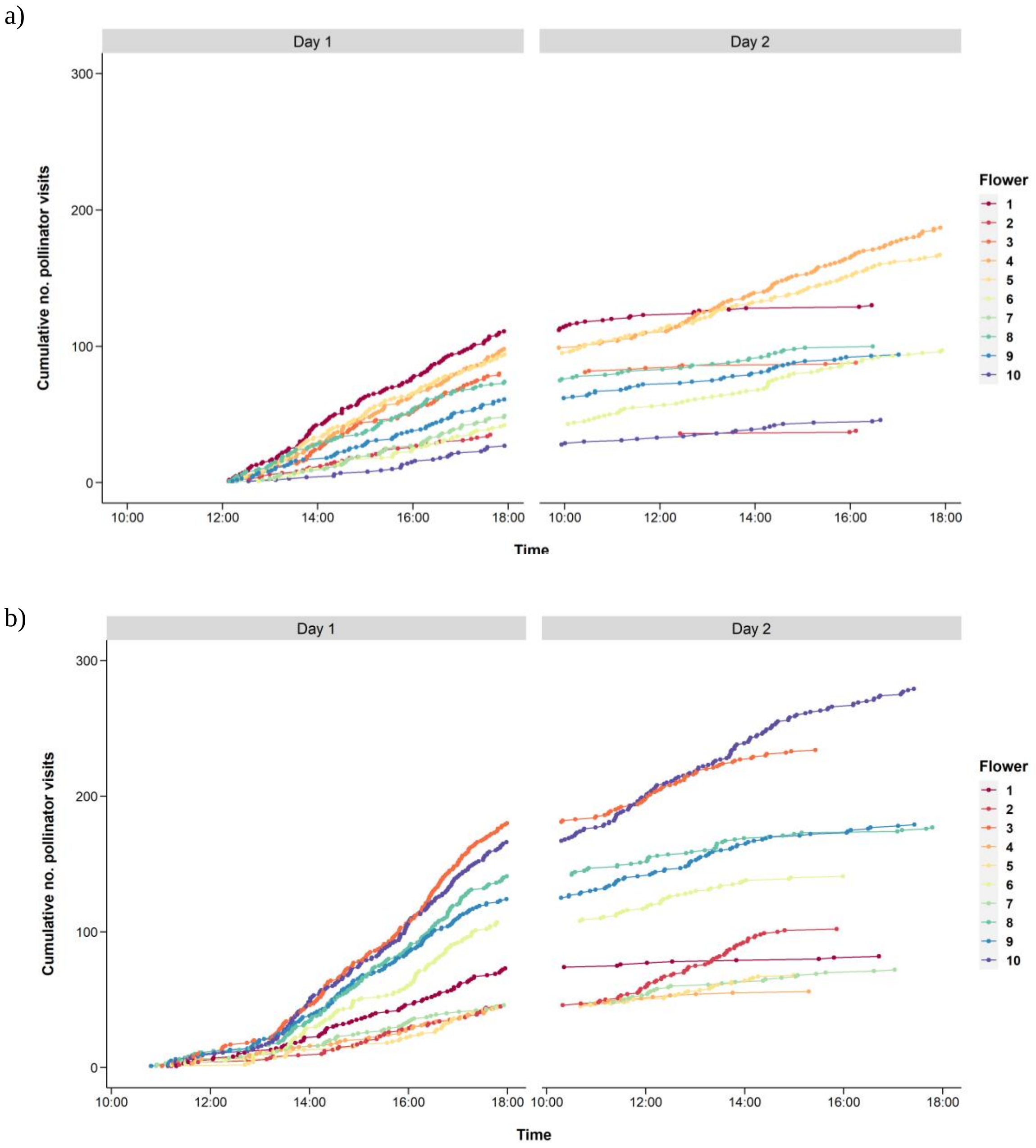

Figure 5.4 Cumulative number of visits observed to each of 10 marked raspberry flowers over two consecutive days of Raspberry Pi video footage for a) Flower set 1 and b) Flower set 2

The number of visits to flowers in Flower Set 1 per 20 minutes was significantly lower on the second day since flower opening compared to the first day (Chisq=218.29, df=1, p<0.0001) (Est=-0.814, SE=0.166, z=-4.893, p<0.0001) (Figure 5.5a). There was no significant interaction effect between observation day and time of day (Chisq=3.163, df=1, p=0.075). Time of day also did not significantly predict visit number per flower (Chisq=0.3995, df=1, p=0.527). Between 12:10 and 5:50, there were 655 visits to the 10 flowers on the first day of filming. Each flower received between 26 and 108 visits with a mean of 65.5 visits per flower (±8.94). During the same hours of observation as the first day (12:10 and 5:50), on the second day each flower received between 0 and 75 visits with a mean of 23.1 visits per flower (± 7.78) (Figure 5.4a).

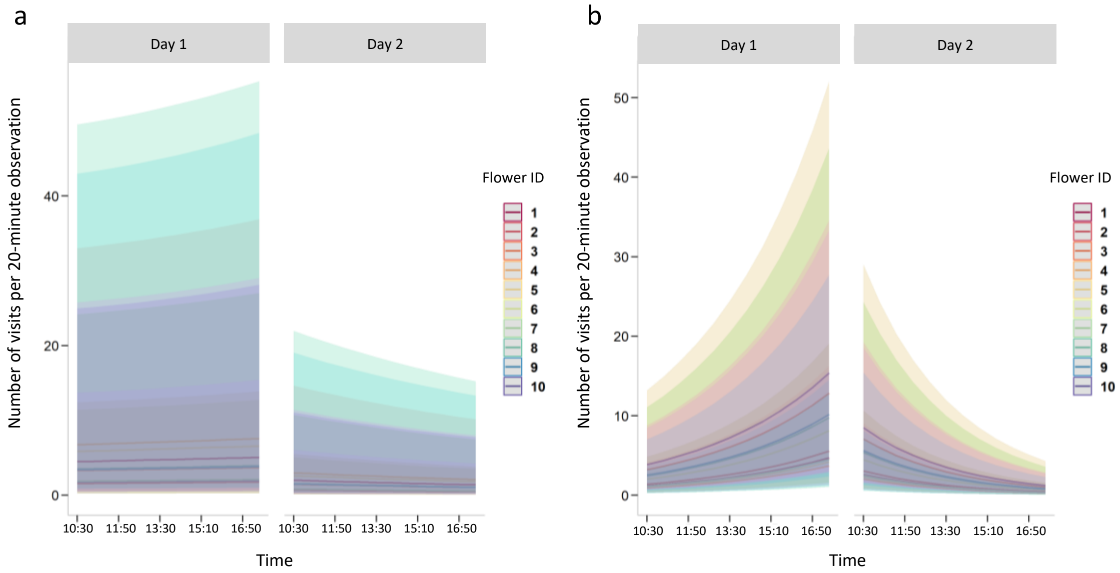

Figure 5.5 Predicted mean number of visits observed to each of 10 marked raspberry flowers over two consecutive days of Raspberry Pi video footage for a) Flower set 1 and b) Flower set 2. Shaded areas represent the bootstrapped 95% confidence intervals.

The number of visits to flowers in Flower Set 2 per 20 minutes was significantly predicted by the interaction between observation day and the time of day (Chisq=151.400, df=1, p<0.0001) (Est=-0.15, SE=0.012, z=-12.304, p<0.0001) (Figure 5.5b). The number of visits per flower per 20 minutes increased by 0.063 visits with each sequential 20-minute timed flower count throughout the first day since flower opening but decreased by 0.088 visits with each sequential 20-minute timed flower count throughout the second day since flower opening. There were 946 visits to the 10 flowers on the first day of filming. Each flower was visited between 44 and 176 times in this first day with a mean of 94.6 visits (±16.4)(Figure 5.4b).

Each flower was visited by honey bees alone for a combined total of 3.9-19.9 minutes (mean 9.0 minutes ± 1.7) over 13 hours of observations for Flower Set 1 and 5.4-21.3 minutes (mean 11.3 minutes ± 1.6) over 14 hours and 20 minutes of observations for Flower Set 2. The duration of honey bee visits to flowers in Flower Set 1 was significantly predicted by the interaction between observation day and the time of day (Chisq=4.5801, df=1, p=0.032) (Figure 5.6a). The predicted duration of honey bee visits decreased by 0.84 seconds (12.9% decrease) between the first and last 20-minute observation on day 1 and by 2.47 seconds (42.3% decrease) on day 2. The predicted visit duration at the start of day 2 was 0.63 seconds shorter than the visit durations at the start of day 1. Model outputs are presented in table S2 of the supplementary material. The duration of honey bee visits to flowers in Flower Set 2 was also significantly predicted by the interaction between observation day and the time of day (Chisq=20.706, df=1, p<0.0001) (Figure 5.6b). The predicted duration of honey bee visits decreased by 0.56 seconds (9.1% decrease) between the first and last 20-minute observation on day 1 and by 3.45 seconds (66.2% decrease) on day 2. The predicted visit duration at the start of day 2 was 0.96 seconds shorter than the visit durations at the start of day 1. Model outputs are presented in table S5.2 of the supplementary material.

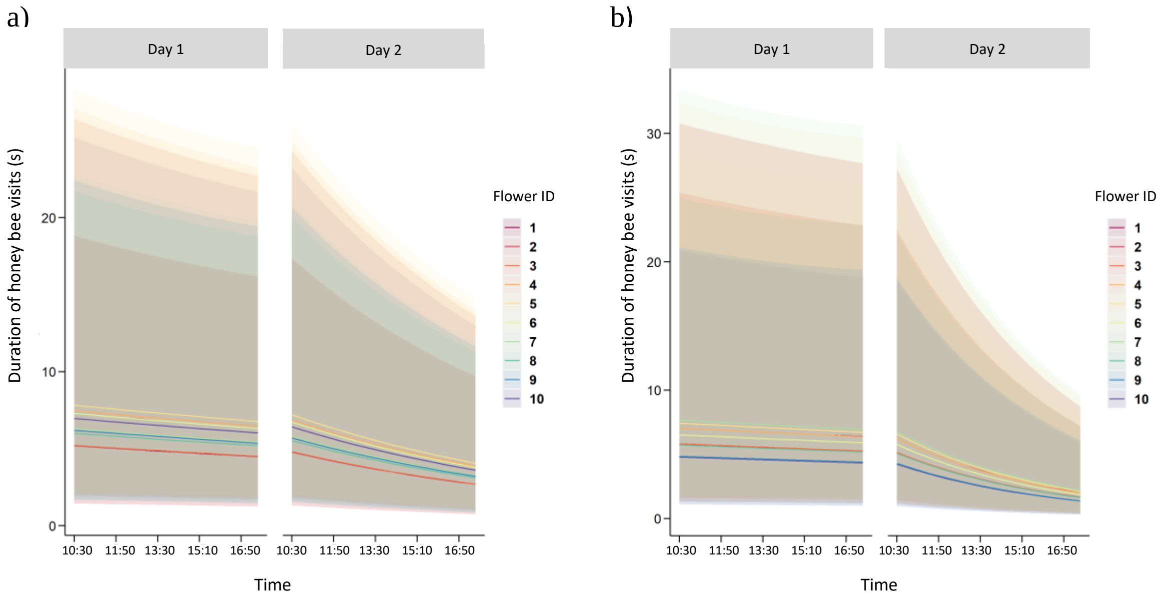

Figure 5.6 Predicted mean duration of honey bee visits observed to each of 10 marked raspberry flowers over two consecutive days of Raspberry Pi video footage for a) Flower set 1 and b) Flower set 2. Shaded areas represent the bootstrapped 95% confidence intervals.

Pollinator visit duration varied significantly with the interaction between pollinator group and observation day (Chisq =19.076, df=1, p<0.0001). Honey bee visits to raspberry flowers were significantly longer than bumble bees on both day 1 (HB: 6.99 seconds ± 0.14, BB: 3.62 seconds ± 0.15) (Est=0.133, SE=0.014, p<0.0001) and day 2 (HB: 5.23 seconds ± 0.17, BB: 1.91 seconds ± 0.22) (Est=0.331, SE=0.049, p<0.0001). Visit duration was also longer for both honey bees (Est=-0.048, SE=0.006, p<0.0001) and bumble bees (Est=-0.247, SE=0.051, p<0.0001) on day 1 than it was on day 2 (Table S5.3; Figure 5.7)

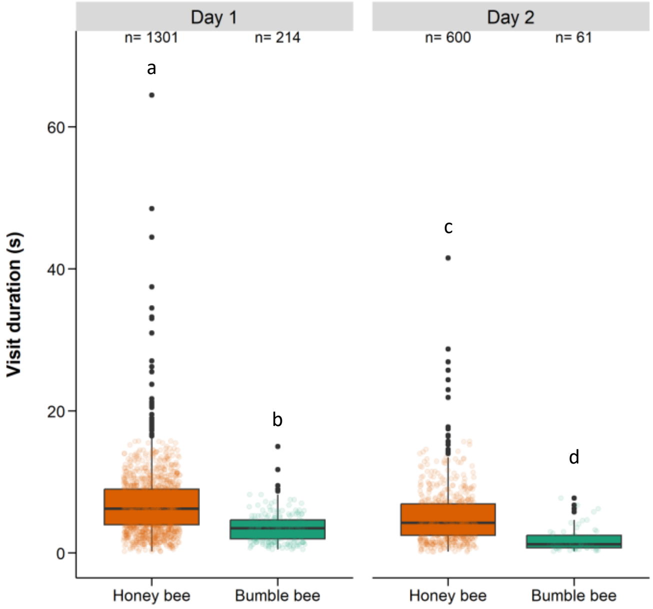

Figure 5.7 Pollinators visit duration during the first and second day since flower opening from Raspberry Pi video footage of two sets of 10 marked raspberry flowers. Letters show significant differences.

The mean interval time between a honey bee visit and the previous insect visit to the same flower was 5.0 minutes (± 0.24) on day 1 and 10.4 minutes (± 1.05) on day 2 for Flower Set 1. This mean visit interval varied greatly between flowers: 3.3-13.9 minutes on day 1 and 5.0-67.9 minutes on day 2. For Flower Set 2, the mean interval time between a honey bee visit and the previous insect visit to the same flower was 4.1 minutes (± 0.25) on day 1 and 8.6 (±0.75) on day 2 varying between 2.1-10.2 minutes per flower on day 1 and 4.1-45.3 minutes on day 2 (Fig 5.8).

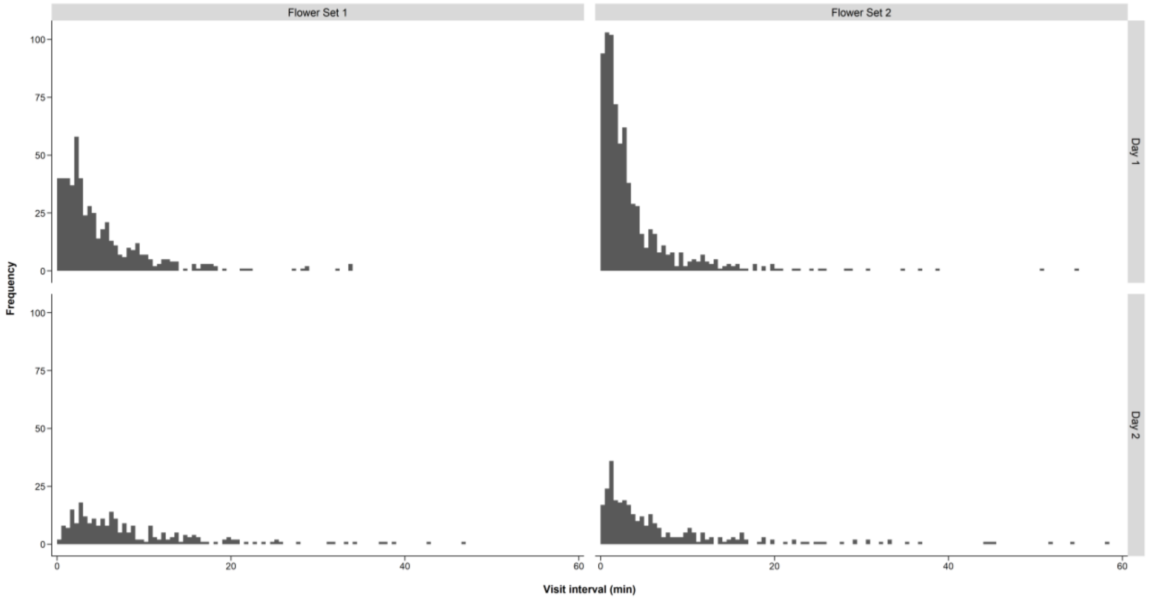

Figure 5.8 Distribution of seconds to honey bee visit after a previous insect visit to two sets of 10 marked flowers during two consecutive observation days of Raspberry Pi video footage. 10 honey bee visits that occurred between 60-190 minutes after the previous visit were omitted to enable the histograms to be viewed more clearly.

There was no significant difference in the minutes since the previous visit with previous visitor identity (Chisq=1.540, df=1, p=0.2147). The interaction between observation day and identity of the previous visitor was also not significant (Table S5.4). There was a significant effect of observation day (Chisq=124.2405, df=1, p<0.0001) (Fig 5.9). Visit intervals were twice as long on the second day of observation (Est=2.022, SE=1.07, p<0.001).

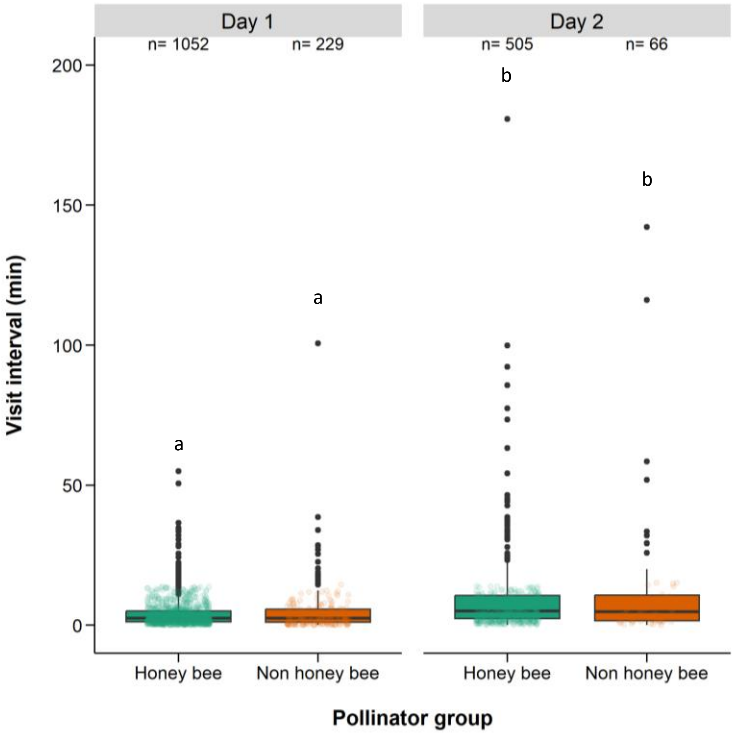

Figure 5.9 Seconds to honey bee visit after a previous insect visit to two sets of 10 marked flowers during two consecutive observation days of Raspberry Pi video footage. Letters show significant differences

5.4.4 In person timed flower counts vs Raspberry Pi video analysis

The mean number of insect visits to 10 flowers observed over 20 minutes was 44.42 ± 4.45 using eight hours of Raspberry Pi video footage between 10:43 and 18:43 (24 x 20-minute counts). Using the bootstrapped subsample t-tests we found that 0.1% of the 1000 iterations found a significant difference between the subsample (n=3 20-minute counts) mean number of visits and the mean number of visits for the remaining observations (n=21 20-minute pollinator counts). Figure 5.10 shows the distribution of observed p-values obtained. This indicates that a sample of three counts across the day, used for the empirical in-person counts, is a reliable indicator of the visitation rate throughout the day.

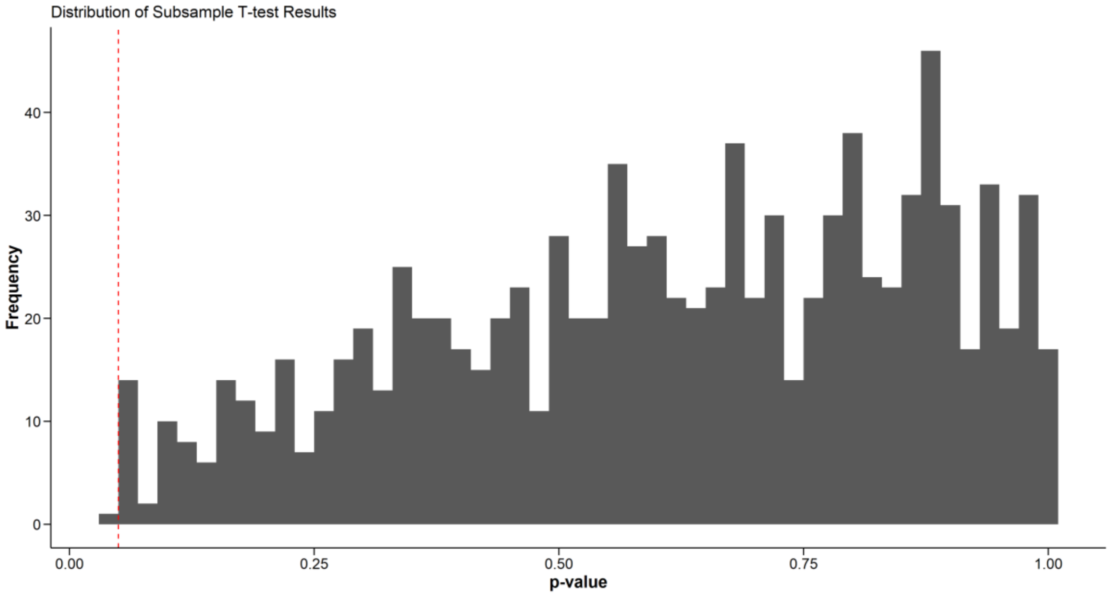

Figure 5.10 Frequency of p-values observed from bootstrapped Welch's t-tests comparing the mean number of insect visits observed to 10 marked flowers during three 20-minute timed flower counts and the day-long mean number of insect visits observed using eight hours of Raspberry Pi video footage. The dashed red line shows the significance threshold of alpha=0.05.

5.5 Discussion

Both human field observations using short, timed flower counts and day-long counts of pollinator visits to crop flowers using manual coding of video footage revealed that raspberry crop flowers at our study site received at least 50 visits on average over two days during their receptive period. This is at least five times higher than the pollinator requirements for yield maximisation determined by other studies (Saez *et al.,* 2014, 2018; Andrikopoulos and Cane, 2018; Chagnon *et al.,* 1991) and 25 times higher than the estimated pollinator requirement in this system (Chapter 3). Most of these visits were by managed honey bees and so the cumulative number of visits flowers received in this study highlights that they are significantly overstocked in this system.

Although the mean visitation rate of the short, timed flower counts was not significantly different from the day-long mean (Figure 5.11), insects visited crop flowers for longer than the six hours of pollinator activity suggested for determining raspberry pollinator requirements on farms (Garibaldi *et* *al.,* 2020). The earliest flower visit observed in this study was 9:52am, 1 minute after the start of the second day-long Raspberry Pi observation for Flower Set 1, and the latest was 18:39, 3 minutes before the end of filming on the first day of observation for Flower Set 2. The actual visitation rate required to maximise yield is therefore likely to be much lower per hour due to the long days of pollinator activity observed in this study. This may be because raspberry crops in the UK flower during the summer months when honey bee colony sizes are high. The longer visitation period could also be due to the fact that polytunnels provide a warmer environment than outdoor environments (Harmanto *et al.,* 2006; Hall *et al.,* 2020), extending the foraging period for insect pollinators of protected crops.

The visitation rate to crop flowers was significantly lower on the second day after the flowers opened (Figures 5.6, 5.7, 5.8 and 5.9). Therefore, when monitoring visitation rates to recently opened flowers, target visitation rates should be higher than the target given by Garibaldi *et al.* (2020), who spread the required number of visits to flowers evenly over the 3-day receptive period. For Flower Set 2 there was a positive effect of time of day on the visitation rate to crop flowers on the first day since flower opening, while there was a negative effect of time of day on the second day. This could be due to pollinators responding to temperature increases throughout the day on day 1 (Nielsen *et al.,* 2017; Tan *et al.,* 2012) but being nectar limited on day 2 as the day progresses and nectar resources are depleted (Schmidt *et al,* 2015; Willmer *et al,* 1994). This effect was not seen for Flower Set 1. This could be due to the observations only starting after midday on the first day of observation and so only the high visitation rates during the afternoon were captured. The difference between flower sets could also be due to the difference in location or environmental variables such as temperature and humidity (Hall *et al,* 2020). More replicates of different flower sets are needed to enable the drivers behind the differences in visitation rate to be identified. To do this, automated methods such as machine learning or artificial intelligence are needed to enable more data to be processed in a shorter length of time. Using BORIS to manually code each visit in this study meant that analysing more than four days of data was not feasible as it took several hours to analyse a single hour of video data. Two Raspberry

Pi's short circuited during filming, we think this may have been due to overheating and is therefore a limitation for using the setups in polytunnels or in warmer conditions.

The duration of honey bees' visits on crop flowers declined with days since flower opening and with time of day. The effect of time of day was much stronger in the second day with a ~50% decline across both flower sets between 10:30 and 5:30. This is likely due to reduced nectar availability as visit duration is strongly correlated with the volume of nectar in the flowers nectaries (Chagnon *et al.,* 1992: Willmer *et al.,* 1994). Although nectar is secreted throughout the day until at least 6pm (Willmer, *et al.,* 1994), the early morning nectar levels, achieved from secretion overnight, are not achieved during the day when being continually foraged. When measuring visitation rates to crop flowers, it is therefore important to survey at different times in the day to collect an accurate day-long visitation rate to the crop flowers.

Crop flowers in this study received at least 3.9 minutes of visitation from honey bees alone, with some receiving up to 21.3 minutes of visitation over two days of observation. Chagnon *et al.* (1991) found that pollination and fruit set reached its maximum after ~150 cumulative seconds of visit time or 5-6 visits in a single day by *Apis mellifera* while Andrikopoulos and Cane (2018) found a mean cumulative visit time of ~127 seconds over two prolonged visits, one on each of two consecutive days, from either a honey bee or bumble bee was sufficient for maximal fruit set. The flowers in this study were therefore receiving more than enough visit time to maximise their fruit set. The honey bee visits observed during the first day since flowers opened in our study were 6.99 (± 0.14) seconds on average and 5.23 (± 0.17) seconds on average during the second day. These are much shorter visits than recorded by Chagnon *et al.,* (1991) and Andrikpoulos and Cane (2018). Crop flowers in this study may therefore have been visited for longer durations at the beginning of the day, before observations started at 10:30, when prolonged foraging on large quantities of nectar is possible. The absence of these prolonged visits in our data suggests that this may have occurred, and thus the visits recorded during our observations between 10:30-5:50 are additional visits above what is required. Therefore, although the raspberry flowers in this system are getting more than enough visits to maximise their yield in terms of visit number and cumulative duration of visits in comparison to estimates of requirements (Garibaldi *et al.,* 2020; Chagnon *et al.,* 1991; Andrikopoulos and Cane, 2018), perhaps recording the first visits of the day using focal flower observations or camera set ups would be of more value than day-long observations for estimating pollination service provision. Pollinator exclusion bags could also be placed on flowers before they open, and their first visit recorded as in Andrikopoulos and Cane (2018) to capture these visits.

Chagnon *et al.,* (1991) found that fewer longer visits yielded more drupelets than many visits, suggesting that the duration of insect pollination visits, rather than the quantity of visits, is of more value to raspberry crops. Whereas, in our study Bumble bees visited flowers for significantly less time than honey bees throughout the observation periods (Figure 5.7) but provided a greater marketable fruit weight after a single visit than honey bees and open pollination (Chapter 3; Table 3.6). Chagnon *et al.,* (1991) observed the benefit of prolonged visitation for honey bees and did not test this for bumble bees. It is therefore possible that bumble bees are more effective on a per visit rate than honey bees and that for honey bees to produce high yields from raspberry flowers, longer visit durations are required. Willmer *et al.,* (1994) also found that bumble bees were more effective pollinators of raspberry and that this was likely due to the greater foraging rate recorded. Andrikopoulos and Cane. (2018a) found that a single visit from a native US *Bombus* sp. to a raspberry flower yielded berries that did not significantly differ in the number of drupelets from those that received a single honey bee visit. The effectiveness of *Bombus* sp. for raspberry pollination is therefore likely to vary between raspberry cultivars, as seen in Andrikopoulos and Cane (2018a) and *Bombus* species.

The time between insect visits and the next honey bee visit in some cases was less than 1 minute (Figure 5.8) suggesting that flowers were not being avoided based on how recently they had been visited. The greater interval between visits on day 2 since flower opening is therefore likely to be due to a lack of attractiveness due to reduced nectar availability and that this is potentially being detected before insects land on the flowers. The length of time before the next honey bee visit was not predicted by the identity of the previous visitor, suggesting that either there was no active avoidance of flowers that had been visited by either conspecifics or other pollinators. This conflicts with the finding that honey bees foraging on *Borago officinalis* rejected flowers that had been recently by a conspecific (Williams, 1998) and honey bee foraging on *Rubus fruticosus* rejected flowers that had recently been visited by any pollinator (Reader *et al.,* 2005). This was initially thought to be due to bees detecting repellent scent marks left by previous visitors to nectar-depleted flowers (Giurfa and Núnez, 1992; Wilms and Eltz, 2008). More recently it has been suggested that this could be due to the detection of the bee altered electric fields of flowers (Clarke *et al,* 2013). Williams (1998) found that honey bees rejected the borage flower when a honey bee had visited the same flower less than 20 seconds previously and so there may have been too few visits in our study to observe this effect. Although raspberry flowers do not always produce greater quantities of nectar than blackberry flowers (Schmidt *et al.,* 2015) the cultivars in our study could be producing large quantities of nectar, reducing the need to partake in the selective foraging documented by Reader *et al*. (2005) or reducing the repellence period (Stout and Goulson, 2002). There were a higher number of rejections in the second day, where bees hovered around flowers but did not land and then moved on to another flower or out of frame (pers. obs). This suggests that honey bees are using direct assessment of the flower without attempting to forage rather than detection of previous visits as found by Goulson *et al.,* (2001). The open raspberry flowers, with easily accessible nectar may therefore be freely foraged upon when nectar is available and rejected only when this stock is depleted as nectar secretion reduces late into the second day since flower opening.

20-minute timed flower counts did not differ from day-long observations in the mean number of visits estimated per 20 minutes (Figure 10). The choice between day-long video observations and in-person timed flower counts therefore depends on the scope and objectives of the study. In person counts allow data to be collected in real time. Where the minimum observation time required is short, multiple replicates of flower observations can be made during the same day, making timed flower counts potentially more appropriate. Using BORIS to manually code visits is not time efficient, and although video footage allows for the collection of a greater amount of data and for multiple sites simultaneously, it takes a long time to process this data. The use of Raspberry Pi video setups and visit observations in BORIS is therefore only suitable where simultaneous sampling is important, or where accurate visit durations and intervals between durations are desired. Continuous recording provides large amounts of visitation data and can be left to record visits to crop flowers while surveyors collect other data. However, the data processing time makes the large amounts of data impractical without automated visit identification. Algorithms to detect insect visits from video are usually bespoke and require computer science expertise to create. There is a trade-off between more comprehensive data or faster data collection.

5.6 Conclusion

We have shown that pollinator visitation rates to and foraging duration on raspberry flowers are lower on the second day of flowering and decline throughout this second day. This shows that when monitoring pollinator service provision to raspberry, the service is unlikely to be uniform across the three-day receptive window. Therefore, when monitoring pollinator visitation, the majority of the required number of visits should be achieved in this first day after flower opening. Monitoring pollinator visitation rates using short, timed flower counts is accurate in determining the number of visits flowers receive on the first day of opening and is a more accessible and time efficient method of recording this compared to video analysis. However, the minimum required length of observation required should be determined to ensure the accuracy of these estimates. The use of longer observation periods to calculate this may be facilitated by video analysis if in-person observations over multiple hours cannot be undertaken.

5.7 References

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5.8 Supplementary material

Table S5.1. Results of Linear Mixed Models (GLMMS) for a response of **number of pollinator visits per 20-minute observation** for (a) Flower Set 1 with a poisson error distribution and (b) Flower Set 2 with a negative binomial error distribution

Variable	Estimate	Standard Error	z value	P value
a)				
(Intercept)	1.1574	0.1966	5.887	**<0.0001**
Day:2	-0.8143	0.1664	-4.893	**<0.0001**
Time of day	0.0054	0.0080	0.680	0.4968
Day:2 x Time of day	-0.0217	0.0122	-1.779	0.0753
b)				
(Intercept)	0.5590	0.1864	2.999	**0.0027**
Day:2	0.7747	0.1210	6.402	**<0.0001**
Time of day	0.0619	0.0056	11.087	**<0.0001**
Day:2 x Time of day	-0.1483	0.0101	-14.604	**<0.0001**

Table S5.2. Results of Linear Mixed Models (LMMS) with normal error distributions for a logged response of **pollinator visit duration** for (a) Flower Set 1 and (b) Flower Set 2

Variable	Estimate	Standard Error	t value	P value
a)				
(Intercept)	1.8734	0.1017	18.419	**<0.0001**
Day:2	-0.0824	0.1232	-0.669	0.5036
Time of day	-0.0066	0.0059	-1.121	0.2625
Day:2 x Time of day	-0.0196	0.0092	-2.140	0.0327
b)				
(Intercept)	1.8240	-0.1034	17.644	**<0.0001**
Day:2	-0.1215	0.1183	-1.027	0.3050
Time of day	-0.0045	0.0055	-0.826	0.4090
Day:2 x Time of day	-0.0470	0.0103	-4.550	**<0.0001**

Table S5.3. Results of Linear Mixed Models (GLMMS) for a response of **pollinator visit duration** with a Gamma error distribution.

Variable	Estimate	Standard Error	t value	P value
(Intercept)	0.2759	0.0137	20.117	**<0.0001**
Day:2	0.2466	0.0505	4.879	**<0.0001**
Pollinator:Bumble bee	-0.1338	0.0140	-9.475	**<0.0001**
Pollinator:Bumble bee x Day:2	-0.1983	0.0509	-3.894	**<0.0001**

Table S5.4. Results of Linear Models (GLMS) for a response of **honey bee visit interval** with a log normal error distribution.

Variable	Estimate	Standard Error	t value	P value
(Intercept)	0.90755	0.10442	8.691	**<0.0001**
Day:2	0.70427	0.06945	10.140	**<0.0001**
Pollinator:Non-HB	-0.13227	0.09137	-1.448	0.148
Pollinator:Non-HB x Day:2	0.14017	0.18738	0.748	0.455

6.0 Conclusions

Pollinators play a vital role in global food production (Klein *et al.*, 2007). While wind-pollinated crops including wheat, rice, maize, and barley provide about 50% of globally produced calories for human consumption (D'Odorico *et al.*, 2014), animal-pollinated crops contribute significantly to our daily intake of essential nutrients (Eilers *et al.*, 2011). Without pollinators such as bees, hoverflies, butterflies, and moths, we would therefore struggle to produce the diverse and nutritious foods we rely on. Managing pollinators effectively is crucial for maximizing yields from animal-pollinated crops. In some systems, such as seed production in leek (Fijen *et al.,* 2018), pollination is an agricultural input as important to yield as fertilizer or water. It is therefore important that pollinators are monitored in agricultural systems and that clear empirical based guidance is available to inform the pollinator management of insect pollinated crops.

This thesis has investigated the significance of insect pollinators, specifically *Apis mellifera* and *Bombus terrestris,* for two previously unstudied cultivars of red raspberry in the UK. The project aimed to determine the importance of insect pollinators and the ability of these two species to provide pollination services to the crop. Additionally, it examined the potential effects of protected cropping on the uniformity of pollination service provision to crop flowers and the temporal variation in crop flower visitation rates to provide insights into how pollinators should be monitored for crop production.

6.1 Summary of key thesis findings

In Chapter 2, we examined the impact of year, cultivar and yield metric on raspberry yields and pollinator dependence valuations. Our aim was to understand the factors that contribute to the variation in pollinator dependence valuations for the same crop, as seen in the meta-analysis in section 1.5. Our findings revealed that insect pollinators were responsible for 64% of total marketable yield of raspberries in grams over three years and two cultivars, indicating that raspberries have a strong need for pollination. This aligns with the findings of Klein *et al.* (2007). Furthermore, our study demonstrated that the choice of yield metric used in pollinator exclusion studies has as a significant impact on pollinator dependence valuations. Previous raspberry pollinator studies (e.g. Andrikopoulos and Cane, 2018a; Cane, 2005; Chagnon *et al.,* 1991; Prodorutti and Frilli, 2008) used yield metrics that relate poorly to the market value of raspberry crops such as number of drupelets or total fruit weight. We argue that these are inappropriate for measuring the value of pollinators to crop yields. When evaluating the impact of pollinators on crop yields, it is crucial to consider crop-specific marketability criteria such as size, shape, minimum weight, protein or sugar content. This is because only marketable quality fruits contribute to the economic yield of a crop. This will provide a more accurate understanding of the contribution of pollinators to crop yields and help inform decisions regarding the management and conservation of pollinator populations. For raspberry specifically, we recommend considering the uniformity of drupelet size within berries as this is a marketability criterion imposed by UK supermarkets. While the rise in popularity of "wonky" fruit and vegetables may change this criterion in the future, for now, excluding these fruits from marketable yield is more appropriate for evaluating the contribution of insect pollinators to raspberry economic yields.

Chapter 3 investigated the effectiveness of two common pollinators, *Apis mellifera* and *Bombus terrestris,* for raspberry pollination. While previous studies have explored the pollinator ability of *Bombus terrestris* in terms of pollen transfer and foraging rate (Willmer *et al.,* 1994), their single visit pollinator effectiveness in terms of yield production has not been established. Given the high numbers of *Bombus terrstris* in the study system (Chapter 4) and across the UK, they represent an important potential alternative pollinator to *Apis mellifera.* Understanding their ability to provide marketable yields in the absence or reduction of honey bee input is important for reducing reliance on managed honey bees for crop pollination and minimizing potential negative impacts associated with honey bee presence on the foraging behaviour (Nielsen *et al.,* 2017; Lindstrom *et al.,* 2016; Angelella *et al.,* 2021) and health (Goulson and Sparrow, 2008; Elbgami *et al.,* 2014; Manley *et al.,* 2015) of bumble bees and other wild pollinator groups . The study found that two visits from either *Apis mellifera* or *Bombus terrestris* were sufficient to produce marketable fruit weight that did not differ from flowers that were openly pollinated. Additionally, a single visit from *Bombus terrestris* resulted in greater marketable fruit weight than unrestricted insect visitation, suggesting that the high visitation rates documented in Chapters 4 and 5 could have had a negative impact on fruit yields in this system. Saez *et al.* (2014) found that high rates of both *Apis mellifera* and *Bombus terrestris* resulted in increased stigma and style damage or loss, resulting in lower numbers of drupelets being produced. By determining the required number of visits for optimal marketable yields, target values of visitation rates can be established to enable monitoring of pollination service provision and prevent yield losses from both insufficient and excessive pollinator visits.

In Chapter 4, we investigated how visitation rates vary within fields that are under protected cropping. Our goal was to gain insight into the potential variability of pollination services across the field and how this information could be used to effectively monitor visitation rates. Our findings showed that, on average, flowers received more than 60 times the required two pollinator visits at honey bee stocking densities of 1 hive/ha. Interestingly, we found that visitation rates were highest at the corners of the fields, lowest in the centre, and intermediate along the non-corner edges. This suggests that monitoring of crop flower visitors should focus on the centre of fields to ensure sufficient pollination across the whole field. We speculate that the physical barrier presented by polytunnels may be responsible for the observed variation in visitation rates within the crop fields.

In Chapter 5, we examined the potential of video cameras to monitor pollinator visitation rates and compared the results with traditional short, timed flower counts. Our findings revealed that the average visitation rate recorded over three 20-minute point counts did not differ significantly from the average visitation rate observed throughout the day. In cases where insect visitation rates are high, shorter observations may be sufficient (Fijen and Kleijn, 2017). Given that growers already monitor several crop growing conditions, such as soil nutrients, soil water content, and pest abundance, adopting short, timed flower counts to assess pollinator needs before implementing managed pollinators could prevent the overstocking of managed bees (Garibaldi *et al.*, 2020). The financial insecurity bee keepers are likely to face in providing hives to farms on short notice and for short term periods may hinder the implementation of this approach. Nevertheless, monitoring pollinators in this way could motivate growers to implement more wild pollinator conservation measures by enabling them to monitor the results and provide them with the confidence to reduce honey bee stocking densities if excessive visits have been determined.

6.2 Commercial applications

This thesis reveals that even a minimal number of pollinator visits, specifically 2 visits from honey bees or bumblebees, can lead to the production of marketable fruit for two raspberry cultivars. Consequently, growers might consider reducing honey bee stocking densities in locations where flowers receive many more than 2 visits required from social bees. Reducing the use and reliance on honey bees for crop pollination will improve the long-term stability of crop yields and help alleviate the negative impacts of high honey bee densities on wild pollinator communities. In systems where honey bees are paid for per hive, reducing the stocking density of honey bees will also reduce input costs.

However, it is crucial to note that the required number of visits may not be universally applicable across all growing conditions and raspberry cultivars, underscoring the need for caution. Therefore, while growers can use this as a rough guide, they should also rely on their own pollinator and yield monitoring for more accurate insights, especially for other cultivars. In cases where growth conditions, such as low fertiliser levels, limit yields, any benefit of increased pollinator levels may be inhibited (Chen *et al.*, 2022). Identifying limiting growth conditions is therefore essential before increasing pollinator densities.

Growers should carefully monitor the interplay between stocking densities, visitation rates, and yield to ensure optimal, yet not excessive, pollination for their crops. In cases where a pollinator deficit is identified, growers can implement short-term solutions involving managed pollinators while concurrently implementing measures for wild pollinator conservation to safeguard long-term pollination service provision for crop flowers.

For site-specific assessments of pollinator requirements, growers can employ the exclusion studies and visitation rate observations detailed in this thesis. These surveys serve not only to identify potential pollination deficits but to evaluate existing visitation rates and track the effects - both positive and negative - of alterations in pollinator management practices and conservation efforts. Additionally, conducting these studies long term will provide valuable insights into the stability of pollination service provision within and between growing seasons to help predict pollinator requirements. While there are barriers to adopting the advice and findings in this thesis, such as associated financial costs of managed pollinators and wild pollinator conservation, growers are encouraged to capitalize on financial incentive schemes for wild pollinator conservation to reduce pollinator costs long term. Given the lack of clear practical recommendations for managing insect-pollinated crops, growers can enhance yields by managing and monitoring pollinators at a farm or field level, similar to approaches for pests and fertilizers. The guidance provided here will be disseminated to enable growers to conduct this monitoring and interpret the results effectively.

6.3 Research limitations

This research is subject to several limitations that warrant acknowledgement to contextualise and interpret the findings appropriately. The omission of key abiotic factors – soil fertility, fertilizer application, water availability, and pest control –from the evaluation of pollinator dependence, pollinator deficits and single-visit pollinator efficiency introduces uncertainty into the calculated values of yield attributed to pollinators. The known interactions between these conditions and pollination treatments (Tamburini *et al.*, 2019), combined with their variability within and between fields, restricts confidence in extrapolating the results beyond the specific conditions studied and hinders a nuanced understanding of the intricate interplay between these factors and their effects on yield. Although the highly-managed nature of the studied system may alleviate some abiotic constraints, the lack of direct measurements diminishes confidence in extrapolating results to diverse agricultural contexts. To enhance relevance and transferability, future research should incorporate abiotic data to achieve a more comprehensive understanding of factors influencing yield. Recording and sharing growth conditions experienced by study plants would enable practitioners and researchers to assess the findings' applicability to their systems and identify potential non-pollinator-related limitations on yield.

The inability to manipulate honey bee densities as desired, owing to the risk averse pollination strategies of collaborating growers, poses challenges in fully understanding the relationship between pollinator densities and crop yield. This common scenario in high value crops, where growers prioritize pollination security (Sulewski, and Kloczko-Gajewska., 2014), underscores the complexity of experimental control in real-world agricultural settings. An alternative to manipulating the density of managed hives at a single farm would have been to carry out studies on multiple farms across a gradient of honey bee stocking densities though my contract with industry partner BerryWorld impeded this. The absence of experimental manipulation of honey bee stocking densities within or between farms impedes a nuanced understanding of the relationship between honey bee stocking densities, pollinator visitation rates, and crop yields. Consequently, guidance on managed pollinator stocking is constrained to a trial-and-error approach, placing the responsibility on growers to fine-tune stocking densities based on their own pollinator visitation rate studies. However, the effectiveness of this approach hinges on the precision of the required number of visits to produce marketable fruit and the applicability of our findings to their specific farming context, introducing inherent uncertainties into the practical implementation of such recommendations

The study's exclusive focus on the biophysical aspects of farming neglects the socioecological dimension inherent in agricultural systems. Although informal conversations with beekeepers and growers provided valuable insights into motivations behind observed stocking densities and on-farm wild pollinator conservation measures, the absence of formal farmer and beekeeper interviews or a comprehensive quantitative survey impacts the wider applicability and depth of the findings. The study lacks clarity on how representative the observed honey bee stocking densities are for UK raspberry growers. Without insights from surveys, it remains uncertain whether the practices observed in this specific farm are reflective of broader industry trends, potentially varying across regions or under different environmental conditions. Understanding these variations would be pivotal in tailoring recommendations and insights to specific regional contexts, ensuring that any proposed changes or guidelines are reflective of the broader agricultural reality. Additionally, understanding the economic influences on stocking densities is crucial, especially given that this particular farm's decisions were primarily driven by the potential loss of yield rather than input costs, which differs from farms that pay per hive of honey bees. Without farmer surveys, the research lacks a comprehensive understanding of whether there are strategies adopted within the industry to mitigate the need for extensive managed pollinator input. Additionally, the absence of farmer perspectives leaves a significant gap in knowledge regarding the upper and lower bounds of honey bee stocking densities that growers, including this particular farm, would tolerate for raspberry cultivation. Insights into these thresholds are crucial for providing practical recommendations that align with growers' comfort levels and economic considerations.

6.4 Future directions

A much-needed update to the global valuation of pollinators to crop production has just been published. The previous review by Klein *et al.*, (2007) is widely cited but was not based on a meta-analysis and the method for study inclusion and calculating pollinator dependence values was flawed. The study highlights the need for pollen supplementation within pollinator exclusion studies to ensure that maximum yields and pollinator deficits are captured. The new review by Siopa *et al.* (2023) found that 80% (instead of less than 50% indicated in the review by Klein *et al.,* (2007)) of animal pollinated crops depend highly on pollinators. The authors also state that pollen limitation was detected in 51.5% of the studies included. The comprehensive advice on how to carry out pollinator exclusion studies to maximise their use for informing pollinator management will hopefully aid in the required expansion of studies covering different crop cultivars and geographic regions. The rapid expansion of uses of AI and automated monitoring shows promise for the ability for future studies to expand the data on pollinator ecology through streamlining data collection and improving the reliability and repeatability of methods used to monitor pollinators (Besson *et al.,* 2022; Bierge *et al.,* 2023). Examples of new technology being used within ecological science includes the use of remote sensing for analysing habitat and floral cover Barnsley *et al.* (*2022*) and acoustic monitoring for environmental policy needs such as determining ecosystem 'health', ecosystem services and function (August *et al.*, 2022). ~380 studies within the fields of ecology and evolution have been published between the beginning of 2019 and the beginning of 2022, have adopted deep learning employing artificial intelligence techniques such as neural networks and supervised learning (Borowiec *et al.*, 2022). I am currently involved in the development of an AI tool aimed at automating flower visitation rate and visitor identity observations for raspberries through machine learning from video data I coded in BORIS and analysed in Chapter 5. The tool identifies the presence of an insect pollinator on a flower, recording the start and end time of the visit. We are also hoping to train the tool to identify different pollinator groups. The goal is to create a user-friendly and efficient method of monitoring pollination rates from video footage, with the potential to adapt the tool for use with other crops using test footage. Using this technology, we hope to simplify data collection and facilitate the comparison of visitation rates with other environmental variables, such as temperature and humidity, measured over whole days. This approach would enable the simultaneous survey of multiple locations over extended periods, allowing for more in-depth analysis of the drivers behind visitation rates and pollinator abundance. The tool would eliminate the need for expertise in identifying broad pollinator groups. If the tool proves to be accurate in estimating the number of visits crop flowers receive and the proportion of visits made by managed honey bees and different groups of wild pollinators, cameras could be set up on farms to run semi-continuously, enabling automated monitoring of pollination service provision. This could inform the need for managed pollinators and the effectiveness of pollinator conservation efforts for short-term and long-term pollination service provision. This is just one example of the ways that computer scientists and ecologists can come together to improve the efficiency of data collection and make it accessible to people outside of ecology.

6.5 References

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