

SYRPHINE HOVERFLIES ARE EFFECTIVE POLLINATORS OF COMMERCIAL STRAWBERRY

Dylan Hodgkiss^{1,2}, Mark J.F. Brown¹, Michelle T. Fountain²

¹School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, UK.

²NIAB EMR, New Road, East Malling, Kent, UK.

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

Keywords: Crop yield, ecosystem services, *Fragaria*, fruit quality, pollination, Syrphidae

INTRODUCTION

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

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

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

Strawberries are aggregate fruits with each flower receptacle containing multiple carpels (Free 1993). During fruit development the flesh around each achene, or seed, only expands once the achene has been fertilised with a pollen grain (Carew et al. 2003). Thus, poor pollination is one of the main reasons for malformations to occur. Carew et al. (2003) suggest that for fruit to develop properly, at least 70-80% of carpels must be pollinated. Due to their less specialised characteristics, such as radial symmetry, disc shape, easily accessible nectar and exposed anthers, strawberry flowers are visited by a wide range of pollinating insects (Nye & Anderson 1974; Albano et al. 2009a). Research into the effectiveness of various strawberry pollinators has shown that several insects are more or less equally important in the creation of high-quality fruit, and indeed that visits from pollinators with diverse morphologies and behavioural habits tend to produce fruit more frequently and with fewer malformations (Chagnon

Received 2 November 2017, accepted 4 February 2018

*Corresponding author: dylan.hodgkiss.2014@live.rhul.ac.uk

et al. 1993; Albano et al. 2009b). Therefore, multiple visits from insect pollinators are necessary in order to achieve full pollination (Free 1993).

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

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

MATERIALS AND METHODS

Pollination effectiveness of a mix of hoverfly species on strawberry flowers

To determine the pollination effectiveness of a mixture of aphidophagous hoverfly species, 18 nylon mesh cages (47.5 × 47.5 × 93.0 cm; BugDorm, Taichung, Taiwan) were constructed and arranged on the ground in a 3 × 6 grid under a polytunnel at the NIAB EMR research institute, Kent, UK (51.286034° N, 0.449165° E, elevation: 35 m). The study site was surrounded by horticultural land which was comprised of other strawberry crops and arable fields, with mixed native hedgerows. Given that the cages were arranged in columns of six on each of three longitudinal drip irrigation lines, two sets of 3 × 3 randomly-generated Latin square designs were used to allocate treatments to the cages, with six cages, or replicates, per treatment. This method ensured that each treatment was represented in every row and twice in each column, reducing bias that may have resulted from distance from the drip irrigation source or from the sides of the tunnel. Ten cv. 'Finesse' strawberry plants in black plastic pots (11 × 11 × 12 cm; Soparco, Condé-sur-Huisne, France) were placed in each cage. All plants were watered and supplied with fertiliser (Ferticare 22-4-22, NutriAg Ltd., Toronto, Canada) at 06:00 and 18:00 daily for five minutes with individual drippers for each pot. The pollination period was started as soon as open flowers were present in each cage: 2 September – 9 October 2015.

The experiment had three treatments: (1) hand pollination (positive control, optimal pollination); (2) insect-exclusion (negative control); and (3) hoverfly visitation. For the hand pollination treatment, a size 12 paintbrush (Major Brushes Ltd., Cardiff, UK) was used to transfer pollen from dehisced strawberry anthers onto the entire receptacle of each open flower in the hand pollination cages. Hand-pollinated cages were visited ten times, approximately twice weekly, over the course of the pollination period and all open flowers were

brushed once with pollen on each visit. Pollinator-excluded cages were left undisturbed throughout the experiment to allow only self- or wind-pollination to occur.

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

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

Comparison of pollination effectiveness of hand pollination and two hoverfly species

Because the hand-pollinated plants in the mixed-species experiment did not yield better-quality fruit than the hoverfly visitation treatment (see Results), we set up an experiment to determine the optimum frequency of hand pollinating strawberry flowers. Four nylon mesh cages were constructed and arranged on the ground in a single column under a small polytunnel at NIAB EMR to exclude insects from visiting the strawberry flowers. Ten 'Finesse' strawberry plants were arranged in each cage, following the procedure in the mixed-species experiment. Four pollination treatments were compared: (1) control, in which no flowers were pollinated by hand; (2) one brush, in which open flowers were brushed with a paintbrush once; (3) two brushes, in which flowers were brushed twice, with 24–48 hours between brushes; and (4) three brushes, in which flowers were brushed three times, again with 24–48 hours between brushes. Plants in each cage were assigned to the four treatments, so that each treatment was represented in every cage. When a flower was brushed, a felt-tipped marker was used to mark the peduncle so that the number of brushes could be tallied for each fruit.

The same general experimental design as the mixed-species experiment was then used to determine whether single species of hoverfly were effective 'Finesse' strawberry pollinators. Twenty cages were constructed to accommodate five replicates for each of four treatments: (1) *Episyrphus balteatus*; (2) *Eupeodes latifasciatus* (Macquart); (3) hand pollination (based on the results from the hand pollination experiment described above); and (4) pollinator-excluded. A randomised block design was employed, with the 20 cages

split into five blocks of four cages, with each treatment represented in each block. Both *Episyrphus balteatus* and *Eupeodes latifasciatus* are common visitors to strawberry flowers, and are common in the southeast of England, where the study took place (Ball & Morris 2015).

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

Fruit quality assessments

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

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

The other half of each berry was used for Brix measurement and counts of fertilised achenes. To measure the Brix, 1-2 drops of juice were squeezed onto a digital refractometer (Palette, Atago, Tokyo, Japan) and soluble solids concentration recorded to the nearest tenth of a percent. To separate achenes from the flesh of the fruit, each berry was placed in a blender (Minipro, Tefal, Rumilly, France) with 200 ml of water and blended for 20 seconds. The contents were then transferred to a 500 ml beaker and allowed to settle. All floating achenes were removed by gently pouring away the supernatant. The sunken achenes were collected by pouring the remaining contents through a sieve. These achenes were then transferred to a petri dish and dried overnight in an incubator at 20°C. The following day, the number of

fertilised achenes per fruit half was counted and recorded for each strawberry. In the latter two experiments, rather than pouring out unfertilised seeds and drying the fertilised achenes in a petri dish, sunken fertilised seeds were simply counted by lifting the glass beaker and counting the achenes that had collected at the bottom. Lastly, strawberries with a minimum diameter of 18 mm and a shape score of 1 or 2 were classed as marketable (Conti et al. 2014; Klatt et al. 2014).

Data analysis

All analyses were carried out in R version 3.3.3 (R Core Team 2017). Average values were calculated for all fruit quality measurements and are presented as mean \pm standard error. For fertilised seed counts from fruit halves, the mass of the fruit half divided by the mass of the whole fruit was calculated and used to weight the calculation of mean seed counts. Linear mixed models were then used on all normally-distributed fruit quality measurements in hoverfly experiments. Response variables were transformed where necessary. When transformations failed to produce normally-distributed data and in the case of fruit marketability, generalised linear mixed models were used instead. For continuous variables, a gamma distribution was used, and for marketability, a binomial distribution was chosen. Fruit shape score frequency distributions were analysed using cumulative link mixed models with a probit link function, as degree of misshapeness in strawberries is a latent continuous variable that was artificially separated into the four shape scores (Christensen 2015).

For all fruit quality measures apart from fruit yield, cage column, cage row, and the interaction between fruit growth position and pollination method were selected as fixed effects for the full model of the mixed- and single-species hoverfly pollination experiments. The optimal model was chosen by sequentially removing the least significant fixed effect from the full model and running the ‘drop1’ function on the reduced model to test the significance of the fixed effects (Ekstrom 2012). The optimal model was obtained once the reduced model contained only statistically significant fixed effect terms. The nested random effect for each model was growth position nested within cage, or when this term did not significantly influence the response variable, the random effect was simplified to cage. The significance of the random effect was tested by comparing the optimal model against an identical model that only contained fixed effects using the likelihood ratio test. To determine where the differences lay among levels of a fixed effect, least-square means were calculated with the ‘lsmeans’ function and Tukey-adjusted comparisons were made to reveal any significant differences among factor levels.

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

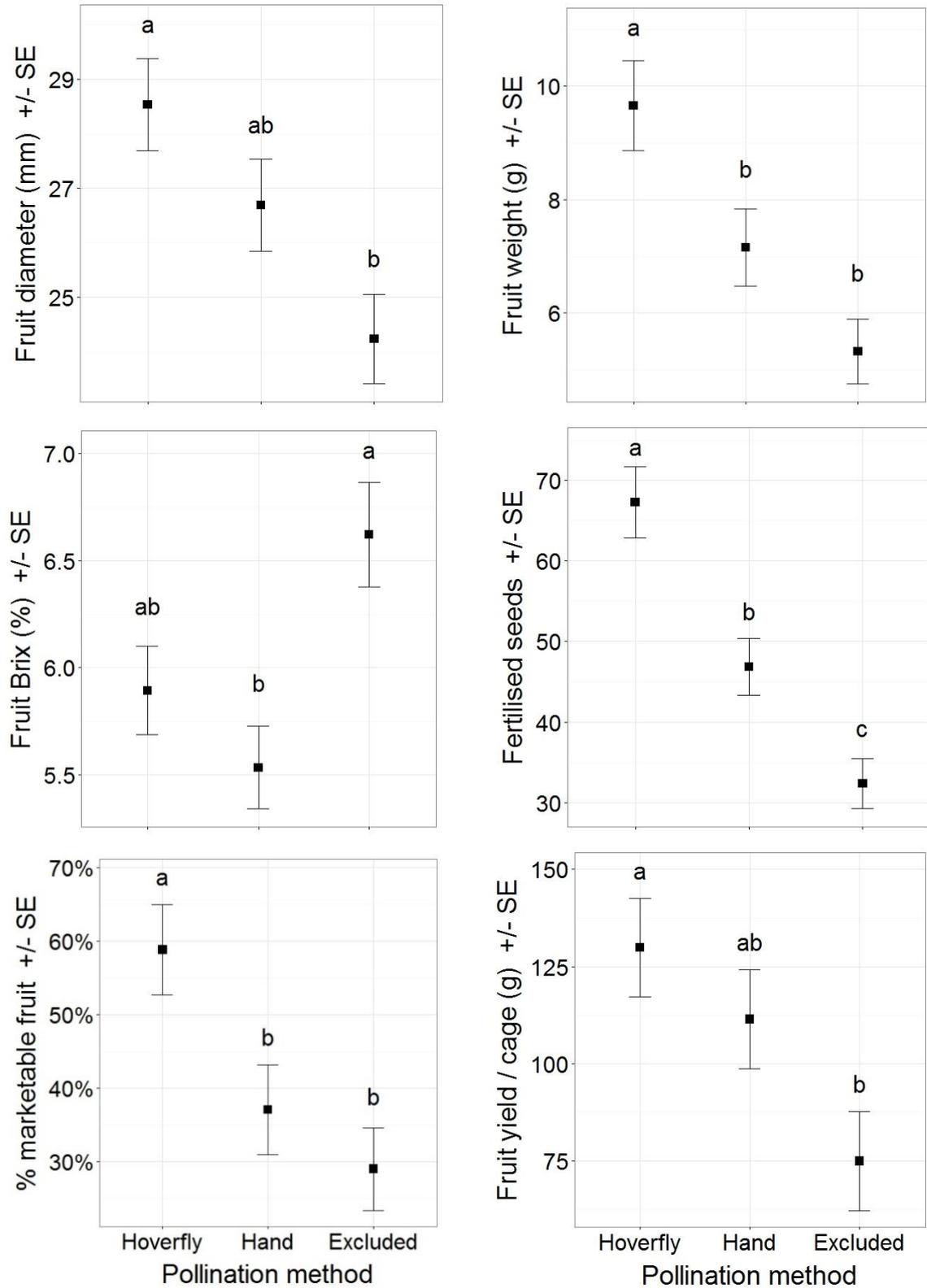


FIGURE 1. Mean fruit diameter, fruit weight, Brix, fertilised seeds per fruit half, proportion of marketable fruit and yield per cage by pollination method. Boxes indicate least square means \pm standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

Finally, for the hand pollination efficacy experiment, generalised linear models were used to account for the unbalanced number of fruit per treatment. Unlike in the

hoverfly pollination experiments, 'cage' was used as a blocking factor in the randomised block design of the hand pollination trial. Therefore, the fixed effects for this experiment were cage and pollination treatment. Response variables were

transformed where necessary, and a binomial distribution was used for fruit marketability. Fruit shape score frequency distributions were compared using cumulative link models with a probit link function. Model selection was again performed using the ‘drop1’ function.

RESULTS

Pollination effectiveness of a mix of hoverfly species

Pollination by the mixed group of hoverflies had significant positive impacts on a range of strawberry quality measures. Across 215 strawberries, fruit diameter varied according to pollination treatment ($\chi^2(2) = 12.67, P = 0.0018$) and growth position ($\chi^2(2) = 21.55, P < 0.001$). Hoverfly-pollinated fruit had the largest mean diameter (28.5 ± 0.84 mm), compared to hand-pollinated fruit (26.7 ± 0.84 mm) and pollinator-excluded (24.2 ± 0.82 mm; Fig. 1). Primary fruit diameter averaged at 29.4 ± 0.64 mm, compared to 26.5 ± 0.60 mm for secondary fruit and 23.6 ± 1.24 mm for tertiary fruit. The interaction between pollination treatment and growth position was not significant.

Pollination method also had a significant effect on fruit weight ($\chi^2(2) = 17.08, P < 0.001$). Hoverfly-pollinated fruit weighed 9.7 ± 0.79 g, compared to 7.2 ± 0.68 g for hand-pollinated fruit and 5.3 ± 0.57 g for pollinator-excluded fruit (Fig. 1). Growth position similarly influenced fruit weight ($\chi^2(2) = 21.11, P < 0.001$), with primary fruit averaging at 9.7 ± 0.60 g, compared to 7.1 ± 0.48 g for secondary fruit and 5.3 ± 0.85 g for tertiary fruit. Again, the interaction between the two variables was not significant.

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

The mean number of fertilised seeds per fruit half (215 berries) was 54.4 ± 2.37 seeds. Pollination method significantly influenced fertilised seed counts ($\chi^2(2) = 31.19, P < 0.001$). Hoverfly-pollinated fruit had the highest seed count (67.3 ± 4.43 seeds) followed by hand-pollinated (46.9 ± 3.56 seeds) and pollinator-excluded fruit (32.3 ± 3.09 seeds; Fig. 1). Cage row also affected the number of fertilised seeds ($\chi^2(5) = 17.82, P = 0.003$), which was lower as row number increased and ranged from 59.4 ± 4.72 to 37.4 ± 5.38 seeds.

A total of 215 strawberries were placed into one of four shape categories (ranging from 1-4). Pollination method was the only fixed effect to have a significant effect on the frequency distribution of shape scores ($\chi^2(2) = 14.60, P < 0.001$). Compared to the hand and insect-excluded

treatments, plants in the hoverfly-pollinated cages tended to produce the least-misshapen fruit (mean shape score = 2.38 ± 0.09), compared to hand-pollinated and pollinator-excluded fruit (mean shape score = 2.77 ± 0.10 and 3.07 ± 0.10 , respectively). Moreover, the frequency distribution of shape scores for hoverfly-pollinated fruit was significantly different to the frequency distributions of both hand-pollinated ($Z = 2.63, P = 0.02$) and pollinator-excluded fruit ($Z = -4.62, P < 0.001$). The shape score frequency distributions of hand-pollinated and pollinator-excluded fruit did not differ significantly from each other ($Z = -2.16, P = 0.08$; Fig. 2).

Overall $41.4\% \pm 0.034\%$ of 215 strawberries were deemed marketable. Plants in the hoverfly-pollinated cages tended to produce the highest proportion of marketable fruit at $58.8\% \pm 6.11\%$, compared to $37.1\% \pm 6.11\%$ for hand-pollinated and $29.0\% \pm 5.61\%$ for pollinator-excluded fruit ($\chi^2(2) = 10.48, P = 0.005$; Fig. 1).

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

The mean fruit firmness (64 strawberries) was 6.0 ± 0.20 Newtons (N) but varied among cage rows ($\chi^2(5) = 12.48, P = 0.029$), with means ranging from 5.3 ± 0.30 N (row 1) to

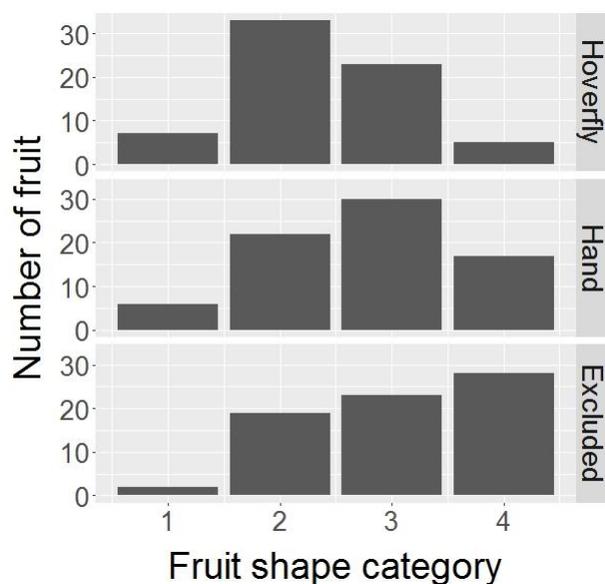


FIGURE 2. Fruit shape category frequency distributions by pollination treatment (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations). Fruit that fell into category 3 or 4 were deemed unmarketable.

6.6 ± 0.47 N (row 3). Pollination method had no effect on fruit firmness ($\chi^2(2) = 2.57$, $P = 0.28$). Lastly, none of the fixed effects affected percent dry matter. However, the random effect of cage significantly influenced fruit percent dry matter ($\chi^2(1) = 11.73$, $P < 0.001$).

Effect of varying brush pollination frequency on fruit quality

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

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

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

In contrast to the effects described above, the number of hand pollination events did not have a significant effect on fruit diameter (mean = 22.1 ± 0.43 mm, $N = 82$; $\chi^2(3) = 5.95$, $P = 0.11$), percent dry matter of strawberries (mean = $8.1\% \pm 0.23\%$, $N = 82$; $\chi^2(3) = 4.26$, $P = 0.24$), the frequency distribution of shape scores (mean shape score = 2.74 ± 0.11 , $N = 82$; $\chi^2(3) = 1.62$, $P = 0.7$) or the proportion of marketable fruit (mean = $46.3\% \pm 0.055\%$, $N = 82$; $\chi^2(3) = 3.07$, $P = 0.4$).

Effect of hoverfly species flower visits on fruit quality

When compared to pollinator-excluded controls, pollination by *Episyrphus balteatus* and *Eupeodes latifasciatus* significantly improved strawberry yields and fruit shape score distributions, but only visits from *Eupeodes latifasciatus* enhanced additional fruit quality measures. Pollination treatment significantly influenced fruit weight ($\chi^2(3) = 9.52$, $P = 0.023$). Hand-pollinated fruit were the heaviest (4.5 ± 0.21 g), followed by fruit pollinated by *Eupeodes latifasciatus* (4.4 ± 0.22 g), *Episyrphus balteatus*-pollinated fruit (4.2 ± 0.21 g) and finally insect-excluded fruit (3.6 ± 0.21 g; Fig. 4). Growth position also had a significant effect on fruit weight ($N = 1083$; $\chi^2(2) = 231.67$, $P < 0.001$), with primary fruit larger (5.6 ± 0.19 g) than secondary fruit (4.4 ± 0.13 g) and tertiary fruit (2.8 ± 0.12 g). The random effect of cage

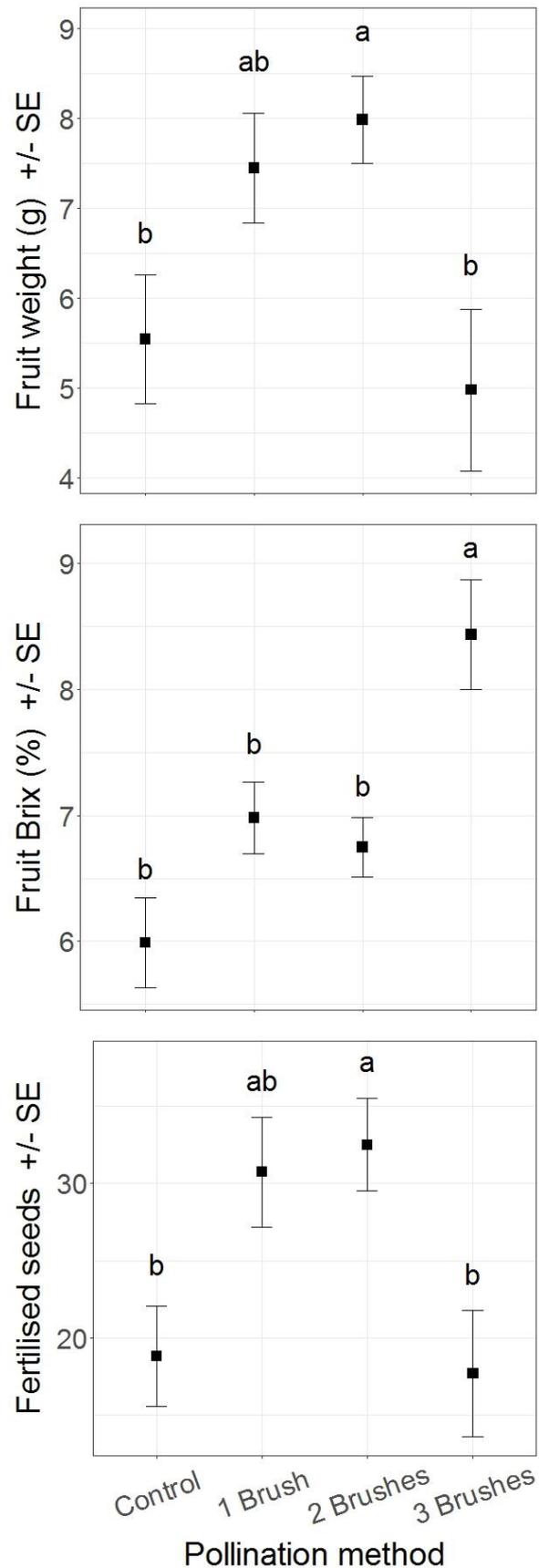


Figure 3. Mean fruit weight, Brix and number of fertilised seeds per fruit half by pollination method. Boxes indicate least square means \pm standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

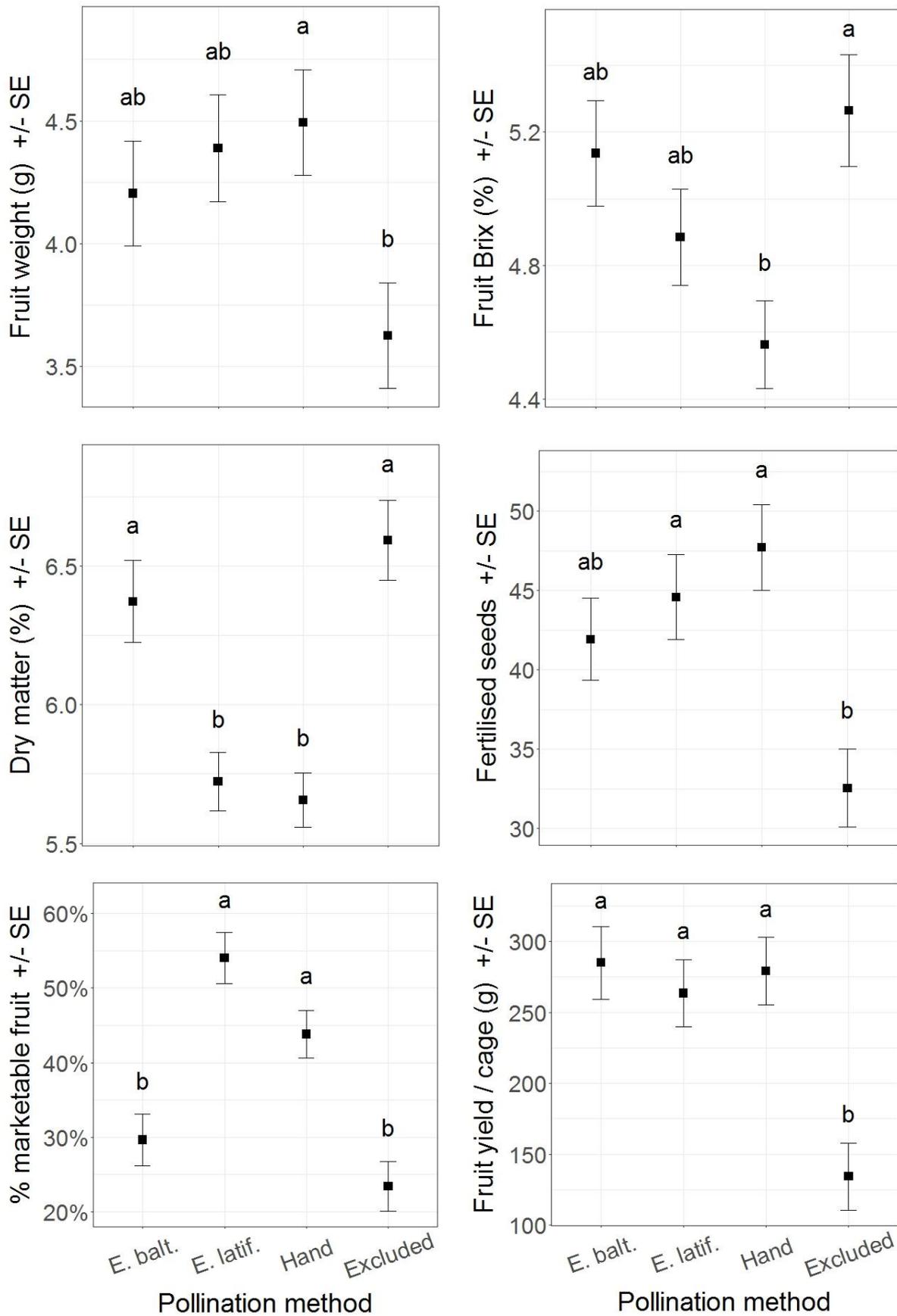


FIGURE 4. Mean fruit weight, Brix, percent dry weight, fertilised seeds per fruit half, proportion of marketable fruit and yield per cage by pollination method. “*E. balt.*” is an abbreviation of *Episyrphus balteatus*. “*E. latif.*” is an abbreviation of *Eupeodes latifasciatus*. Boxes indicate least square means \pm standard error. Means sharing the same letter are not significantly different (Tukey-adjusted comparisons).

also significantly influenced fruit weight ($\chi^2(1) = 6.95, P = 0.008$).

Pollination method also significantly affected Brix ($\chi^2(3) = 12.58, P = 0.006$). Pollinator-excluded fruit had higher Brix ($5.3\% \pm 0.17\%$), compared with fruit pollinated by *Episyrphus balteatus* ($5.1\% \pm 0.16\%$), *Eupeodes latifasciatus* ($4.9\% \pm 0.14\%$) or hand-pollinated fruit ($4.6\% \pm 0.13\%$; Fig. 4). Cage column affected fruit Brix ($N = 1076; \chi^2(2) = 7.70, P = 0.021$), with fruit from the central column of cages possessing the highest mean Brix ($5.2\% \pm 0.13\%$), followed by fruit from the column nearest the irrigation source ($4.9\% \pm 0.13\%$) and the column farthest from the irrigation source ($4.8\% \pm 0.13\%$). Primary fruit tended to have higher sugar concentrations ($5.3\% \pm 0.11\%$) than secondary ($5.0\% \pm 0.083\%$) or tertiary fruit ($4.7\% \pm 0.088\%$; $\chi^2(2) = 34.03, P < 0.001$). Lastly, the random effect of cage also significantly influenced fruit Brix ($\chi^2(1) = 9.52, P = 0.002$).

Pollinator-excluded fruit had the highest percent dry weight ($6.6\% \pm 0.15\%$), followed by fruit pollinated by *Episyrphus balteatus* ($6.4\% \pm 0.15\%$), *Eupeodes latifasciatus* ($5.7\% \pm 0.11\%$) and hand-pollinated fruit ($5.7\% \pm 0.10\%$; $\chi^2(3) = 16.68, P < 0.001$; Fig. 4). Fruit from the central column had the highest percent dry matter ($6.3\% \pm 0.097\%$), followed by the column nearest the irrigation source ($6.0\% \pm 0.091\%$) and the column farthest from the irrigation source ($5.9\% \pm 0.11\%$; $N = 1075; \chi^2(2) = 7.72, P = 0.021$). Analysis of the influence of cage row revealed that percent dry weight generally decreased as distance from the irrigation source increased ($\chi^2(6) = 16.72, P = 0.010$).

Hand-pollinated fruit had the highest mean seed count (47.7 ± 2.70 seeds) compared with fruit pollinated by *Eupeodes latifasciatus* (44.6 ± 2.68 seeds), *Episyrphus balteatus* (41.9 ± 2.58 seeds) and pollinator-excluded fruit (32.5 ± 2.45 seeds; $\chi^2(3) = 15.90, P = 0.0012$; Fig. 4). Primary fruit had greatest number of fertilised seeds (51.5 ± 2.38), followed by secondary (44.7 ± 1.91) and tertiary fruit (29.8 ± 1.68 ; $N = 1141; \chi^2(2) = 45.74, P < 0.001$). However, the random effect of growth position nested within cage also significantly influenced fertilised seeds counts ($\chi^2(2) = 21.66, P < 0.001$).

Strawberries pollinated by *Eupeodes latifasciatus* had the best mean shape score (2.43 ± 0.054), compared to hand-pollinated fruit (2.46 ± 0.046), fruit pollinated by *Episyrphus balteatus* (2.63 ± 0.048) and pollinator-excluded fruit (2.99 ± 0.057). Moreover, the frequency distribution of shape scores for *Eupeodes latifasciatus*-pollinated fruit significantly differed from that of *Episyrphus balteatus*-pollinated fruit ($Z = 3.42, P = 0.004$). In contrast, the shape score distribution for hand-pollinated fruit was not significantly different from either of the hoverfly-pollinated treatments (hand-*Episyrphus balteatus* comparison: $Z = 2.06, P = 0.17$; hand-*Eupeodes latifasciatus*: $Z = -1.75, P = 0.30$). However, the shape score distribution for pollinator-excluded fruit differed significantly from all other treatments (excluded-*Episyrphus balteatus*: $Z = -4.25, P < 0.001$; excluded-*Eupeodes latifasciatus*: $Z = -8.30, P < 0.001$; excluded-hand: $Z = -7.31, P < 0.001$; Fig. 5). Cage row significantly influenced shape score ($\chi^2(6) = 24.69, P <$

0.001), with mean scores ranging from $2.47 - 2.80$ across cage rows;. Primary fruit had the highest mean shape score (2.81 ± 0.060), followed by secondary (2.55 ± 0.037) and tertiary fruit (2.54 ± 0.045 ; $\chi^2(2) = 18.14, P < 0.001$).

Pollination method significantly affected the proportion of marketable fruit ($N = 1071; \chi^2(3) = 26.11, P < 0.001$). Plants in the *Eupeodes latifasciatus*-pollinated cages produced the highest proportion of marketable fruit ($54.0\% \pm 3.42\%$), compared to hand-pollinated ($43.8\% \pm 3.21\%$), *Episyrphus balteatus*-pollinated ($29.7\% \pm 3.44\%$) and pollinator-excluded fruit ($23.4\% \pm 3.31\%$; Fig. 4). Proportions of marketable fruit across cage rows varied from $19.1\% - 48.7\%$ ($\chi^2(6) = 20.65, P = 0.002$). Finally, secondary fruit possessed the highest proportion of marketable fruit ($45.4\% \pm 2.37\%$), followed by primary ($36.7\% \pm 3.28\%$) and tertiary fruit ($29.8\% \pm 2.67\%$; $\chi^2(2) = 20.29, P < 0.001$).

Pollination treatment also significantly influenced fruit yield per cage ($F_{3,10} = 9.26, P = 0.003$), with *Episyrphus balteatus*-pollinated cages producing the highest yields (285.0 ± 25.73 g), compared with hand-pollinated (279.1 ± 23.76 g), *Eupeodes latifasciatus*-pollinated (263.4 ± 23.53 g) and pollinator-excluded cages (134.2 ± 23.58 g; Fig. 4). In addition, cage row had a significant effect on the yield of strawberries per cage ($F_{6,10} = 6.83, P = 0.004$).

Finally, growth position was the only fixed factor to have a significant effect on fruit diameter ($N = 1082$ strawberries; $\chi^2(2) = 252.53, P < 0.001$). Primary fruit were larger (22.9 ± 0.26 mm) compared to secondary fruit (20.7 ± 0.20 mm) and tertiary fruit (17.9 ± 0.24 mm). Pollination method did not affect fruit diameter ($\chi^2(3) = 5.90, P = 0.12$). In addition to growth position, the random effect of cage also influenced fruit diameter ($\chi^2(1) = 6.52, P = 0.011$).

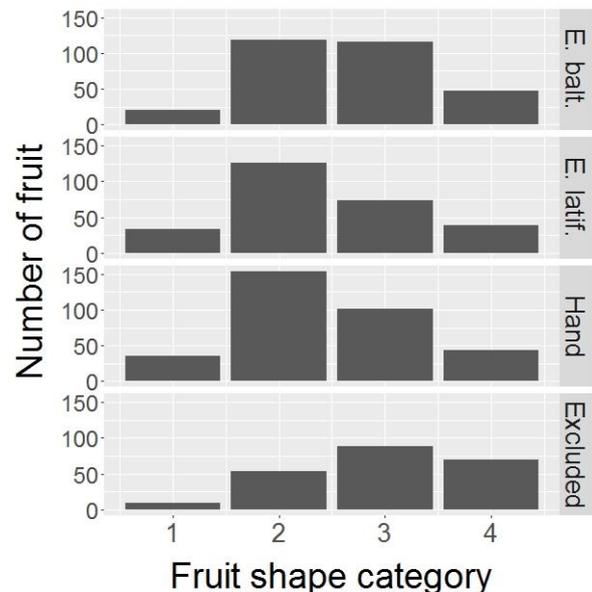


FIGURE 5. Fruit shape category frequency distributions by pollination treatment (1 = highly symmetrical fruit with no malformations; 2 = slightly asymmetrical fruit with minimal malformations; 3 = fruit with clear asymmetry and/or some malformations; 4 = fruit with major malformations). Fruit that fell into category 3 or 4 were deemed unmarketable. “E. balt.” is an

abbreviation of *Episyrphus balteatus*. "E. latif." is an abbreviation of *Eupeodes latifasciatus*.

DISCUSSION

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

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

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

Although intended to serve as a positive control, the hand pollination treatment in the mixed-species experiment did not produce more marketable fruit. For most fruit quality

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

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

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

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

When the pollination efficacy of single species of hoverfly is compared against the results from the mixed-species experiment, several similarities emerge in the effect that the insects have on fruit quality parameters. Most notably, fruit yields were significantly augmented by both mixed-species

assemblages of hoverflies and visits from only *Episyrphus balteatus* or *Eupeodes latifasciatus*. In the mixed-species experiment, fruit yields grew by 73.1% in hoverfly-pollinated cages when compared to controls, while the difference was even more pronounced in the single-species experiment. In that trial, introducing *Episyrphus balteatus* and *Eupeodes latifasciatus* to cages resulted in yield increases of 112.4% and 96.3%, respectively. The mean proportion of marketable fruit in mixed-species and in *Eupeodes latifasciatus* cages was over double that of pollinator-excluded cages in both experiments: mixed species of hoverflies increased proportions of marketable fruit by 102.8%, and *Eupeodes latifasciatus* enhanced rates of marketable fruit by 130.8%. By contrast, *Episyrphus balteatus* did not significantly improve fruit marketability when compared to the pollinator-excluded controls. In terms of pollination success rates, visitation from a mixed of hoverfly species led to a 108.4% increase in the number of fertilised seeds, while visits from *Eupeodes latifasciatus* improved pollination success rates by 37.2% over pollinator-excluded controls. Research by Klatt et al. (2014) documented a 61.7% rise in the number of fertilised achenes when bee-pollinated fruit were compared against self-pollinated controls using different strawberry cultivars; therefore, syrphine hoverflies may be as effective strawberry pollinators as bees.

Moreover, though *Eupeodes latifasciatus* outperformed mixed-species assemblages of hoverflies in enhancing yields and fruit marketability, visits from a group of hoverfly species resulted in a larger increase in numbers of fertilised achenes, when compared against fruit from control cages. Although these results seem to indicate slight differences in the pollination efficacy of *Eupeodes latifasciatus* as compared to a mixed group of hoverfly species, in order to uncover true differences, future research should compare single- and multiple-species assemblages in the same experiment.

The findings of this study provide the first evidence to suggest that hoverflies with aphidophagous larvae are effective pollinators of strawberry. Given that aphids are the primary prey of syrphine larvae (Rotheray & Gilbert 2011), these hoverflies may be capable of delivering both pollination and pest control ecosystem services for strawberry growers. Syrphine hoverflies have been shown to pollinate other crops, such as oilseed rape (Jauker & Wolters 2008; Jauker et al. 2012; Garratt et al. 2014) and apple (Garratt et al. 2016). Though these studies found that aphidophagous hoverflies were less effective pollinators than bees, syrphines may nonetheless supplement bee pollination and provide pest control services in these and other crops.

The main limitation of this study is that, as a cage trial, these results provide evidence that syrphines are capable of pollinating strawberry flowers; however, whether hoverflies pollinate strawberries effectively in the field remains to be demonstrated. Hoverflies may not visit strawberry flowers as frequently in the field and therefore their potential value as pollinators may not be as high as our findings imply (Albano et al. 2009b). Furthermore, although syrphine hoverflies are able to improve fruit quality and yields in cages, other flower-visiting taxa may prove to be even more effective pollinators of strawberry. Previous research has shown that honeybees,

bumblebees, halictid bees and cristaline hoverflies are also effective strawberry pollinators (Albano et al. 2009b; Gibson 2012). In order to assess the pollination efficacy of syrphines in relation to other taxa, one method that may prove useful is comparing the pollination success and fruit quality after a single visit from flower visitors (King et al. 2013). Such single visit deposition rates can then be coupled with flower visitation rates in the field to obtain a more complete picture of the pollination effectiveness of different species groups, as was done by Albano et al. (2009b) using honeybees, halictid bees and cristaline hoverflies as focal taxa.

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

ACKNOWLEDGEMENTS

This research was funded jointly by the East Malling Trust and Royal Holloway University of London. We would like to thank Dilly Rogers for providing glasshouse space and the polytunnel used in these experiments; Roger Payne for his assistance with the installation of the irrigation system; Alvaro Delgado for helping transfer plants between glasshouses and cages; and Phil Brain and Mark Jitlal for their advice on the statistical analyses.

APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. List of hoverfly species used in mixed-species experiment

REFERENCES

- Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2008) Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Current Biology* 18:1572–1575.
- Albano S, Salvado E, Duarte S, Mexia A, Borges PAV (2009a) Floral visitors, their frequency, activity rate and Index of Visitation Rate in the strawberry fields of Ribatejo, Portugal: selection of potential pollinators. Part 1. *Advances in Horticultural Science* 23:238–245.
- Albano S, Salvado E, Duarte S, Mexia A, Borges PAV (2009b) Pollination effectiveness of different strawberry floral visitors in Ribatejo, Portugal: selection of potential pollinators. Part 2. *Advances in Horticultural Science* 23:246–253.

- Albrecht M, Schmid B, Hautier Y, Müller CB (2012) Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society B: Biological Sciences* 279:4845-4852.
- Ball S, Morris R (2015) *Britain's Hoverflies: A Field Guide*, 2nd edn. Princeton UP, Princeton.
- Blitzer EJ, Gibbs J, Park MG, Danforth BN (2016) Pollination services for apple are dependent on diverse wild bee communities. *Agriculture, Ecosystems & Environment* 221:1-7.
- Carew JG, Morretini M, Battey NH (2003) Misshapen fruits in strawberry. *Small Fruits Review* 2:37-50.
- Chagnon M, Ingras J, De Oliveira D (1993) Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). *Journal of Economic Entomology* 86:416-420.
- Christensen RHB (2015) Analysis of ordinal data with cumulative link models - estimation with the R-package 'ordinal'. [online] URL: https://cran.r-project.org/web/packages/ordinal/vignettes/clm_intro.pdf (accessed: September 2017).
- Conti S, Villari G, Faugno S, Melchionna G, Somma S, Caruso G (2014) Effects of organic vs. conventional farming system on yield and quality of strawberry grown as an annual or biennial crop in southern Italy. *Scientia Horticulturae* 180:63-71.
- Csakasi F, Osorio S, Gutierrez JR, Kitamura J, Gialisco P, Nakajima M, Fernie AR, Rathjen JP, Botella MA, Valpuesta V (2011) Gibberellin biosynthesis and signalling during development of the strawberry receptacle. *New Phytologist* 191:376-390.
- Darrow GM (1929) Inflorescence types of strawberry varieties. *American Journal of Botany* 16:571-585.
- Defra (2015) *Horticulture Statistics - 2014*. Department for Environment, Food & Rural Affairs, London.
- Ekstrom CT (2012) *The R Primer*. CRC Press, Boca Raton.
- Ellis JD, Evans JD, Pettis J (2010) Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *Journal of Apicultural Research* 49:134-136.
- FAO (2017) *FAOSTAT crops statistics*. [online] URL: <http://www.fao.org/faostat/en/#data/QC> (accessed: September 2017).
- Free JB (1993) Chapter 56: Rosaceae: *Fragaria*. In: *Insect Pollination of Crops*. Academic Press, London, pp 425-430.
- Gallai N, Salles J-M, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68:810-821.
- Garibaldi LA, Carvalheiro LG, Leonhardt SD, Aizen MA, Blaauw BR, Isaacs R, Kuhlmann M, Kleijn D, Klein AM, Kremen C, Morandin L, Schepers J, Winfree R (2014) From research to action: enhancing crop yield through wild pollinators. *Frontiers in Ecology and the Environment* 12:439-447.
- Garratt MPD, Breeze TD, Boreux V, Fountain MT, Mckerchar M, Webber SM, Coston DJ, Jenner N, Dean R, Westbury DB (2016) Apple pollination: demand depends on variety and supply depends on pollinator identity. *PloS One* 11:e0153889.
- Garratt MPD, Coston DJ, Truslove CL, Lappage MG, Polce C, Dean R, Biesmeijer JC, Potts SG (2014) The identity of crop pollinators helps target conservation for improved ecosystem services. *Biological Conservation* 169:128-135.
- Gibson RH (2012) *Pollination Networks and Services in Agroecosystems*. Biological Sciences, University of Bristol.
- Gómez JM, Bosch J, Perfectti F, Fernández J, Abdelaziz M (2007) Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* 153:597-605.
- Hoehn P, Tschamtké T, Tylianakis JM, Steffan-Dewenter I (2008) Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B: Biological Sciences* 275:2283-2291.
- Jauker F, Bondarenko B, Becker HC, Steffan-Dewenter I (2012) Pollination efficiency of wild bees and hoverflies provided to oilseed rape. *Agricultural and Forest Entomology* 14:81-87.
- Jauker F, Wolters V (2008) Hover flies are efficient pollinators of oilseed rape. *Oecologia* 156:819-823.
- King C, Ballantyne G, Willmer PG (2013) Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4:811-818.
- Klatt BK (2013) *Bee Pollination of Strawberries on Different Spatial Scales—from Crop Varieties and Fields to Landscapes*. Niedersächsische Staats- und Universitätsbibliothek Göttingen.
- Klatt BK, Holzschuh A, Westphal C, Clough Y, Smit I, Pawelzik E, Tschamtké T (2014) Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences* 281:20132440.
- Klein A-M, Steffan-Dewenter I, Tschamtké T (2003) Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:955-961.
- Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tschamtké T (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274:303-313.
- Mallinger RE, Gratton C (2015) Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology* 52:323-330.
- Martins KT, Gonzalez A, Lechowicz MJ (2015) Pollination services are mediated by bee functional diversity and landscape context. *Agriculture, Ecosystems & Environment* 200:12-20.
- Nye WP, Anderson J (1974) Insect pollinators frequenting strawberry blossoms and the effect of honey bees on yield and fruit quality. *Journal of the American Society for Horticultural Science* 99:40-44.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25:345-353.
- R Core Team (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org>.
- Rotheray GE, Gilbert F (2011) *The Natural History of Hoverflies*. Forrest Text, Cardigan, UK.
- Smith P, Ashmore M, Black H, Burgess P, Evans C, Hails R, Potts SG, Quine T, Thomson A (2011) Chapter 14: Regulating Services. In: *UK National Ecosystem Assessment*. UNEP-WCMC, Cambridge, pp 535-596.
- Ssymank A (2009) Flower Flies (Syrphidae). In: Ssymank A, Hamm A, Vischer-Leopold M (eds) *Caring for Pollinators: Safeguarding Agro-biodiversity and Wild Plant Diversity*. BfN, Skripten, pp 159-162.
- Ssymank A, Kearns CA (2009) Flies – Pollinators On Two Wings. In: Ssymank A, Hamm A, Vischer-Leopold M (eds) *Caring for Pollinators: Safeguarding Agro-biodiversity and Wild Plant Diversity*. BfN, Skripten, pp 39-52.
- Ssymank A, Kearns CA, Pape T, Thompson FC (2008) Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity* 9:86-89.

Williams IH (1994) The dependence of crop production within the European Union on pollination by honey bees. *Agricultural Zoology Reviews* 6:229-257.



This work is licensed under a [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/).