Article

Buzzing benefits: How multi-species pollination boosts strawberry yield, quality, and nutritional value

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Abstract—A diverse assemblage of insect visitors can provide functional complementarity within plant pollination due to differences in characteristics such as their physical traits, visitation rate and foraging time of day or year. In a horticultural context, greater functional complementarity may play a crucial role in enhancing fruit yield and quality by improving pollination. We tested whether the identity of the crop pollinators (bumblebee *Bombus terrestris* and hoverfly *Eupeodes corollae)* independently and additively influenced commercial strawberry yield, quality, and nutritional parameters such as vitamin C and sugar concentration. *Fragaria* x *ananassa* "Malling Champion" plants received pollination treatments of either a) "control": self-pollination where pollinators were excluded, b) "bee": bumblebee *Bombus terrestris*, c) "hoverfly": *Eupeodes corollae*, d) "combined": both *B. terrestris* and *E. corollae*. Hoverflies and bumblebees exhibited distinct visitation patterns throughout the day, establishing a functional complementary relationship that enhances pollination success and crop output as well as vitamin C concentrations. Strawberries from plants receiving pollination by bumblebees, or bumblebees and hoverflies combined, had higher yields of higher marketable quality. They also had measurably higher vitamin C content than strawberries from plants pollinated by hoverflies alone, or the control (self-pollinating) plants. This study advances our understanding of niche complementarity and its impact on fruit yield and quality. By elucidating the behavioural and temporal dynamics of pollinators, we provide valuable insights for optimizing pollination strategies in agricultural contexts. Our findings highlight the significance of behavioural factors, such as handling time and number of visits, in determining fruit quality.

Keywords—Niche complementarity, Hoverflies, Bumblebees, Fruit production, Vitamin C

Introduction

It has been widely accepted within current research that a higher pollinator species richness and diversity of pollinators provides a higher efficiency of pollination due to the complementarity between species (Fontaine et al. 2005; Balvanera et al. 2006; Bartomeus et al. 2013; Fründ et al. 2013; Herrmann et al. 2019). Previously, many studies have linked pollinator diversity and the functional differences between species to increased crop yields and quality (Bommarco et al. 2012; Brittain et al. 2013; Garibaldi et al. 2016; Hodgkiss et al. 2018). For example, even when strawberry crops were predominantly pollinated by just two species of bee, honeybees (*Apis mellifera*) and red mason bees (*Osmia bicornis*), fruits were redder in colour and had higher mean berry mass when compared to self-pollination controls (Klatt et al. 2014). However, the mechanisms which best promote crop pollination are still unknown.

Strawberry (*Fragaria* x *ananassa*) has also been shown to benefit from pollination from both hoverflies (*Eupeodes corollae* and *Episyrphus balteatus*) and bees (*Bombus terrestris* and *Bombus impatiens*) (Hodgkiss et al. 2018; Herrmann et al.

2019). Both taxa are used commercially in Europe, primarily *Bombus terrestris* colonies deployed in the crop (Martin et al. 2019), but sometimes also hoverflies (Van Oystaeyen et al. 2022), and there may be scope to combine these for improved results. Other studies have revealed that pollination by a higher diversity of wild bees led to greater yields compared to honeybees, despite no noticeable difference in pollen loads (MacInnes et al. 2019), indicating that the functional mechanisms within pollination warrant investigation to establish nutritional and quality assessments.

Over the past decade, several studies have provided evidence that pollinators have a beneficial impact on nutrition security through increasing the availability of macro- and micronutrients (Chaplin-Kramer et al. 2011; Klatt et al. 2014; Samnegård et al. 2019). This highlights the importance of investigating modes of pollination on nutrient availability, such as differences between pollinators like bees and hoverflies compared to self-pollination.

Pollinator-dependent crops are the primary source of many micronutrients that humans are unable to synthesize themselves. Thus, these micronutrients, including also vitamins, can only be obtained by consumption and are essential for human nutrition. Vitamin C in particular exhibits many biological activities including reducing carcinogenesis, reducing the risks of cardiovascular diseases, and stimulating the immune system in the body (Yahia et al. 2019).

Strawberries contain a high concentration of vitamin C (Woodward, 1972), which consists of ascorbic acid (AA), which is the reduced form of vitamin C, and its oxidised form dehydroascorbic acid (DHA). In plants, AA is also involved in various plant processes such as cell division, expansion, hormone signalling and acquiring reactive oxygen, thus protecting DNA, protein, and lipids from oxidative damage during development or abiotic stress (Davey et al. 2000; Fenech et al. 2019). AA is a potent antioxidant, neutralizing reactive oxygen species (ROS) and free radicals, thus protecting plant cells from oxidative stress (Akram et al. 2017). DHA can be taken up by cells through different mechanisms, often facilitated by glucose transporters due to its structural similarity to glucose (Fenech et al. 2019). DHA itself is not an antioxidant, but it can be readily reduced back to AA within the cell, thus continuing the antioxidant cycle.

The quantity of AA available in strawberries has been previously linked to the plant's growing conditions (Lee et al. 2000), but how pollination affects the content of ascorbic acid in commercial strawberries remains unknown. We conducted trials in polytunnels and cages in a glasshouse, with fruit grading and HPLC analysis to investigate how pollinator species influence fruit quality, sugar ratios, and vitamin C concentration in commercial strawberry crops. The aim was to evaluate whether pollinator species impacted fruit nutrition, and therefore could be considered more efficient pollinators in these environments, and whether combining species provided economic and nutritional benefits. We characterised total ascorbic acid (TAA) as the sum of ascorbic acid (AA) and its oxidised form dehydroascorbic acid (DHA). Both forms are essential in protecting plants against various environmental stressors such as drought, salinity, and pathogen attack (Akram et al. 2017). The impact of pollination modality on AA, DHA, and their ratio, as well as the concentration of glycosides, may have an important bearing on fruit quality attributes as well as plant defence and resistance. The ability to convert DHA back to AA helps the fruit mitigate oxidative stress, maintaining cellular health, and prolonging shelf life (Dewhirst et al. 2018). Here, we examined the behaviour of hoverflies and bumblebees on strawberry flowers in terms of visitation and diurnal activity and investigated how single-species and combined-species pollination influenced fruit yield parameters, quality, firmness, colour, vitamin C (TAA) and its two components (AA and DHA) and sugar content (glycosides).

MATERIALS AND METHODS

Two repeated three-month experiments were conducted over two years. The first was conducted in polytunnels and recorded species visitation behaviour on commercial strawberry *Fragaria* x *ananassa* ("Malling Champion"). Yield and quality were assessed in different pollinator treatments of single species of hoverfly (*Eupeodes corollae*) and bumblebee (*Bombus terrestris*) and a combination of both, compared to each other and a self-pollinating control treatment where pollinators were excluded. For the second experiment, the same treatments were applied in cage/greenhouse trials and fruit quality, yield, sugar, and vitamin C concentrations were determined in strawberries produced.

LOCATION AND ENVIRONMENTAL CONDITIONS

Experiments in year 1 were undertaken as semi-field trials which were conducted from May-August 2021 in polytunnels. Temperature and humidity were recorded using dataloggers (Lascar EL-USB-2LCD) located at the centre of each compartment. Campark cameras (Campark Wildlife, 32MP 1296P IP66) were located at each end of the polytunnels except for the control and the number of visits, time of day, species, and handling time were determined from the recordings. We recorded time of day to determine if pollinators changed their visitation rate, handling times, or behavioural pattern in accordance with the time of day or changes in temperature and humidity, and what effect this may have on pollination success.

Experiments in year 2 were undertaken as small-scale greenhouse trials, conducted from May-August 2022. Inside the greenhouse, four treatment BugDorms™ (90 cm x 90 cm x 60 cm) contained the same pollinator species as in year 1 (bees, hoverflies, control, and bees and hoverflies combined). Additionally, day/night lengths were monitored, and temperature and humidity were recorded using dataloggers (Lascar EL-USB-2LCD) located at the centre of each compartment/dorm to observe if the behaviour of each species varied diurnally. Campark cameras (Campark Wildlife, 32MP 1296P IP66) were located in each of the Bugdorms except in the control. The position of each treatment dorm was alternated randomly between each of the eight replicates, with each allowing pollinators to forage for 1 week and concluding when resulting fruit was at least 75% red (Hodgkiss et al. 2018).

POLYTUNNEL TRIALS

Two polytunnels (12 m x 1.5m x 2 m) were used, located at Niab, East Malling, Kent, UK ME19 6BJ (51.286034° N, 0.449165 ° E, elevation: 35 m). Each tunnel was divided using 1.35mm nylon woven mesh (Veggiemesh®) to prevent pollinators from accessing other compartments, resulting in four experimental compartments (bees, hoverflies, bee and hoverfly and a control with no pollinators). To achieve two replicates in each compartment, half of the plants were covered with exclusion netting for the first four weeks, following which they were then uncovered for a new replicate, while the previously tested plants were covered instead.

The number of individual pollinators in each test compartment was kept consistent throughout the trial period (Table 1), to reflect population densities used in commercial fruit production to avoid over-pollination or damage to flowers (such as blackened stigmas). Based on previously reported trials where 5-8 colonies of *B. terrestris* (Biobest®) containing 50-80 individuals per colony were deployed per hectare (Hall et al. 2019; Martin et al. 2019), the number of individuals released were scaled down accordingly to reflect the size of the polytunnels used. Where pollinators were combined, the numbers of individuals of each species were scaled down, so the same overall number of individual insects in each treatment were used. New individual insects were exchanged from a host bee colony/ host hoverfly pot into each polytunnel and within treatments to prevent pseudoreplication. Campark cameras placed at each end of the polytunnel compartments recorded visits between 08:00 and 18:00 daily for 2 months.

Table 1. Release and sample replacement schedule for semi-field trial treatment compartments

Treatment	Number of individuals	Individuals changed	Pot/ hive changed
Bee	6	Weekly	Every 3 weeks
Hoverfly	12 (6 ♀ + 6 ♂)	Weekly	Every 2 weeks
Bee + Hoverfly	3 bees, 6 hoverflies (3♀ + 3 ♂)	Weekly	Every 3 weeks, Every 2 weeks

Yield and Quality Assessments

For each pollinator treatment, each individual strawberry was weighed on a digital scale (Fisherbrand™ Precision Balance) within 60 minutes of picking and recorded to the nearest ±0.1 g. Fruits were graded (with the treatment group masked from assessor) using the criteria classification acquired from the EU Marketing Standards for Fresh Horticultural Practice: Marketing Standards for Commercial Strawberries (Department for Environment, Food & Rural Affairs, 2011). When comparing the fresh weight of samples and their marketable grading, any fruit with a grading of 3 or 4 (unmarketable) were removed from our data to compare the marketable weight between treatments.

Measurements for height and width were taken using digital callipers to ±0.1 mm. Each measurement was repeated three times for each strawberry to calculate an average. Height was taken from the base of sepals to the distal tip of the strawberry, and width was taken at the widest point of the strawberry. These measurements were used as a secondary form of assessment, as these values represent the evenness in shape and size and therefore reflect the class grading, density, volume, and marketability.

A TA. XT plus texture analyser (Stable Micro Systems, UK) was used to assess the firmness of fruit picked using a 5 mm diameter probe which measures the resistance force required to pierce the fruit in grams. Assessments used the following parameters: pre-test speed 5.0 mm/s, test speed 1.0 mm/s and post-test speed 8.0 mm/s; to a penetration a depth of 6 mm. Due to the number of strawberries needed for ascorbic acid analysis, we were unable to conduct this analysis with the greenhouse trials.

A handheld colourimeter (Minolta CR-400 Chronometer; Konica Minolta Sensing, Osaka, Japan) was used to take colour assessments of individual strawberries which measure the hue, darkness-lightness, and colour absorbance of each strawberry. L*(Lightness) a*(red/green coordinate) b*(yellow to blue coordinate) hue angle is calculated from a* and $b^* = \tan^{-1}(b^*/a^*)$ (See supplementary Table S1 for values). Software (Spectra Magic, CM-S100w-NX Pro) was used to calibrate, analyse, and assess values given during assessments. Three repeated measurements per strawberry were undertaken by rotating to three separate areas beneath the calyx to give an average value for L*, a* and b* for each fruit which were averaged and provide a mean score per fruit. Fruits were only assessed if undamaged and at least 75% red (Hodgekiss et al. 2018)

Greenhouse trials

Small-scale greenhouse trials were conducted from May-August 2022, located at the University of Greenwich, Gillingham, Kent, UK ME4 4TB (51.39743° N, 0.54155° E, elevation: 13 m). Trials were conducted using 256 plants of strawberry "Malling Champion" in four treatment cages, with eight complete replicates (see supplementary Table S2). One coir grow bag of 8 plants at a time was placed in one of four 90 x 60 x 90 cm BugDorm™ cages (purchased from Watkins and Doncaster, UK) in a shared greenhouse compartment. Each replicate had its own grow bag, and none were reused. Each cage contained one Botanicoir Precision Plus-Ultra© growbag with eight individual strawberry plants, receiving 50 ml water supply via 2 drip feeders from a fertigation line, active for 15 minutes twice daily at 08:00 and 15:00 throughout trials, and an additional 15 minutes at 12 pm in peak summer temperatures (over 28°C). All strawberries arrived as root stock and were planted at the same time for each replicate, receiving the same watering schedule. With each replicate, each Bugdorm and associated treatment moved randomly on the benches throughout a total of eight replicates to avoid positional bias.

Both, bumblebees (*Bombus terrestris audax*) and hoverflies (*Eupeodes corollae*), were obtained from Biobest Ltd. (de Weerts, Netherlands) supplied via Agralan Ltd. (Wiltshire, UK). Bumblebees were kept in the colony until required in NRI (Natural Resources Institute) insectary laboratories in Kent and kept at a 12 hr:12 hr light/dark cycle, between 21-25 °C, and a humidity between 55-65 %. Worker bumblebees in good condition and of average size were removed individually into plastic (33.5 x 21 x 14 cm) microcolony boxes and kept for 24 hours inside the laboratory, then transferred to the compartment to acclimate before the box was placed in the cage and the entrance opened. Hoverflies were supplied as pupae and eclosed in a Bugdorm (40 x 40 x 60 cm) in a non-climate-controlled room, before individual adults were sexed and transferred to the treatment cages in the greenhouse.

ASCORBIC ACID (AA) SAMPLE PREPARATION

The methodology was adapted from Van De Velde et al. (2012), who provided optimised design and extraction techniques for vitamin C determination in strawberries. Fruit were weighed and graded, then freeze dried, homogenised, and stored at -80°C. Overall, each replicate used 5 g of the total dried samples from each treatment for a total of eight replicates throughout the trial; the results from each replicate per treatment were analysed separately and used to calculate an average. After samples had been freeze-dried and homogenized into a powder form, 2.5 g was weighed out and added to 27.5 ml of the extraction solution (metaphosphoric acid 30 g L^{-1}, acetic acid 80 g L^{-1}, and 0.1 g ascorbic acid powder). The mixture was vortexed for 1 minute, then sonicated in an ultrasonic bath for 15 minutes, then centrifuged at 3500 rpm for 20 minutes at 4°C. A total of 2 ml of the supernatant was removed and diluted with mobile phase (0.03 M sodium acetate/acetic acid buffer, 5% methanol) to achieve a final volume of 6 ml. The mixture was centrifuged again at 3000 rpm for a further 5 minutes and filtered through a 0.45 μm Millipore membrane syringe and the content of ascorbic acid (AA) was quantified by HPLC-UV.

TOTAL ASCORBIC ACID (TAA) AND DEHYDROASCORBIC ACID (DHA) SAMPLE PREPARATION

The method progressed as described for AA, until after the first centrifugation. 2 ml of supernatant was then added to 0.2 ml of DTT solution (5 mg L^{-1} dithiothreitol (DTT) prepared in 2.58 M potassium phosphate dibasic) which was kept in the dark at room temperature for 2 hours. This solution was then made up to the volume of 6 ml with mobile phase, centrifuged for a further 5 minutes at 3000 rpm, the filtered through a 0.45 μm Millipore membrane syringe before being quantified by HPLC-UV.

HPLC AA AND TAA QUANTIFICATION

Samples' AA and TAA content was determined using an Agilent HPLC system (Agilent Technologies, Santa Clara, United States) consisting of a 1260 series quaternary pump, 1260 series autosampler, 1200 series column oven and 1200 series photodiode detector. Samples were injected as 10 μl aliquots on to a Phenomenex Gemini C18 column (250 mm × 4.6 mm i.d., 5 μm particle size) with a guard column and separated with a flow rate of 0.5 ml/min, and a mobile phase consisting of 95% sodium acetate (0.03 M) buffer and 5% methanol, adjusted to pH = 5.8 with acetic acid. Samples were quantified by UV at a wavelength of 251 nm (±4 nm) and calibrated with standard solutions of ascorbic acid (0.5, 0.4, 0.3, 0.2 and 0.1 mg mL^{-1}). This analysis provided concentrations for both AA and TAA; DHA content was then calculated as the difference between these two values.

SUGAR EXTRACTION

A subsample of 500 g of fresh strawberries were selected at random from each replicate. Within 2 hours of picking, samples were then freeze dried for 48 hours and homogenized into a fine powder, and dried samples were stored at -8 °C until analysed. 0.2 g of dried sample was added to 2 ml Eppendorf tubes and made up to a volume of 1.6 ml with 80% ethanol. Each sample was then mixed using a vortex mixer and placed into a shaking water bath for 2 hours at 70 °C, before being centrifuged at 3000 rpm for 10 minutes at 4 °C and then filtered through a 0.45 μm syringe filter before HPLC analysis (Lee et al. 2018).

HPLC GLYCOSIDE QUANTIFICATION

The concentration of glycosides was determined using Agilent HPLC system (Agilent Technologies, Santa Clara, United States) consisting of a 1200 series quaternary pump, 1200 series autosampler, 1200 series column oven and 1200 series refractive index detector (RID). Samples were stored at 5 °C, injected as 5 μl aliquots on a Phenomenex Luna Sugar column (150 mm × 4.6 mm i.d., 3 μm particle size) at 35 °C with a guard column and separated with a flow rate of 1 ml/min, and a mobile phase consisting of 75 % Acetonitrile 25 % H_2O. Samples were quantified by RI response and calibrated with standard glycoside solutions of D-(+)-fructose, D-(+)-sucrose, and D-(+)-glucose in water at 50, 100, 500, 1000, and 5000 ppm.

STATISTICAL ANALYSIS

Treatment groups were used as independent factor variables in the subsequent analysis: each dependent variable (fruit weight, fruit width, fruit height, total ascorbic acid, sucrose, glucose, and fructose composition) and each independent variable (treatment, temperature, time of day, and species) analysed separately using a one-way ANOVA in SPSS version 26. Strawberry fruit size data were input as individual fruits, with replicate as a mixed effect model; sugars and total ascorbic acid data required pooling of several fruits to provide material for the analysis, meaning that each data point was a representative value for several fruits within the same treatment and replicate where average values per batch and treatment were used in final analysis. All data were checked visually for normality. A one-way ANOVA was used to compare the DHA (mg mL^{-1}) in all treatment replicates. DHA concentration was calculated as the difference in concentration between the TAA and AA readings and presented in concentrations (Fig. 5A) and as a percentage of TAA (Fig. 5B).

Means for fruit measurements were compared using a post-hoc Tukey test based on a linear mixed effect model (R, lme4 (Bates et al. 2015)) with treatment as a fixed effect and batch as a random component. Analysis of the number of visits for both species used a negative binomial generalised linear model with a log link (R, MASS package) for a three-way analysis of variance, with species (bee or hoverfly), treatment (combined or single species) and time of day (5 2-hour time bins spanning the observation period) included in the model. Means and standard errors are model predictions.

RESULTS

POLYTUNNEL TRIAL: POLLINATOR BEHAVIOUR

Observations for the polytunnel treatments were recorded from 24/06/2021 to 08/08/2021 with a total of 2054 bee visits and 1831 hoverfly visits recorded within the combined treatment alone. The hoverfly treatment had a total of 1464 total visits, and the bee treatment having a total of 2142 visits during the trials. There was a significant difference between the number of visits between treatments (ANCOVA, F_3 =14.68, P = 0.0001), with bees having a higher number of visits in both the combined and singular treatments compared to hoverflies. There was also a difference between time of day and the number of visits by each treatment (P = 0.044) and species (P = 0.006) where hoverflies had a higher number of visits in the combined treatment compared to the singular hoverfly treatment, with the highest proportion of visits between 16:00 – 18:00 (Fig. 1). Analysis, via a negative binomial GLM, showed that the pattern of the bee visits throughout the day was significantly different to the diurnal distribution of hoverfly visits (Fig. 1) (chi-square = 12.8, P = 0.00033).

FRUIT QUALITY AND YIELD

A total of 2071 strawberry fruits were used for yield and quality assessments for all treatment groups. Fruits from the combined treatment led to significantly larger fruits (Fig. 2), with strawberries having a greater width, weight, height, and grading score out of all four treatments in polytunnel trials, but not in the greenhouse trials. There was a significant difference in weight between treatments (ANOVA, F_3 = 16.75, P = 0.001), with fruits produced in the combined treatment significantly heavier than all other treatments (Fig. 2) (Tukey's P = 0.0001). There was no difference between the bee and hoverfly treatment (Tukey's, P = 0.121) and the control plants produced significantly heavier fruits than the hoverfly-pollinated plants (Tukey's, P = 0.022).

Weight differed significantly between the first and second replicate (One way ANOVA, F = 84.78, P = 0.0001), with the second replicate producing overall heavier fruits. This was to be expected as the second replicate was grown in the warmer summer conditions.

Fruits from the hoverfly treatment had on average a significantly smaller width and height compared to all other treatments (Tukey's P = 0.001). Fruits from the combined treatment were on average significantly longer (ANOVA, F_3 = 54.25, P = 0.0001) (Fig. 2) and wider (ANOVA, F_3 = 34.37, P = 0.0001).

TEXTURE

A total of 264 individual strawberries were analysed per treatment to obtain firmness values (Fig. 3). Overall, firmness differed between treatment groups (ANOVA, F_3 = 6.89, P = 0.0001). Fruits from the bee treatment showed the highest average firmness of all treatments and were significantly firmer than those from the combined treatment and control. No significant difference of

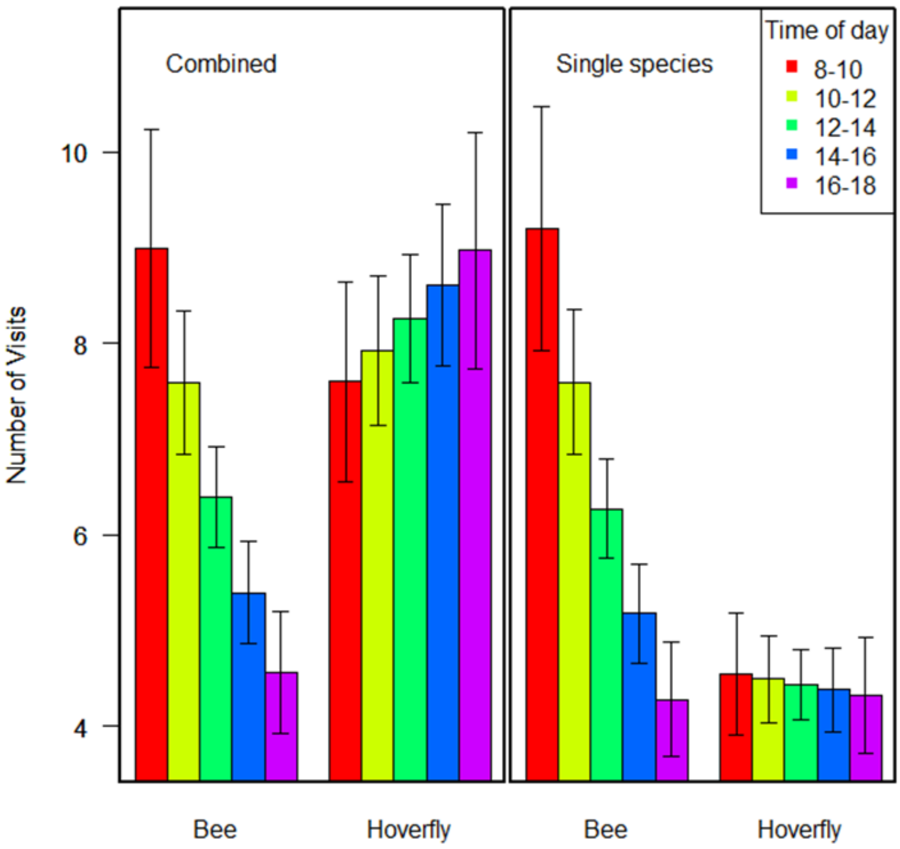

Figure 1. Average number of total visits by each [Bees(B), and Hoverflies (H)] individual in the combined treatment (left) and single species treatment (right) throughout the day (each column refers to a two-hour timebin (2 hours calculated from visitation between 08:00-09:59, 10:00-11:59, 12:00-13:59, 14:00-15:59, 16:00-17:59

firmness was observed between the hoverfly and bee treatment (Tukey's post hoc test $P = 0.703$).

COLOUR

A total of 556 strawberries per treatment were used to analyse colour space. We observed a significant difference in L* (ANOVA, $F_3 = 14.69$, $P = <0.0001$) and a* (ANOVA, $F_3 = 21.29$, $P = <0.0001$) between treatments, with fruits from the combined treatment having redder and darker colour (Fig. 4) compared to all other treatments (Tukey's post-hoc, $P = <0.0001$).

GREENHOUSE TRIALS

Between May and August 2022, 280 total fruits were collected (control: 14.8 %, bee: 26.6 %, hoverfly: 25.9 %, and combined: 32.7 %). Over this period, seven batches were collected throughout the season where all fruits were picked to calculate total yield and percentage of marketable fruits (Table 2). All pollinator treatments increased fruit weight compared to the control (bee, an increase of 48 %, hoverfly, 57 %, combined, 148 %). Overall, the combined treatment produced heavier fruit weights compared to other treatments (Fig. 2) but this was not significant between treatments (ANOVA, $F_7 = 2.508$, $P = 0.072$) or replicates throughout the trial.

There was a significant difference between marketable fresh weight between groups (GLMM, $F_3 = 6.06$, $P = 0.0001$). The combined treatment resulted in a higher overall marketable weight than the control (Fig. 2) (Tukey's, $P = 0.001$), but there was no difference between the bee or hoverfly treatments and the control (Tukey's, $P = 0.079$). The control treatment also produced the highest amount of non-marketable fruits overall compared to all other treatments one-way, ANOVA, $F_3 = 6.06$, $P = 0.0001$.

Compared to the control, the bee treatment, hoverfly, and combined treatments all had a higher percentage of DHA (mg/ml) (32.23 %, 36.69 %, and 47.44 % higher respectively). Fruits from both the bee and combined treatment had significantly higher concentrations of DHA compared to the control (Tukey's, bee: $P = 0.005$, combined: $P < 0.001$). Similarly, fruits from the combined treatment had the highest concentration of DHA compared to all other treatments (See supplementary Table S2), followed by the bee treatment (Fig. 5A).

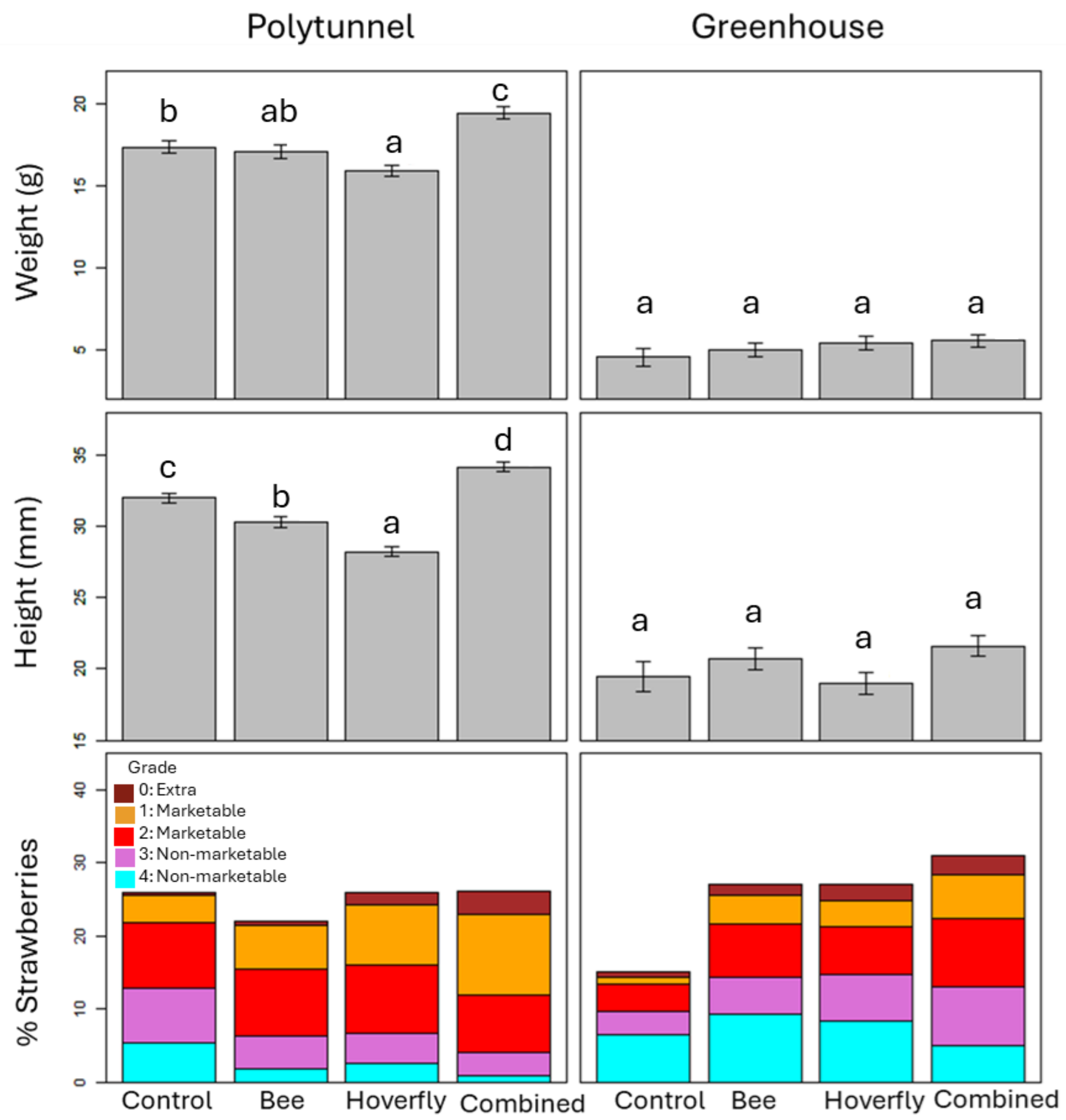

Figure 2. Polytunnel and Greenhouse values for average weight, height, and % grading for all strawberries across the four treatments used in each assessment across all replicates.

Collectively, all pollinator treatments resulted in fruits with a significantly higher concentration of DHA (Fig. 5B) as a percentage of AA (Fig. 5C) compared to the control (ANOVA, F_3 = 6.56, P = 0.002), Fruits from the hoverfly treatment had a higher concentration of AA than the control treatment (Fig. 5C), (Tukey's, P = 0.046).

The linear mixed effect models (using replicate as a random factor component), accounted for around 10% of the variation but found no significant effect of replicate (batch) on weight (F = 2.508, P = 0.07), width (F = 2.02, P = 0.12), or height (F = 1.28, P = 0.30). However, the DHA did vary between replicates (F = 6.56, P = 0.002).

Discussion

Strawberries benefit from insect pollination, with the value of bee pollination to strawberry quality and storability in Europe estimated at US $1.44 billion in 2014 (Klatt et al. 2014) but likely to be considerably higher in 2024. Despite strawberries' ability to self-fertilise, this is usually restricted to between 60-70% of achenes being

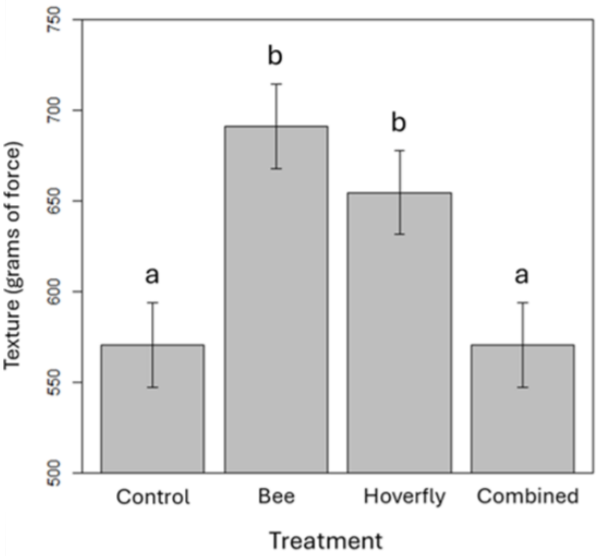

Figure 3. Average texture values of individual strawberries produced in each of the treatments in the polytunnel trials.

fertilised by dehiscing and wind pollination alone (Nitsch, 1950; Pion et al. 1980). Both bumblebees (*Bombus* spp.) and hoverflies (e.g. *E. corollae*, *Episyrphus balteatus*, *Eristalis tenax*) can be effective pollinators of this crop, as well as a range of solitary bee species (e.g. *Lasioglossum* spp., *Osmia* spp. and *Andrena* spp.). We explicitly measured for the first time the benefits from combining controlled numbers of two commercially available crop pollinators, *Bombus terrestris* and *Eupeodes corollae*, on strawberry production in polytunnel and greenhouse environments. We found that the combination of pollinators gave better results than deploying hoverflies alone in both experiments, and may outperform the use of bumblebees alone in polytunnels. Our results were not related to abundance of individual pollinators. Conversely to the findings of Herrmann et al. (2019), size and quality of fruits produced increased in the combined treatment of hoverflies and bees compared to all other treatments in polytunnel trials. Throughout trials we observed that bees effectuate shorter but more frequent visits, similar to Willmer et al. (2011). Bees will visit at a higher rate during the morning, in between 06:00 – 10:00 (Herrera 1990; Vaudo et al. 2015; Hall et al. 2019). Conversely, hoverflies peak in activity between 10:00 – 13:00 and 16:00 – 18:00 (Venjakob et al. 2016; Sánchez et al. 2022). Strawberry flowers only remain receptive to pollination for four days, restricting the time available for cross-pollination.

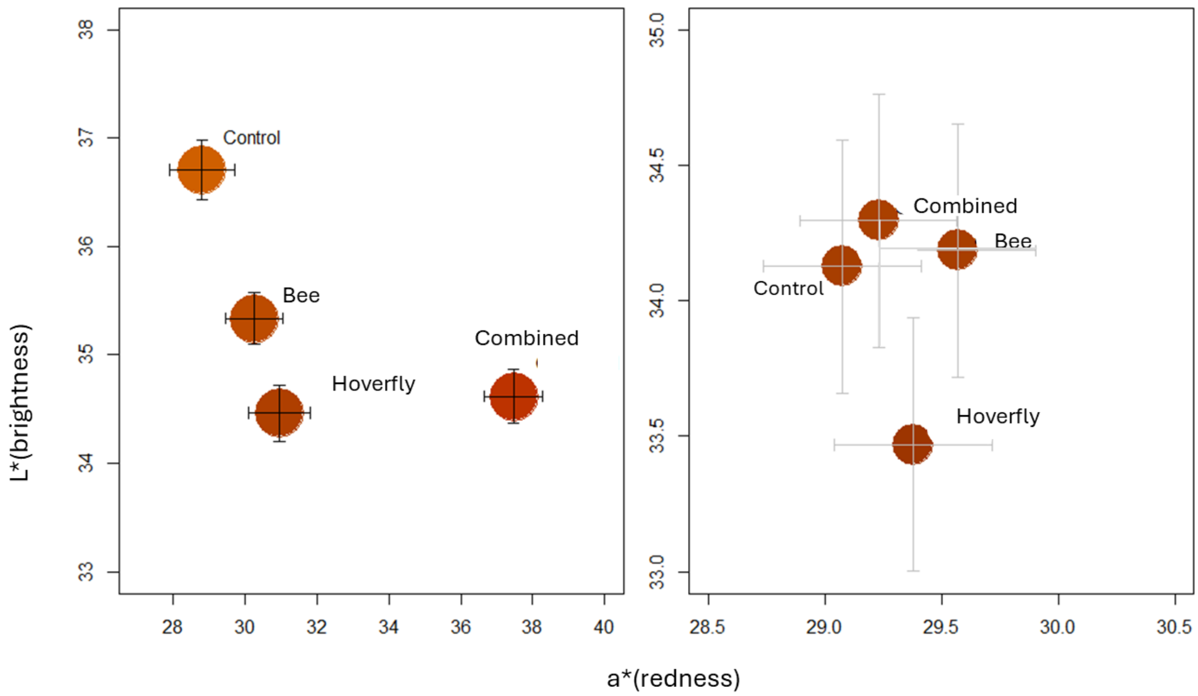

Figure 4. Colour value spatial analysis for each treatment including all replicates in the polytunnel trials (left) and greenhouse trials (right).

Treatment	N	% Of Total Yield	% Marketable	%Non-Marketable
Control	42	15.6	33.3	66.6
Bee	69	25.6	44.9	55
Hoverfly	75	27.8	48	52
Combined	83	30.8	59	40.9

Table 2. Total number of fruits produced in each treatment, percentage of total yield and of marketable and non-marketable fruits.

Thus, providing pollinator activity throughout the day with the combined system increases the chances of effective pollination. The effect appears to be mediated by a change in hoverfly behaviour rather than a change in bee behaviour; in the combination treatment in the polytunnel trials, the bees behaved as they did in the single-species treatment, but the hoverflies changed their flower-visitation behaviour in terms of the frequency of visits. This could suggest that hoverflies change their flower-visitation behaviour in response to heterospecific presence as hoverflies appear to visit flowers more readily when there is a decline in bee visits or exhibit learning behaviour but further research is required to investigate this principle.

This study compared the effects of different pollinator treatment types on strawberry yield, quality, and nutritional composition in closed system polytunnel and greenhouse cage trials. Self-pollinated strawberries had high numbers of malformed fruit (similar to Hodgkiss et al. 2018), but in both our experiment and Hodgkiss's adding pollinators (including hoverflies, especially multiple pollinator species) improved this. We also confirm that commercially-used *B. terrestris* increases the quality and yield of strawberries compared to self-pollination when pollinators were excluded, similar to Martin et al. (2019). However, compared to the combined treatment, our results from the polytunnel trials suggest that, there is potential to improve on the performance of *B. terrestris* alone by adding other pollinators to enhance fruit size and quality. The increase in strawberry yield when multiple pollinators are present has previously been shown to increase crop yield and quality (Gudowska et al. 2024, Martin et al. 2019). Our study adds to literature from other crops demonstrating that increasing pollinator richness (in most cases by allowing wild pollinators access to the crop) improves crop yield and quality parameters (Pisanty et al. 2022; Hoehn et al. 2008; Garibaldi *et al*, 2013). The combined treatment of bee and hoverfly in this study enhanced the yield and quality when compared to self-pollinated plants. Conversely, other studies (Herrmann, et al. 2019; MacInnis et al. 2019) observed lower fruit weights from plants pollinated by multiple pollinator species, so there are clearly other factors influencing the process, perhaps related to abundance of bumblebees or other crop growth conditions. Conversely to our results, Herrmann et al. (2019), found the quality of strawberries was not higher in a combined treatment compared to a control, this could however be an indicator of species differences as the approach used a solitary bee and green bottle fly in their trials. *L. sericata* adult females require a higher intake of protein for oogenesis and oviposition much like many fly species; however, they can obtain this from carrion, faeces, and pollen producing flowers (Cook 1991). This variety introduces a myriad of issues when considering foraging behaviour, and their pollination efficiency. Due to the plethora of protein sources for adult females, this will likely impact their motivation and constancy in floral visitation (Goulson et al. 1998) compared to hoverfly species such as *E. corollae*, whose adults forage solely on flowers for diet resources, which suggests they may not be as effective pollinators in commercial scale crops.

The combination pollination treatment was superior in terms of fruit yield, quality, and vitamin C content compared to the control, and higher in vitamin C concentration to the single species pollinator treatments. While this was a cage trial, and the fruits were overall of small size and differed from commercially produced fruit on farms, this outcome and the other yield observations have implications for commercial production. On farms in Europe, pollination services for strawberry are typically provided by managed bumblebees (Kleijn et al. 2015) or honeybees, with wild pollinator visits usually too infrequent to provide reliable pollination services

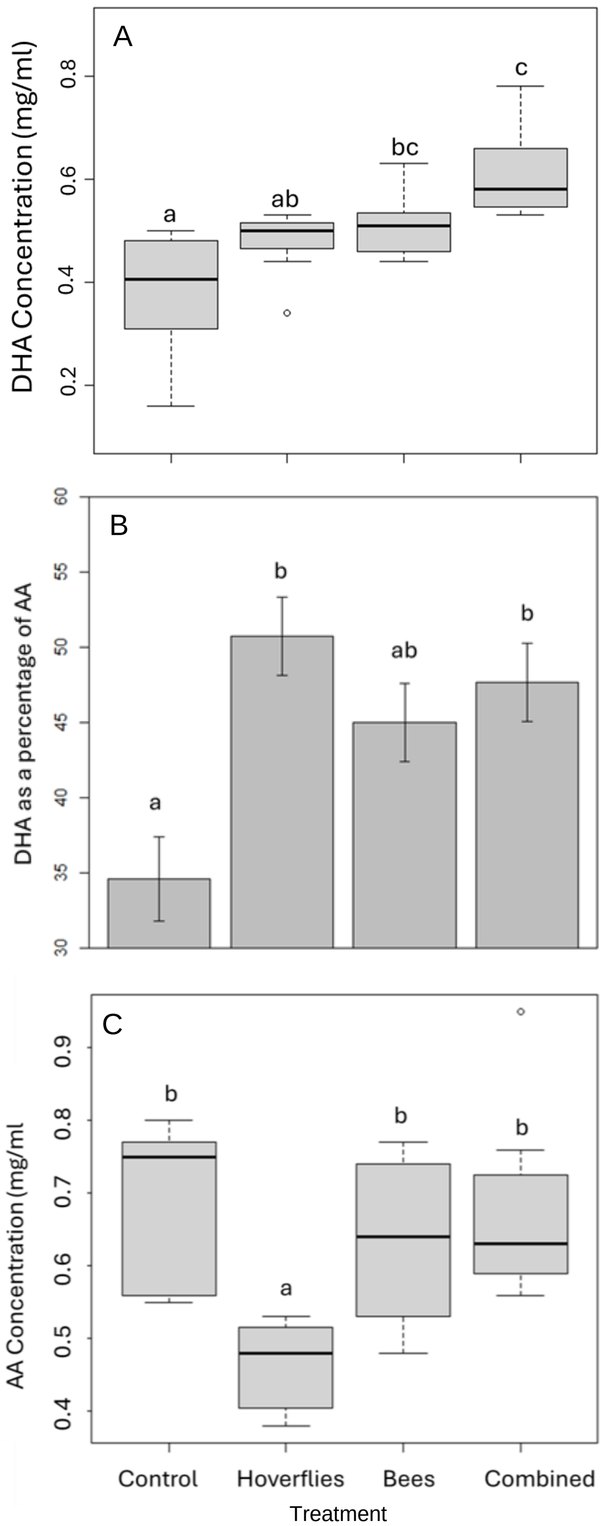

Figure 5. (A) A Mean concentration of DHA (mg/ml) taken from AA and TAA concentrations of each pollinator treatment and combined replicates in greenhouse trials. (B) Concentration of DHA taken from AA as a percentage from all treatments and combined replicates in greenhouse trials. (C) The AA concentrations of strawberries from all treatments and combined replicates in greenhouse trials.

without supplementation (Martin et al. 2019). Hoverflies are currently not widely deployed on purpose as part of the pollination system in outdoor and semi-protected crops. However, various suppliers make them commercially available now, and they are starting to be adopted in some vertical farming systems (pers. com. Olombria Ltd., thriveagrifood.com,).

The mechanism for this improvement in fruit size, quality and nutritional content derived from a multi-species system is likely to stem from the differences in behaviour when complementary species forage in parallel, compared to using a monospecific system and varying morphological differences. When multiple species of visitors are present visits may be shorter (Jeavons et al. 2022), and visitors may move between flowers more often (Jeavons et al. 2022). The presence of more species is also more likely to give rise to a pollinator community with complementary behaviour patterns on flowers (Miñarro et al. 2018) as well as contrasting morphologies providing more even pollen deposition.

Fertilised achenes release auxin which is important for fruit ripening and quality (Wietzke et al. 2018). Effective pollination influences the synthesis of auxins in the fruit during development (Wietzke et al. 2018). Consequently, more even pollen coverage on the stigma of the flower will result in more consistently fertilised achenes and lead to higher auxin levels in the fruit, improving development and ripening. Links between auxin and vitamin C synthesis pathways in fruit have been suggested (Lima-Silva et al. 2012; Lin et al. 2022). Pollination processes appear to have further-ranging consequences than perhaps fully appreciated previously.

The redox state of vitamin C (the balance between AA and DHA) in strawberries influences their post-harvest physiology. High levels of AA are associated with better quality and longer shelf life, as AA helps to reduce oxidative damage during storage. Research on strawberries has shown that the balance between AA and DHA changes in response to various stress conditions (Dewhirst et al. 2018). For example, during periods of high oxidative stress, the conversion of AA to DHA increases, indicating the utilization of AA to counteract the stress. The higher concentrations of DHA available in fruits we assessed in the greenhouse trials could suggest a better capability of plants to react to oxidative, pest, or even disease stress or damage. Previously, the applications of ascorbic acid have shown to supress the growth and abundance of grey mould (*Botrytis cinerea*) (Zhao et al. 2022), the most prolific fungal pathogen of strawberry. The increase in available DHA and higher concentrations of ascorbic acid may play a role in supressing fungal pathogens, as well as the increase in available antioxidants may prove beneficial to overall plant defence. This warrants further investigation, as the mechanisms of plant defence, enzyme function and vitamin C synthesis are still not fully understood.

High-quality and nutritious fruit benefit growers by commanding better prices and reducing waste (low-quality fruit can result in rejection of entire shipments) and consumers by conferring better value for money and nutrition. Finding that optimising pollination can improve fruit size by 27% relative to bee pollination alone and 148% relative to self-pollinated controls, and vitamin C concentration by 22% and 47% respectively, demonstrates the importance of pollination in food production and nutritional security. Additionally, the redox state of vitamin C can enhance the plant's innate defence systems. Ascorbic acid participates in the synthesis of various defensive compounds and the strengthening of cell walls, which can make it more difficult for pathogens like powdery mildew to invade and establish themselves. This increased rate of DHA observed could suggest more availability for conversion to oxidised AA not only for plant defence, but also in high stress environments such as drought or increased salinity. Further investigation of the underlying metabolic processes is warranted, as is quantification of vitamin C concentrations in fruit grown with wild pollinator communities and in managed monocultures applying hand pollination.

Conclusion

Our findings demonstrate that combinations of different pollinator species can be more effective pollinators of strawberry than single species alone, increasing the marketable yields and quality. These results suggest that multiple species of pollinators provide more effective pollination, leading to dual benefits of larger and higher quality fruits, and increased vitamin availability in fruits. Though our results illustrate the additional benefits to human health and pollinator management, future studies could compare the pollination effectiveness of open pollination with that of conventionally managed pollinators, including multi-species hoverfly assemblages, and of wild and managed solitary bees.

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Author contribution

The authors confirm contribution to the paper as follows: Conceptualization: K. James, S. Arnold, and R. Colgan; Data curation: K. James, S. Springate, S. Harte & D. Farman; Visualisation and Validation: K. James, S. Arnold; Writing: K. James. All authors contributed critically to the manuscript review and editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data used to write this article are available as supplement in the online version of this article (Semi-fieldMastersheet_James et al.xlsx)

Appendices

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

Table S1. Mixed model effect output table for strawberry quality

Table S2. Multiple comparisons of strawberry DHA mg/ml

Table S3. Mixed model effect output table for strawberry quality

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