

**Appendices to J Poll Ecol 37(20), James et al.**

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Materials and methods

Polytunnel trials

*Fragaria* x *ananassa* (Malling Champion) was selected as a recent variety developed by the East Malling Strawberry Breeding Club (EMSBC) program in Kent, UK (UK). *Fragaria x ananassa* (Malling Champion) is a standard, commercially available everbearing variety (flowers appear repeatedly over the cropping season and are less sensitive to day-length). It was selected as a modern variety that normally produces >85% marketable fruit and has reasonable resistance to crown rot (*Phytophthora cactorum*) and Verticillium wilt (*Verticillium dahliae*) and moderate resistance to powdery mildew (*Podosphaera aphanis*) (Cockerton et al. 2021) and is regularly grown on the experimental site.

50L coir bags (Botanicoir, precision plus ultra ©, [Botanicor, London, UK]) each held 8 *Fragaria* x *ananassa* (Malling Champion) (Berry Plants Ltd) and were supplied with fertigation lines (fertigation supplied for 15 minutes every 2 hours). Plants were introduced as root stock into 10 individual grow bags per compartment in May 2021 and trials began once at least 75% of the total plants were in flower. Fruits were picked between the hours of 09:00 and 10:30 and they were transferred to the lab and assessed within five hours of picking.

Data for each compartment was then recorded for each individual at a later date. A visit was only recorded if an individual contacted the apical region of the flower for more than 2 seconds. In each compartment (except the control with no-pollinators), we recorded the number of visits, handling time, time of day, temperature, humidity, and species visiting individual flowers.

Glasshouse trials

A soil moisture reader was used daily to maintain soil moisture between 40-45% optimum. Fertiliser mix was comprised of a mix of two fertilisers NPK (nitrogen, phosphorus, and potassium) of 27 – 4 – 15 and 17 – 4 – 21. Both fertilisers were mixed at a 1:1, 60 ml of each diluted into 35 L water tank which fed irrigation lines. Nitric acid (0.1 M) was used to lower the pH of the fertigation mix, as previous experimentation has shown that a pH of between 5.5-6.0 pH is optimum for nutrient uptake (Ikegaya *et al*., 2020) in addition to reduction of pathogens and root rot when kept between pH 5 and 7 (Fang *et al*., 2012).

Assessments of fruit were undertaken at the Natural Resources Institute post-harvest laboratory located at the University of Greenwich.

Results

Table S Mixed model effect output table for strawberry quality: Density (weight, height, width), weight, width, and ratio (height and width)

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| Statistical analysis Date October 22, 2024 |
| Data file | Berrieschked |
| R script | chkdata5-5-2023 |
| Analysis description | mixed effect models by batch |
| Density |
| [1] Estimated p-values, effective N from ICCC= 0.0654 npar Sum Sq Mean Sq F value pvaluetreat 3.0 40.48038 13.49346 3.914994 0.01183347Residuals 74.7  Var1 Freq mns ses SD1 Bee 75 1.182141 0.4277186 3.7041522 BH 86 1.047792 0.3994289 3.7041523 Control 42 1.187167 0.5715630 3.7041524 Hoverfly 75 2.094592 0.4277186 3.704152> |
| Linear mixed model fit by REML ['lmerMod']Formula: density ~ treat + (1 | batchfac)REML criterion at convergence: 1140.4Random effects: Groups Name Variance Std.Dev. batchfac (Intercept) 0.2411 0.4911  Residual 3.4466 1.8565 Number of obs: 278, groups: batchfac, 7Fixed effects: Estimate Std. Error t value(Intercept) 1.03094 0.29124 3.540 (this is bee)treatBH -0.06011 0.29766 -0.202 (these are differences from bee)treatControl -0.19294 0.36431 -0.530treatHoverfly 0.82301 0.31682 2.598 |
| Weight |
| [1] Estimated p-values, effective N from ICCC= 0.1378 npar Sum Sq Mean Sq F value pvaluetreat 3.0 81.016 27.00533 2.508011 0.07263948Residuals 39.9  Var1 Freq mns ses SD1 Bee 75 4.985200 1.0431793 9.0341982 BH 86 5.531512 0.9741826 9.0341983 Control 42 4.543810 1.3940070 9.0341984 Hoverfly 75 5.426400 1.0431793 9.034198 |
| Linear mixed model fit by REML ['lmerMod']Formula: weight ~ treat + (1 | batchfac)REML criterion at convergence: 1456.2Random effects: Groups Name Variance Std.Dev. batchfac (Intercept) 1.721 1.312  Residual 10.768 3.281 Number of obs: 278, groups: batchfac, 7Fixed effects: Estimate Std. Error t value(Intercept) 5.1220 0.6382 8.026treatBH 0.7176 0.5282 1.358treatControl -0.3324 0.6455 -0.515treatHoverfly 1.2151 0.5645 2.153 |
| Width |
| [1] Estimated p-values, effective N from ICCC= 0.0948 npar Sum Sq Mean Sq F value pvaluetreat 3.0 245.9383 81.97942 2.024808 0.1209276Residuals 55.5  Var1 Freq mns ses SD1 Bee 75 19.22307 1.711437 14.821482 BH 86 21.17174 1.598241 14.821483 Control 42 18.81429 2.287004 14.821484 Hoverfly 75 20.51293 1.711437 14.82148 |
| Linear mixed model fit by REML ['lmerMod']Formula: width ~ treat + (1 | batchfac)REML criterion at convergence: 1817.1Random effects: Groups Name Variance Std.Dev. batchfac (Intercept) 4.238 2.059  Residual 40.488 6.363 Number of obs: 278, groups: batchfac, 7Fixed effects: Estimate Std. Error t value(Intercept) 19.7366 1.0977 17.980treatBH 1.8636 1.0223 1.823treatControl 0.2437 1.2503 0.195treatHoverfly 2.2630 1.0906 2.075 |
| Ratio |
| [1] Estimated p-values, effective N from ICCC= 0.0782 npar Sum Sq Mean Sq F value pvaluetreat 3 0.4038109 0.1346036 2.574388 0.06145189Residuals 65  Var1 Freq mns ses SD1 Bee 75 1.100833 0.05561873 0.48167232 BH 86 1.040230 0.05194006 0.48167233 Control 42 1.074156 0.07432365 0.48167234 Hoverfly 75 0.968908 0.05561873 0.4816723 |
| Linear mixed model fit by REML ['lmerMod']Formula: ratio ~ treat + (1 | batchfac)REML criterion at convergence: -6.4Random effects: Groups Name Variance Std.Dev. batchfac (Intercept) 0.004434 0.06659  Residual 0.052286 0.22866 Number of obs: 278, groups: batchfac, 7Fixed effects: Estimate Std. Error t value(Intercept) 1.09715 0.03745 29.297treatBH -0.04496 0.03670 -1.225treatControl -0.03937 0.04490 -0.877treatHoverfly -0.10794 0.03911 -2.760 |
| Height |
| [1] Estimated p-values, effective N from ICCC= 0.2895 npar Sum Sq Mean Sq F value pvaluetreat 3.0 135.7159 45.23862 1.282449 0.3093244Residuals 18.8  Var1 Freq mns ses SD1 Bee 75 20.74667 2.929033 25.366172 BH 86 21.59616 2.735304 25.366173 Control 42 19.47143 3.914085 25.366174 Hoverfly 75 18.98267 2.929033 25.36617 |
| Linear mixed model fit by REML ['lmerMod']Formula: height ~ treat + (1 | batchfac)REML criterion at convergence: 1786.2Random effects: Groups Name Variance Std.Dev. batchfac (Intercept) 14.37 3.791  Residual 35.28 5.939 Number of obs: 278, groups: batchfac, 7Fixed effects: Estimate Std. Error t value(Intercept) 21.2966 1.6099 13.228treatBH 1.2401 0.9589 1.293treatControl -0.9781 1.1700 -0.836treatHoverfly 0.3702 1.0268 0.361 |

Supplementary Table S Multiple comparisons of strawberry DHA mg/ml with treatment as the fixed factor, batch as a random factor and DHA as the dependant factor.

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| **Multiple Comparisons** |
| Simultaneous Tests for General Linear HypothesesMultiple Comparisons of Means: Tukey Contrasts |
| Fit: lmer (formula = DHA ~ treat factor + (1 | batch factor)) |  |
|  | Estimate Std. | Error | z value |  Pr(>|z|)  |
| Linear Hypotheses: |
| H - C == 0  | 0.13286 | 0.05121 | 2.594 | 0.04656 \*  |
| B - C == 0  | 0.16714 | 0.05121 | 3.264 | 0.00585 \*\* |
| BH - C == 0  | 0.27143 | 0.05121 | 5.301 | < 0.001 \*\*\* |
| B - H == 0  | 0.03429 | 0.05121 | 0.67 | 0.9086 |
| BH - H == 0  | 0.13857 | 0.05121 | 2.706 | 0.03429 \*  |
| BH - B == 0  | 0.10429 | 0.05121 | 2.037 | 0.1746 |
| Signifiance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 |  |  |
| (Adjusted p values reported -- single-step method) |

Estimated the cross-section area by counting graph paper squares to be width x length x 0.833.

The volume was calculated as pi x length of each fruit x (0.883/2\*width of each fruit)2/1000 in cubic centimetres. Calculation and method conducted by Stephen Young, outputs of volume given in Figure S1 and density in Figure S2.

Table Mixed model effect output table for strawberry quality: Volume (weight, height, width and density)

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| --- |
|  Source Df SumSq MeanSq F pValue star treat 3 1.81 0.6 21.71 <0.0001 \*\*\* Residuals 2067 57.39 > asd$lets<-lt3> print(asd) treat Freq means se lets1 bee 457 1.0411047 0.007794841 b2 BH 540 1.0586390 0.007170815 b3 control 537 1.0605574 0.007190817 b4 hoverfly 537 0.9887523 0.007190817 a |
|  A graph of a number of bars  Description automatically generated with medium confidenceFigure Sa Fruit volume calculated as an average of each individual strawberry produced through polytunnel trials  |
|  Source Df SumSq MeanSq F pValue star treat 3 13411.74 4470.58 44.81 <0.0001 \*\*\* Residuals 2067 206207.16 > asd$lets<-lt3> print(asd) treat Freq means se lets1 bee 457 1.730939 0.07087257 b2 BH 540 1.022628 0.06519877 c3 control 537 1.110470 0.06538064 b4 hoverfly 537 1.543746 0.06538064 a |
| A graph of a number of bars  Description automatically generatedFigure S Fruit density of each treatment given as an average of each individual strawberry value given throughout polytunnel trials |
|  Source Df SumSq MeanSq F pValue star treat 3 2.90 0.97 15.59 <0.0001 \*\*\* Residuals 1792 110.93 > asd$lets<-lt3> print(asd) treat Freq means se lets1 bee 376 1.0117008 0.01283110 bc2 BH 507 0.9388005 0.01104979 a3 control 489 0.9751599 0.01125132 ab4 hoverfly 424 1.0450125 0.01208300 c |

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Figure S Colour space values for colourimetry analysis, representing L\*, a\*, and b\* values as lightness/darkness, hue, chroma/saturation levels (Mouw 2018).

References

Cockerton HM, Karlström A, Johnson AW, Li B, Stavridou E, Hopson, KJ, Whitehouse AB, and Harrison, RJ (2021) Genomic Informed Breeding Strategies for Strawberry Yield and Fruit Quality Traits. Frontiers in Plant Science, 12. <https://doi.org/10.3389/fpls.2021.724847>

Mouw T (2018) Tolerancing part 3: Color space vs. color tolerance. [online] (Accessed on the 22/07/2024). <https://www.xrite.com/blog/tolerancing-part-3>