# DOI: 10.1002/jsf2.70012

## SHORT COMMUNICATION

Revised: 6 May 2025



# Contribution of pollinators to delivering fruit quality in commercial sweet cherry orchards

Zeus Mateos-Fierro<sup>1,2</sup> | Michael P. D. Garratt<sup>3</sup> | Michelle T. Fountain<sup>4</sup> | Kate Ashbrook<sup>2</sup> | Duncan B. Westbury<sup>2,5</sup>

<sup>1</sup>Department of Entomology, Purdue University, West Lafayette, Indiana, USA <sup>2</sup>School of Science & the Environment, University of Worcester, Worcester, UK

<sup>3</sup>School of Agriculture, Policy and Development, University of Reading, Reading, UK

<sup>4</sup>NIAB, East Malling, UK

<sup>5</sup>Land and Property Management, Royal Agricultural University, Cirencester, UK

Correspondence

Zeus Mateos-Fierro, Department of Entomology, Purdue University, West Lafayette, IN, USA. Email: zmateosf@purdue.edu

Funding information University of Worcester; Berry Gardens Ltd.; Waitrose & Partners

#### Abstract

**Background:** Pollinators provide an essential ecosystem service to many crops, including sweet cherry (*Prunus avium*), which can be quantified in terms of fruit number and/or quality. Most studies in sweet cherry have explored the extent to which fruit set relies on pollinators but have neglected pollinators' contribution to fruit quality. We investigated the impact of pollinators on fruit set (2018–2019) and fruit quality (2017–2019). In 10 commercial sweet cherry orchards under polytunnels, we conducted insect-exclusion experiments comparing insect-excluded blossoms (mesh-bagged blossoms) to blossoms exposed to floral visitors (open blossoms). We then investigated relationships between fruit set and fruit quality.

**Results:** Pollinators were key to underpinning commercial fruit set (15.4% fruit set from open blossoms compared to 1.1% with bagged blossoms), equivalent to a contribution of 92.8%. Pollinators were also essential to achieving higher cherry fruit quality. With open blossoms, fresh mass, width, dry matter, and flesh/pit ratio of cherries increased by 19.8%, 7.9%, 19.8%, and 10.5%, respectively, compared to cherries from bagged blossoms. In contrast, firmness was similar between both pollination treatments. We did not find a significant relationship between fruit set and quality, suggesting trees did not carry an excessive fruit burden.

**Conclusion:** Our results highlight the importance of pollinators, not only for underpinning commercial yields in terms of fruit set, but also for higher fruit quality. We recommend growers adopt effective pollinator management practices to help underpin commercially viable yields consisting of fruit with a higher marketable potential.

KEYWORDS bees, dry matter, fruit mass and size, insect-exclusion experiment, pollination services, polytunnels

# INTRODUCTION

Pollination delivered by pollinators is essential to underpin commercial production in many pollinator-dependent crops, including the globally cultivated sweet cherry (*Prunus avium* L.).<sup>1-3</sup> Crops can benefit from pollination services not only in terms of fruit set, but also with regard

to fruit quality.<sup>2</sup> To help secure commercially viable cherry yields, especially in self-incompatible cultivars, growers typically use managed pollinators, including honey bees (e.g., *Apis mellifera*), bumble bees (e.g., *Bombus terrestris*) and/or mason bees (e.g., *Osmia cornuta*).<sup>4-6</sup> Although fruit set is typically the main factor determining yields, fruit quality is also an important factor in cherry profitability.<sup>7</sup>

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Larger cherry fruits are often preferred by consumers<sup>8</sup> and consequently, fruit of reduced mass, size, and shape can negatively impact the proportion of marketable fruit, reducing commercial output.<sup>9</sup> Thus, to further enhance fruit set and fruit quality, in some regions, growers use protective coverings (i.e., open-ended polytunnels).<sup>10,11</sup> Polytunnels protect blossoms and fruit from environmental factors such as rainfall, which can reduce yields.<sup>10,12</sup> Growers may therefore combine both management practices (pollinators and polytunnels) to achieve commercial yields, including cherries of greater quality. Consequently, it is essential to explore the contribution of pollinators to both fruit set and fruit quality.

A number of studies have explored the impact of pollinators on sweet cherry fruit set.<sup>5,6,13,14</sup> However, the extent to which pollinators affect cherry fruit quality has been largely neglected.<sup>3</sup> Fruit quality has been explored with supplemental managed pollinators, but not in the absence of pollinators,<sup>15</sup> the critical role of pollinators cannot be therefore ascertained. Mateos-Fierro et al.<sup>6</sup> demonstrated that pollinators provide a critical pollination service for cherry fruit set and significantly enhance fruit width (diameter), but not fruit mass. Yet, this was based on only one year of data and consequently, more research is needed to determine the contribution of pollinators to fruit quality in sweet cherry. In this paper, we combine three years of fruit quality data and assess other cherry quality parameters deemed important in fruit quality (e.g., dry matter<sup>16</sup>), not only for fresh-fruit industry<sup>17,18</sup> but also for breeding programmes.<sup>19,20</sup> The aims of this study were to quantify the contribution of pollinators to fruit quality and explore relationships between different quality parameters and fruit set. We also provide further evidence of the essential role pollinators play in fruit set. This will help commercial growers manage sweet cherry orchards more effectively to deliver economically viable, high-quality, marketable fruit.

# MATERIALS AND METHODS

#### Study sites

The study was conducted in 10 commercial, conventionally managed, sweet cherry orchards in the West Midlands, UK between 2017 and 2019. The size of the orchards varied from 1.3 to 7.5 ha (mean 3.3 ha  $\pm$  SD 1.8) while the distance between orchards varied from 0.03 to 92.9 km (mean ~ 50.9 km  $\pm$  SD 36.5). All 10 orchards contained a maximum of six cultivars per orchard, including the self-incompatible cultivar Kordia (i.e., the focal cultivar used in this study) and at least one compatible pollinizer cultivar (Table S1). Cultivars were planted in separate rows (one cultivar per row), but the alternating row order varied among orchards. Polytunnels were used by growers prior to the cherry blooming period (April) until after harvest (September). The pollinator management applied by growers consisted of a combination of *A. mellifera* and *B. terrestris*, except for one orchard where only *A. mellifera* was used. The floral visitor community (managed + wild) visiting cherry flowers was previously explored in these orchards by

conducting walking transect surveys along three different Kordia rows for each of the 10 orchards<sup>6</sup> and a summary of the floral visitors is presented in Table S2. We refer to "floral visitors" when their contribution to pollination cannot be ascertained and to "pollinators" as those floral visitors, which visits successfully transferred pollen and resulted in fruit being produced, that is, pollination.

#### Fruit set and fruit quality assessments

To measure the extent to which fruit set and fruit quality were affected by pollinators, we conducted an insect-exclusion experiment. We measured fruit set in 2018-2019 and fruit quality in 2017-2019 (fruit set was not explored in 2017). In 2017-2018, we used eight trees for each of the three Kordia rows where we investigated flower visitor communities (i.e., 24 trees per orchard; 240 total trees in the 10 orchards per year). Trees were selected at 9.5 m intervals along the row from the orchard edge to account for potential edge effects.<sup>21</sup> In 2019, we used four trees (at 19 m intervals) per Kordia row (i.e., 12 trees per orchard: 120 total trees in the 10 orchards). On each tree, prior to the cherry blooming period, we randomly selected two spurs ( $\sim$ 30 cm long from the tip), with at least 20 buds each (mean 69.8 ± SD 24.7), at 1.5-2.0 m above the ground. One spur received an insect pollination exclusion treatment (bagged; absence of floral visitors), using PVC mesh bags (mesh gauge 1.2 mm<sup>2</sup>), which allowed pollen movement but prevented insect visits. These spurs were mesh-covered prior to the cherry blooming period and uncovered afterward. Blossoms in the second spur were left open for insect pollination (open; presence of floral visitors). All blossoms were counted in 2018-2019.

In July,  $\sim$ 2 days prior to commercial harvest, fruit set was determined, and cherries were harvested. In 2017, we randomly harvested, among the available cherry fruits, a maximum of 10 cherries per spur (to ensure sufficient statistical power), but in 2018-2019, following power analysis (data not presented), we harvested, also randomly, a maximum of three. Fruits were stored at 6°C until assessments and evaluated within 48 h from harvest. Prior to assessments, cherry stalks were removed. To assess fruit quality, we measured fresh mass, height, width, length, firmness, dry matter, and mass, height, width, and length of pits (endocarp, in which the seeds are enclosed), and calculated flesh (mesocarp)/pit ratio.<sup>22</sup> We did not measure fruit length and pit length in 2018. An electronic scale (Precision Balances Entris®, model 822-1SUS) was used to measure mass, whilst an electronic digital calliper was used to record dimensions. A firmness tester (Agrosta® 100USB) was used to measure fruit firmness. We measured each cherry fruit on two perpendicular sides to obtain a mean firmness value per cherry. We report firmness as Durofel units, which indicate resistance from 1 to 100 (soft to firm).<sup>18</sup> A stainless-steel cherry pitter was used to manually extract the pits. Then, an industrial oven was used for 48 h at 65°C to fully dry the cherries, which were then weighed. Lastly, we weighed and measured all pits.

#### Statistical analysis

Data were analyzed with the software R (version R-4.4.0)<sup>23</sup> using Generalized Linear Mixed-Effect Models (GLMER) and Linear Mixed-Effect Models (LMER) ("Ime4" package<sup>24</sup>). Data visualization was done using the function ggplot ("ggplot2" package<sup>25</sup>). Fruit set was analyzed using a GLMER with binomial error distribution (function = glmer; family = binomial), considering the proportion of fruits that set, whilst fruit quality parameters were analyzed using LMERs (function = Imer). Bound Optimization By Quadratic Approximation (BOBYQA) optimizers were used to minimize convergence error. All models (fruit set and the 11 fruit quality parameters) included pollination treatment (bagged or open) as a fixed factor. Random factors included (i) year and (ii) nested Kordia tree. Kordia row, and orchard to account for potential environmental, annual variation, and location variability, respectively (Table S3). Additionally, to account for the imbalance in fruit numbers between bagged versus open pollination treatments, the 11 fruit quality parameters also analyzed 10 subsamples (function = sample; N = 300) randomly selected for each pollination treatment level (Table S4).

Flesh/pit ratio was calculated for each cherry as:

$$Flesh/pit ratio = \left(\frac{Fresh mass - Pit mass}{Pit mass}\right).$$

We averaged values of fruit set and fruit quality parameters (from bagged and open blossoms, respectively) per Kordia row and orchard (across all Kordia trees within each Kordia row and years) to calculate the pollinators' contribution (at Kordia row level):

Pollinators contribution = 
$$\left[1 - \left(\frac{\text{Bagged pollination}}{\text{Open pollination}}\right)\right] \times 100.$$

To further investigate cherry quality, we performed linear regressions (we confirmed the models' residuals were normally distributed with normal probability plots) to explore the relationships between different metrics using geom\_smooth (method = "lm"; formula =  $y \sim x$ ) based on quality measures used by the industry, that is, fruit set and mass and size.<sup>7,26,27</sup> We averaged values per orchard and year (as fruit set was not measured in 2017) across all trees and explored the relationships between fruit set with (i) fresh mass, (ii) width, and (iii) dry matter in 2018–2019, and between fresh mass with (i) width, (ii) dry matter, and (iii) flesh/pit ratio in 2017–2019.

## RESULTS

#### Fruit set

We counted a total of 50,286 blossoms in 2018 and 2019 (25,595 bagged blossoms and 24,691 open blossoms). There was a significant effect of pollination treatment (Z = 45.51, p < 0.001; Figure 1a; Table S3) with 15.4% fruit set (3,794 fruits) from open blossoms

compared to 1.1% fruit set (279 fruits) from bagged blossoms. Across both years, we calculated an overall pollinators' contribution to fruit set of 92.8% ( $\pm$ SD 3.6).

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## Fruit quality

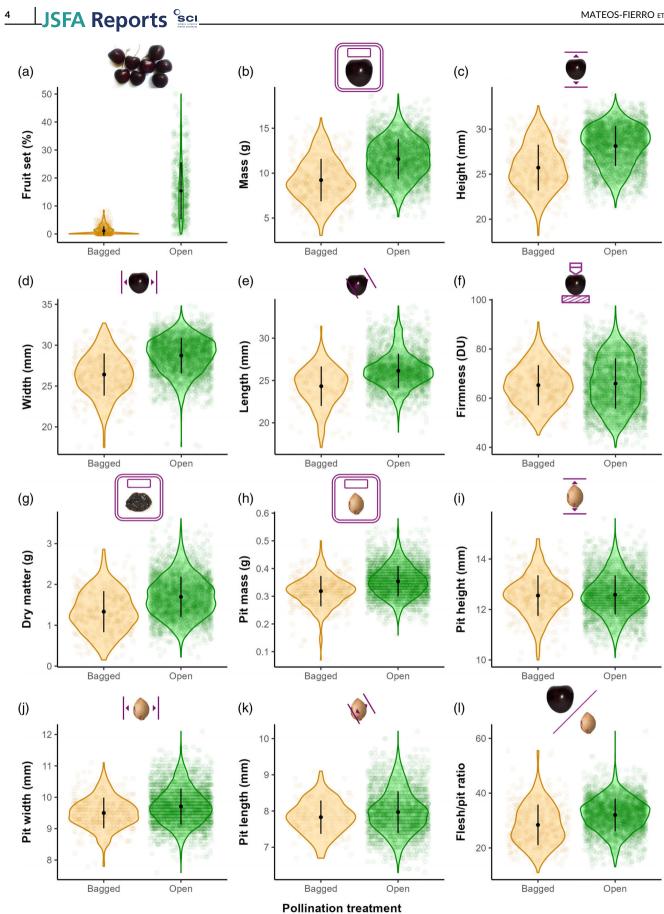
We assessed a total of 3,604 Kordia fruits between 2017 and 2019 (364 from bagged blossoms and 3,240 from open blossoms). Pollination treatment influenced most cherry quality parameters; values were significantly greater with open blossoms compared to bagged blossoms, including mass, height, width, length, and dry matter by 2.3 g, 2.4 mm, 2.3 mm, 1.3 mm, and 0.4 g, respectively, but not firmness (Figure 1b-g; Table S3). Pit mass and size (width and length but not height) and the flesh/pit ratio were also significantly greater with open blossoms compared to bagged blossoms (Figure 1h-l; Table S3). Pollinators' contribution to fruit quality parameters was consistent with greater values recorded with open blossoms (pollinator presence increased quality up to 20%), except for firmness and pit height and width, which increases were  $\leq 2\%$  (Table S3). For either pollinator treatment, the relationships between fruit set with fresh mass, width, and dry matter between 2018 and 2019 were not significant (Figure 2a-c), whilst the relationships between fresh mass with width, dry matter, and flesh/pit ratio between 2017 and 2019 were positively significant (Figure 2d-f).

# DISCUSSION

Our study demonstrates the importance of insect pollinators<sup>6</sup> for overall sweet cherry production with regard to fruit mass and size. It is evident that pollinators are not only essential for achieving commercial yields in terms of the amount of fruit produced, but they also significantly contribute to improving cherry fruit quality.

The low number of cherries from bagged blossoms (1.1%) that set compared to open blossoms (15.4%) was expected and highlights the important role pollinators play in securing fruit set,<sup>5,6,13</sup> particularly in self-incompatible cultivars where cross-pollination is essential.<sup>28,29</sup> Additionally, the 15.4% fruit set from open blossoms in our study is consistent with the fruit set reported in other studies investigating open pollination in Kordia: 17.7% (Kordia + Regina) with 1 year of study in Germany,<sup>13</sup> 17.6% across 3 years in Serbia,<sup>30</sup> 17.4% across 2 years in Poland.<sup>31</sup> However, the pollinators' contribution in sweet cherry in our study (92.8%) is moderately lower compared to the 96.4% reported by Osterman et al.,<sup>3</sup> although the latter percentage was averaged across four studies and multiple cultivars. Regionality, year (variability in environmental factors), and cultivar could account for differences in fruit set.<sup>3,30</sup>

In this study, we found cherry fruit quality (except for firmness) to be greater with open pollination. This highlights the importance of insect pollination for fruit quality, including important parameters such as mass, width, and dry matter. The overall greater fruit quality achieved with pollinators we show in sweet cherry is a consistent



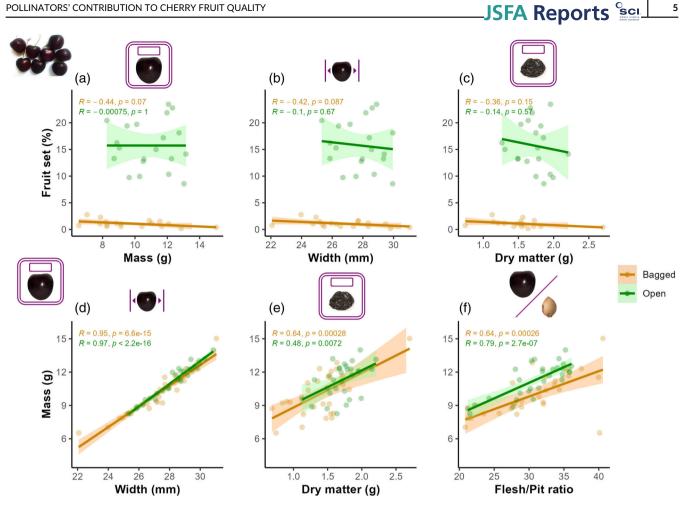


FIGURE 2 Linear regressions. Linear regressions (Im) between fruit set (%) with (a) fresh mass (g), (b) width (mm) and (c) dry matter (g) in 2018-2019, and between fresh mass with (d) width (mm), (e) dry matter (g) and (f) flesh/pit ratio in 2017-2019 for each pollination treatment. Coloured dots represent average values per orchard and year.

finding observed in other pollinator-dependent crops, including strawberry,<sup>9</sup> apple,<sup>32</sup> and pear.<sup>33</sup> Globally, fruit guality is increased by  ${\sim}23\%$  with animal pollination, but contribution to fruit quality parameters can differ among crops.<sup>34</sup> For example, pollinators increased mass in strawberry and apple but not in pear, although this could also be influenced by environmental factors and the cultivars investigated.<sup>9,32,33</sup> Additionally, in some of these studies, fruits were also firmer with insect pollination, but not in our study. Firmness could have been more affected by the polytunnels than the other parameters, since covered trees produce larger but less firm cherries than open orchards.<sup>18</sup>

Commercially, fruit mass and width are two of the most important attributes of cherries<sup>7,26,27</sup> and a mass of 10-12 g and widths of 28-30 mm are standard fruit characteristics in Kordia.<sup>31,35,36</sup> However, due to annual and regional variation, mass and width can be as low as

8.9 g<sup>37</sup> and 26.4 mm,<sup>38</sup> or as high as 13.8 g and 30.7 mm.<sup>39</sup> Our fruit mass and width of 11.6 g and 28.7 mm, respectively, from open blossoms fell into the commercial standards for Kordia, but values from bagged blossoms (9.2 g and 26.4 mm) fell into the lowest values, which could negatively impact the amount of marketable fruit.9,27 Thus, our study shows the important contribution of pollinators to mass and size, providing a pollinators' contribution in mass-width quality of 13.9% (Table S3), an ecosystem service value, which, to the best of our knowledge, has never been previously demonstrated. Our firmness at  $\sim$ 65 Durofel units with both pollination treatments and dry matter with open pollinated blossoms of 1.7 g (=14.7 g/100 g of fresh mass) were similar to other studies on Kordia at 62.4-63.8 Durofel units and 16.1 g/100 g of fresh mass, respectively.<sup>16,37,38</sup> Since the pollination treatment did not affect firmness, a percentage difference in firmness between bagged and open blossoms (0.2%) was not

FIGURE 1 Fruit quality measurements. Mean ± SD (black dots and lines) of (a) fruit set (%) recorded between 2018 and 2019 and fruit quality parameters including (b) fresh mass (g), (c) height (mm), (d) width (mm), (e) length (mm), (f) firmness (Durofel units), (g) dry matter (g), (h) pit mass (g), (i) pit height (mm), (j) pit width (mm), (k) pit length (mm) and (l) flesh/pit ratio of Kordia cherry fruits assessed between 2017 and 2019 (length and pit length were not measured in 2018) according to pollination treatment. Coloured dots represent individual cherries/pits.

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expected. Although mass and width may be important quality parameters in cherry, the impact of insect pollination on other quality parameters (e.g., nutritional composition and soluble solids concentration) still needs to be quantified.<sup>16,22,31</sup>

Interestingly, however, the non-significant relationship between fruit set and quality in either pollination treatment (Figure 2a-c) suggests trees did not carry excessive fruit burden (crop load), because greater fruit burdens on trees usually lead to the production of smaller fruit (i.e., negative relationship between fruit set with mass and size).<sup>7</sup> Trees failing to produce a greater number of fruits might have been as a result of pollination deficits (i.e., insufficient pollination), which were recorded in these orchards.<sup>6</sup> Additionally, the highly significant positive relationships between fresh mass with width, dry matter, and flesh/pit ratio suggest that fruits were proportionally produced (i.e., the greater mass, the greater size, the greater flesh), with more edible flesh from open pollinated blossoms even though pit size from bagged blossoms was smaller than with open blossoms.

## CONCLUSION

Knowing to what extent pollinators contribute, not only to fruit set but also to fruit quality in sweet cherry, facilitates decision making with regard to pollinator management. Thus, due to this valuable contribution insect pollinators make to sweet cherry fruit set and quality, we recommend sweet cherry growers apply effective pollinator management strategies in their orchards to maximize marketable yields. These management strategies could include supplying floral resources outside the main cherry blooming period<sup>6,40</sup> and habitat for nesting.<sup>41</sup>

#### ACKNOWLEDGMENTS

We would like to thank Waitrose & Partners, Berry Gardens Ltd., and the University of Worcester for funding this project, the growers for allowing us to use their orchards, and the East Malling Trust for the use of facilities for fruit quality assessments.

#### DATA AVAILABILITY STATEMENT

Data are available on GitHub at https://github.com/ZeusMF/Cherryfruit-quality\_Mateos-Fierro.

#### ORCID

Zeus Mateos-Fierro <sup>1</sup> https://orcid.org/0000-0002-6970-6533 Michael P. D. Garratt <sup>1</sup> https://orcid.org/0000-0002-0196-6013 Michelle T. Fountain <sup>1</sup> https://orcid.org/0000-0002-1317-4830 Kate Ashbrook <sup>1</sup> https://orcid.org/0000-0001-6555-8791 Duncan B. Westbury <sup>1</sup> https://orcid.org/0000-0001-7094-0362

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Mateos-Fierro Z, Garratt MPD, Fountain MT, Ashbrook K, Westbury DB. Contribution of pollinators to delivering fruit quality in commercial sweet cherry orchards. JSFA Reports. 2025. <u>https://doi.org/10.</u> <u>1002/jsf2.70012</u>