Title:

Influence of seed set on apple symmetry assessed by image analysis: an overlooked effect of pollination on fruit shape

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Highlights:

- An image analysis tool to asses pollination effect on apple symmetry was developed
- The pollination effect on symmetry was positive but commercial class dependent
- Image analysis is more precise to asses symmetry than previous used methods
- Pollination has stronger link with fruit symmetry than with fruit weight

Abstract:

The role of pollination ecosystem service on crop yield and some quality parameters have been largely studied. Nevertheless, the effect of pollination on fruit appearance is still a poorly explored field and it has been assessed by means of oversimplified measurements. Here, we developed an image analysis tool to test the hypothesis that pollination greatly affects the symmetry of one of the most consumed fruit worldwide: the apple. The hypothesis was tested for three commercial classes separately, to understand whether the pollination effect was consistent among classes. We also compared the performance of the image analysis tool with a simplified symmetry measurement that has been previously used. Finally, the effect of pollination on apple symmetry was compared with the effect on weight. We quantified the amount of non-symmetrical area of harvested Gala cultivar apples using two-dimension images of fruits sectioned along the longitudinal plane. We showed that increasing pollination led to significant increase of apple symmetry. The image analysis tool resulted more precise to assess the pollination effect on fruit symmetry than the previously used symmetry measurement, leading to the conclusion that pollination effect on apple shape has been probably overlooked because of the use of oversimplified symmetry measures. The effect of pollination on apple symmetry was not consistent among commercial classes since it resulted significant for little and medium size apples but unimportant for large size apples. Differently from the symmetry, apple weight appeared as not influenced by the pollination. Our findings suggest that the effect of pollination on fruit production should be assessed taking into account different variables and different commercial classes of apple.

Keywords:

Fruit growth, *Malus domestica*, Gala, fruit quality, fruit shape, axi-symmetric fruit

Abbreviations

ES: ecosystem services

SDR: Symmetry difference ratio

1. Introduction

Intensive agriculture has reached high crop production by suppling a heavy amount of inputs (e.g. fertilizers, water, pesticides) (Pretty, 2008; Tilman et al., 2002). However, the challenge of increasing yields to satisfy growing food demand, should be matched with the urgent need to minimize the impact on the environment and on human health due to the excessive use of such agricultural inputs (Godfray et al., 2010; Pretty, 2008). This challenge can be addressed by an “ecological intensification”, that means maintaining and integrating ecosystem services (ES) (e.g. soil fertility, water retention, pollination, etc.) to support crop production (Bommarco et al., 2013). ES can replace or even boost benefits from agricultural inputs (Blaauw and Isaacs, 2014; Davis et al., 2012). To promote more sustainable agricultural practices, broadening knowledge on ES effects on production is needed. This issue is more effectively addressed by means of commercial variables (e.g. fruit grading, colour, quality etc.) that are straightforward and thus they are capable to convince farmers of ES benefits (Kleijn et al., 2019). Focusing on the horticultural sector, one important parameter is the fruit appearance. Fruit size, shape and colour are features that influence consumer choice (Djekic et al., 2019; Loebnitz and Grunert, 2018; Wendin et al., 2019), and are increasingly taken into account by farmers when they plan agricultural management. Indeed, a good fruit appearance may reduce the amount of fruits excluded from sale and thus, food waste.

Among ES, pollination supports the production in about 75% of most important crops (Garibaldi et al., 2013; Klein et al., 2007). On the other hand, pollen limitation has been linked to yield loss and quality reduction of different crops (Bommarco et al., 2012; Garratt et al., 2014; Klatt et al., 2013; Tamburini et al., 2016). Worldwide, the expansion of pollination-dependent crops has accelerated (Aizen et al., 2019); simultaneously, the overall amount of pollination ES has dropped because of the decline of pollinators, particularly the bees (Herrera, 2020; Potts et al., 2010; Zattara and Aizen, 2021). This mismatch between pollination demands and pollination ES provisions has raised general concern on effects on global food production (Potts et al., 2016; Vanbergen et al., 2013). However, the relative importance of pollination ES in ensuring yield and quality of pollinator-dependent crops is not ubiquitous, but it depends on crop and cultivar traits (Garibaldi et al., 2013; Geslin et al., 2017) and on the interaction between pollination and other agronomic and abiotic factors that contribute to modify plant physiology (Tamburini et al., 2019).

As pollination ES is among the critical factors regulating fruit grown, it affects also fruit appearance and its marketability. Traditionally, pollination effect on fruit appearance has been evaluated as fruit size that is usually strongly positively correlated with fruit weight (e.g. Garratt et al., 2014 for apple; Ryder et al., 2020 for cherry; Quinet and Jacquemart., 2017 for pear and Sáez et al., 2019 for kiwifruit). Nevertheless, few studies considered fruit shape and malformations as pollination-dependent parameters (e.g. Andersson et al., 2012 for strawberry; Xiao et al., 2009 for tomato and Sheffield, 2014 for apple).

Apple is a pollination-dependent crop cultivated worldwide, and it is one of the four most-consumed fruits (Musacchi and Serra, 2018). It is also widely used as a model in studies focused on fruit development (Eccher et al., 2014). Apple seed fertilisation through cross-pollination is needed for fruit development, as parthenocarpy and self-fertilization are unusual (Galimba et al., 2019; Musacchi and Serra, 2018). The number of fertilized seeds per apple is a measure of pollination ES extent. Fertilized seeds are producers of phytohormones involved in fruit abscission (Ferrero et al., 2015), and they are also associated with fruit growth. Apple fruit growth is a complex process in which many endogenous and exogenous factors are involved (Musacchi and Serra, 2018). Despite this complexity, several studies pointed out a positive effect of pollination on apple weight (Buccheri and Vaio, 2006; Webber et al., 2020) and on apple appearance (Buccheri and Vaio, 2006; Garratt et al., 2014). The majority of the studies focusing on the pollination effect on apple appearance have explored the differences between deformed and regular fruits (Buccheri and Vaio, 2006; Elsysy et al., 2019; Herrmann et al., 2019; Matsumoto et al., 2012; Wu et al., 2021), whereas quantitative measures to assess the regularity of fruit shapes have been rarely adopted (Carisio et al., 2020;

Sheffield, 2014). In these last cases, fruit shape measurements were oversimplified (e.g. ratio between maximum and minimum fruit heights), thus they may be not capable to account for overall irregularities of fruit shape. In addition, the pollination effect, either on apple weight or on apple appearance, has been seldom analysed among commercial classes (but see Webber et al., 2020). From farmer's perspective, it is pivotal to understand if pollination may be a relevant factor to obtain apples fitting the attributes of the most valuable commercial class.

A more precise tool to analyse fruit shape is image analysis. Image analysis have been developed to measure agricultural products (Sabliov et al., 2002). Focusing on fruits, it has been adopted to measure volume and estimate mass (Vivek Venkatesh et al., 2015), to characterize the cultivars of different crops (e.g. common bean (Kara et al., 2013), cherry (Beyer et al., 2002),) including apple (Currie et al., 2000; Malladi et al., 2020) and also to investigate apple growth process (Jing and Malladi, 2020). However, to our knowledge, image analysis has never been used to explore the pollination effect on apple appearance. Moreover, most of the analysis on fruit image have been developed by the use of sophisticated equipment or implementing not-open-access software code. These factors can be considered serious obstacles to reproducing results and replicating experiments in agricultural science.

In this study, we implemented an image analysis tool using R language and R libraries by means of scanner pictures, with the aim of exploring the effect of pollination, intended as number of seeds per apple, on apple symmetry. Notably, we tested for the following hypothesis:

H1) Pollination has a positive effect on fruit symmetry, thus we expect that the number of seeds increases with fruit symmetry, and we tested the hypothesis between different commercial classes to understand whether the pollination effect is consistent among classes.

H2) The image analysis is more precise to assess pollination effect on symmetry than biometric measurements feasible in field data recording and we tested this hypothesis comparing our symmetry measurement with a Symmetry index used by (Sheffield, 2014) which is based on calliper measurements.

H3) The strength of pollination effect on symmetry is different from that on fruit weight, thus we expect that the pollination effect on symmetry is stronger and more evident than that on weight.

2. Materials and methods

2.1 Pollination and fruit samples

We used apples of Gala cultivar which has been one of the most adopted in apple pollination studies (Carisio et al., 2020; Elsysy et al., 2019; Garratt et al., 2014; Webber et al., 2020). The number of seed per fruit, namely the seed set, has been largely used as proxy of pollination efficiency. Accordingly, from now on, we will refer to a higher or lower pollination as equal to a greater or lower number of seed per apple.

We used for the analysis apples of three commercial classes: 104 apples of waste class (diameter < 65 mm, low colour intensity), this class is destined to juice industry, 133 of medium class (70 mm < diameter < 75 mm, good colour intensity), and 88 of premium class (85 mm < diameter < 90 mm, good colour intensity). The three commercial classes have respectively the lowest, the intermediate and highest price in the Italian market. Fruits were provided in November 2020 by a large-scale apple distribution industry and stored in a refrigerated room until analysis, which was carried out in the last two weeks of November. Before performing the fruit image analysis, all apples were weighted.

2.2 Fruit image and photo processing

Apples were sectioned along the longitudinal plane. The vertical plane incision passed through the sepal-end cavity and the pedicel-joint (Fig. 1a). We counted total amount of seeds per apple (between 0 and 10 in Gala cultivar). Each apple sections was placed, separately, on an HP 4520 printer and scanned. The image was saved in jpg format with 300 dpi resolution. After preliminary analysis, it was verified inconsistency between pixel counts of the same apple section (see 2.3 for the details on measurements), due to section position over the scanner surface. To adjust scanner measurements, we used a calibrated blue square of known area that was pinned under the apple cut surface before scansion (Fig. 1a). In this way, pixel counts of the same apple section became irrelevant, regardless its position on the scanner. Extraneous objects present in scansion, such as dust, were removed by the function “subtract background” of ImageJ (1.53e). Then per each image, we added two green dots, one over the pedicel-joint and the second on the sepal-end (Fig. 1a). These were aimed to adjust apple rotation and to identify apple central axis in the image analyses process. A mismatched fruit orientation would affect measurement of apple symmetry. After obtaining two images per apple, we performed apple image analysis by means of R language code (see Carisio, 2021 for analysis code). The analysis was executed in R 4.0.3, using the packages OpenImageR (Mouselimis et al., 2020) for the photo editing (crop and rotate) and countcolor (Weller, 2019) to isolate the pixels of the scanned objects (apple and the reference square). The image analysis steps are graphically summarized in Fig 1 as follows: 1) photo was rotated to have the y-axis passing across the green dots. 2) Pixels belonging to apple image and blue square were selected through the identification of specific RGB colour frequencies ranges. (Fig 1b). All selected pixels were counted. The measure of apple surface in cm^2 was calculated by the formula:

$$S = (k * Pa + Ps) / Ps$$

Where k is the known area of the attached square (4 cm^2), Pa and Ps are the number of pixels that belong to apple and to the square surfaces respectively. 3) We identified apple central axis as the line between the green dots (Fig 1a). The photo was cropped on the apple central axis and the right image cut was flipped in order to have the two photos with the same orientation on x and y axes (Fig 1c) 4) Apple pixels were transformed in data points (a pixel for each data point) of x and y coordinates for both images and stored in two different databases (Fig 1d). The height of each of apple halves was measured by the difference of y axis values between the highest and the lowest point of the database. This procedure simulates the measure of apple heights by means of a calliper 5) We contrasted the two databases of right and left halves and we isolated all points which were unique for one of the databases (Fig 1e). These selected points correspond to the no-overlapping area between the left and the right apple halves. The amount of no-overlapping points were counted and transformed in a cm^2 measure by the formula:

$$OV = (k * Po) / Ps$$

Where *k* is the known-measure of the attached square, *Po* and *Ps* are the number of pixels of the no-overlapping area and of the square surfaces respectively.

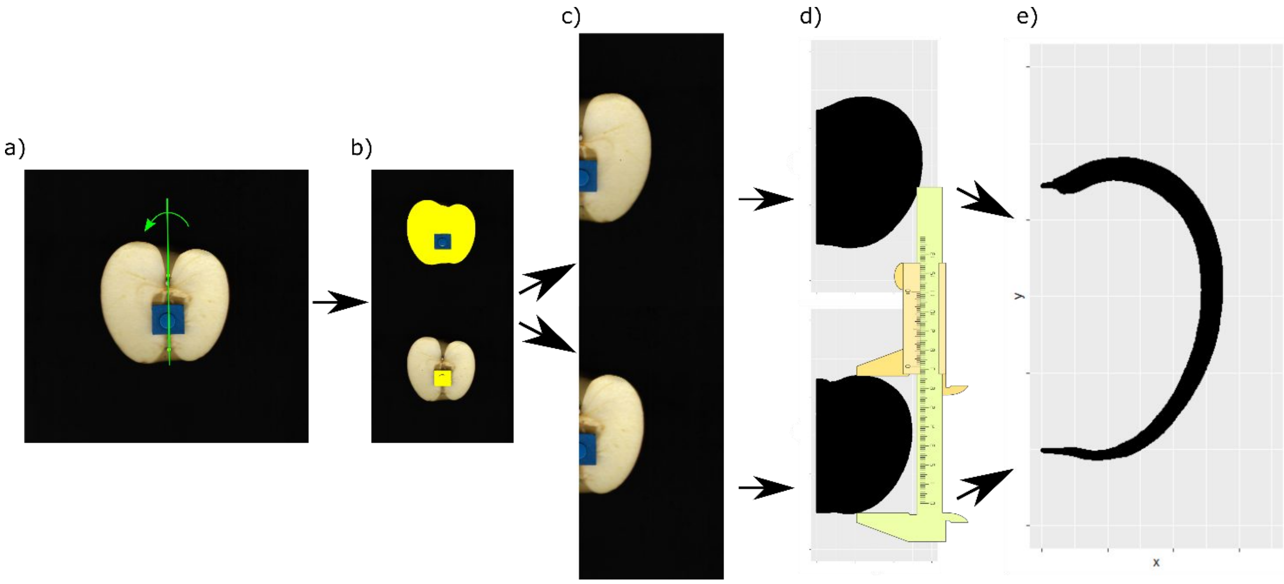

Fig 1. Photo analysis process: a) rotation of apple; b) identified pixel (yellow colour) of the apple and of the square respectively; c) apple photo cropping and flipping to same axis orientation; d) transformation of pixels to data points and measurement of apple height simulating calliper measurement; e) isolation of no-overlapping area between the apple halves.

2.3 Variables and Statistical analysis

To assess the effect of the seed set on symmetry and the difference in symmetry between apple classes we considered three variables: the symmetry index according to Sheffield, 2014, the no-overlapping area and the symmetry difference ratio (SDR). The Sheffield's symmetry index is calculated as the ratio between minimum and maximum apple height. This index has been proved as the most correlated to seed number and it is easy to measure in field experiments since is based on calliper measurements (Sheffield, 2014). The second variable is the measure in cm^2 of the no-overlapping area between the left and the right apple halves; the third variable, SDR, is calculated as the percentage ratio between the no-overlapping area and the total scanned apple surface. A 0% SDR indicates a perfect symmetry and correspondence between apple halves, otherwise, a value of 100% means total miss-match between halves. This last variable was adopted in order to normalise the measure of symmetry between apple classes despite their differences in volume. Since we measured the three variables for both sections of each apple, we used for the analysis the averaged measures between the two observations. This allows to reduce the errors due to apple cut impreciseness. To test the effect of pollination on symmetry and if the effect is different between apple commercial classes, we used generalized mixed models (glm) with Gaussian distribution and logarithmic link function. In these, SDR was used as explanatory variable and seed set as fixed effect. At first, model was implemented using all data, and then we implemented a model per each apple class. To test the differences in SDR, no-overlapping area and seed set between apple commercial classes, we adopted a glm with Gaussian distribution and logarithmic link function except for seed set which was analysed using a Poisson distribution. The difference between classes were validated by a Tuckey post-hoc test. The effect of seed set on the symmetry index and the relationship between the symmetry index and SDR were tested by a glm with Gamma distribution and logarithmic link function. The effect of seed set on apple weight was analysed by a linear model per each apple class. The diagnostic of all models was performed checking for patterns in model residuals. All statistical analyses were performed with R 4.0.3 (see Carisio, 2021 for statistical analysis code).

3. Results

Overall, the pollination affected apple symmetry as we found a negative correlation between SDR and seed set (Tab1 a, Fig. 4a). However, when we distinguished the effect by each apple class, we found that seed set had a significant effect on the waste and the medium classes, whereas it was not influential in premium class (Fig 2, Tab1.). The pollination effect was more evident in the waste class in which the increase of the number of seeds caused an estimated reduction of SDR from 18.50 % to 4.88 % and less pronounced for medium class, as SDR decrease ranged from 9.32 % to 4.88 %.

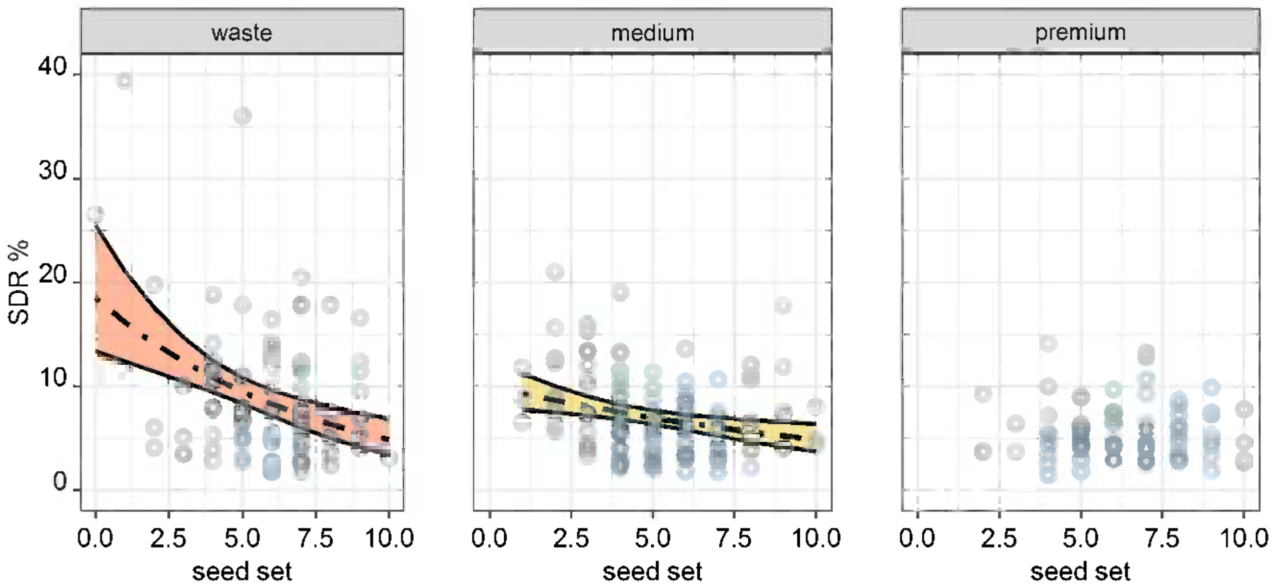

Fig 2. Scatterplot between seed set and SDR in waste, medium and premium apple classes. For significant relationships (waste and medium), the marginal effect was added. Coloured areas indicate 95 % confidence intervals.

Tab1. Generalized mixed model outputs for model with all data a), and models with only Premium b), Medium c) and Waste d) apple class data.

	Estimate	Std. Error	t value	*P*	R^2
a) Overall data					0.700
Intercept	2.4166	0.093	25.88	<0.0001	
seed set	-0.0818	0.017	-4.72	<0.0001	
b) Premium					0.015
Intercept	1.770	0.174	10.15	<0.0001	
seed set	-0.012	0.026	-0.43	0.664	
c) Medium					0.578
Intercept	2.305	0.115	20.06	<0.0001	
seed set	-0.072	0.023	-3.06	0.0027	
d) Waste					0.986
Intercept	2.918	0.164	17.74	<0.0001	
seed set	-0.133	0.031	-4.25	<0.0001	

The SDR showed significant differences between apple classes (Fig. 3a): waste apple class had the greatest SDR (mean ± sd = 8.89 ± 6.41 %) followed by the medium class (mean ± sd = 7.02 ± 3.78 %) and by the premium class (mean ± sd = 5.46 ± 2.59 %). The no-overlapping area was similar between the three classes (respectively mean ± sd = 2.89 ± 1.40 cm2, 2.39 ± 1.27 cm2, 2.41 ± 1.75 cm2), and only the difference between medium and premium class was slightly significant (Fig. 3b). Seed set was higher for premium class apples (mean ± sd = 6.35 ± 1.91) and it was significantly different (Fig. 3c) from seed set of medium class apples (mean ± sd = 5.06 ± 2.05). Waste class apples had greater seed set (mean ± sd = 5.88 ± 1.92) than the medium class, but did not differ from the premium class.

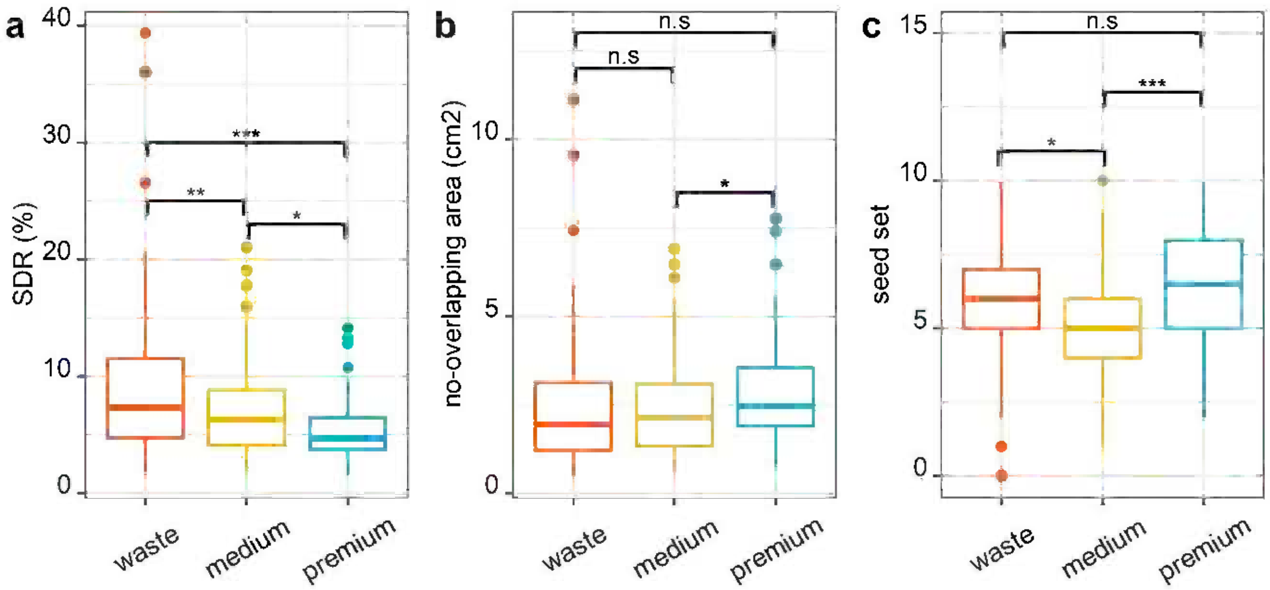

Fig 3. Differences between commercial classes for the variables: a) SDR, b) no overlapping area and c) the total amount of seeds per apple.

The symmetry index was significantly affected by the seed set (β = 0.007 ± 0.001, p value < 0.0001, Fig. 4b). The relationship between the symmetry index and SDR was strong (β = 0.012 ± 0.000, p value < 0.0001, R^2 = 0.899 Fig 4c). However, the model that adopted SDR as symmetry measure was more explanatory than the model that used the symmetry index (R^2 = 0 .700 for SDR and R^2 = 0 .053 for symmetry index).

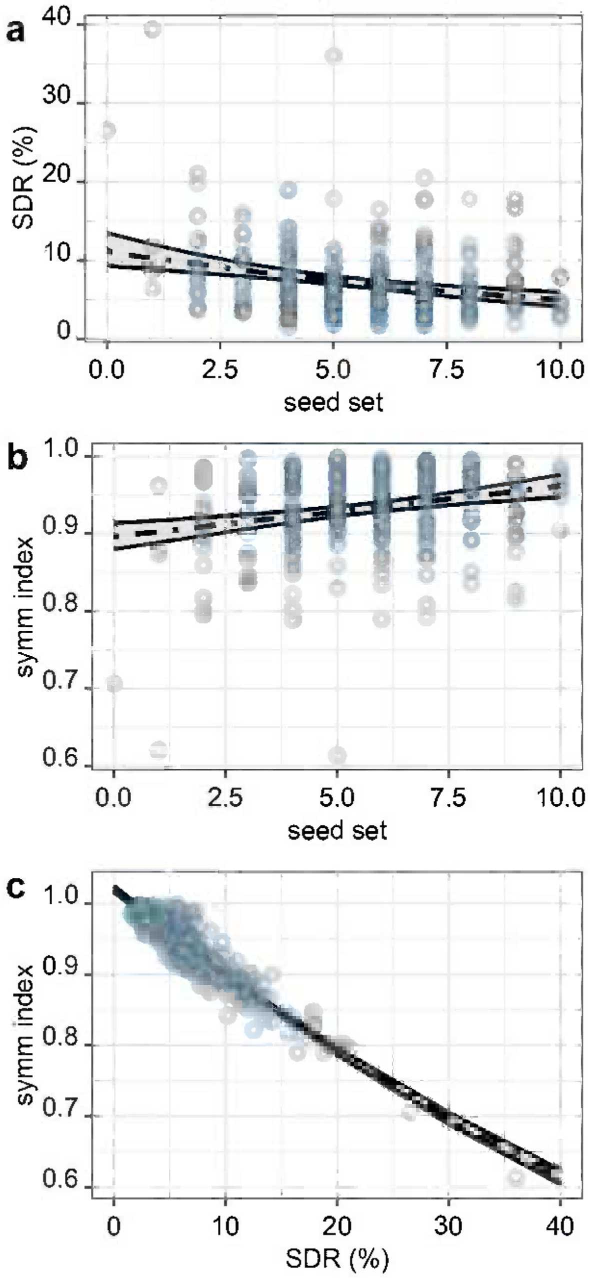

Fig4. Marginal effect of seed set a) over SDR and b) over the symmetry index using all data. c) Relationship between the symmetry index and SDR.

Apple weight was not significantly influenced by seed set for all the three apple classes (Tab. S2, Fig S1).

4. Discussion

Image analysis has been employed for measurement of many agricultural products (Sabliov et al., 2002; Vivek Venkatesh et al., 2015). This study constitutes the first attempt to assess the effect of pollination ecosystem services in fruit symmetry by means of an image analysis tool. This tool was implemented aiming to provide an open access software code that allows to potentially reproduce the symmetry analysis on a wide range of axi-symmetric fruits. The advancement of a low-impact farming trough ecological intensification needs a better understanding of the positive effects of ecosystem services on agricultural production. Our study demonstrated a strong effect of pollination on fruit symmetry. In particular, we found that an increasing number of fertilized seeds led to significant increase of apple symmetry. When seed number was maximum, apples had in average 5% of asymmetrical scanned surface, whereas under low pollination, the non-symmetrical area was about the double. This result aligns with previous studies that have identified a positive effect of seed number and seed distribution in apple symmetry for 'McIntosh', 'Cortland' (Sheffield, 2014) and 'Fuji' (Wu et al., 2021), although the effects detected by the two studies were slight. In addition, our result differs from a previous report of lack of pollination effect on 'Gala' symmetry (Carisio et al., 2020). Due to either low or null effect, these existing studies suggest that pollination affects fruit symmetry minimally. In contrast, our study proved a strong effect of seed set on 'Gala' apples symmetry, leading to the conclusion that the role of pollination on defining apple shape has been probably overlooked. The concept of an overlooked effect of pollination is supported by the comparison between our image analysis measurement and the ratio between minimum and maxim apple height (symmetry index), which has been employed as symmetry measurement on previous studies (Carisio et al., 2020; Sheffield, 2014). In particular, we found that the correlation between the image analysis measurement with seed set was greater than that between the symmetry index and the seed set. This result indicates that the symmetry index is inaccurate to assess pollination effect on fruit symmetry. Although it has not been tested, it is likely a lack of reliability for further apple symmetry measurements, as for example the subjective distinction between deformed and regular apples that have been employed in several studies on apple pollination (Matsumoto et al., 2012; Wu et al., 2021). Despite the impreciseness in assessing fruit symmetry, the symmetry index was found lightly correlated with seed set so it could still be a valuable approach for field measurements.

Surprisingly, we found that the positive effect of the seed set on fruit symmetry was not consistent among the three commercial classes employed for the analysis. All except one, the greatest size commercial class (here called premium), had the symmetry influenced by the seed set. Premium apple class showed also an average standardized non-symmetrical area significantly lower than the other two commercial classes. We advance two hypotheses to explain such a lack of effect of seed set on the premium commercial class. The first one is that premium class apples have been not influenced because they had an extraordinarily low rate of apple with few seeds. Premium apples showed in average a greater number of seed than medium size apple; in addition, 94% of premium apples had more than three seeds. Therefore, in premium class, low seed set apples were rare, as well as in non-symmetric apples. The second hypothesis is that the premium class had a growing process less influenced by pollination. Pollination benefits on yield and on fruit growth may be additive, synergic, or replaced by the effect of other fruit growth factors (Tamburini et al., 2019). In the case of premium apple, it is likely that a trade-off between pollination benefits and benefits due to other growth factors has occurred, and therefore pollination resulted as not influential. For example, the flower position as growing factor may neutralize pollination effect: central fruitlet within the 4:6-fruitlets corymb cluster, has shown to dominate the lateral fruitlets in resources intake, regardless the number of fertilized seeds (Bangerth, 2000; Jakopic et al., 2015). Furthermore, another aspect of apple growth process might have been influential. After seeds fertilization, apple developing is exponential with intensive cells production (Bain and Robertson, 1951; Jing and Malladi, 2020). Then, the growth slows down and most of the apple development is due to cell expansion (Malladi and Johnson, 2011). However, the cell expansion can reach a plateau since it continues until space between cells is still available. As it has been observed by Bain and Robertson (1954), larger apples differing each other in volume, had the same space between cells. This means that in case of larger size fruit, such as the premium class, a cell packing threshold is reached. Therefore, we speculate that premium apples, that were non-symmetrical in the first step of growth process, became symmetrical during cell expansion because of a compensation process: higher cell expansion in apple parts where cell-packing was not reached.

In our study, we found that the average amount of the non-symmetrical surface between commercial classes was either not different or lightly contrasting, although differences between classes were significant when the symmetry measurement was normalized with overall apple scanned surface. The lowest-size apples had the highest average amount of normalized non-symmetric surface. Furthermore, this commercial class had fruit symmetry most affected by seed set. For lowest-size class, a symmetric shape is less important from a commercial standpoint, since it is destined to juice industry.

We are aware that observed symmetry differences between apple commercial classes might be the result of a sampling bias and that our data do not allow to understand which were the biological processes that led to the symmetry differences. However, our results revealed potential differences in pollination effect on fruit growth process between different sizes of apples. In addition, they suggest that pollination is important for having symmetric low-medium size apples, whereas it is not fundamental regarding larger size apples. We believe that an approach distinguishing between commercial classes is pivotal to provide clear indications to farmers on the benefits of pollination ecosystem service.

In our study, we did not find an effect of seed set on apple weight. A positive effect of pollination on apple weight was found in several studies, some of which focused on 'Gala' (Buccheri and Vaio, 2006; Sheffield, 2014; Webber et al., 2020), whereas other did not find such a positive effect (Carisio et al., 2020; Wu et al., 2021). This confirms that seed set has and indirect link with apple weight. Indeed, seed set affects the crop load and thus growth resource distribution by regulating the fruitlet abscission, which usually targets fruitlets that were poorly pollinated (Ferree and Warrington, 2003). In addition, many low seed number apples are likely of being removed through the season by hand thinning because they should be more subjected to poor development (Garratt et al., 2014). As a result, the weight of harvested apples appears as marginally affected by seed set because most of low seed number apples were previously discarded. This leads to a pollination effect on apple weight that it is usually, as in our study, not detectable. This result underlines that a communication to farmer on pollination benefit only focused on weight gain could be misleading, since the effect on harvested fruits is not commonly evident. We suggest including in to-farmer communications also other variables such as quality, grading and shape fruit parameters which contribute in determining crop production value (Kleijn et al., 2019). Although apple commercial classes in Europe do not take into account fruit shape and symmetry, in grading process deformed fruits are usually excluded from market (Musacchi and Serra, 2018). Our results highlight that pollination is a critical pre-harvest factor in fruit development and it should be properly managed to reduce production losses.

5. Conclusions

The challenge to maintain/increase fruit production, and at the same time to reduce agricultural negative impacts, requires an extensive understanding of the benefits given by ecosystem services. The benefits of pollination ecosystem service to yield and quality of different fruits have been well studied. However, focusing on the quality parameter of apple symmetry, the effect of pollination has been tested by simple measurement or visual assessments. Here, we showed through a precise and simple image analysis approach that pollination greatly affects the symmetry of Gala apples and that the effect was not consistent across different commercial classes. The image analysis tool developed as an open-access resource within this study could be applied on a wide range of axi-symmetric fruits testing different hypotheses. The biological reason that has led to an effect of pollination on apple symmetry that changed according to commercial classes should be investigated in future researches. Moreover, we encourage the research community to evaluate ecosystem service benefits taking into account a broad number of fruit quality variables and distinguishing the effects between different commercial classes. This approach could allow a more practical understanding of ecosystem service that it may be more effectively transferred to farmers.

Acknowledgements

We thank D. Nari for providing technical support, G. Rivoira for the help to get experimental material and B. Moroni for proof-reading the manuscript.

Authors contribution

Conceptualization, LC, SS, AM; Data curation, LC; Formal Analysis, LC, AM; Funding acquisition MP; Investigation, LC; Fieldwork LC; Project administration, LC, MP; Supervision, MP; Visualization, LC; Writing – original draft, LC; Writing – review & editing, LC, SS, AM, MP.

Funding

This work was funded by Department of Agricultural, Forestry and Food Science, University of Turin.

Conflicts of interest/Competing interests The authors have no conflict of interest to declare that are relevant to the content of this article.

Availability of data and material The dataset generated and analysed during the current study is available in the OSF repository, https://osf.io/y3q5s/.

Code availability R code generated during the current study is available in the OSF repository, https://osf.io/y3q5s/.

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