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Pollination as a key management tool in crop production: Kiwifruit orchards as a study case

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ABSTRACT

Despite the key role of pollination in the production of many crops, this is still one of the least understood factors in the orchard context. Pollen limitation, and impacts on crop production, is influenced by several potentially interacting factors such as crop or crop variety degree of dependence on cross pollination and on pollinators, diversity and abundance of the pollinator communities, landscape context and climate conditions. Understanding the pollination needs within an orchard is key to determine if the pollination is optimal or if it needs improvement. In some crops, such as kiwifruit, artificial pollination may be required where and when natural pollination cannot be improved or there is lack of pollen. In this study we quantified improvements to productivity resulting from artificial pollination and the efficiency of the technique for kiwifruit production and monetary gain on seven orchards distributed over the production range of this crop in Portugal. For that, we quantified orchard yield under 1) current natural pollination services, including pollination provided by wind and naturally occurring pollinator communities, 2) after artificial pollination and 3) under optimal pollination services. We characterized fruit production and key fruit traits such as fruit weight, size and caliber, and used production values, fruit caliber and respective market values to calculate the economic impact of pollination improvement. Results showed that pollen supply improved kiwifruit production in most orchards, either by changes in fruit set, fruit weight or both, and/or changes in fruit distribution by caliber and category. However, artificial pollination was not always efficient and/or needed. Contrarily to the expected, the artificial pollination treatment did not increase fruit weight but, in some orchards, it resulted in higher proportion of high-quality market fruits and/or lower proportion of unmarketable fruits. The changes in fruit traits translated into a tendency towards a small to moderate increase in monetary gain in four out of the seven orchards. This study reinforces the need to understand the current status of pollination services within the orchard context and to look at artificial pollination as a management tool in kiwifruit production. We conclude that pollination services in the study region might be sufficient to attain profitable yields; however, artificial pollination could be a useful tool under unpredictable pollination scenarios, but reviews of the efficiency of the methodologies used in this region are still necessary.

1. Introduction

Pollination is a key feature in the production of many crops being an important part of the economy of crop production (Garratt et al., 2014; Klein et al., 2007). Pollination can be defined as the delivery of pollen from the anthers to the stigma, by biotic or abiotic vectors, involving the removal of pollen from the anthers, its transportation, and its deposition on a receptive flower (Calderone, 2012). Pollen limitation can be the result of pollinator limitation, asynchrony between plant flowering and pollinator activity, insufficient pollen production or lack of synchrony between pollen release and stigma receptivity, and can be an important limiting factor to crop production and quality worldwide (Vaissière et al., 2011; Wilcock and Neiland, 2002). However, and despite the increasing number of studies on the importance of pollination and pollinators for crop production and quality (e.g. Blitzer et al., 2016; Garibaldi et al., 2013; Garratt et al., 2014; Klein et al., 2007; Miñarro and Twizell, 2015; Nicholson and Ricketts, 2019), pollination in the orchard context is still one of the least understood management factors (Goodwin, 2012). In this context, there is a number of potentially interacting factors influencing the levels of pollen limitation such as crop species or

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variety, degree of dependence on cross pollination and on pollinator communities' diversity and abundance, landscape context and climate conditions (Connelly et al., 2015; Klein et al., 2007). Understanding pollination needs within the orchard is key to determine if this service is optimal or if it can be improved. Management practices promoting wild pollinators and/or using managed pollinator species have been shown to enhance crop pollination (Garibaldi et al., 2014; Maes and Jacobs, 2017). For instance, management practices aimed at incrementing the richness of flowering plants, such as adding flower strips or margins, and/or reducing the use of pesticides have been shown to promote the diversification of wild pollinators, thereby providing supplemental pollination (Brittain et al., 2010; Garibaldi et al., 2017, 2014; Karamaouna et al., 2019; Pywell et al., 2015). Among managed pollinator species, Apis mellifera is the most widely used, but other social bees, such as some specific species of the genus Bombus, and solitary bees, such as species of the genus Osmia, have also been used to enhance crop pollination (Garibaldi et al., 2017; Pomeroy and Fisher, 2002; Ryder et al., 2020). When and where natural pollination cannot be improved or lack of pollen is observed, artificial pollination may be vital to reach the commercial goals. Although it usually implies high costs and labor and/or mechanical force, this management practice has been used in several crops (e.g. kiwifruit (Tacconi et al., 2016; Tacconi and Michelotti, 2018), pistachio (Karimi et al., 2017), almond (Vaknin et al., 2001), apple, cherry, walnut (Pinillos and Cuevas, 2008), olive tree (Pinillos and Cuevas, 2008; Tacconi and Michelotti, 2018), hazelnut (Ellena et al., 2014)) to overcome pollen limitation.

Kiwifruit (*Actinidia* spp., Actinidiaceae) is a dioecious crop, *i.e.*, male and female flowers grow up on different vines, and pollination is mainly provided by insects and, to a less extent, by wind (Miñarro and Twizell, 2015; Testolin *et al.*, 1991). Fruit size in kiwifruit is correlated with the number of seeds produced, which is dependent on the number and quality of the pollen grains reaching the stigmas (Ferguson, 2013). High quality market fruits (>100 g) have over 1,000 seeds, and to ensure good pollination each female flower should receive estimated numbers of 2, 000-3,000 viable pollen grains (Ferguson, 1984; Tacconi *et al.*, 2016; Testolin *et al.*, 1991). Therefore, efficient pollination is a key aspect in kiwifruit production and economic viability. However, kiwifruit pollination is highly dependent on weather conditions during flowering time, which often compromise insect pollination, as reported by several studies (*e.g.* Miñarro and Twizell, 2015; Oliveira *et al.*, 2009; Testolin *et al.*, 1991; Castro *et al.* submitted). Additionally, the short flowering period, the lack of synchrony between male and female plants and the low number and/or inadequate distribution of males inside orchards may also reduce pollination success (Gonzalez *et al.*, 1998). Management practices such as the use of hail net to reduce damages by wind and decrease the susceptibility to diseases also reduces pollinator activity and visitation rates, affecting pollination levels and fruit production (Evans *et al.*, 2019; Tacconi and Michelotti, 2018). Susceptibility to diseases, such as *Pseudomonas syringae* pv. *actinidiae*, particularly of male plants, can also reduce the vigor of male plants and consequently, the amount of pollen available within the orchards, being dependent on the severity of the outbreak each year (Tacconi and Michelotti, 2018; Castro *et al.*, submitted). In kiwifruit, inefficient pollination leads to unsatisfactory fruit size, shape and uniformity, leading to high percentages of unmarketable fruits (Oliveira *et al.*, 2009; Tacconi *et al.*, 2016), and consequently to reduced market values. As a result, artificial pollination of kiwifruit vines became a widespread practice over the last decade (Tacconi and Michelotti, 2018). In New Zealand and Italy, two of the leading countries in kiwifruit production, various studies have evaluated the amount of pollen needed and the methodology for pollen collection and application, coupled with guidance practices to producers (*e.g.* Goodwin and McBrydie, 2013; NZKGI, 2016; Tacconi *et al.*, 2016; Tacconi and Michelotti, 2018; Testolin and Ferguson, 2009; Williams *et al.*, 2020). In countries with lower but still significant production of kiwifruit, such as Portugal, these practices are far less used (APK, 2007; Oliveira *et al.*, 2009), and very little is known about its need and efficiency at the orchard level.

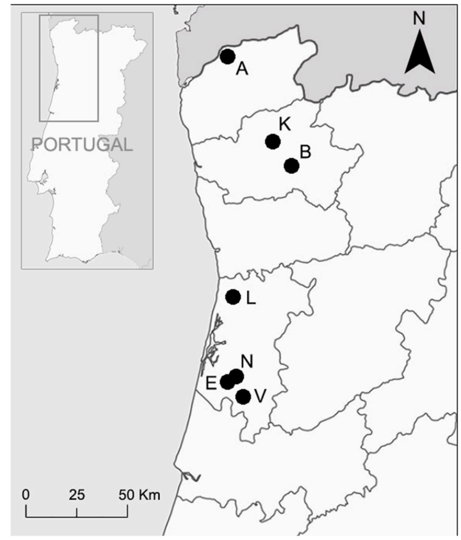

Fig. 1. Distribution of sampling orchards.

In Portugal, the area used for kiwifruit production has grown over the last decades, being currently around 2,736 hectares, and the main areas of production are located in the coastal regions of the North (70%) and Centre (29%) (INE, 2019). Kiwifruit production in Portugal in 2018 reached 34,000 tons and 49% of these were exported, with a value of 19,920 M euros (INE, 2019). The study by Castro *et al* (submitted) has shown that while for many orchards natural pollination is sufficient to produce good quality fruits, others suffer from pollen limitation, and require additional measures to attain higher productivity and monetary gain. In this study, we evaluate improvements to productivity resulting from artificial pollen application and the efficiency of the technique for kiwifruit production and monetary gain on seven orchards distributed over the production range of this crop in Portugal. We hypothesize that if current pollination services are inadequate and artificial pollen application is done efficiently, fruit set and fruit caliber will be improved by artificial pollination, and this will be reflected on overall orchard production and monetary gain. To test our hypothesis, we set up a classical pollination experiment, quantifying orchard yield under 1) current natural pollination services, including pollination provided by wind and naturally occurring pollinator communities; 2) artificial pollination; and 3) optimal pollination services, and characterized fruit production and key fruit traits such as fruit weight, size and caliber. Finally, production values, fruit caliber and respective market values were used to calculate economic impact of artificial pollination.

2. Methods

2.1. Study area

The study was conducted in 2019 in seven kiwifruit orchards in the Centre and North of Portugal representing the production area (Fig. 1). Orchard characteristics such as kiwifruit varieties, ratio of male to female plants, age, area and management practices are provided in Appendix 1. Artificial pollination system followed the dry procedure in all selected orchards and represents the procedure more commonly used in the study region. Commercial pollen was applied by the producers according to the recommendations given by field technicians and consisted of 200 g of pollen per hectare mixed with an inert dispersant, usually *Lycopodium* spores, 400 g per hectare, and applied in cloudy days, with cool mornings, when the relative humidity is over 70%. The application was fast, maintaining a uniform and constant rhythm, and dusting the pollen into the air at 50-60 cm from the flowers to be pollinated. The pollen was applied once during flowering (with > 70% flowers open; field observations) in green pulp kiwifruit and repeated multiple times in yellow pulp kiwifruit.

2.2. Experimental design

The experimental design included three pollination treatments: 1) open pollination (O), which received natural pollination thereby quantifying services provided by natural pollination vectors; 2) artificial pollination (AP), in which, in addition to receiving natural pollination, flowers also received additional pollen, applied in the orchards by the producer using appropriate machinery; and 3) supplementary pollination (S), in which, in addition to receiving natural pollination, female flowers were supplemented by hand with pollen from orchards' male flowers, thereby quantifying yield under optimal pollination services. By combining these three treatments it was possible to quantify improvements to productivity resulting from artificial pollination (open vs. artificial pollination) and the efficiency of the artificial pollination technique (artificial pollination vs. supplementary pollination).

For each orchard and variety, at peak of orchard flowering, we selected 30 female plants separated by at least 3 m, in a line at the middle of the orchard, covering the entire length of the orchard. In each of the 30 female plants, 3 flowers located on the same branch to guarantee similar resource availability were marked and each assigned to a given treatment in the immediate days before artificial pollination. Two receptive flowers with the petals starting to fall and with sticky stigmatic surfaces (Tacconi *et al.*, 2016) were selected for both artificial and supplementary pollinations ensuring that they were receptive at the moment of artificial pollination. Supplementary pollinations were performed by gently rubbing anthers of up to eight freshly collected male flowers with dehiscent pollen (visible by whitish anther pores and visible clouds of pollen when rubbing the flowers) from the available male varieties in the orchard. One flower with stigmatic surfaces no longer receptive (dry and slightly brownish stigmas) was selected for open pollination treatment ensuring that it was no longer receptive when artificial pollination was undertaken. In two of the seven orchards (namely, orchards E and V) it was not possible to have an open treatment because the producers decided to do the artificial pollination before our visit.

In the study region, fruit harvesting starts at end of October until beginning of December. Fruit collection was coordinated with each orchard manager and collected, on average, two days before harvest date indicated by the producer of each orchard. All fruits were weighed and measured for short diameter (at the equatorial zone) and long diameter, as well as observed for deformations and skin damages. According with New Zealand Kiwifruit Growers Incorporated (NZKGI, 2016), these are the most relevant fruit traits in kiwifruit commercial grading.

2.3. Commercial grading and monetary gain

Each sampled kiwifruit was assigned to class I or class II, and within classes they were assigned to one of 11 caliber categories (namely, 18, 20, 23, 25, 27, 30, 33, 36, 39, 42, 46) based on their weight, following standard grading tables provided by the Portuguese Association of Kiwifruit producers (APK). Kiwifruits were assigned to class I or class II based on short to long diameter ratio (S:L), deformation and skin damages. Fruits with S:L below 0.75, deformed or bearing skin damage were assigned to class II. For further analyses fruits were grouped into four groups: calibers 18-30 (C18-30), including fruits weighing over 95 g; calibers 33-46 (C33-46), including fruits weighing between 65 and 95 g; unmarketable (fruits weighing lower than 65 g); and Class II.

We assessed the gain in productivity resulting from artificial pollination by comparing yields under natural pollination (open pollination) and under artificial pollination. To assess if artificial pollination was efficient, we compared production resulting from artificial pollination with that resulting from optimal pollination (supplementary pollination). Orchard production values were provided by the producer and represent the production resulting from artificial pollination; these values were used as basis to estimate production under open and supplementary pollination. First, we used the index of pollen limitation provided by Larson and Barrett (2000): PL = 1 – Treatment1/Treatment2, which provides a measure of the increment in fruit weight provided by Treatment 2 in comparison with Treatment 1, and calculated the increment in fruit weight provided by artificial pollination in comparison with open (PL = 1 – O/AP) and the increment in fruit weight provided by supplementary pollination in comparison with artificial pollination (PL = 1 – AP/S). Production under open pollination was then estimated taking into account the loss in fruit set in open-pollinated fruits compared to artificial pollination and the pollen limitation values calculated from open and artificial pollinated fruits, obtained for each orchard, according to the following equation: Po = Pa - (Pa*PL) - (Pa*Fa), where Po is the production under open pollination, Pa is the production under artificial pollination, PL is the pollen limitation, and Fa is the difference in fruit set between artificial and open pollination treatments. Production under supplementary pollination was estimated taking into account the gain in fruit set in supplementary-pollinated fruits compared to artificial pollination and the pollen limitation values calculated from supplementary and artificial pollinated fruits, obtained for each orchard, according to the following equation: Ps = Pa + (Pa*PL) + (Pa*Fs), where Ps is the production under supplementary pollination, Pa is the production under artificial pollination, PL is the pollen limitation and Fs is the difference in fruit set between artificial and supplementary pollination treatments.

We assessed the increase in monetary gain by comparing the monetary gain under natural pollination and under artificial pollination. Monetary gain was obtained by calculating the amount, in Euros, corresponding to each orchard production, both under open and artificial pollen application, considering the percentage of fruits in each class and caliber, and the corresponding monetary value. Average prices paid to the producer according to class and caliber assignments were provided by APK. Calculations were done according to the following equation:

Monetary gain (euros) = $\sum$ P*Ci*Ei, where P is the production (t/ha) of a given field corresponding to the production value provided by producers (artificial pollination) or natural (calculated as described above), Ci is the proportion of fruits in each class and caliber combination, and Ei is the price, in Euros (€), payed per kg of fruit for each class and caliber combination.

To assess if artificial pollination was efficient, we compared monetary gain resulting from artificial pollination with that resulting from optimal pollination (supplementary pollination), applying the methodology described above.

Using monetary gains obtained with open and artificial pollination and considering an average of 700 €/ha as the cost of pollen acquisition (values obtained from field technicians for green pulp kiwifruit) we calculated the potential economic benefit of using artificial pollination. Due to lack of information, it was not possible to consider the cost for human resources associated with artificial pollination.

2.4. Statistical analyses

Generalized linear models (GLM) were used to explore differences between pollination treatments within orchards. The effect of pollination treatment on fruit set, fruit weight, distribution of fruits per class and caliber categories, production and monetary gain across orchards was analyzed using generalized linear mixed models (GLMM), with pollination treatment as fixed factor, and with orchard and kiwifruit variety nested within orchard, as random factors. Fruit weight, production and monetary gain were analyzed using a Gaussian distribution with the identity link function and fruit set and distribution of fruits per class and caliber categories were analyzed using a binomial distribution with the logit link function. Model validation was performed on the residuals by checking heteroscedasticity and normality (Zuur *et al.*,

Table 1
Fruit set (%) from open (O), artificial pollination (AP) and supplementary pollination (S) for each orchard and variety within the surveyed orchards. X^2 and P values resulting from Generalized Linear Model analysis are shown. Statistically significant differences are highlighted in bold and differences among pollination treatments within orchard at $P < 0.05$ are indicated by different letters. *$P = 0.053$

Orchard	Variety	Open	AP	Supplement	X^2 and P values
A	BoErica	100.00	100.00	100.00	$X^2_{2,\ 87} = 0.00$; $P = 1.000$
B	BoErica	96.77	83.87	96.77	$X^2_{2,\ 90} = 4.61$; $P = 0.100$
	Hayward	93.33	80.00	83.33	$X^2_{2,\ 87} = 2.58$; $P = 0.276$
E	*A. chinensis*	-	80.65	87.10	$X^2_{1,\ 60} = 0.48$; $P = 0.276$
K	BoErica	93.33	93.33	93.33	$X^2_{2,\ 87} = 0.00$; $P = 1.00$
L	**BoErica**	**53.33 (a)**	**63.33 (a)**	**96.67(b)**	$\mathbf{X^2_{2,\ 87} = 18.55}$; $\mathbf{P < 0.001}$
	Hayward	**36.67 (a)**	**63.33 (b)***	**90.00(c)**	$\mathbf{X^2_{2,\ 87} = 19.92}$; $\mathbf{P < 0.001}$
N	**Hayward**	**86.49 (b)**	**53.33 (a)**	**80.00(b)**	$\mathbf{X^2_{2,\ 94} = 9.92}$; $\mathbf{P = 0.007}$
V	Hayward	-	90.00	96.67	$X^2_{1,\ 58} = 1.12$; $P = 0.290$

2009). All analyses were done using R version 3.3.2 (Core Development Team, 2016) using the package "car" for Type-III analysis of variance (Fox and Weisberg, 2019) and "lme4" for generalized linear models (Bates and Mächler, 2015), and TukeyHSD for Multiple comparisons after analysis of variance. "Psych" was used to obtain mean and standard error values (Revelle, 2020).

3. Results

3.1. Pollination treatments

Overall, pollination treatment significantly affected fruit set ($X^2_2 = 18.37$; $P = 0.0001$), with supplementary pollination resulting in higher fruit set than open pollination and artificial pollination (mean ± SE: 91.5 ± 1.9%, 80.28 ± 2.7% and 76.8 ± 2.9%, respectively). Individual analyses at the orchards level showed that, within orchard, significant differences were obtained in orchards L and N (Table 1). Artificial pollination resulted only in a marginally significant increase in fruit set in orchard L for 'Hayward' variety compared to open pollination treatment, and supplementary pollination resulted in higher fruit set than the remaining treatments in orchard L for both varieties (Table 1).

Pollination treatment also affected fruit weight significantly ($F_{2,\ 499} = 14.30$; $P < 0.001$), with supplementary pollination resulting in higher fruit weight than open pollination and artificial pollination (mean ± SE: 106.17 ± 1.40 g, 96.92 ± 1.68 g and 97.93 ± 1.63 g, respectively). Individual analyses at the orchards level revealed significant differences for the orchards K, L (in both varieties), N and V (Fig. 2, Appendix 2). In these orchards three different patterns were observed: artificial pollination was similar to supplementary pollination, and in both treatments significant increases in mean fruit weight were observed when compared to open pollination (orchard L for 'BoErica'; Fig. 2-LBoE); supplementary pollination resulted in significantly heavier fruits than open pollination, and artificial pollination presented intermediate values non significantly different from the other two treatments (orchards K and N; Fig. 2); finally, supplementary pollination resulted in significantly heavier fruits than artificial pollination (orchards V and L for 'Hayward' variety; Fig. 2), with open pollination treatment presenting intermediate values non significantly different from the other two treatments (orchard L; Fig. 2).

Pollination treatment significantly affected the distribution of the fruits by caliber and class categories (C18-30: $X^2_2 = 18.02$, $P = 0.0001$;

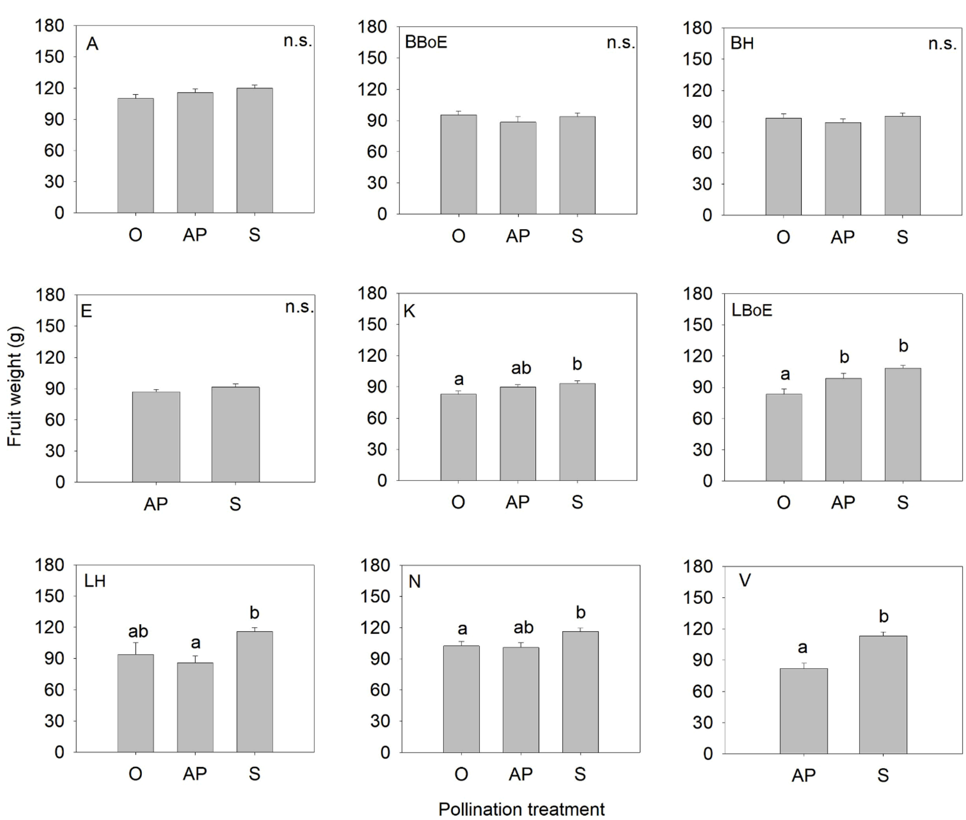

Fig. 2. Mean fruit weight of kiwifruits (g) resulting from open (O), artificial (AP) and supplementary (S) pollination treatments applied in each orchard and variety within the seven orchards surveyed. A - orchard A; BBoE - orchard B, variety 'BoErica'; BH - orchard B, variety 'Hayward'; E - orchard E; K - orchard K; LBoE - orchard L, variety 'BoErica'; LH - orchard L, variety 'Hayward'; N - orchard N; V - orchard V. Significant differences ($P < 0.05$) resulting from GLM analyses of the effect of pollination treatment on fruit weight are indicated by different letters.

Table 2
Generalized mixed-effect model analysis of the effect of pollination treatments on fruit distribution by caliber and class categories for each orchard and variety within the surveyed orchards. Statistically significant differences are highlighted in bold and significant differences among pollination treatments within orchard at $P < 0.05$ are indicated.

Orchard		C18-30	C33-46	Class II	Unmarketable
A		X_2 = 74.326; P = 0.176	X_2 = 68.280; P =0.301	-	X_2 = 17.537; P = 0.439
B	BoErica (BoE)	X_2= 116.75; P =0.359	X_2 = 116.75; P = 0.359	X_2 = 22.325; P = 0.007 O > (AP,S)	X_2 = 109.11; P = 0.110
	Hayward (H)	X_2 = 100.44; P =0.776	X_2 = 105.74; P = 0.711	X_2 = 44.088; P = 0.026 S > AP	X_2 = 36.575; P = 0.804
E	-	X_2 = 67.085; P =0.333	X_2 = 70.002; P = 0.342	-	$X2$ = 17.182; P = 0.979
K	-	X_2 = 95.752; P =0.652	X_2 = 112.83; P = 0.530	X_2 = 46.764; P = 0.491	X_2 = 26.276; P = 0.003 O > (AP, S)
L	BoErica (BoE)	X_2 = 67.551; P < 0.001 S > (O, AP)	X_2 = 67.350; P = 0.034 O > S	-	X_2 = 37.552; P = 0.005 O > S
	Hayward (H)	X_2 = 55.28; P < 0.001 S > (O, AP)	X_2 = 38.955; P = 0.026 AP > S	-	X_2 = 44.875; P = 0.005 O > S
N	-	X_2 = 66.865; P = 0.003 S > (O, AP)	X_2 = 60.032; P = 0.010 AP > S	X_2 = 8.7687; P = 0.437	X_2 = 19.505; P = 0.078
V	-	X_2 = 66.865; P = 0.003 S > AP	X_2 = 43.423; P = 0.010 AP > S	-	X_2 = 48.058; P =0.001 AP > S

C33-46: $X^2{}_2$ = 4.88, P = 0.087; Class II: $X^2{}_2$ = 0.48, P = 0.788; Unmarketable: $X^2{}_2$ = 14.02, P = 0.0009), with supplementary pollination resulting in higher percentage of fruits in C18-30 when compared to open pollination and artificial pollination (mean ± SE: 66.3 ± 3.4%; 47.4 ± 3.8% and 47.5 ± 4.0%, respectively), and in lower percentage of unmarketable fruits than the other two treatments (mean ± SE: 2.1 ± 1.0%; 12.1 ± 2.5% and 11.3 ± 2.5%, respectively). When analyzed individually, the orchards lacking differences in fruit weight (orchards A, B and E), overall, also did not present differences in fruit distribution among calibers and classes (Table 2, Fig. 3). As expected, the differences in fruit weight among treatments resulted in significant differences in fruit caliber in orchards K, L, N and V (Table 2, Fig. 3). Overall, supplementary pollination increased the proportion of fruits in high quality market calibers (C18-30) in comparison with artificial pollination and open pollination treatments (Table 2, Fig. 3); no significant differences in the proportion of fruits in high quality market calibers (C18-30) as a result of artificial pollination, were observed (Table 2), although a tendency for better calibers in artificially pollinated fruits, in comparison to open pollinated, was observed in some orchards (namely in orchards L in 'BoErica', B in 'Hayward', and K; Fig. 3). Artificial pollination did significantly reduce the proportion of unmarketable fruits in orchard K and of fruits in class II in orchard B (for both varieties) when compared to open pollination (Table 2, Fig. 3) and, although not significant, a similar trend was observed in other orchards (L and N) (Fig. 3).

3.2. Linking pollination to orchard production and market value

Overall, artificial pollen application did not contribute to a clear increase in kiwifruit total production per hectare, although there was a slight tendency towards an increase in productivity as a result of artificial pollination in orchards K and L, when compared to open pollination (Fig. 4). The same trend was observed with estimated monetary gain (Fig. 5). The results also show a tendency towards an increase in production and monetary gain as result of supplementary pollination when compared to artificial pollen application in all surveyed orchards (Figs. 4 and 5). As a consequence, in 3 out of the 5 orchards for which artificial and open pollinations treatments were made, monetary gains due to the application of artificial pollination were observed (Fig. 5), spanning from 756 €/ha (in orchard A) to 4240 €/ha (in orchard K).

Fig. 6.

4. Discussion

Efficient pollination is a key aspect in kiwifruit production and economic viability (Ferguson, 2013). However, kiwifruit pollination is influenced by several interacting factors such as pollen availability, pollinator diversity and behavior, and weather conditions during flowering, that often makes insect pollination challenging and results in pollination deficits (*e.g.* Miñarro and Twizell, 2015; Oliveira *et al.*, 2009; Testolin *et al.*, 1991; Castro *et al.*, 2021). An inefficient pollination leads to unsatisfactory fruit size, shape and uniformity that reduces market value (Oliveira *et al.*, 2009; Tacconi *et al.*, 2016), which may lead producers to resort to artificial pollination (Tacconi and Michelotti, 2018). Here, we evaluated potential improvements to productivity resulting from artificial pollination and the efficiency of the technique for kiwifruit production and monetary gain on seven orchards distributed over the production range of this crop in Portugal. Overall, our results showed that artificial pollination was not always efficient and/or needed, but pollen supply improves kiwifruit production in most orchards, through low/moderate changes in fruit set and fruit weight that mostly drive changes in fruit distribution by caliber and category.

Overall, artificial pollination did not result in increased fruit set or fruit weight, but rather in a higher proportion of high-quality market fruits and/or lower proportion of unmarketable fruits in comparison with natural pollination, at least in some orchards. The results for fruit set and fruit weight are similar to those obtained by Razeto *et al.* (2005) and Gonzalez *et al.* (1998) in kiwifruit orchards in Chile and Spain, respectively, where no significant differences were obtained when comparing fruits from flowers that received natural pollination with those that received artificial pollination. In contrast, King and Ferguson (1991) in New Zealand kiwifruit orchards, found a significant increase in fruit weight resulting from artificial pollination (using dry pollen) in comparison to natural pollination, which was then reflected in an increase in the proportion of fruits in higher calibers. These overall patterns may suggest a contrasting success of natural pollination in these regions. The large kiwifruit orchards, largely concentrated over a single region within New Zealand (Testolin and Ferguson, 2009), leading to landscape simplification, are expected to have significantly lower pollinator communities, resulting in poor natural pollination, as shown for other crops (*e.g.*, strawberry, apple; Connelly et al., 2015; Martins et al., 2015) and in significant increments after supplementary (hand or mechanical) pollination (Connelly et al., 2015; King and Ferguson, 1991; Martins et al., 2015; Tacconi et al., 2016). In contrast, orchards in our study region are small and integrated within a heterogeneous landscape which may provide abundant pollinator communities (Gaspar, 2020; Castro et al., submitted). Indeed, this was the case in orchards A and B, where mean fruit weight of kiwifruits resulting from artificial pollination, open pollination and supplementary pollination was not significantly different, supporting that in the study year the natural pollination in these orchards was sufficient and artificial pollination seemed to be overall unnecessary. Diverse and abundant pollinator communities were shown to correlate positively with productivity in many crops (e.g., Connelly et al., 2015; Martins et al., 2015; Nicholson and Ricketts, 2019), and the availability of insects both in number and diversity were shown to contribute to larger fruits and to higher

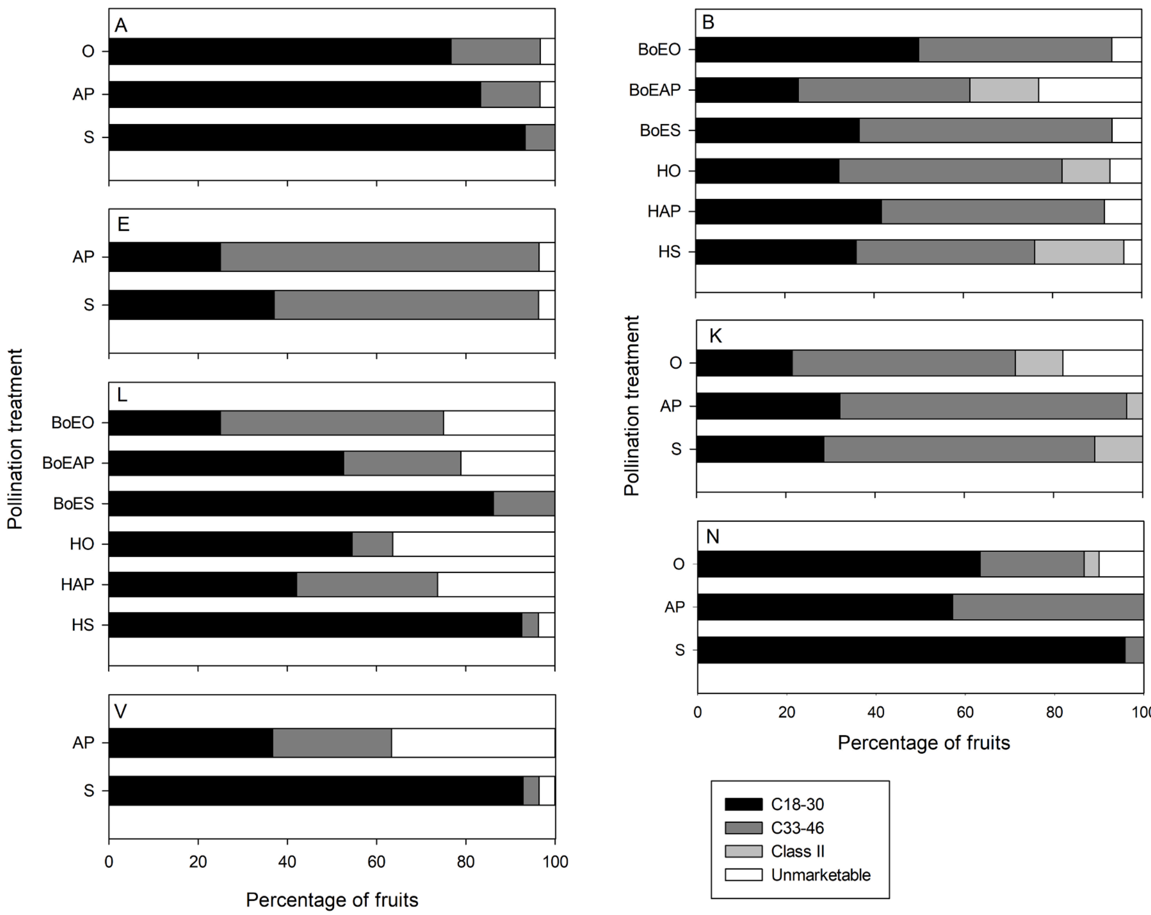

Fig. 3. Distribution of kiwifruits resulting from open (O), artificial (AP) and supplementary (S) pollinations by caliber groups for each orchard (A, B, E, K, L, N and V), and variety (BoE - 'BoErica'; H - 'Hayward') within the seven orchards surveyed.

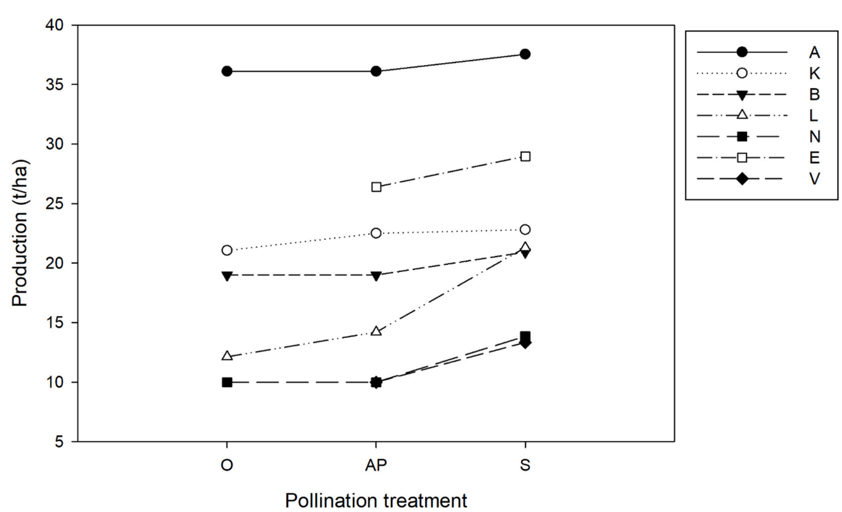

Fig. 4. Kiwifruit production (t/ha) corresponding to open (O), artificial and supplementary (S) pollination treatments for the seven orchards surveyed (A, B, E, K, L, N and V).

homogeneity in fruit weight in kiwifruit, playing a bigger role (from an efficiency perspective) than artificial pollination (Sáez et al., 2019). Nevertheless, in orchard A there was a slight increase in the proportion of fruits in high quality market calibers and a decrease in the proportion of unmarketable fruits in the artificial pollination treatment compared to the open pollination, which led to a slight increase on estimated monetary gain after artificial pollination.

Previous studies have shown that some orchards in our study region suffer from pollen limitation to varying degrees (results herein; Castro *et al.*, 2021). Therefore, we expect that, if well applied, artificial pollination would directly result in an increase in mean fruit weight with subsequent positive effects on fruit caliber in orchards with pollen limitation. In fact, in the orchards with higher pollen limitation levels (indicated by significantly higher fruit weights after supplementary

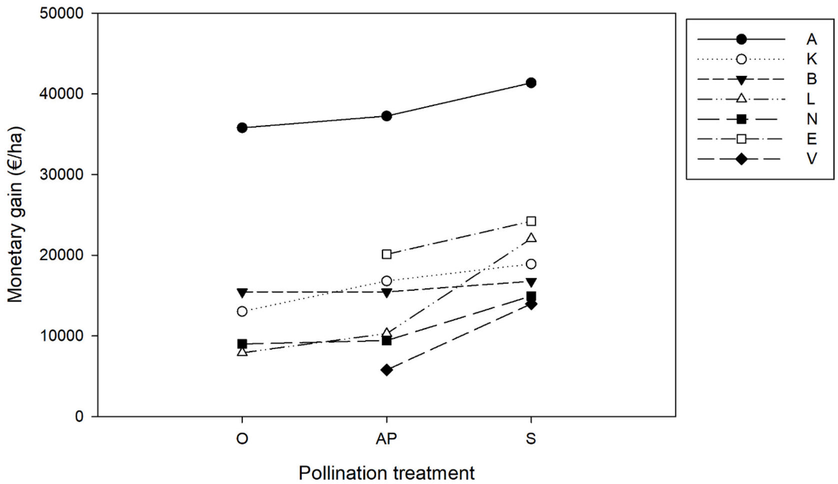

Fig. 5. Monetary gain (Euros/ha) corresponding to kiwifruit production under open (O), artificial and supplementary (S) pollination treatments for the seven orchards surveyed (A, B, E, K, L, N and V).

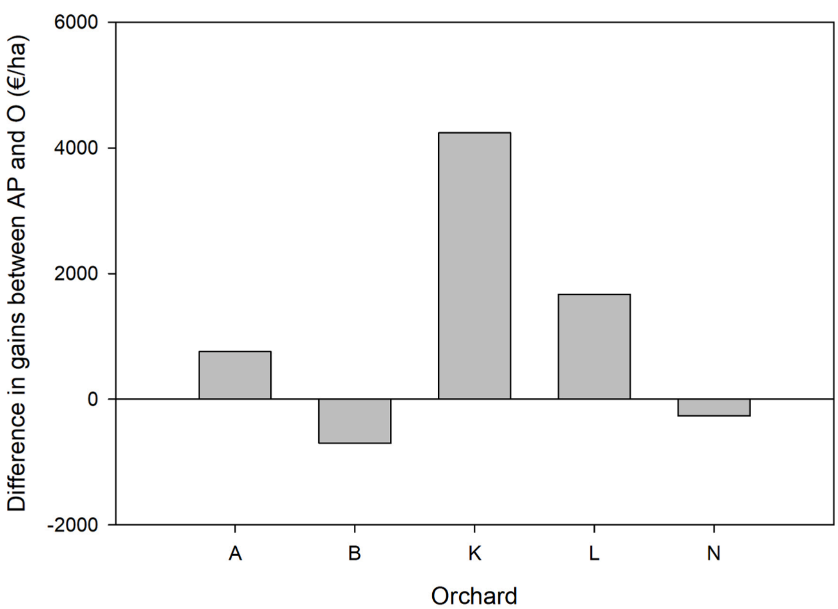

Fig. 6. Differences in monetary gain (in Euros) between artificial and open pollination, taking into account the costs associated with pollen acquisition (around 700 Euros/ha, on average for green kiwifruit) for the five orchards for which both treatments were available.

compared to open pollinations) such as orchard L (variety 'BoErica') and K, artificial pollination had positive impacts, which resulted in increased monetary gains. As stated above, poor pollination levels can be driven by insufient pollen availability in the orchard, due to insuficient male plants or asynchrony between male and female flowering, and/or pollinator limitation, and management practices (Antunes *et al.*, 2007; Gonzalez *et al.*, 1998; Miñarro and Twizell, 2015, Castro *et al.*, 2021). For example, orchard K is the orchard with the lowest male to female ratio and may suffer from limited pollen availability; orchard L is an orchard covered with hail net, which is an important factor affecting natural pollination as these structures reduce ventilation and pollen movement, restrict movements from beneficial insects and change or reduce visual cues necessary for their orientation, with significant impacts in production (Evans *et al.*, 2019; Tacconi and Michelotti, 2018).

Overall, the occurrence of pollination deficits in some orchards suggest that artificial pollination could be a useful tool to improve productivity levels, particularly when the conditions for successful pollination are poor. However, while artificial pollination may be a useful tool, it is important to ascertain its efficiency so that its costs/benefits as management tool are overall positive (Tacconi and Michelotti, 2018). Our results suggest that in some orchards the success of the pollen application was lower than desirable to attain good production levels. In green pulp kiwifruit orchards L, N and V, fruit set and/or mean fruit weight resulting from artificial pollination was significantly lower than that obtained after supplementary pollination (and similar to natural pollination) indicating that artificial pollination was largely insufficient. Previous studies evaluating the efficiency of various artificial pollen techniques showed that hand pollination often yields better results than pollen application using machinery (*e.g.*, Gonzalez *et al.*, 1998; Razeto *et al.*, 2005). For example, higher efficiencies of hand pollinations have been attributed to the fact that many machines blow the pollen towards the female flowers but not directly at the stigma, reducing the efficiency of artificial pollination (Goodwin and McBrydie, 2013). Among the factors affecting the success of the artificial pollination are the protocol of pollen application itself, the number of times and timing of pollen application and the phenology of the plants within the orchard (Oliveira et al., 2009; Tacconi et al., 2016; Tacconi and Michelotti, 2018). The efficiency of pollen application protocol directly depends on parameters such as pollen quality (germinability, humidity and conservation), pollination system (dry or liquid), use of coadjuvants to dilute pollen (dry or liquid) or to help germination, and adequate use of the technique (Oliveira *et al.*, 2009; Tacconi *et al.*, 2016; Tacconi and Michelotti, 2018). In the study region, and in green pulp kiwifruit orchards, artificial pollination is undertaken only once per orchard and thus, the producer must decide the moment when the maximum number of female flowers are receptive to maximize pollination success. Thus, flower stage is a key factor determining pollination success, and pollen application is advisable to be undertaken at 90% open flowers (Tacconi *et al.*, 2016). However, the criteria between regions and field technicians may vary and the application in an incorrect timing may be one of the factors that strongly contributed to the low success of artificial pollination. In addition, during the study year the flowering season in orchards L and N presented high heterogeneity in flowering. This heterogeneity led to a mismatch in flowering period between female and male plants (flowering peak of female flowers was earlier than that of male plants) and, in orchard N, to a high heterogeneity in flower maturation (*i.e.*, the presence of closed buds and wilted flowers, sometimes fruits all in the same plant; author's field observations; Castro *et al.*, 2019). This flowering heterogeneity was most probably produced by the occurrence of very hot days intermingled by cold days previous to the flowering period in the spring of 2019, as temperature was shown to have a strong effect on budbreak and flowering of kiwifruit vines, leading to year to year and regional variations in flowering timings (McPherson *et al.*, 1994). A high flowering heterogeneity within the orchard makes the decision of field technicians on the most suitable moment for pollination highly difficult, as it is difficult to have a high percentage of simultaneously receptive flowers, consequently impacting the success of the pollen application. Despite this, considering that in orchards L and N artificial pollination led to a decrease in the proportion of unmarketable fruits, a mild positive impact on estimated monetary gain was still observed as a result of this management practice.

Although we found that, in general, artificial pollination resulted in no increase or small increases in fruit weight, often with no statistical significance when compared to open pollination, in some orchards these differences translated into increases in the percentage of fruits falling into higher payed caliber categories and/or a reduction in the percentage of unmarketable fruits. Indeed, in three of the five green pulp kiwifruit orchards where both artificial and natural pollination were tested, monetary gain resulting from artificial pollination was positive. Although we did not take into account losses and other costs that producers may have and that may result in a decrease in the mean price received per kilogram of kiwifruit, our results suggest that in those orchards, even after considering the costs associated to pollen acquisition (around 700 Euros/ha, on average for green kiwifruit), the application of artificial pollen was still beneficial. Artificial pollen application may prove to be important in increasing monetary gain, particularly when natural pollination is not optimal, for example in years with adverse weather conditions (Gonzalez *et al.*, 1998; Miñarro and Twizell, 2015) and/or in orchards that are covered with hail net (Evans *et al.*, 2019; Tacconi and Michelotti, 2018), due to the negative impact of these factors in insect activity and phenological patterns (Evans *et al.*, 2019; Gonzalez *et al.*, 1998; Miñarro and Twizell, 2015; Tacconi *et al.*, 2016; Testolin *et al.*, 1991).

Nevertheless, artificial pollination has high costs and the potential for dissemination of the *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit and currently the most severe disease of this crop, causing significant economic losses in the main kiwi producing countries, including Portugal (Donati et al., 2018; Garcia et al., 2018; Tacconi et al., 2016). Strategies to promote pollination, such as pollinator friendly practices, that have been shown to promote pollinator communities and increase pollination services within the field in several crops, with impacts the productivity (Garibaldi et al., 2014; Pérez-Méndez et al., 2020), and the installation of managed bees, a tool frequently used in crop pollination (Rollin and Garibaldi, 2019), and the appropriate ratio and distribution of male plants (Gonzalez et al., 1998) can help to minimize pollination deficits. This, however, does not solve issues related with the lack of pollen in result of flowering asynchronies. Therefore, the costs and benefits of all these management practices need to be evaluated at the orchard level and according to factors determining the success of pollination, not only each year but also at long term.

5. Conclusions

While several orchards in the study region might profit from appropriated conditions for successful pollination and attain profitable yields, artificial pollination could still be a useful tool under unpredictable pollination scenarios or in orchards with strong determinants of pollinator communities (e.g., covered orchards). Pollinator friendly practices are advisable to maintain and promote wild pollinator communities and diminish the need to resort to the use of artificial pollination, but pollen application has still produced increments in fruit caliber and monetary gains. Finally, the discrepancies between artificial and supplementary pollinations suggest that reviews of the efficiency of the methodologies used in this region would be recommended.

Authors' contributions

All authors contributed to data collection and to the manuscript preparation and approved the publication of the manuscript in this form.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2021.110533.

References

Antunes, M.D.C., Oliveira, M., Teixeira, M., Veloso, A., Veloso, F., Panagopoulos, T., 2007. Evaluation of the effect of complementary pollination on actinidia deliciosa "Hayward" in northwest Portugal. Acta Hortic. 753, 347–352. https://doi.org/10.17660/ActaHortic.2007.753.43.

APK, 2007. Manual do kiwicultor. APK- Associação Portuguesa de Kiwicultores.

Bates, D., Mächler, M., 2015. Package ' lme4 ': Linear mixed-effects models using eigen and S4. J. Stat. Softw.
Blitzer, E.J., Gibbs, J., Park, M.G., Danforth, B.N., 2016. Pollination services for apple are dependent on diverse wild bee communities. Agric. Ecosyst. Environ. 221, 1–7. https://doi.org/10.1016/j.agee.2016.01.004.
Brittain, C.A., Vighi, M., Bommarco, R., Settele, J., Potts, S.G., 2010. Impacts of a pesticide on pollinator species richness at different spatial scales. Basic Appl. Ecol. 11, 106–115. https://doi.org/10.1016/j.baae.2009.11.007.
Calderone, N.W., 2012. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. PLoS One 7, 24–28. https://doi.org/10.1371/journal.pone.0037235.
Castro, H., Loureiro, J., Castro, S., 2019. A fenologia da floração dos pomares de kiwi em Portugal. Boletim da Associação Portuguesa de Kiwicultores 59, 3–4.
Castro, H., Siopa, C., Casais, V., Castro, M., Loureiro, J., Gaspar, H., Dias, M.C., Castro, S., 2021. Spatiotemporal variation in pollination deficits in an insect-pollinated dioecious crop. Plants 10, 1273. https://doi.org/10.3390/plants10071273.
Connelly, H., Poveda, K., Loeb, G., 2015. Landscape simplification decreases wild bee pollination services to strawberry. Agric. Ecosyst. Environ. 211, 51–56. https://doi.org/10.1016/j.agee.2015.05.004.
Core Development Team, R., 2016. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.
Donati, I., Cellini, A., Buriani, G., Mauri, S., Kay, C., Tacconi, G., Spinelli, F., 2018. Pathways of flower infection and pollen-mediated dispersion of Pseudomonas syringae pv. actinidiae, the causal agent of kiwifruit bacterial canker. Hortic. Res. 5 https://doi.org/10.1038/s41438-018-0058-6.
Ellena, M., Sandoval, P., Gonzalez, A., Galdames, R., Jequier, J., Contreras, M., Azocar, G., 2014. Preliminary results of supplementary pollination on hazelnut in South Chile. Acta Hortic. 121–127. https://doi.org/10.17660/ActaHortic.2014.1052.15.
Evans, L.J., Cutting, B.T., Jochym, M., Janke, M.A., Felman, C., Cross, S., Jacob, M., Goodwin, M., 2019. Netted crop covers reduce honeybee foraging activity and colony strength in a mass flowering crop. Ecol. Evol. 9, 5708–5719. https://doi.org/10.1002/ece3.5154.
Ferguson, A.R., 2013. Kiwifruit. The wild and the cultivated plants. Adv. Food Nutr. Res. 68, 15–32. https://doi.org/10.1016/B978-0-12-394294-4.00002-X.
Ferguson, A.R., 1984. Kiwifruit: A Botanical Review. In: Janick, J. (Ed.), Horticultural Reviews. AVI Publishing Company, INC, pp. 1–64.
Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third edit. Sage, Thousand Oaks CA.
Garcia, E., Moura, L., Abelleira, A., Aguín, O., Ares, A., Mansilla, P., 2018. Characterization of Pseudomonas syringae pv. actinidiae biovar 3 on kiwifruit in north-west Portugal. J. Appl. Microbiol. 125, 1147–1161. https://doi.org/10.1111/jam.13943.
Garibaldi, L.A., Carvalheiro, L.G., Leonhardt, S.D., Aizen, M.A., Blaauw, B.R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A.M., Kremen, C., Morandin, L., Scheper, J., Winfree, R., 2014. From research to action: enhancing crop yield through wild pollinators. Front. Ecol. Environ. 12, 439–447. https://doi.org/10.1890/130330.
Garibaldi, L.A., Requier, F., Rollin, O., Andersson, G.K., 2017. Towards an integrated species and habitat management of crop pollination. Curr. Opin. Insect Sci. 21, 105–114. https://doi.org/10.1016/j.cois.2017.05.016.
Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhoffer, J. H., Freitas, B.M., Ghazoul, J., Greenleaf, S., Hipolito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K.M., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlof, M., Seymour, C.L., Schuepp, C., Szentgyorgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N., Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339 (80), 1608–1611. https://doi.org/10.1126/science.1230200.
Garratt, M.P.D., Breeze, T.D., Jenner, N., Polce, C., Biesmeijer, J.C., Potts, S.G., 2014. Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. Agric. Ecosyst. Environ. 184, 34–40. https://doi.org/10.1016/j.agee.2013.10.032.
Gonzalez, M.V, Coque, M., Herrero, M., 1998. Influence of pollination systems on fruit set and fruit quality in kiwifruit (Actinidia deliciosa). Ann. Appl. Biol. 132, 349–355. https://doi.org/10.1111/j.1744-7348.1998.tb05210.x.
Goodwin, M., 2012. Pollination of Crops in Australia and New Zealand.
Goodwin, R.M., McBrydie, H.M., 2013. Use of Pollen Blowers and Pollen Dispensers to Pollinate Kiwifruit Artificially. A Report Prepared for. Zespri Group Ltd.
Karamaouna, F., Kati, V., Volakakis, N., Varikou, K., Garantonakis, N., Economou, L., Birouraki, A., Markellou, E., Liberopoulou, S., Edwards, M., 2019. Ground cover management with mixtures of flowering plants to enhance insect pollinators and natural enemies of pests in olive groves. Agric. Ecosyst. Environ. 274, 76–89. https://doi.org/10.1016/j.agee.2019.01.004.
Karimi, H.R., Mohammadi, N., Estaji, A., Esmaeilizadeh, M., 2017. Effect of supplementary pollination using enriched pollen suspension with Zn on fruit set and fruit quality of pistachio. Sci. Hortic. (Amsterdam). 216, 272–277. https://doi.org/10.1016/j.scienta.2017.01.027.
King, M.J., Ferguson, A.M., 1991. Collection and use of dry pollen for pollination of kiwifruit. New Zeal. J. Crop Hortic. Sci. 19, 385–389. https://doi.org/10.1080/01140671.1991.10422881.
Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/rspb.2006.3721.
Larson, B.M.H., Barrett, S.C.H., 2000. A comparative analysis of pollen limitation in flowering plants 69, 503–520.
Maes, J., Jacobs, S., 2017. Nature-Based Solutions for Europe's Sustainable Development. Conserv. Lett. 10, 121–124. https://doi.org/10.1111/conl.12216.
Martins, K.T., Gonzalez, A., Lechowicz, M.J., 2015. Pollination services are mediated by bee functional diversity and landscape context. Agric. Ecosyst. Environ. 200, 12–20. https://doi.org/10.1016/j.agee.2014.10.018.
Miñarro, M., Twizell, K.W., 2015. Pollination services provided by wild insects to kiwifruit (Actinidia deliciosa). Apidologie 46, 276–285. https://doi.org/10.1007/s13592-014-0321-2.
Nicholson, C.C., Ricketts, T.H., 2019. Wild pollinators improve production, uniformity, and timing of blueberry crops. Agric. Ecosyst. Environ. 272, 29–37. https://doi.org/10.1016/j.agee.2018.10.018.
NZKGI, 2016. Kiwifruit book.
Oliveira, M., Veloso, A., Teixeira, M., Veloso, F., Antunes, D., 2009. Avaliação do efeito da polinização complementar em actinídea deliciosa CV . " Hayward " na Região de Entre – Douro e Minho, in: Ramos, A., Martins, J.M.S. (Eds.), I Simpósium Nacional de Fruticultura. Associação Portuguesa de Horticultura, pp. 97–101.
Pérez-Méndez, N., Andersson, G.K.S., Requier, F., Hipólito, J., Aizen, M.A., Morales, C.L., García, N., Gennari, G.P., Garibaldi, L.A., 2020. The economic cost of losing native pollinator species for orchard production. J. Appl. Ecol. 57, 599–608. https://doi.org/10.1111/1365-2664.13561.
Pinillos, V., Cuevas, J., 2008. Artificial Pollination in Tree Crop Production, in: Horticultural Reviews. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 239–276. https://doi.org/10.1002/9780470380147.ch4.
Pomeroy, N., Fisher, R.M., 2002. Pollination of kiwifruit (Actinidia deliciosa) by bumble bees (Bombus terrestris): Effects of bee density and patterns of flower visitation. New Zeal. Entomol. 25, 41–49. https://doi.org/10.1080/00779962.2002.9722093.
Pywell, R.F., Heard, M.S., Woodcock, B.A., Hinsley, S., Ridding, L., Nowakowski, M., Bullock, J.M., 2015. Wildlife-friendly farming increases crop yield: Evidence for ecological intensification. Proc. R. Soc. B Biol. Sci. 282 https://doi.org/10.1098/rspb.2015.1740.
Razeto, B., Reginato, G., Larraín, A., 2005. Hand and Machine Pollination of Kiwifruit. Int. J. Fruit Sci. 5, 37–44. https://doi.org/10.1300/J492v05n02_05.
Revelle, W., 2020. psych: Procedures for Psychological, Psychometric, and Personality Research.
Rollin, O., Garibaldi, L.A., 2019. Impacts of honeybee density on crop yield: A meta-analysis. J. Appl. Ecol. 56, 1152–1163. https://doi.org/10.1111/1365-2664.13355.
Ryder, J.T., Cherrill, A., Prew, R., Shaw, J., Thorbek, P., Walters, K.F.A., 2020. Impact of enhanced Osmia bicornis (Hymenoptera: Megachilidae) populations on pollination and fruit quality in commercial sweet cherry (Prunus avium L.) orchards. J. Apic. Res. 59, 77–87. https://doi.org/10.1080/00218839.2019.1654062.
Sáez, A., Negri, P., Viel, M., Aizen, M.A., 2019. Pollination efficiency of artificial and bee pollination practices in kiwifruit. Sci. Hortic. (Amsterdam). 246, 1017–1021. https://doi.org/10.1016/j.scienta.2018.11.072.
Tacconi, G., Michelotti, V., 2018. Artificial Pollination in Kiwifruit and Olive Trees. In: Segneanu, A.E., Orbeci, C., Lazau, C., Sfirloaga, P., Vlazan, P., Bandas, C., Grozescu, I. (Eds.), Pollination in Plants. InTech, p. 29. https://doi.org/10.5772/intechopen.74831.
Tacconi, G., Michelotti, V., Cacioppo, O., Vittone, G., 2016. Kiwifruit pollination: The interaction between pollen quality, pollination systems and flowering stage. J. Berry Res. 6, 417–426. https://doi.org/10.3233/JBR-160138.
Testolin, R., Ferguson, A.R., 2009. Kiwifruit (Actinidia spp.) production and marketing in Italy. New Zealand J. Crop Horticult. Sci. https://doi.org/10.1080/01140670909510246.
Testolin, R., Vizzotio, G., Costa, G., 1991. Kiwifruit pollination by wind and insects in Italy. New Zeal. J. Crop Hortic. Sci. 19, 381–384. https://doi.org/10.1080/01140671.1991.10422880.
Vaissière, B., Freitas, B., Gemmill-Herren, B., 2011. Protocol to detect and assess pollination deficits in crops: a handbook for its use. Pollination Serv. agriculture - Field Manuals.
Vaknin, Y., Gan-Mor, S., Bechar, A., Ronen, B., Eisikowitch, D., 2001. Improving pollination of almond (Amygdalus communis L., Rosaceae) using electrostatic techniques. J. Hortic. Sci. Biotechnol. 76, 208–212. https://doi.org/10.1080/14620316.2001.11511352.
Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. Trends Plant Sci. 7, 270–277. https://doi.org/10.1016/S1360-1385(02)02258-6.
Williams, H., Nejati, M., Hussein, S., Penhall, N., Lim, J.Y., Jones, M.H., Bell, J., Ahn, H. S., Bradley, S., Schaare, P., Martinsen, P., Alomar, M., Patel, P., Seabright, M., Duke, M., Scarfe, A., MacDonald, B., 2020. Autonomous pollination of individual kiwifruit flowers: Toward a robotic kiwifruit pollinator. J. F. Robot. 37, 246–262. https://doi.org/10.1002/rob.21861.
Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R, Statistics for Biology and Health. Springer, New York, New York, NY. https://doi.org/10.1007/978-0-387-87458-6.