

Field trials to enhance kiwifruit production using *Osmia bicornis* and supplementary flowering plants

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Abstract

Kiwifruit has a strong dependence on insect pollinators, and yet the relatively low attractiveness of kiwi flowers to European pollinators may lead to deficits in production. In Italy, due to the scarce efficacy of the honey bee in pollinating kiwifruit, the most used strategy to mitigate this issue is artificial pollination with mass-produced pollen, which incurs high costs and increases the risk of disease; alternative strategies are thus direly needed. The aims of this study are i) to quantify pollination deficit and pollinator contribution to kiwifruit production and quality, and ii) to investigate the release of red mason bees *Osmia bicornis* (L.) (synonym *Osmia rufa*) (Hymenoptera Megachilidae) and the use of flower strips (supporting both wild pollinators and the released red mason bees) as strategies for the improvement of kiwifruit pollination. Through manipulative experiments in 3 golden kiwifruit orchards in Northern Italy, we verified that kiwifruit production in the area was significantly improved by insect pollinators, but also suffered from pollination deficit, with average reductions of 9% in fruitlet set, 15% in final fruit set, and 30% in seed number. Our findings confirmed successful population establishment of managed *O. bicornis* in most sites, and analyses of pollen collected in their provisions indicated that these bees effectively gathered kiwi pollen. While the presence of *O. bicornis* had limited but statistically significant positive effects on fruit production and quality, the introduction of flower strips had a distraction effect on *O. bicornis* and other pollinators, leading to some negative consequences on the target crop. This suggests a need for careful management of floral resources to balance the benefits of supporting pollinator populations with the goal of maximizing kiwifruit production; at the same time, the introduction of managed pollinators such as *O. bicornis* might be especially beneficial to this crop in agroecosystems that are poor in alternative flower resources.

Key words: pollination deficit, *Actinidia chinensis*, red mason bee, flower strips.

Introduction

Agriculture highly relies on insect pollination, with the production of one-third of crops depending on it to a great extent (Aizen *et al.*, 2009) and an increasing trend of dependence at a global scale (Garibaldi *et al.*, 2011a). In species requiring cross-pollination to set fruit, pollination is often a limiting factor in productivity both in terms of quantity and quality (Bartomeus *et al.*, 2014; Wietzke *et al.*, 2018). The well documented global trend of the decline in diversity and abundance of insect pollinators (Potts *et al.*, 2010; Marshman *et al.*, 2019) results in a pauperisation of pollination services affecting also agricultural ecosystems (Cameron *et al.*, 2011; Potts *et al.*, 2016). This can lead to the phenomenon named pollination deficit, corresponding to inadequate pollination that reduces the plant reproductive success, causing a lower fruit production than the one expected in adequate pollination services conditions (Aizen and Harder, 2007; Garibaldi *et al.*, 2013). Kiwifruit (*Actinidia chinensis* Planchon) has been identified among the 13 major crops for which insect-mediated pollination results crucial (Klein *et al.*, 2007); its fruit size strongly correlates with the number of seeds (Brundell, 1975; Schmid, 1978; Hopping Monselise, 1986; Vaissière *et al.*, 1991; González *et al.*, 1997) and seeds number directly depends on pollination rate. This correlation results in different classes of marketable fruits in terms of size ("Extra" Class is 90 g, for Class I is 70 g and for Class II is 65 g., UNECE, 2017), resulting from the fact that several pollinator visits

are required to reach the highest marketable classes (Broussard *et al.*, 2022). Considering that, the current decline of pollinators makes it more difficult to maintain an economically sustainable production in this crop. The biology of *A. chinensis* partially explains the vulnerability of kiwifruit production linked to pollination services. Kiwifruit is a dioecious crop, and its flowers are generally less attractive for insect pollinators than other co-flowering species because neither flower sex produce nectar, and even if female plants produce flowers with well-developed anthers, these contain non-viable pollen (Schmid, 1978). Regarding that, Meroi Arcerito *et al.* (2021) demonstrated that fragrance addition can improve attractiveness of kiwi flowers toward honey bees, with positive effects on kiwifruit quality. Common strategies used to mitigate crop pollination deficit in kiwifruit may involve the manipulation of plant density and male/female ratio in orchards (Meroi Arcerito *et al.*, 2024) or, more frequently, the management of pollinators themselves. The latter may include the introduction of managed pollinators (Fulton *et al.*, 2015), mainly honey bees and bumblebees, and/or the enhancement of wild pollinator communities by preserving and promoting natural or semi-natural areas (Garibaldi *et al.*, 2011b). In Italy, kiwifruit producers follow two different main approaches: i) pollinator supplementation by the introduction of the European honey bee *Apis mellifera* L. (Howpage *et al.*, 2001) and the buff-tailed bumblebee *Bombus terrestris* L., often combined with the practice of ventilation consisting in the generation of directed air streams

by a fan moved across the orchard; *ii*) artificial pollination (Abbate *et al.*, 2021), performed manually or mechanically. This method normally involves mass produced male pollen available on the market, while rarely pollen might be locally collected and used on the same orchard. Artificial pollination represents so far, the most used strategy (Castro *et al.*, 2021), mainly due to the limited supply of honey bees and their scarce efficiency in kiwi pollination. Artificial pollination removes the constraints of kiwi production from pollinating insects (Abbate *et al.*, 2021), delegating pollination to a human intervention, but it overlooks the reasons at the base of the impoverishment of pollination services. This practice is therefore expensive and time consuming (Hii, 2004) and its efficiency compared to insect pollination is debated (Sáez *et al.*, 2019). Furthermore, pollen has been identified as a vector of *Pseudomonas syringae* pv. *actinidiae*, the pathogenic agent of kiwifruit bacterial canker (Lopes *et al.*, 2020) and its massive production and dispatch over several orchards dramatically increases the risk of disease spreading.

In our study we aimed to *i*) assess the pollination service and quantify the pollination deficit suffered by golden kiwifruit (Zespri Gold G3®) orchards in Northern Italy and *ii*) evaluate an innovative approach to agricultural ecosystem management to help mitigate the pollination deficit affecting this kiwifruit variety in the study area. To quantify pollination deficits in environments like orchards or crop fields, controlled pollination experiments are commonly used, which compare fruit set or yield parameters between flowers exposed to natural pollination services and those where pollination has been maximized manually (Garratt *et al.*, 2014). Yield is typically measured by fruit number per hectare or per vine, with the fruit set commonly providing insights into pollination deficits. Quality parameters, such as fruit weight, size, shape, seed count, and dry matter, can also be evaluated. Additionally, pollinator visitation rates and their correlation with yield are often assessed. In the proposed agroecosystem we aimed to increase the insect pollinator community acting on two levels: *i*) by releasing and establishing a population of a managed solitary bee species, the red mason bee *Osmia bicornis* (L.) (synonym *Osmia rufa*); *ii*) by sowing a cover crop mix including species with good nectar production flowering simultaneously with *Actinidia* to attract wild pollinators and to sustain the population of the red mason bee *O. bicornis*, in particular during the pre-nesting period.

O. bicornis represents a good candidate as a supplementary pollinator in kiwi orchards for several reasons. First, it shows an efficient foraging behaviour due to its high visit legitimacy on fruit trees and high rate of fruit set in flowers receiving a single visit (Bosch and Blas, 1994; Monzón *et al.*, 2004; Garratt *et al.*, 2016; Vicens and Bosch, 2000; Eraerts *et al.*, 2020). Second, red mason bees can also fly with cooler temperatures compared to honey bees, thus extending the daily foraging period. This is particularly relevant when the blooming of the target species has a short duration, as for *Actinidia*, and the bloom occurs under harsh weather conditions as ones characterizing northern Italy during spring season. Red mason bees also have a shorter foraging home range than

honey bees and bumblebees (Guédot *et al.*, 2007; Bidinger *et al.*, 2013) thus they are more likely to collect pollen in the proximity of the provided nesting station and, as other solitary bees, they need to collect a conspicuous amount of pollen that represents the main food source for offspring development. Compared to other species belonging to the genus *Osmia* used in pollination services such as *Osmia cornuta* (Latreille) (Ladurner *et al.*, 2002; Maccagnani *et al.*, 2003; 2007), *O. bicornis* naturally emerges later in the season (Bak *et al.*, 2003) and its reproductive phase is more likely to be synchronized with kiwi flowering. However, flower plants were sown in proximity of the *Osmia* nests in order to foster the establishment of our released population. The introduction of supplemental floral resources in cultivated fields, on the other hand, might be beneficial to both managed and wild bees and is a growing practice aimed to conserve pollinators in agricultural landscapes (Winfrey, 2010; Tuell *et al.*, 2014). However, the effectiveness of this practice is often context dependent and controversial, as introducing additional flowering plants in orchards with flowers that are less attractive to pollinators, might produce a distraction effect (Holland *et al.*, 2015).

Materials and methods

The study has been carried out in 3 golden kiwifruit (Zespri Gold G3®) orchards (areas and coordinates available in supplemental material table S1) located in the Emilia-Romagna region (Northern Italy). The experimental orchards have been identified collaborating with cooperatives of local producers (Apofruit and Agrintesa) among farms with similar management in terms of adopted pollination practices: selected orchards were not applying artificial pollination but all of them were performing ventilation and pollinator supply by temporarily introducing bumblebees or honey bees right before the flowering of kiwifruit.

Field work

Pollination deficit experiment

Pollination deficit has been estimated in each orchard through a manipulative pollination experiment in plants located far from the *Osmia* nesting station (>100 m) and the edge of the field (>10 m). In each orchard, before flowering and when floral buds were visible, 10 kiwi female trees were randomly selected, and 9 floral bud clusters were chosen for each plant and divided into 3 groups of 3 clusters each. Bud clusters groups were involved in 3 different pollination treatments: *i*) pollinator exclusion, *ii*) open pollination and *iii*) hand pollination. In the exclusion treatment, the bud cluster was bagged in a nylon mesh (1 mm mesh) to prevent pollinator visits but still allowing the anemophilous pollination. Bags have been removed only at the end of kiwifruit petal fall. The hand pollination treatment has been performed at peak blossom by using flowers of male trees collected in the same orchard showing evident dehiscent anthers, brushing them on the female stigma of the buds already bloomed of the target floral cluster. This treatment has

been repeated twice during the peak of kiwi flowering to ensure the pollen saturation level and to increase the probability to match the receptivity peak of the stigma. Open pollination floral clusters were simply marked and let open to allow spontaneous pollination by insects as much as by the wind. Experimental floral bud clusters have been monitored during the entire season and data about the initial number of floral buds (late April), fruit-lets produced (June) and final fruit set (September) have been collected. At harvest, we collected fruit quality parameters on a subset of 2 fruits for each treatment per tree, when available, in some cases, individual fruits were collected, or none if the branch did not have any. Fruits have been weighed in laboratory, their size and shape have been evaluated with a calliper by measuring maximum diameter, orthogonal diameter (to calculate the minimum/maximum diameter ratio “MMR”), and fruit length. Dry matter has been determined from a slice 5 mm in width, sampled from the central part of each fruit (ranging from 15 to 20 grams per slice). Initial fresh weight of each slice was recorded using an analytical balance and slices were placed in a drying oven and let dry for 48-72 hours at 65 °C, after that, samples final dry weight has been recorded. Dry matter content of each sample has been calculated as the percentage of the dry weight with respect to the initial fresh one. Finally, we estimated the seeds quantity by taking a picture of the 4 sections obtained from this cut: the two sides of the slide and the exposed section of the two remaining parts of the fruit. Seeds have been counted from pictures by using the counter tool of ImageJ bundled with 64-bit Java 7 (<https://imagej.net/>).

Red mason bees

In mid-April 2023, prior to kiwifruit flowering, one nesting station of *O. bicornis* was placed in each experimental site, for a total of nine sites. Experimental sites were distributed in the 3 orchards, close to a female kiwifruit plant, in order to minimize the border effect (>10 m from the orchard edge) and minimize the overlapping of the flight range of the red mason bees (>200 m between each site) (Hofmann *et al.*, 2020). Because of the different shape and area of the three orchards it was not possible to equally distribute the experimental sites in each orchard (supplemental material table S1). Osmipro nesting station was used as nesting material, consisting of a plastic shelter containing 160-180 reeds of *Arundo donax* L. (total length: 30 cm; inside diameter: ca 8 mm). In each nesting shelter 550-600 *O. bicornis* cocoons (M:F sex ratio: 1.5:1) were released. Nesting stations were placed *in situ* a week before the blooming expected date, held 1.5 m above the ground, fixed to the supporting pole of the row and below the vines of the respective kiwi plant (supplemental material figure S1). Cocoons of *O. bicornis* were released 3 days before the kiwi flowering, which started on May 6, 2023. Throughout the kiwi flowering period, we monitored the activity of *O. bicornis* to evaluate the success of their release, observing their flight patterns, mating behaviour, pollen collection, and nesting activities. Right after kiwi petal fall, while *O. bicornis* nesting activity was still occurring, 3 nested reeds with the mud plug were collected from each nesting

station and transferred to the laboratory for pollen identification. Reeds have been opened to collect pollen samples, and the percentage of kiwi pollen in provisions destined to offspring has been quantified from the provision occurring in the first cells, which were assumed to have been collected during the peak of kiwi flowering. Pollen provisions were gently extracted from the reeds and placed individually in a 2 ml Eppendorf centrifuge tube. Pollen pellets were suspended in 1 ml of distilled water and when necessary diluted to observe the grains at the optical microscope, to ensure the homogeneous suspension of the pollen grains the mixture had been vortexed before each withdrawal of aliquots of liquid. For each sample, an aliquot of 50 µl of the suspension was placed on a microscope slide and let dry on a hot plate before covering it with a drop of Kaiser's glycerol gelatine and protected it with a glass coverslip. Slides were observed at 400× magnification to allow identification of pollen grains. 100 grains of pollen have been observed for identification by following a boustrophedon pattern organized in 3 virtual parallel lines, separated by an observation field at least, where only the grains insisting on the centre of the observation field have been counted. Identification of pollens was at species level, when possible, but never lower than the family one, according to the melissopalynological nomenclature currently in use (Persano Oddo and Ricciardelli D'Albore, 1989). After the conclusion of the kiwi flowering and the nesting activity of *O. bicornis*, the nesting stations were removed from the orchards and stored in a non-climatized room to keep them exposed to natural temperature fluctuation. To assess reproductive success, in early autumn, reeds were opened to record the number of cocoons.

Flower strips

In autumn 2022, in the proximity of 5 on 9 experimental sites, a mixture of cover crop species, including *Brassicaceae* and *Leguminosae* plants has been sown for 25 m along a tree row below each nesting station (12.5 m left and right to the station, width = 1 m, supplemental material figure S1). Spontaneous vegetation was allowed to grow at the remaining four sites, where, following the standard orchard management, it was removed by mowing a few days before the start of kiwifruit flowering. The seed mixture used was represented by the commercial mix “APISTICO AUTUNNALE” (MAS Seeds Company - MAÏSADOUR Group) integrated with a 3% in weight of *Camelina sativa* (L.) Crantz (supplemental material table S2). This specific mixture was selected to be attractive to most insect pollinators and ensure a long flowering period, starting from early April to the end of May, in order to include the kiwi flowering and a few weeks before and after it. Kiwi flowering in our latitudes occurs in a relatively narrowed timespan, it starts between the end of April/early May, and it lasts around two weeks. Suggested seeding dosage has been increased 3 times: from 30 kg/ha (3 g/m²) to 100 kg/ha (10 g/m²) to minimize the possible lack of germination. Since the success of the germination resulted variable among the different sites and considered the presence of some spontaneous vegetation, the percentage of flower coverage in proximity of each nesting station has been evaluated

twice during the kiwi flowering by taking 3 pictures of a 1×1 m plot: one right below the station, and two at the distance of 10 m from the station in opposite directions, average percentage values had been calculated daily for each experimental site.

Assessing the effect of red mason bees and flower strips on pollinator visits and fruit production and quality

In each row hosting an experimental site, 6 trees at increasing distances from the nesting station (0, 20, 40, 60, 80 and 100 m) were chosen as experimental trees (supplemental material figure S1). For each experimental tree, 10 floral bud clusters have been individually labelled and monitored until the harvest. Of each cluster we collected data on the number of floral buds (late April), the number of fruitlets (June), and the final fruit set (September). At harvest of the kiwifruit, 6 fruits from the tagged branches were collected (324 in total) and moved to the laboratory to assess the same fruit quality parameters previously described for the fruits involved in the assess of the pollination deficit (weight, maximum diameter, orthogonal diameter, length, dry matter and number of seeds). Pollinator diversity and density have been assessed by performing observational transects along each one of the rows hosting the nesting stations, starting from the station, and ending at the experimental tree placed at 100 meters of distance; distance has been covered at a constant pace in a time of 20 minutes. Only insects interacting with kiwi flowers have been counted. Each transect was divided in 5 subunits of 20 m each where data have been collected separately. Pollinators were classified under the following categories: *O. bicornis* (released pollinator); *A. mellifera*; *Bombus* spp.; wild bees; hoverflies (Diptera Syrphidae); other pollinators. At each experimental site, the pollinator survey was conducted twice during the peak flowering period of kiwifruit (May 6th and 8th). Observations were carried out during the warmest hours of the day, starting at 10:00 and concluding no later than 14:00.

Phytosanitary management of the study orchards

The orchards in this study were managed according to the phytosanitary practices used in the production of Zespri Gold G3® kiwifruit. Below, the practices are described with a focus on the period surrounding flowering. Copper-based treatments to prevent bacterial canker (*Pseudomonas syringae* pv. *actinidiae*) were typically applied one week before flowering and prior to the release of *O. bicornis* in the study orchards. At the onset of flowering (10-20% of flowers open), a fungicide treatment combining cyprodinil and fludioxonil (commercial product: Switch) was applied to control *Botrytis* and *Sclerotinia*. The only insecticide treatment used on the study orchards was against the brown marmorated stink bug *Halyomorpha halys* (Stal), including etofenprox (commercial product: Trebon Up) and deltamethrin (commercial product: Decis Evo), that was applied in late July, after the removal of the red mason bee nesting stations from the orchards.

Statistical analysis

For fruit production variables in the pollination deficit experiment, we calculated two Generalized Linear Mixed-Effects Models (GLMMs) assuming a binomial distribution, testing the effects of the pollination treatment. The dependent variable was represented respectively by the percentage of flowers per cluster that turned into fruitlets (early fruit set) and by the percentage of flowers that turned into fruits (final fruit set). Site, transect and plant identity were included as nested random factors. We also calculated six Linear Mixed-Effects Models (LMMs) testing the effect of pollination treatment on fruit quality variables (shape, MMR, length, weight, dry matter, number of seeds), once again including site and plant identity as nested random factors. MMR was arcsine-root transformed (Gotelli and Ellison, 2004), and weight was log-transformed in order to meet model assumptions. We initially intended to use a GLMM with either Poisson, negative binomial or quasi-Poisson distribution for the model involving the number of seeds, but severe violations of the assumptions for the latter made us prefer the LMM. For models that detected significant differences, we conducted post-hoc tests with Tukey adjustments to compare the different pollination treatments. In one case (shape) in which the LMM detected a significant effect of the pollinator treatment, but the Tukey test only detected near-significant differences between treatments, we used Fisher's Least Significant Differences (LSD) test (Williams and Abdi, 2010), which is less conservative but more powerful than Tukey, as a post-hoc test. We also calculated the pollination deficit for each experimental plant according to Garratt *et al.* (2014). The deficit was calculated for early and final fruit set, fruit weight, fruit length and average number of seeds per fruit, according to the formula:

$$\text{deficit output} = (H - O) / H$$

where H is the output under hand pollination and O is the output under open pollination. In cases where the output achieved following open pollination conditions was greater than for hand pollination, the deficit was calculated as a proportion of the output under open pollination. Thus, the deficit is a proportion of maximum crop output allowing both positive and negative values to account for possible limitations due to insufficient pollination (positive deficit values) but also risks of suboptimal output due to over pollination (negative deficit value) causing rising costs and/or lower fruit quality (Garratt *et al.*, 2014). We used GLMMs with Poisson distribution to test the effects of flower strip floral cover and distance from experimental site, as well as their interaction, on the number of pollinator visits to kiwi flowers, and on their species/morphospecies richness. Site and transect identity were included as nested random factors. As a proxy for red mason bee visitation rate of kiwi flowers, we used a weighted kiwi pollen collection index. First, we calculated the average percentage of kiwi pollen present in the provisions collected in a subset of *O. bicornis* nests. Then, we multiplied that value by the total number of *O. bicornis* cells built in the corresponding nesting station. We ran binomial GLMMs to test the effects of flower strip floral cover, distance from experimental sites and weighted kiwi pollen collection by red mason bees,

as well as their interactions, on early and final fruit set. Site, transect and plant identity were included as nested random factors. Additionally, we calculated LMMs testing the effects of flower strip floral cover, distance from stations, kiwi pollen collection and their interactions on the six fruit quality variables, with site, transect and plant identity as nested random factors. In all cases, when interactions between explanatory variables were not significant ($p > 0.05$), we removed the interaction and re-ran the model in order to avoid overfitting and correctly interpret the main effects (Engqvist, 2005; Lami *et al.*, 2021). Once again, MMR was arcsine-root transformed in order to meet model assumptions, and we opted for LMM for the model involving the number of seeds, due to assumptions problems with the Poisson, negative binomial and quasi-Poisson GLMMs. We also calculated a Pearson correlation between the floral coverage of flower strip and the number of cells recorded in the examined red mason bee nest in each experimental transect, to check the effect of the flower strips on red mason bee reproductive success. Finally, we calculated for completeness a Pearson correlation between the percentage of kiwi pollen grains and the percentage of oak pollen grains (*Quercus* L.) found in *Osmia* cells, as *Quercus* pollen is known to be very attractive for *Osmia* bees, and the availability of oaks in the landscape might therefore act as a distraction from the crop (Hansted *et al.*, 2014; Splitt *et al.*, 2021). All analyses were performed using packages lme4 v1.1-35.1, emmeans v1.9.0 and DHARMA v0.4.6 in R v4.4.1.

Results

Pollination deficit

The average pollination deficit overall for the early fruit set was 8.6%, ranging from -13.3% to 73.3%. For the final fruit set, the overall mean deficit was 14.6% (ranging from -56.4% to 100%). For fruit weight, fruit length and number of seeds, the mean overall pollination deficit was respectively 11.0% (ranging from -17.7% to 36.4%), 5.0% (ranging from -2.9% to 19.3%) and 29.6% (ranging from -10.8% to 91.6%) (table 1). Pollination treatment had a significant effect on early fruit set, final fruit set, and on the quality metrics of shape, length, weight, dry matter and number of seeds (table 2). Post-hoc tests revealed that pollinator exclusion significantly lowered all of these indicators when compared to the other treatments (figure 1), except for shape (figure 1c), for which higher values correspond to lower quality, and for which exclusion and open pollination were not significantly different. Except for dry matter, hand pollination also had significantly higher values (or lower, in the case of shape) than open pollination (figure 1a-e, g).

Red mason bees and flower strips

The successful release of *O. bicornis* was confirmed by the field observation of activities such as flight, mating, and pollen collection. Pollen analyses of samples collected from nesting stations once kiwi flowering ended allowed for a semi-quantitative assessment of kiwi pollen in provisions, calculated as percentage, was on average

Table 1. Mean values (%) and standard deviations (SD) of pollination deficit calculated on yield parameters of early fruit set and final fruit set, and quality parameters of weight, length and seeds number. Values of pollination deficit are reported separately for each orchard and cumulated.

	Orchard 1 (Cesena)		Orchard 2 (Faenza)		Orchard 3 (Ravenna)		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Early fruit set	3	4	25	27	-3	6	9	20
Final fruit set	7	9	31	41	6	16	15	28
Mean weight	10	9	11	16	12	13	11	13
Mean length	3	3	6	6	6	6	5	5
Mean number of seeds	25	21	32	35	32	30	30	28

Table 2. Results of the Generalized Linear Mixed-Effects Models (GLMMs) testing the effect of pollination treatment on early and final fruit set, and of the Linear Mixed-Effects Models (LMMs) testing the effect of pollination treatment on fruit quality metrics (shape, MMR, dry matter, length, weight and number of seeds).

Dependent variable	Fixed effect	d.f.	χ^2	p	Sample size
Fruit production					
Fruitlets/flowers	Pollination treatment	2	133.87	<0.001	268
Fruits/flowers	Pollination treatment	2	115.89	<0.001	268
Fruit quality					
Shape	Pollination treatment	2	7.46	0.02	178
MMR ^a	Pollination treatment	2	4.89	0.09	178
Dry matter	Pollination treatment	2	44.31	<0.001	178
Length	Pollination treatment	2	115.35	<0.001	178
Weight ^b	Pollination treatment	2	131.34	<0.001	178
Number of seeds	Pollination treatment	2	254.67	<0.001	178

^a = Arcsine-root transformed in order to meet model assumptions.

^b = Log-transformed in order to meet model assumptions.

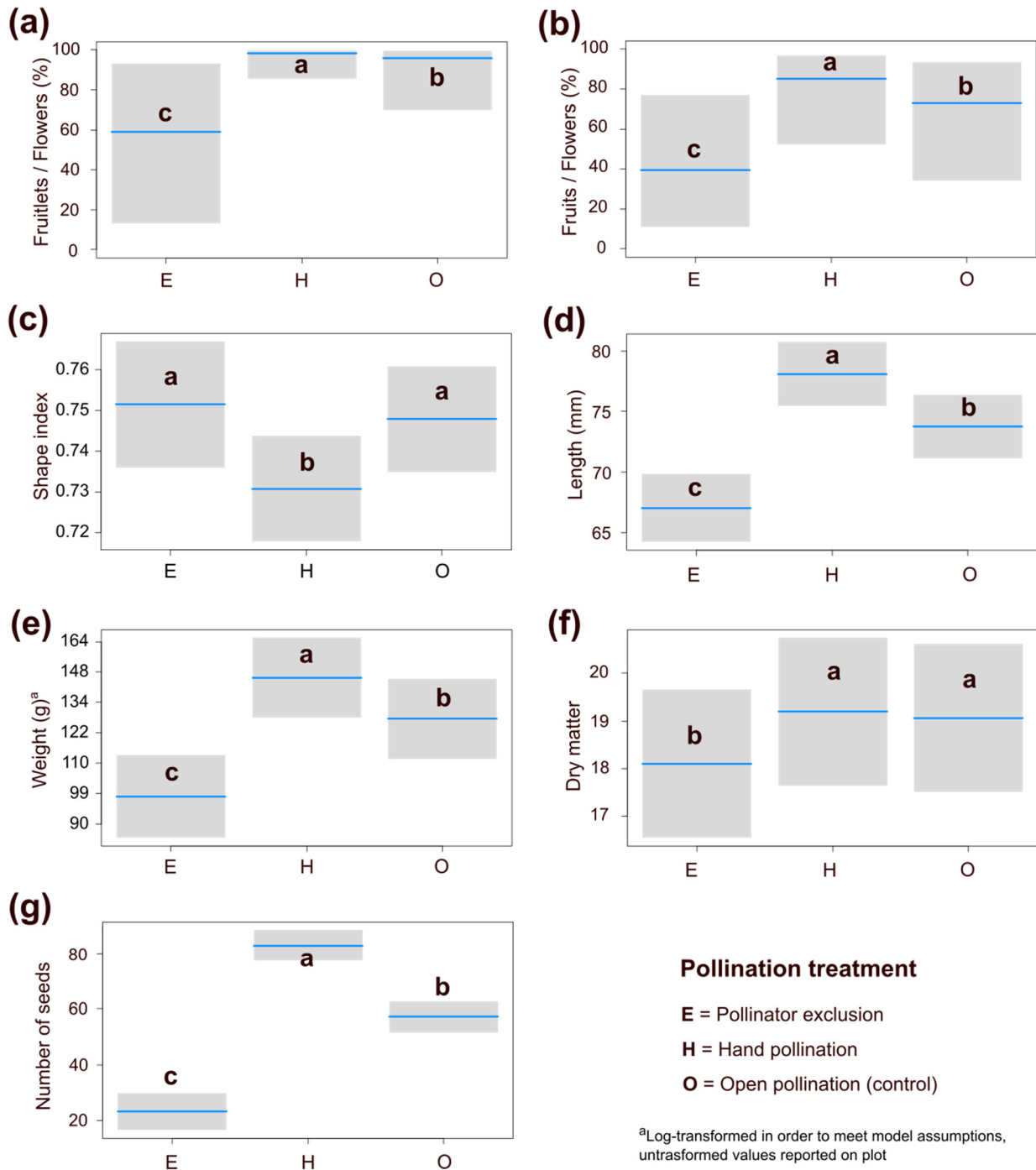


Figure 1. Effect of the pollinator treatment on early fruit set (a), final fruit set (b), fruit shape (c), fruit length (d), fruit weight (e), fruit dry matter (f), and number of seeds (g), with the prediction line in blue and Wald-type 95% confidence intervals. Different letters indicate statistically significant differences as detected by post-hoc pairwise comparisons with Tukey adjustment ($p < 0.05$); in the case of shape (c) we used the less conservative but more powerful LSD test, as the Tukey test could only detect quasi-significant differences while the LMM detected a significant effect of the pollination treatment. Plots were generated using the *visreg* package in R.

29%, ranging from 0% to 99% (supplemental material table S3). The reproductive success, intended as the number of developed cocoons of *O. bicornis*, was highly variable among nesting stations, ranging from 19 to 895 (supplemental material table S4). Average floral cover in experimental sites, on the other hand, ranged from 0% to 23.3%,

and is reported in detail in supplemental material table S5. Flower strip floral cover had no significant relation with the number of cells in red mason bee nests ($r_p = 0.54$, $p = 0.13$), while the percentage of collected *Quercus* pollen had a significant negative relation with the percentage of collected kiwi pollen ($r_p = -0.94$, $p < 0.001$).

Pollinator visits to kiwi flowers

The number of recorded visits of pollinators on kiwi flowers was scarce, with a total of 146 recorded visits during our 18 transects (over a total duration of 6 hours of observations). Overall, 40.41% of visits were *Bombus* spp., 27.40% were other wild bees (except *O. bicornis*), 13.70% were *A. mellifera*, 12.32% were hoverflies, and 1.37% of total observations were of *O. bicornis* (the remaining 4.79% were other pollinators). Data on insect pollinators recorded in each observational transect are available as supplementary material (supplemental material table S6). Distance from the experimental site and floral cover in flower strips (table 3) had an interactive effect on the total number of pollinator visits to kiwi flowers. In sites with low floral cover in the strips, the

number of visits were higher closer to the experimental site itself, while there was an opposite trend when the floral coverage in its proximity was higher (table 3, figure 2a). Furthermore, the richness of pollinators observed interacting with kiwi flowers showed a negative correlation with the floral coverage of flower strips (table 3, figure 2b), while distance from experimental site and its interaction with floral coverage had no significant effect on the diversity of floral visitors.

Effects of red mason bees and flower strips on kiwifruit production and quality

There was a significant three-way interaction between flower strip floral cover, kiwi pollen collection by red mason bees and distance from experimental sites, influ-

Table 3. Results of the Generalized Linear Mixed-Effects Models (GLMMs) testing the effect of distance from experimental site, kiwi pollen collection by *O. bicornis* and flower strip floral cover on the abundance and richness of kiwi flower visitors and on early and final fruit set, and of the Linear Mixed-Effects Models (LMMs) testing the effect of the same variables on fruit quality metrics (shape, MMR, dry matter, length, weight and number of seeds).

Dependent variable	Fixed effects	d.f.	χ^2	p	Sample size
Pollinators					
Pollinator visits	Distance from site	1	7.14	<0.01	88
	Strip floral cover	1	13.76	<0.001	
	Distance × Cover	1	7.03	<0.01	
Pollinator richness	Distance from site	1	0.04	0.84	88
	Strip floral cover	1	5.49	0.02	
Fruit production					
Fruitlets/flowers	Distance from site	1	1296.40	<0.001	524
	Weighted kiwi pollen collection by <i>Osmia</i>	1	596963.00	<0.001	
	Strip floral cover	1	41150.10	<0.001	
	Distance × Pollen	1	2844.40	<0.001	
	Distance × Cover	1	1816.70	<0.001	
	Pollen × Cover	1	21261.70	<0.001	
Fruits/flowers	Distance × Pollen × Cover	1	8412.00	<0.001	507
	Distance from site	1	0.53	0.46	
	Weighted kiwi pollen collection by <i>Osmia</i>	1	2.15	0.14	
	Strip floral cover	1	0.75	0.39	
Fruit quality					
Shape	Distance from site	1	3.88	0.04	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.00	0.97	
	Strip floral cover	1	1.44	0.23	
MMR ^a	Distance from site	1	4.84	0.03	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.54	0.46	
	Strip floral cover	1	1.36	0.24	
Dry matter	Distance from site	1	0.18	0.67	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.49	0.48	
	Strip floral cover	1	4.95	0.03	
	Pollen × Cover	1	7.31	<0.01	
Length	Distance from site	1	0.35	0.56	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.61	0.44	
	Strip floral cover	1	2.83	0.09	
Weight	Distance from site	1	0.09	0.76	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.13	0.72	
	Strip floral cover	1	1.52	0.22	
Number of seeds	Distance from site	1	0.01	0.94	318
	Weighted kiwi pollen collection by <i>Osmia</i>	1	0.83	0.36	
	Strip floral cover	1	0.18	0.67	
	Distance × Cover	1	4.74	0.03	

^a = Arcsine-root transformed in order to meet model assumptions.

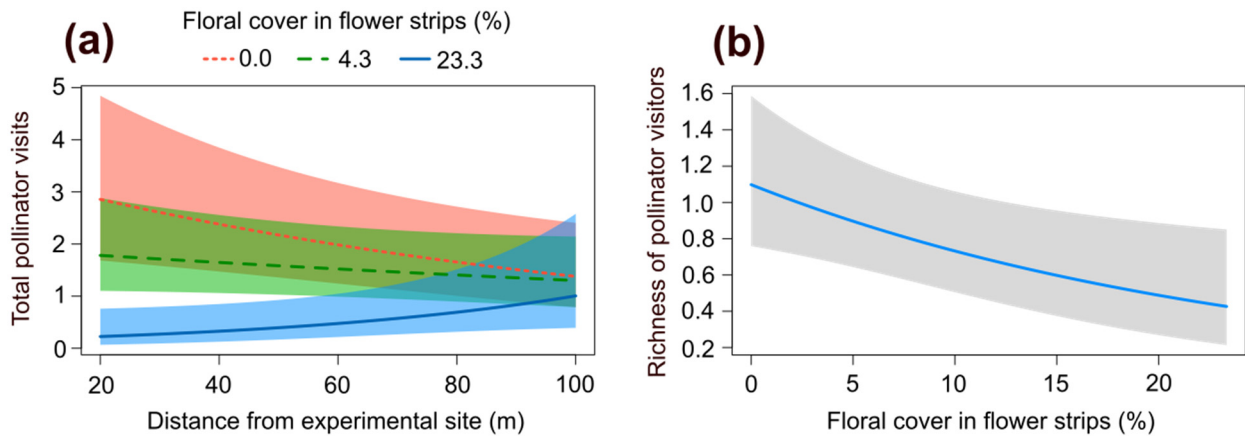


Figure 2. Effect of floral cover in flower strips and distance from experimental sites on total pollinator visits on kiwi flowers (a) and their species and morphospecies richness (b), with prediction lines and Wald-type 95% confidence intervals. Only significant effects are depicted. Plots were generated using the *visreg* package in R.

encing early fruit set (fruitlets/flowers) (table 3). Kiwi pollen collection by red mason bees in all conditions improved early fruit set; in scenarios of high kiwi pollen collection and no flower strip flowering, the plants closer to the experimental site showed an increased early fruit set compared with distant plants. In scenarios of high flower coverage in the flower strip, the effect of distance was reversed, with a higher early fruit set in plants distant from the experimental site (figure 3, table 3). Regarding the quality parameters, we found a significant interaction between floral cover in flower strips and kiwi pollen collection by red mason bees, influencing dry matter in ki-

wifruits: high kiwi pollen collection caused a slight increase in dry matter, but only in scenarios with higher floral cover (figure 4a, table 3). For the number of seeds, on the other hand, there was a significant interaction between floral cover in flower strips and distance from experimental site, with number of seeds decreasing with distance when floral cover was low and increasing with distance when floral cover was high (figure 4b, table 3). Finally, distance from experimental sites was also positively correlated with the value of the shape index (figure 4c, table 3) and negatively correlated with MMR (figure 4d, table 3).

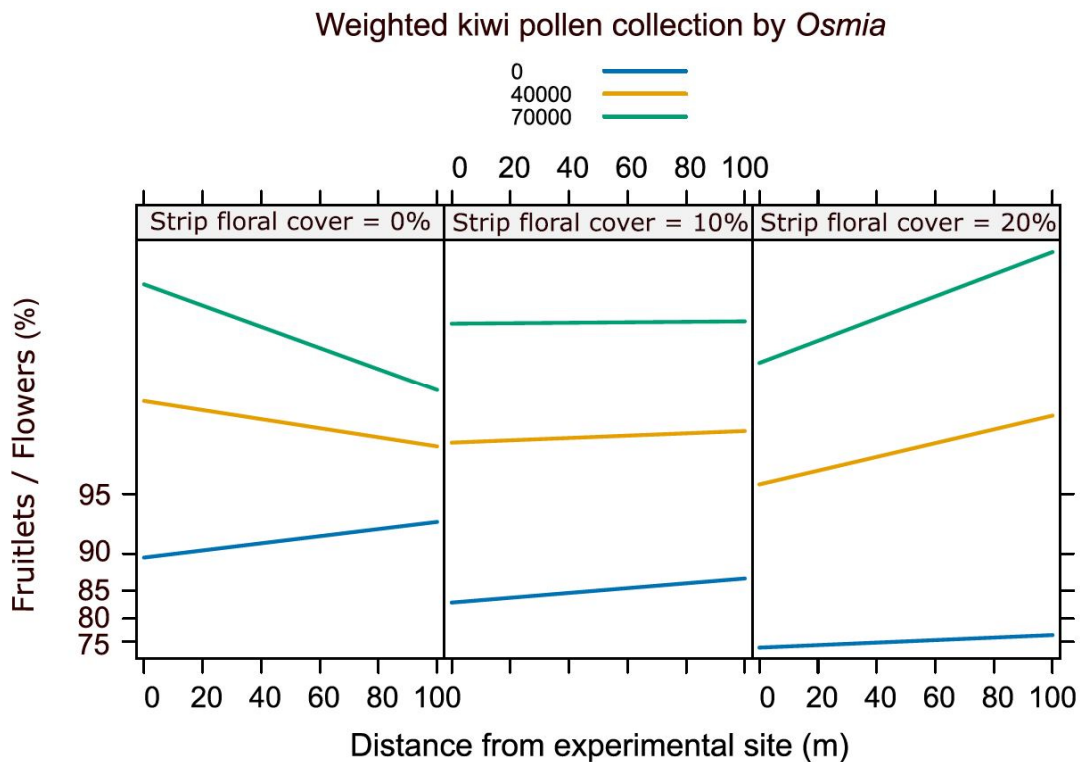
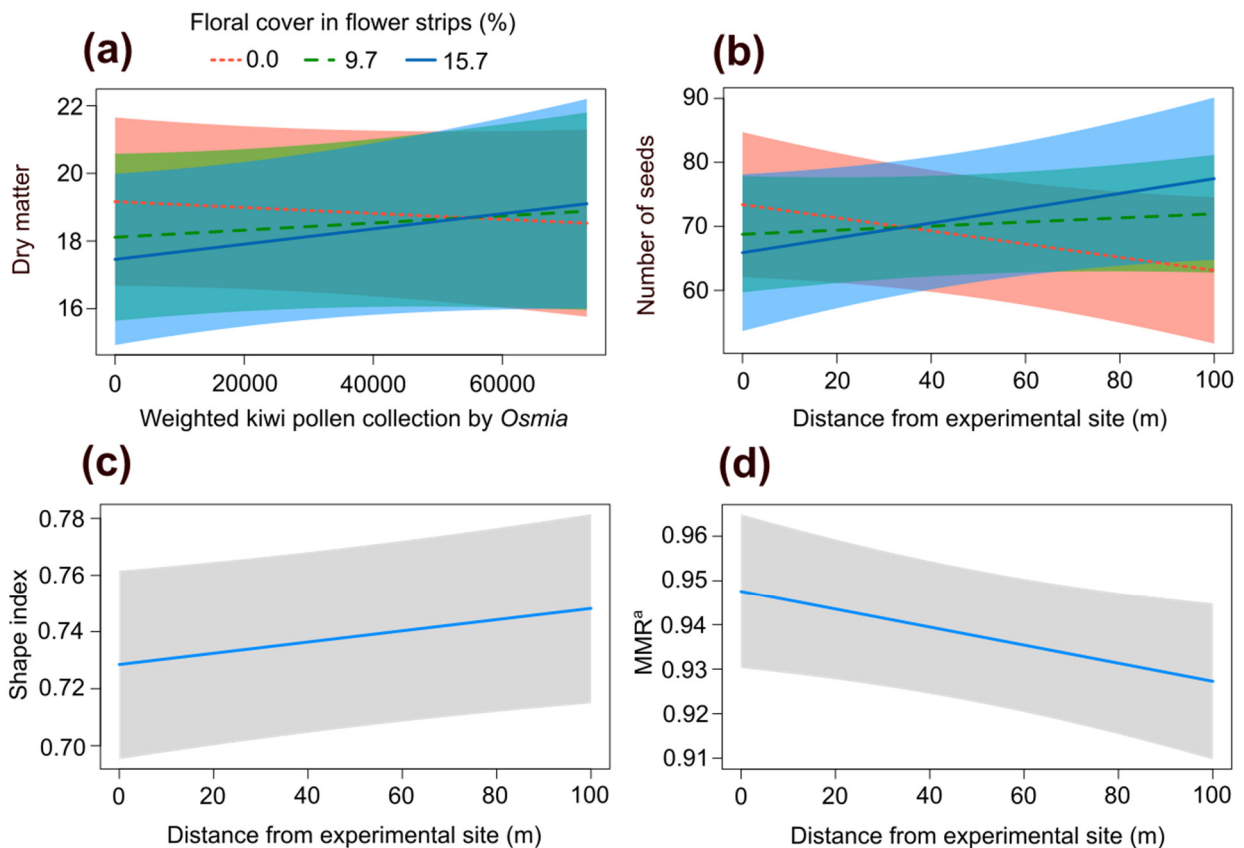


Figure 3. Effect of the interaction between flower strip floral cover, distance from experimental site and kiwi pollen collection by red mason bees (as a proxy for kiwi flower visitation) on the production of fruitlets from flowers (early fruit set). The vertical axis is printed on the scale of the response variable. Plots were generated using the *effects* package in R.



^aArcsine-root transformed in order to meet model assumptions, untransformed values reported on plot

Figure 4. Effect of flower strip floral cover, distance from experimental site and kiwi pollen collection by red mason bees (as a proxy for kiwi flower visitation) kiwifruit quality parameters dry matter (a), number of seeds (b), shape (c) and MMR (d), with prediction lines and Wald-type 95% confidence intervals. Only significant effects are depicted. Plots were generated using the *visreg* package in R.

Discussion and conclusions

Pollination deficit and contribution of insect pollinators in kiwifruit production

This study focuses on kiwifruit, a dioecious crop emerging in Mediterranean Europe, and examines the limitation on its production related to inadequate pollination. Despite extensive research demonstrating the dependence on pollinators of crops in Europe, methods for accurately quantifying pollination deficits remain underutilized. Available results for fruit crops are particularly limited, with most research focusing on apples, another crop heavily relying on insect pollination (Garratt *et al.*, 2014). Pollination deficits for apple fruit set in Europe have been reported to average around 30% (Olhnuud *et al.*, 2022), however, the usefulness of direct comparisons is questionable, as kiwifruit orchards in our study implemented mechanical ventilation to improve pollination, a technique not commonly used in apple cultivation. Our pollinator manipulation experiment revealed that insect pollinators significantly improve kiwifruit production in terms of both quantity and quality. However, it also showed the existence of a pollination deficit impacting several aspects of kiwifruit yield and quality, likely due to the relatively low attractiveness of the kiwi flower for European pollinators (Schmid, 1978),

possibly exacerbated by the widespread pauperization of pollinator communities (Potts *et al.*, 2016). The average values of pollination deficit observed for kiwifruit in this study were 9% for fruitlet set, 15% for fruit set, 30% for seed number and 11% for weight. Similar findings were reported in Portugal by Castro *et al.* (2021), who estimated a pollination deficit in kiwifruit weight of 6-11%, varying by year. In contrast, Portugal kiwifruit plantations are generally composed of smaller-scale orchards within mosaic landscapes rich in semi-natural habitats, which may enhance abundance and diversity of both wild and managed pollinators, potentially mitigating pollination deficits. In our case the study area is characterized by a landscape largely dominated by intensive farming, mostly employing conventional agronomic practices, where the large use of chemicals is likely to compromise the overall biodiversity and abundance of pollinators. Pesticides used in agroecosystem have been shown to affect development and reproduction of wild pollinators, including solitary bees (Phan *et al.*, 2024).

Red mason bee populations establishment in kiwifruit orchards

Due to the high costs of artificial pollination, the risks correlated and its controversial efficacy, it is especially important to investigate new ways to improve insect

pollination in kiwifruit, including the use of alternative managed pollinators and flower strips to enhance the presence of wild pollinators. Currently, honey bees and bumblebees are the primary managed pollinators introduced into kiwi orchards to support effective fruit sets. In certain kiwifruit varieties, such as the green-fleshed 'Hayward', it has been observed that an optimal fruit set may require up to 40 honey bee visits per flower (Goodwin, 1995). In contrast, yellow-fleshed varieties, as the one involved in our study, generally require around six honey bee visits to achieve sufficient pollination (Goodwin *et al.*, 2017). A comparative study of kiwifruit pollinators in New Zealand further indicates that *Bombus* spp. and *A. mellifera* exhibit comparable efficiency in pollinating kiwifruit (Broussard *et al.*, 2022). On the other hand, it seems that honey bee legitimacy is lower compared to other pollinator groups in apple fruit, such as the solitary bees belonging to the genus *Osmia* (Lyu *et al.*, 2023). Some *Osmia* species have been largely studied as possible managed pollinators in orchards and other agricultural crops (Bosch and Kemp, 2002; Maccagnani *et al.*, 2003; 2007; Ryder *et al.*, 2020) but, as far as we know, our study is the first that assessed the efficacy of *Osmia* as a pollinator of kiwifruit. Firstly, our results showed that it is possible to establish populations of *O. bicornis* in kiwifruit orchards. We observed an increase in populations in most experimental sites; only two sites in the orchard located in Cesena showed critically low reproductive success (3.45% and 25.09% respectively), measured by the number of viable cocoons relative to the initial number of released individuals. This phenomenon may be attributed to the mowing practices that were mistakenly conducted in sync with the mating of released red mason bees. Given that this species often mates on the ground the timing of the mowing could have disrupted this critical phase. In the other study sites, the average reproductive success was 110.85%, ranging from 77.27% to 162.72%. These fluctuations in reproductive success might be partially attributed to the higher dispersal rates of adults due to extreme weather conditions, in the Emilia-Romagna region during spring 2023 (Arrighi and Domeneghetti, 2024). It is well known that reproduction success in this species is maximized under optimum environmental conditions (Ivanov, 2006; Giejdasz *et al.*, 2016). Noteworthy, even under the harsh weather conditions occurred right before the kiwi flowering, the red mason bees nesting activity resulted in a satisfying reproductive success in most of the experimental sites.

Effect of managed red mason bees and flower strips on kiwifruit farming

While the number of red mason bees (and pollinators in general) observed during transects was very low, this species did visit kiwi flowers, as evidenced by the collected kiwi pollen found in the nests. However, we found a pronounced difference in pollen composition of provisions collected by the red mason bees among the experimental sites, probably due to the availability of other more attractive pollen sources in the surrounding environment compared to kiwi flowers. Results suggest that the availability of *Quercus* trees might be quite distracting, with the pollen from these trees likely to be preferred by red mason

bees if they are within their flight range (Hansted *et al.*, 2014; Splitt *et al.*, 2021). In any case, red mason bee presence and overall rate of flower visitation seem to have a partially positive impact on kiwifruit production (but only on the fruitlet stage), and on some aspects of fruit quality such as number of seeds, dry matter, and shape (both shape index and MMR). In contrast, flower strips, presumably because they included more attractive flowers, seem to have played a distracting effect on both red mason bees and other pollinators, reducing the number of visits from both individual pollinators and morpho-groups. This distraction partially negatively impacted kiwifruit production and quality, particularly in terms of early fruit set and number of seeds. Distraction from crops is a well-recognized risk when employing flower enhancements to support pollinators (Nicholson *et al.*, 2019). Additionally, the Pearson correlation revealed that flower strips did not enhance the reproductive success of red mason bees, measured as the number of cells in the surveyed nests. Based on these data, it is therefore not advisable to use flower strips in kiwi agroecosystems if the goal is to improve production of the crop itself, and not to sustain wild pollinators; on the other hand, the use of red mason bees might help improving kiwifruit production and quality, especially in those kiwifruit orchards that are poor in alternative floral resources. However, more research is needed to confirm these results and to find a balance point where floral resources could be planted in crop fields without distracting insects from pollinating surrounding plants, without renouncing to the general benefits offered by cover crops (Holland *et al.*, 2015). As red mason bees seem to visit and benefit kiwi flowers closer to their nesting stations (at least when distracting flower sources are absent), a sound strategy to provide pollination to the entire crop would be to install multiple evenly spaced red mason bee nesting stations across the orchard.

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