



# Evaluating honey bee foraging behaviour and their impact on pollination success in a mixed almond orchard

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**Abstract** – Although *Apis mellifera* is the dominant managed pollinator used to enhance crop production, the variation of its foraging behaviour among crop-cultivars is not considered as a factor influencing pollination success and, thus, yield. Almond production is highly dependent on cross-pollination. Herein, we examined honey bees foraging behaviour and pollen limitation in ‘Nonpareil’ and ‘Independence’ cultivars. Nectar and pollen-foragers differed in the flower handling behaviour, regardless of the cultivar. However, most bees on ‘Independence’ flowers collected pollen in the morning and bees exhibited less floral constancy in this cultivar, potentially promoting cross-pollination. Also, supplementation of ‘Nonpareil’ flowers with ‘Independence’ pollen had a positive effect in nut-set, suggesting that higher visitation rates are needed to increase yield. Our study underlines the importance of integrating behavioural knowledge of pollinators to optimize practices in pollinator-dependent crops.

*Apis mellifera* / *Prunus dulcis* / foraging behaviour / floral constancy / pollen limitation

## 1. INTRODUCTION

Cultivated areas of pollinator-dependent crops are in continuous expansion around the globe (Aizen et al. 2019) and mainly rely on the managed honey bee *Apis mellifera* L. to increase crop yield (McGregor 1976; Klein et al. 2007). Despite an increasing demand for pollination services, the global stock of honey bee colonies is growing at a

slower pace than current needs (Aizen and Harder 2009; Breeze et al. 2014). Although the contribution of wild insects to pollination has been recently recognized for a wide variety of crops (Garibaldi et al. 2013; Mallinger and Gratton 2015; Rader et al. 2016), the abundance and diversity of species varies over space and time, and also with agricultural intensification (Kremen et al. 2002; Klein et al. 2012; Mandelik et al. 2012).

The high dependence on biotic pollination in crops like apples, pears and almonds resides on the presence of self-incompatibility systems

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(Socias I Company 1998; Klein et al. 2007). If flowers receive a deficient quantity of compatible pollen to fertilize all their ovules, pollination will be limited, and the crop will produce less fruits or nuts than it would have with adequate pollen transfer (Knight et al. 2005). Although foraging behaviour of pollinators will ultimately affect the quantity and quality of pollen deposited on flower stigmas, and consequently crop yield (Brittain et al. 2013), it is not always weighted as an important aspect influencing pollination success when planning an orchard. Honey bees are considered efficient pollinators of fruit and nut trees, transferring pollen among varieties while foraging for nectar and pollen (Thomson and Goodell 2001; Henselek et al. 2018). Previous studies in orchards with multiple cultivars show, on the one hand, that the great majority of captured bees foraging on almond trees carried pollen from many varieties on their bodies (DeGrandi-Hoffman et al. 1992); but on the other hand, that most honey bees established foraging areas on a single or a few trees in apple or pear orchards, rarely moving across rows between different cultivars (Free 1966; Quinet and Jacquemart 2017). Such fidelity to a single cultivar can be altered in the presence of non-*Apis* bees, in which case honey bees increased the proportion of movements between rows, therefore, enhancing cross-pollination (Brittain et al. 2013). Additionally, different varieties might differ in their attractiveness to honey bees (e.g. in their floral morphology, odours, and rewards) and thus influence their foraging behaviour (Wright and Schiestl 2009). Previous studies in pollinator-dependent crops with dimorphism between genotypes like sunflower show that honey bees exhibit high fidelity to a single variety, hindering the transfer of pollen between cultivars with different floral whole disc diameter and plant height (Susic and Farina 2016; Estravis Barcala et al. 2019).

Almond (*Prunus dulcis* (Mill.) D.A. Webb) production is highly dependent on cross-pollination and growers rely on managed honey bees to ensure pollination success (Delaplane and Mayer 2000; Klein et al. 2007). Almond global production keeps steadily growing, reporting a total crop production over 1.3 million metric tons (kernel basis) in 2019/2020, 26% above the

previous 10-year average (International Nut and Dried Fruit Council Foundation 2020). California state, USA, accounts for 77% of the world crop share and demands an increasing number of beehives each season (CDFA 2020). Almond trees start to bloom in late winter, being the earliest temperate crop tree to flower, at a time when wild bee populations are scarce (Gradziel 2009; Klein et al. 2012). Moreover, most orchards in the main production areas in California are distant from natural habitats (Champetier 2011). Thus, almond orchards can be considered as a homogeneous monoculture environment in which honey bees are the single dominant pollinator species. In order to maximize cross-pollination and, consequently, crop yield, 1:1 or 1:1:1 intercultural planting schemes are preferred (Micke 1996; Delaplane and Mayer 2000). However, different studies made in 'Nonpareil', the leading almond cultivar in the USA, found signs of pollen limitation in mixed plantations with 'Padre', 'Carmel' and 'Price' cultivars (Klein et al. 2015; Cunningham et al. 2016). In such pollen-limited systems, from a pollinator behavioural perspective, any differences in honey bee foraging patterns (i.e. handling time, foraged resource, floral constancy) among cultivars could modify the quantity or quality of pollen delivered, and eventually translate into differences in fruit set and yield.

Although almond is a predominantly self-incompatible species, breeding of self-compatible cultivars has become of growing interest (Martínez-Gómez et al. 2007). 'Independence' (Independence® (Alm-21 cv.) Patent no.20295) is a self-fertile almond cultivar reported as one of the top ten producing varieties in California (CDFA 2020). It is considered a pollen donor for 'Nonpareil', and both varieties overlap in their bloom time. Some almond genotypes are not influenced by self- or cross-pollination on the fruit set and fruit quality, supporting the feasibility of monovarietal orchards which would require lower number of beehives (Dicenta et al. 2002). However, there is evidence of the positive contribution of honey bees to yield in a self-fertile cultivar marketed as pollinator-independent (Sáez et al. 2020). Studies

contrasting pollinators foraging behaviour on self-fertile and self-incompatible almond varieties are scarce, and generally refer to a single cultivar (Thomson and Goodell 2001; Klein et al. 2012; Cunningham et al. 2016). Moreover, only a few cases evaluate pollinator visits among different varieties or the presence of self- or cross-pollen on their bodies (DeGrandi-Hoffman et al. 1992; Brittain et al. 2013). Therefore, a more thorough understanding of the foraging behaviour of honey bees in a mixed system including self-compatible and self-incompatible varieties could provide useful knowledge for almond growers.

Here, we examined the foraging behaviour of managed *A. mellifera* in an orchard designed with a single-row arrangement of ‘Nonpareil’ and ‘Independence’ cultivars. We assessed the foraging patterns on each cultivar in terms of the number of flowers visited and the resource collected, while we measured movements between cultivars during a single foraging trip. Finally, we evaluated quantity and quality pollen limitation in both cultivars.

## 2. MATERIALS AND METHODS

### 2.1. Study site and managed honey bees

Field studies were performed during the almond blooming season in February–March 2018, in a commercial orchard (Fresno County, CA, 36° 21' 25.64" N 119° 42' 15.54" W). We worked in three plots with trees in their second and third year of production of the varieties ‘Nonpareil’ (self-incompatible cultivar) and ‘Independence’ (pollinizer), planted in alternating rows. The distance between trees in different rows and between those within the same row were 6.4 m and 4.6 m, respectively. The plots were subjected to the same management practices (such as irrigation, fertilizer and pesticide applications, and pruning). The assessment of the phenology of both cultivars over the course of the behavioural studies showed that the blooming of ‘Independence’ (pollinizer) trees progressed slightly slower than the ‘Nonpareil’ trees.

A total of 260 colonies of European honey bees were located along plot edges ( $\approx 5$  hives/hectare,

which is the recommended stocking rate; FAO 2018).

### 2.2. Honey bees foraging behaviour

#### 2.2.1. Visitation rates on almond cultivars

We conducted a total of 82 visit censuses (47 on ‘Independence’ and 35 on ‘Nonpareil’ trees) over 16 consecutive days. In each tree, we randomly selected a group of  $54 \pm 2.5$  flowers (mean  $\pm$  S.E.) on outer branches to be observed during 5 minutes, in which we recorded the number of flowers visited by each visiting pollinator (i.e.  $n \text{ visits} * \text{flower}^{-1} * 5 \text{min}^{-1}$ ). Only legitimate visits, with bees contacting the floral reproductive structures, were considered. All measures were recorded between 10 am and 5 pm, during the period of activity of honey bees.

#### 2.2.2. Number of flowers visited and type of resources exploited

We monitored 185 honey bees during their sequential foraging visits from the moment they landed on a flower until the observer lost sight of the focal bee. For each individual, we registered the number of flowers visited, the time spent on each flower, the cultivar and the time of day (morning, from 9 am to 12 pm, or afternoon, from 2 to 4 pm). We categorized bees with pollen in the corbiculae as “pollen-foragers” and individuals extending their proboscis and without pollen in the corbiculae as “nectar-foragers” (DeGrandi-Hoffman et al. 1992).

#### 2.2.3. Movement of honey bees between cultivars

Since almond production depends on cross-pollination, we evaluated the floral constancy of *A. mellifera* while foraging on each variety. Bees were monitored during their sequential visits while flying from one tree to another for a maximum of 10 minutes, recording if they switched between cultivars or not, to calculate the percentage of bees that showed constancy on each cultivar.

### 2.3. Pollen limitation in almond cultivars

To assess pollen limitation in the self-compatible cultivar ‘Independence’, we randomly selected 10 trees and marked 9 branches per tree to be assigned to the following treatments: (a) the flowers of three branches were exposed to natural pollination (O), (b) the flowers of three different branches were supplemented with pollen from flowers of the same tree (S1) and (c) the flowers of the remaining branches were supplemented with pollen from flowers of different trees of the same variety (S2).

To assess pollen limitation in the self-incompatible cultivar ‘Nonpareil’, we randomly selected 10 trees and marked 6 branches per tree, to be assigned to the following treatments: (a) the flowers of three branches were exposed to natural pollination (Open), and (b) the flowers of the remaining three we supplemented with pollen from ‘Independence’ flowers (Supplemented).

All experimental flowers were supplemented with fresh pollen (collected the same morning), using clean brushes. A total of approximately 1700 flowers were supplemented with pollen at least two times during their lifespan.

At the moment of supplementation, we recorded the number of flowers in each experimental branch for later estimation of the fruit set (i.e. proportion of flowers setting a fruit). In April, about three weeks after the end of bloom, we counted the number of fruit in all branches assigned to the different treatments as an estimation of initial fruit set. In August, prior to harvest and after physiological fruit drop (“June drop”), we counted the number of fruits retained by the trees and calculated the final fruit set.

### 2.4. Statistics

All statistical tests were performed with R v3.6.2 (R Core Team 2019) using *glmmTMB* package (Brooks et al. 2017). To analyse visitation rates, we proposed a generalized linear mixed model (GLMM) to test the influence of almond cultivar (fixed effect) on the number of visits by honey bees (response variable), following a negative binomial error distribution to account for the overdispersion of the data. We included the log-

transformed number of flowers observed in each census as an offset, and the “date” and “tree” as random effects because we sampled the visitation rate repeatedly on the same experimental trees over different days.

To analyse the foraging behaviour of honey bees, we proposed two generalized linear models (GLMs) to test the influence of (1) almond cultivar, time of day and resource exploited (fixed effects) on the number of flowers visited per minute (response variable) following a negative binomial error distribution to account for the overdispersion of the data; and (2) almond cultivar, time of day (fixed factors), on the resource exploited (response variable), following a binomial error distribution. In the first model, we included the observation duration as an offset. In the second model, we included a two-way interaction between cultivar and time of day, and we conducted post hoc comparisons across cultivar with *emmeans* function (Lenth 2020).

The floral constancy was assessed by means of a GLM with a Bernoulli binomial error distribution, considering almond cultivar and time of day (fixed factors) and including the log-transformed observation duration as an offset.

We evaluated the effect of pollen supplementation treatments on initial and final fruit set with two GLMMs, one for each variety. Because response variables were binary (i.e. a flower setting or not a fruit), the models assumed a binomial error distribution with a *logit* link function. Pollen supplementation treatments were included in the models as fixed effect and each “branch” nested within “trees” as a random effect, allowing the intercept to vary among trees/branches. To analyse differences in the number of fruits retained by ‘Nonpareil’ trees (final fruits/initial fruits), we proposed a GLMM following a binomial distribution, considering the supplementation treatments as fixed effect and each “branch” nested within “trees” as a random effect.

Models in general were simplified as follows: significance of the different terms was tested starting from the higher-order terms model using *anova* function to compare between models (Chambers and Hastie 1992). Non-significant terms ( $P > 0.05$ ) were removed (see Supplementary Table 1). We considered the use of GLM and

GLMM because they allow analysing response variables whose errors are not normally distributed, avoiding the transformation of the response variable or the adoption of non-parametric methods (Crawley 2013).

### 3. RESULTS

#### 3.1. Honey bees foraging behaviour

##### 3.1.1. Visitation rates on almond cultivars

We observed a total of 315 flower visits by honey bees on almond flowers during the 82, 5-min observation periods (6.8 h in total). When we compared both cultivars, the rate of *A. mellifera* tended to be higher on ‘Nonpareil’ trees (Figure 1; in *log* scale,  $\beta = 0.49$ ,  $SE = 0.28$ ,  $z = 1.76$ ,  $p = 0.08$ ). On average, honey bees performed (mean  $\pm$  SE)  $1.1 \pm 0.2$  and  $0.7 \pm 0.1$  visits per flower per hour on ‘Nonpareil’ and ‘Independence’, respectively. No other floral visitors were observed during the surveys.

##### 3.1.2. Number of flowers visited, and type of resources exploited

In total, 185 honey bees were monitored while visiting 812 and 904 almond flowers of ‘Independence’ and ‘Nonpareil’ varieties, respectively. Honey bees foraged for nectar as well as for pollen on both cultivars (Figure 2). However, the number of flowers visited per unit of time differed in relation to the resource collected (Figure 2a): pollen-foragers visited more flowers per minute than bees foraging exclusively for nectar (pollen-foragers: (mean  $\pm$  SE)  $6.5 \pm 0.3$  flowers/min; nectar-foragers:  $4.6 \pm 0.2$  flowers/min). This difference in relation to the exploited resource (in *log* scale,  $\beta = -0.46$ ,  $SE = 0.07$ ,  $z = -6.84$ ,  $p < 0.001$ ) was not affected by the almond variety (in *log* scale,  $\beta = -0.05$ ,  $SE = 0.07$ ,  $z = -0.79$ ,  $p = 0.43$ ).

However, the analysis of the foraging preferences throughout the day on each cultivar revealed a significant interaction between the cultivar and the time of day (Figure 2b). While the majority of bees foraged for nectar on ‘Nonpareil’ flowers and this preference was not modified by

the time of day (Supplementary Table 1), most *A. mellifera* (75%) collected pollen during the morning on ‘Independence’ trees (*post hoc* comparison, odds ratio = 6.80,  $SE = 3.24$ ,  $z$  ratio = 4.02,  $p < 0.001$ ).

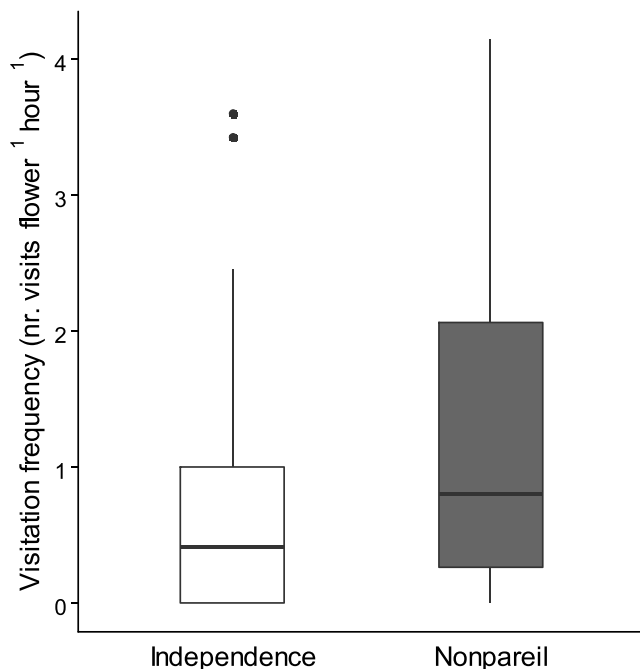
##### 3.1.3. Movement of honey bees between cultivars

When we evaluated the movement of bees between cultivars, we found that honey bees differed in their floral constancy during their sequential visits of almond flowers (Figure 3). Honey bees foraging on ‘Independence’ flowers were more likely to switch to ‘Nonpareil’ trees than those foraging on the latter variety (in *logit* scale,  $\beta = -1.66$ ,  $SE = 0.67$ ,  $z = -2.49$ ,  $p = 0.01$ ). Moreover, this behaviour did not depend on the time of day (Supplementary Table 1).

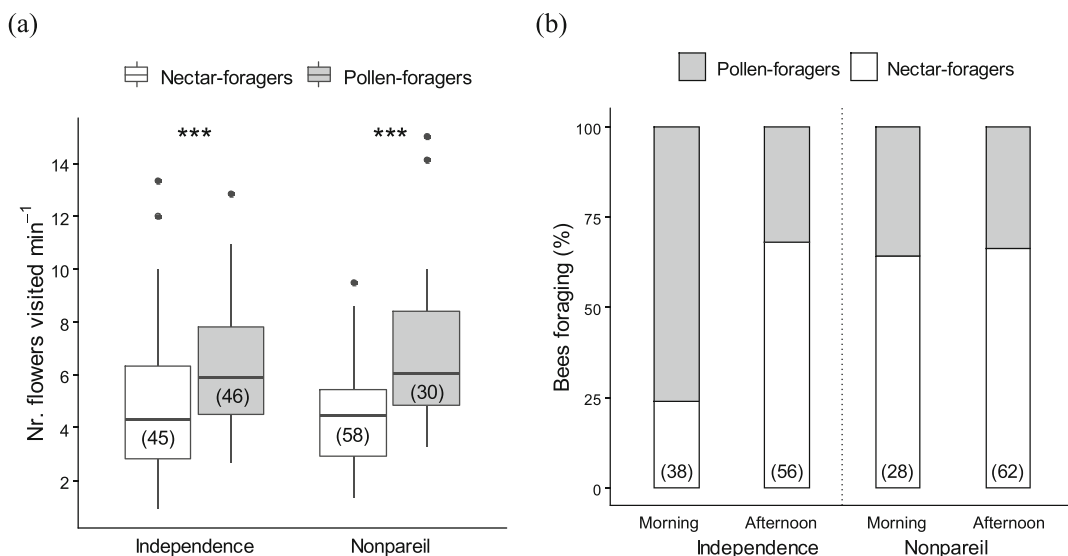
#### 3.2. Pollen supplementation of almond cultivars

The effect of pollen supplementation on ‘Independence’ flowers (self-compatible cultivar) is shown in Figure 4 a. We found no significant differences neither in the initial nut-set of flowers between the different treatments (in *logit* scale,  $\beta = -0.07$ ,  $SE = 0.30$ ,  $z = -0.22$ ,  $p = 0.83$ ; and  $\beta = 0.19$ ,  $SE = 0.31$ ,  $z = 0.61$ ,  $p = 0.54$ ; for comparisons of the initial fruit set between the open-pollinated and self-supplemented, and open and cross-supplemented treatments, respectively) nor in the final fruit set of this variety (in *logit* scale,  $\beta = -0.50$ ,  $SE = 0.42$ ,  $z = -1.17$ ,  $p = 0.24$ ; and  $\beta = -0.11$ ,  $SE = 0.42$ ,  $z = -0.26$ ,  $p = 0.79$ ; for comparisons of the initial fruit set between the open-pollinated and self-supplemented, and open and cross-supplemented treatments, respectively). In this cultivar, fruit set dropped from an initial fruit set of  $0.87 \pm 0.02$  fruits/flowers to a final fruit set of  $0.20 \pm 0.02$  fruits/flowers (mean  $\pm$  SE) before harvest.

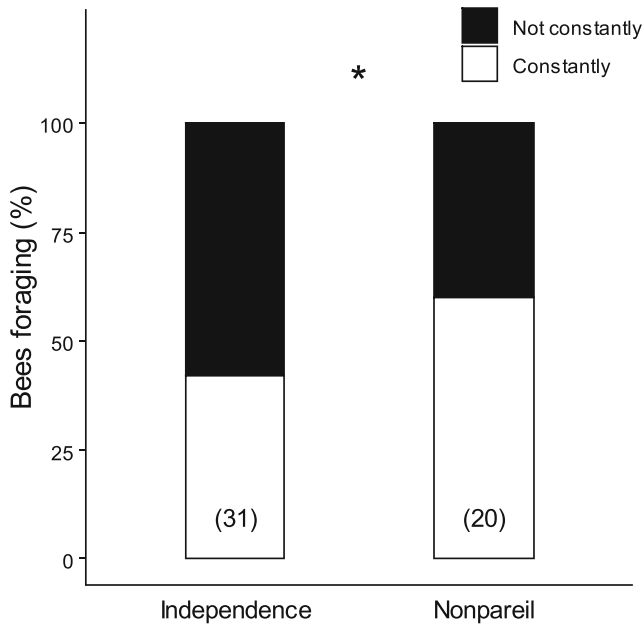
In contrast, the supplementation of ‘Nonpareil’ flowers (self-incompatible cultivar) with pollen from ‘Independence’ variety resulted in a higher proportion of flowers setting nut (Figure 4b). Cross-supplementation had a significant positive effect both in the initial as well as in the final fruit



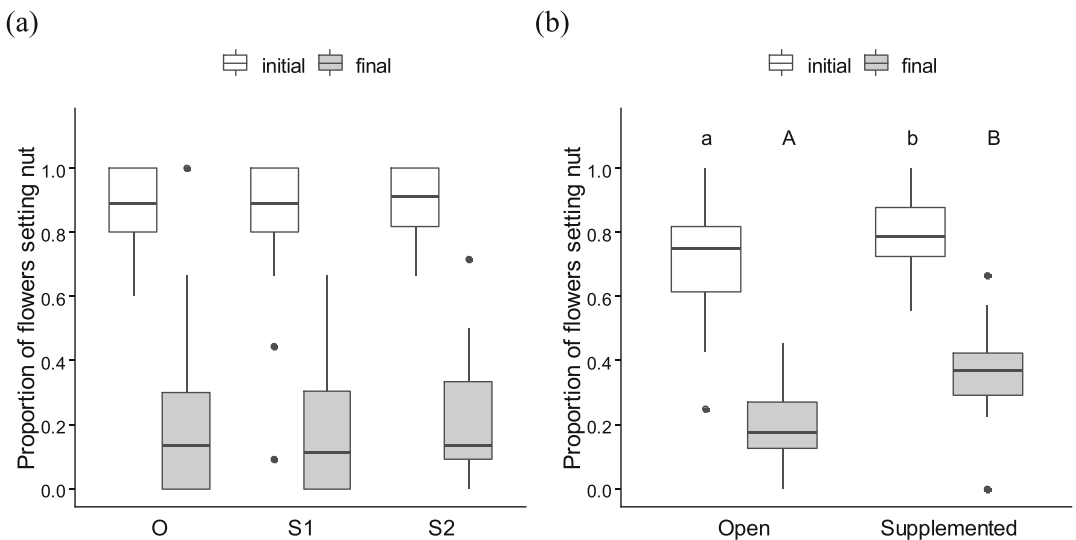
**Figure 1.** Visitation rate of *Apis mellifera* on almond cultivars. Number of visits per flower per hour estimated from 47 and 35 censuses per variety, respectively. (GLMM:  $p = 0.08$ ).



**Figure 2.** Foraging behaviour of *Apis mellifera* on almond cultivars. a, Number of flowers visited per minute per bee for 'Independence' and 'Nonpareil' cultivars by pollen- and nectar-foragers (GLM: \*\*\*,  $p < 0.001$ ). Boxplot shows the median and interquartile range (IQR), with whiskers showing the maximum value within 1.5 IQR, and individual points mark values outside this range. b, Percentage of pollen- or nectar-foragers on each cultivar, during the morning or the afternoon. (GLM:  $p < 0.001$ ). Sample size indicated between brackets.



**Figure 3.** Floral constancy of *Apis mellifera* on almond cultivars. Percentage of bees foraging exclusively on flowers of the same cultivar (constantly) or alternating varieties (not constantly). (GLM:\*,  $p < 0.05$ ). Sample sizes are indicated between brackets.



**Figure 4.** Pollen supplementation of almond cultivars. Proportion of flowers setting a nut three weeks after the end of bloom (initial) and prior to harvest (final). a, ‘Independence’ flowers were either exposed to natural pollination (O) or supplemented with pollen from flowers of the same tree (S1) or of different trees of the same variety (S2). (GLMM:  $p > 0.05$ ). b, ‘Nonpareil’ flowers were either exposed to natural pollination (Open), or supplemented with ‘Independence’ pollen (Supplemented). (GLMM: letters indicate significant differences). Boxplot shows the median and interquartile range (IQR), with whiskers showing the maximum value within 1.5 IQR, and individual points mark values outside this range. (N = 10 trees /treatment).

set (in *logit* scale,  $\beta = 0.51$ ,  $SE = 0.21$ ,  $z = 2.36$ ,  $p = 0.02$ ; and  $\beta = 0.82$ ,  $SE = 0.20$ ,  $z = 4.14$ ,  $p < 0.001$ ; for comparisons of the initial and final fruit set, respectively, between the Open-pollinated and Supplemented treatments). The initial fruit set of 'Nonpareil' cultivar was on average  $0.71 \pm 0.03$  fruits/flowers in open-pollinated trees and  $0.80 \pm 0.02$  fruits/flowers in those supplemented (mean  $\pm$  SE), while the final fruit set was  $0.20 \pm 0.02$  fruits/flowers, and  $0.35 \pm 0.03$  fruits/flowers, respectively, Supplemented 'Nonpareil' flowers showed a 14%-increase in the initial nut-set, and a 78%-increase in the final nut-set.

Even though many fruits abort during development, the number of nuts retained by 'Nonpareil' trees resulted significantly higher in cross-supplemented than in open flowers. On average,  $0.45 \pm 0.03$  fruits were retained in branches assigned to the Supplemented treatment, while  $0.29 \pm 0.03$  fruits (mean  $\pm$  SE) persisted in branches assigned to the Open treatment (on the log odds ratio scale, odds ratio = 0.47,  $SE = 0.10$ ,  $df = 57$ ,  $t$  ratio =  $-3.49$ ,  $p < 0.001$ , for comparison between treatments).

## 4. DISCUSSION

Despite its relevance for pollination success, the foraging behaviour of pollinators on the different cultivars is generally overlooked. Our results revealed differences in *Apis mellifera* foraging patterns between 'Nonpareil' and 'Independence' almond cultivars. The flower handling behaviour differed between nectar- and pollen-gatherers, and it was not influenced by the cultivar identity. However, while most honey bees visiting 'Nonpareil' flowers were nectar-foragers, most bees on 'Independence' flowers (pollinizer trees) collected pollen during the morning. Moreover, honey bees exhibited less floral constancy in this cultivar, promoting pollen flow from this pollinizer variety to the self-incompatible 'Nonpareil'. Nevertheless, supplementation of 'Nonpareil' flowers with

'Independence' pollen increased nut-set by 78%, indicating that pollination is pollen-limited in the system studied.

### 4.1. Visitation rates

When we assessed visitation rates on each cultivar, we found that 'Nonpareil' flowers were marginally more frequently visited by honey bees than 'Independence' flowers (Figure 1). Further studies are needed to determine whether cultivars differ in their floral scent or the nutritional quality of the floral rewards, aspects which could also influence their attractiveness to bees (Wright and Schiestl 2009), but which were not evaluated in the present study. Another aspect to consider is that the amounts of nectar and pollen offered by almond blossoms can vary greatly among cultivars (Hill et al. 1985; Abrol 1995). 'Nonpareil' flowers increase the amount pollen on display over the course of the day (Cunningham et al. 2016) but the diurnal patterns of production of these rewards have not been studied in 'Independence' flowers.

### 4.2. Flower handling behaviour

Pollen-foraging honey bees visited a higher number of flowers per unit time than nectar-foragers, irrespective of the cultivar (Figure 2). Our results are consistent with shorter visitation times of almond flowers reported by Henselek and collaborators (2018) for pollen-collecting than for nectar-collecting *Apis* bees on 'Nonpareil', 'Peerless', 'Monterey', 'Mission' and 'Padre' cultivars. In their study, *Apis* foraging for pollen deposited fewer non-almond pollen grains per visit than nectar-foragers, and though they contacted the stigma more frequently, no significant differences were found in their pollination effectiveness. In our study, pollen-foragers visited more flowers per unit time and could contribute to higher deposition of almond pollen grains considering the



overall pollen-foraging workforce. Forty percent of the foragers actively collected almond pollen, which is considered of high-quality for honey bees (Somerville 2001), boosting their populations and benefitting both beekeepers and almond growers (Delaplane and Mayer 2000).

The cultivar identity became relevant when we considered the time of day on the foraging preferences of honey bees. While most bees gathered nectar on 'Nonpareil' flowers throughout the day, many foragers actively collected pollen on 'Independence' flowers during the morning (Figure 2). This change in the foraging pattern on 'Independence' variety could be explained by pollen depletion. In kiwifruit, it has been shown that higher pollen collection by honey bees during the morning resulted in depletion of pollen supply by the flowers, decreasing pollen gathering by bees during the afternoon (Goodwin 1995). This result may have strong implications for pollination, since pollen-collecting bees are more efficient pollinators per visit than nectar-collecting bees in almond (Bosch and Blas 1994). Percival (1955) studied the pollen presentation of 86 species, including some Rosaceae crops, and found no simple relationship between the amount of pollen available, the timing of its presentation and its collection by honey bees. For this reason, further studies evaluating the pollination efficiency and pollen flow during morning and afternoon are needed to better understand the pollination process in intercultural plantations.

### 4.3. Floral constancy

Our results showed that honey bees exhibited high fidelity to 'Nonpareil' flowers but were more likely to switch between cultivars while foraging on 'Independence' flowers (Figure 3), promoting high-quality pollen transport to the self-incompatible 'Nonpareil' variety. Flower constancy is common in honey bees (Waser 1986) but there are few studies evaluating this behaviour in different varieties

of the same plant species. In the system studied, monovarietal rows facilitates management practices, but may hinder cross-pollination, since honey bees fly more frequently between neighbouring trees down the same row than across rows (Free 1960; Brittain et al. 2013; Quinet and Jacquemart 2017). However, flights across rows might be underestimated because of the difficulty for the observer to follow the focal bee (Free 1960). Moreover, the quantification of cross-pollen on the bee bodies revealed that 90% of foragers captured on almond flowers carried pollen from different varieties, indicating that bees were foraging on different cultivars throughout the day or that pollen could be transferred from one bee to another inside the hive (DeGrandi-Hoffman et al. 1992). Also, flights across rows could be promoted by the presence of non-*Apis* bees in almond orchards (Brittain et al. 2013), though diverse communities of wild pollinators are rare in intensive agroecosystems. In conventionally managed systems with little natural habitat for wild pollinators, flights across rows influenced by other pollinator species can be infrequent, particularly in highly dimorphic crops which offer different rewards between varieties (Susic and Farina 2016; Estravis Barcala et al. 2019). In such scenario, a field design of monovarietal blocks could come into conflict with honey bees foraging patterns, and potentially limit cross-pollination.

### 4.4. Pollen limitation

Our results showed no pollen limitation in 'Independence' cultivar (Figure 4). While the recommended honey bee stocking densities saturate the pollination demand of this variety, it remains to be estimated which densities of honey bees are needed to saturate pollination function of this self-compatible but not fully self-pollinated almond variety (see Sáez et al. 2020; Garibaldi et al. 2020). On the contrary, cross-supplementation of 'Nonpareil' flowers had a

positive effect in nut set, thus confirming the compatibility of ‘Independence’ trees as pollen source for cross-pollination, as well as revealing pollen limitation of ‘Nonpareil’ cultivar in this system under the recommended stocking densities. Moreover, the increase in final nut set is consistent with the fruit set reported for flowers of this variety supplemented with different, but compatible, pollinizers (Klein et al. 2015; Cunningham et al. 2016). Our results, as well as those exposed previously (Klein et al. 2015; Cunningham et al. 2016), suggest that there is still plenty of room to improve honey bees pollination efficiency by a proper management of pollination services. These services may be enhanced not only by an adequate density of honey bee hives (Garibaldi et al. 2020) but also by increasing habitats for wild bees, which can improve the quality and quantity of pollination services (Garibaldi et al. 2014).

#### 4.5. Implications and conclusions

Altogether, our findings showed that honey bees performed efficiently and could promote cross-pollination in an almond orchard designed with a single-row interplant scheme of ‘Nonpareil’ and ‘Independence’ cultivars. Additionally, the pollen supplementation assay results suggest that higher densities of managed as well as of wild bees would contribute to reduce pollination deficits. Almond pollination is affected by several factors including pollen compatibility, weather conditions, water and nutrients availability (Connell 2000; Klein et al. 2015; Cunningham et al. 2016), but also many others which directly influence bees foraging behaviour, such as blooming time of cultivars, their attractiveness to bees and the spatial arrangement of trees. In conclusion, our research underlines the importance of integrating ecological and behavioural knowledge about pollinators to optimize management practices in pollinator-dependent crops.

## SUPPLEMENTARY INFORMATION

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**Code availability** Not applicable.

## AUTHOR CONTRIBUTION

MCEB, AS, PN and WMF conceived the ideas and designed the study. MCEB, AS, MMG, PN and WMF collected and interpreted the data; MCEB and AS analysed the data; MCEB, AS and WMF wrote a first draft of the manuscript. MCEB, AS, MMG, PN, MV and WMF contributed critically in the revisions of it and approved the final manuscript. Funding

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Raw data supporting the findings of this study are available from the corresponding author upon request.

## DECLARATIONS

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

**Évaluation du comportement de butinage des abeilles domestiques et de leur impact sur le succès de la pollinisation dans un verger mixte d'amandiers.**

***Apis mellifera* / *Prunus dulcis* / comportement de butinage / constance florale / limitation du pollen.**

**Bewertung des Sammelverhaltens von Honigbienen und deren Einfluss auf den Bestäubungserfolg in einer gemischten Mandelbaumpflanzung.**

***Apis mellifera* / *Prunus dulcis* / Sammelverhalten / Blütenkonstanz / Pollenlimitierung.**

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