

Hoverfly pollination enhances yield and fruit quality in mango under protected cultivation

M. Sánchez^{a,b,*}, Y. Velásquez^b, M. González^c, J. Cuevas^a

^a Department of Agronomy, University of Almería, ceiA3, La Cañada de San Urbano s/n, 04120, Almería, Spain

^b Polyfly S.L., Avenida de la Innovación 15, 04131, Almería, Spain

^c Experimental Station of Foundation Cajamar, Paraje Las Palmerillas 25, 04710, El Ejido, Almería, Spain

ARTICLE INFO

Keywords:

Mangifera indica L.
Mango pollination
Alternative pollinators
Hoverfly pollination
Eristalinus aeneus
Pollen-pistil interaction

ABSTRACT

Mango (*Mangifera indica* L.) is one of the main tropical fruit crops worldwide. Although mostly grown in open fields, protected cultivation of mango has recently increased. Pollination in mango mainly occurs by flies (myophily) among which the group of eristaline hoverflies (Diptera: Syrphidae) stands out. Greenhouse studies were conducted on the pollination services provided by *Eristalinus aeneus* released at three densities in a protected mango orchard in southern Spain, during two consecutive seasons (2019 and 2020). Medium and high densities (30 and 45 individuals per square meter, respectively) led to higher number of floral visits to the middle and apical portions of the inflorescences where hermaphrodite flowers are more common, which resulted in more pollen grains being transferred to the stigma of fertile flowers. Medium and high densities of *E. aeneus* increased yields and improved fruit quality compared to low density (15 individuals per square meter), no insect release, and conventional pollination management using bumblebees. Yield enhancement achieved by using hoverflies as pollinating agents at medium and high densities implies an economic gain for farmers ranging from 16% to 29% in 2019 and from 212% to 456% in 2020. Therefore, our results suggest that medium and high densities of *E. aeneus* are profitable pollination solutions for mango cultivated under greenhouses.

1. Introduction

Mango (*Mangifera indica* L.) is a tropical fruit crop belonging to the family Anacardiaceae. Mango blooms form very large apical panicles that can bear from a few hundred to several thousand flowers, with a variable proportion of hermaphrodite and male flowers (i.e. mango is andromonoecious). The proportion of hermaphrodite and male flowers varies greatly depending on the season, cultivar, tree, branch, as well as on the environmental and management conditions and especially within panicles with most hermaphrodite flowers developing in the apex (Galán-Saúco, 2014; Geetha et al., 2016; Mukherjee, 1997; Shü, 1999; Singh, 1954; Sukhvibul et al., 1999). Most mango cultivars are considered self-compatible, while some others benefit from cross-pollination. Whatever the case may be, pollen transfer from the anthers to the stigma is always required for normal fruit development (Popenoe, 1917; Singh, 1954; Free and Williams, 1976). In outdoor mango, the flowers are visited by a large number of insects belonging to the group of Hymenoptera, Diptera, Lepidoptera and Coleoptera. Particularly, mango flowers attract a great diversity of flies for pollen transfer, which results

in myophily being the main pollination syndrome for this crop (Ramírez and Davenport, 2012, 2016). In southeastern Spain, some wild insects are observed foraging on mango flowers inside greenhouses. These groups included calliphorid dipterans (*Calliphora* sp., *Chrysomya* sp. and *Lucilia* sp.), sarcophagids (*Sarcophaga* sp.), syrphids (*Eristalinus aeneus*, *Eristalinus taeniops*, *Eristalis tenax*, *Episyrphus balteatus*, *Eupeodes* sp., *Sphaerophoria rueppellii*, *Syrphus* sp., *Volucella zonaria*) and tachinids (*Tachina* sp.). Some hymenopterans such as wasps (*Sceliphron* sp., *Vespa* sp.), bumblebees (*Bombus terrestris*, *Xylocopa* sp.), and bees (*Apis mellifera*, *Osmia* sp.) are also observed during flowering.

The high economic and nutritional value of its fruits makes mango the most cultivated species of the family Anacardiaceae. FAO reports that worldwide area for a group that also includes mangosteen and guava accounts for 5.5 million ha, with an increase of 12% in the last decade (FAO, 2021). In Europe, mango cultivation is concentrated in Spain (SE and Canary Islands) with 4838 ha in open fields and 287 ha under protected cultivation in plastic greenhouses (FAOSTAT, 2021). The most widespread commercial variety in Spain is 'Osteen', accounting for nearly 80% of the cultivated area, due to its favourable

* Corresponding author.

E-mail address: sanchezfernandezmanuela87@gmail.com (M. Sánchez).

<https://doi.org/10.1016/j.scienta.2022.111320>

Received 8 February 2022; Received in revised form 29 June 2022; Accepted 30 June 2022

Available online 9 July 2022

0304-4238/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

harvest dates, high yield, easy handling, and satisfactory postharvest behavior (Calatrava et al., 2013).

In recent years, greenhouse cultivation of mango has aroused great interest worldwide since it enables early harvest and provides protection against pests, diseases and harsh environmental conditions thus improving yield and fruit quality (Akinaga and Hasbullah, 2002; Galán-Saúco, 2015; Hermoso et al., 2018; Lionakis and Loxou, 1997; Medany et al., 2009; Mizuno et al., 2007; Yonemoto, 2018). However, greenhouse production can lead to situations of pollination deficits because wild pollinators cannot access to the crop. Making things worse, none of the commercially available hymenopteran pollinators, such as honeybees or bumblebees, are very effective in mango since its flowers are not much attractive to them and its floral rewards are insufficient to maintain the colonies of these social insects (Du Toit and Swart, 1993; Free and Williams, 1976; Popenoe, 1917; Sung et al., 2006; Usman et al., 2001). For the aforementioned reasons, alternative pollinators for mango in protected cultivation based on bees (Dag et al., 2001; Dag and Gazit, 2000; Mizuno et al., 2007) and flies (Alqarni et al., 2017; Dag and Gazit, 2000; Galán-Saúco et al., 1997; Saeed et al., 2016; Sharma et al., 1998) need to be tested.

In this regard, eristaline hoverflies (Diptera: Syrphidae) have drawn attention because of their abundance and frequent visits to mango flowers (Alqarni et al., 2017; Anderson et al., 1982; Dag and Gazit, 2000; Fajardo et al., 2008; Gajendra Singh, 1997; Kumar et al., 2016; Mohsen, 2019; Rajan and Reddy, 2019; Reddy and Sreedevi, 2016; Sung et al., 2006; Usha et al., 2014; Vishwakarma and Singh, 2017). Among the potential pollinators, *E. aeneus* seems to be a good candidate for pollinating mango under protected cultivation because of its foraging behavior and its adaptability to greenhouse environments (Sánchez et al., 2022).

The aim of this study was to confirm the pollination effectiveness of *E. aeneus* in mango cultivated in a plastic greenhouse, comparing three release densities of said hoverfly. To reach this objective, we analyzed the foraging behavior of this pollinator, the processes involving pollen-pistil interaction after pollen transport as well as fruit set, size and yield.

2. Materials and methods

2.1. Site and plant material

The experiments were carried out in an experimental plastic greenhouse of Grupo La Caña, located in Motril, Granada (Spain) (36°43'47.7 "N, 3°27'28.9 "W). The greenhouse was a galvanized steel multi-tunnel type of 1122 m² covered with a 3-layer of non-photosensitive polyethylene plastic with passive lateral ventilation. The mango plantation included 'Osteen', 'Irwin', 'Keitt', 'Ataulfo' and 'Langra' cultivars from which three-year-old 'Osteen' trees 2 m tall, 2.0 × 2.5 m spaced and trained following an espalier were selected for the trial. 'Osteen' is a medium-late monoembryonic variety, although its cultivation in greenhouses typically accelerates its ripening. It should be noted that in our region, mango often presents an early bloom, before March, scarcely viable due to frequent seed abortion. This early flowering is usually removed manually by the farmers. Following inflorescence removal, a second and more abundant bloom is produced in more suitable dates (Galán-Saúco, 2014; Hermoso et al., 2018).

The experimental design was a randomized block design with four treatments and three replicates (Fig. 1). Twelve cages were set-up with a white insect-proof net (6 × 6 threads/cm²) to compare different hoverfly release densities: high density (HD) with 45 individuals/m², medium density (MD) with 30 individuals/m², and low density (LD) with 15 individuals/m². A control without hoverflies was included in the experimental design. For the hoverfly treatments, three 14 m² (7.5 × 1.8 × 2.2 m) cages with three small trees each were used as replicates, while three 9 m² (5.0 × 1.8 × 2.2 m) cages with two enclosed trees were used for the control (C). The means of each cage were used as the replicated values.



Fig. 1. Greenhouse mango plantation and trees inside the cages used as replicates in the experimental design.

Outside the cages, the greenhouse owners placed two commercial hives of bumblebees (*Bombus terrestris*), at a density of approximately 1 hive/500 m². Since putting a bumblebee hive inside a trial cage was impossible, the bumblebee-pollinated trees ($n = 18$) are not considered as a treatment. However, they were used as a reference of the conventional pollination system.

A single release of *E. aeneus* pupae (Goldfly®, Polyfly, Spain) was made in each treatment and replicate; 630 pupae were introduced per cage in HD, 420 pupae in MD, and 210 pupae in LD. To corroborate that the intended density in each treatment was accurate, the emergence of adult hoverflies was checked days after the introduction of the boxes containing the pupae. The final emergence results exceeded 96% in both years of the experiment, thus reaching the targeted density. The hoverflies were released when the first flowers of the earliest panicles started to bloom: at stage 514 and 515 according to the BBCH scale (Hernández-Delgado et al., 2011). In 2019, the release occurred on March 26, and on March 10 in 2020. Water and sugar supplies were placed in each enclosure in plastic containers and Petri dishes, respectively to ensure that the hoverflies would not miss vital resources.

In 2019, data collection focused on pollen-pistil interaction, crop production and fruit quality, while in 2020, observations were extended including the number of floral visits and pollen transport. During this second year, the course of pollen-pistil interaction at different phenological stages of flowering was compared. The temperature was recorded throughout the flowering period of both years using three dataloggers (Inkbird IBS-TH1 Plus) placed inside the cages. In 2019, the average temperature was 19.1 °C, with a maximum of 36.4 °C and a minimum of 8.9 °C. In 2020, lower temperatures were recorded, with an average of 17.3 °C, a maximum of 34.8 °C, and a minimum of 7.6 °C.

2.2. Floral visits

In 2020, the number of floral visits was recorded one week after the introduction of the pupae. During four non-consecutive days over a two-week period, 5-minute observations on three different panicles, one from each tree, per replicate of each treatment were made to evaluate the number of insect visits and their duration (Fig. 2).

2.3. Pollen transport

In 2020, five hoverflies per replicate and treatment were captured at each stage of the flowering period (early, full and late bloom) and preserved in 1 ml vials containing 70° ethanol. These specimens were later analyzed under microscopy to determine the pollen load on their bodies, following the procedure indicated by Dafni et al. (2005).

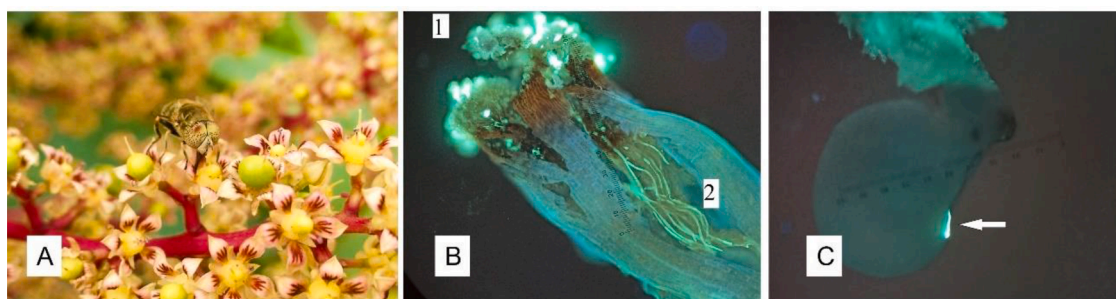


Fig. 2. (A) Visit of *Eristalinus aeneus* nectar-feeding on mango hermaphrodite flower. (B) Adhesion and germination of pollen grains (1); and pollen tube growth (2). (C) Fertilization of mango ovule (right). Arrow shows the pollen tube penetrating the ovule. The ovule was dissected to allow better observation.

2.4. Pollen-pistil interaction

Pollen-pistil interaction was assessed in both years. In 2019, 25 flowers per replicate and treatment (i.e. 75 flowers per treatment) were harvested soon after flowering ended. In 2020, 25 flowers per replicate and treatment were also collected but, this time in three distinct periods: early, full and late flowering (900 flowers total). Flowers were randomly collected from different panicles, when the color of the petals indicated the beginning of senescence (Pérez, 2013). The flowers were preserved in FAE (formalin, glacial acetic acid, and 70% ethanol in a 1:2:17 v/v ratio) until they were analyzed under fluorescence microscopy (Martin, 1959). After fixation, FAE was removed by successive washes with water and afterwards the flowers were immersed in a softening solution (NaOH 0.8 N) for 4 h, followed by a final wash with water for 8 h. The flowers were dissected and stained with aniline blue to observe pollen adhesion on the stigma, pollen tube growth on the style, and the arrival of the pollen tube to the ovule (Fig. 2). Pollen adhesion and germination, pollen tube growth in the transmitting tissue of the style, and ovule fertilization were compared among treatments. Pollen adhesion was assessed by counting the number of pollen grains per flower adhered to the stigma and by establishing the percentage of flowers with pollen grains present on the stigma. A pollen grain was considered germinated when its pollen tube was at least as long as its diameter. Pollen germination was expressed as the ratio of adhered and germinated pollen grains. Pollen tube growth was quantified by the number of pollen tubes reaching the base of the style. Finally, fertilization was estimated by the percentage of flowers showing the penetration of the pollen tube through the side of the only ovule mango flowers contain (fertilization by chalazogamy).

2.5. Fruit set, yield and components

In 2019 and 2020, pollination effectiveness was determined according to the three *E. aeneus* release densities. This was done by quantifying the initial and final fruit set on 10 panicles per tree, corresponding to fruits in stages A (3 weeks after flowering; fruits at 10% of their final size) and B (10 weeks after flowering; fruits at 30% of their final size) (Aubert and Lossois, 1972).

Crop production was estimated at harvest by counting the number of panicles with fruit, the number of fruits per panicle, as well as the number and weight of fruit per replicate and treatment. Finally, the fruits were classified into commercial categories according to their weight, as follows: fruits <300 g; 300–500 g, 500–700 g, 700–1000 g, and >1000 g. Fruits weighing <300 g were deemed non-commercial. Considering the production and the average price of the fruit, the yield (t/ha) and the resulting economic value (€/ha) were calculated for each treatment. The data compiled by the Observatory of Prices and Markets showed that the average price received by farmer was 1.54 €/kg in August 2019, and 1.74 €/kg in August 2020 (Junta de Andalucía, 2021). The economic analysis does not include the cost of releasing this pollinating insect because its industrial mass-rearing is still under

development and definitive economic figures will depend on ongoing field studies in several crops.

2.6. Data analyses

Statistical analysis was performed with R Statistical software (R-Core Team, version 4.0.2, Vienna, Austria). For the analysis of insect activity, represented by the number and duration of visits, Generalized Linear Models (GLM) were fitted. For the model of the number of visits recorded with counts, a GLM with Poisson distribution and log-link for the response variable was used. In the case of the duration of the insect visits, a square root transformation was performed on the dependent variable (time of visits in seconds) to ensure a normal distribution of the response variable. In both cases, for the number and duration of visits, the models contained the independent variables of release densities and of the area of the panicles where the visits took place. A GLM was also used for the determination of insect pollen transport, with the number of pollen grains on the insect body as the dependent variable, and the release density and flowering stage as independent factors. The final models were selected by comparing the AICc (Akaike Information Criterion) value against the value of the full models. To ensure no violation of the normality and homoscedasticity assumption of the residuals, all models were graphically inspected with quantile-quantile plots (Q-Q plots) and histogram graphics. Subsequent separation of means was performed by Tukey's test ($p < 0.05$).

Data on pollen adhesion and germination, pollen tube growth, and percentage of fertilized flowers were analyzed by analysis of variance (ANOVA). In 2020, adhesion and fertilization data were square-root transformed before analysis so as not to violate the assumption of homoscedasticity. The data on the proportion of flowers with pollen adhesion and germination and of fertilized flowers were subjected to angular transformation before analysis. Finally, the separation of means was carried out by Tukey's test ($p < 0.05$).

The initial and final fruit sets, expressed as the number of fruits per panicle, were compared by ANOVA both years for the different treatments. Fruit production in both years was analyzed by analysis of covariance (ANCOVA), with the number of fruits per tree as the dependent variable, the number of panicles per tree as the covariate, and the density of release as the independent factor. Finally, fruit weight was compared among the different treatments and a commercial categorization of fruit by weight was performed. When significant differences were detected, means were separated using Tukey's test ($p < 0.05$).

3. Results

3.1. Floral visits

In 2020, 741 visits by *E. aeneus* were recorded in the 72 panicles kept under observation. The results show that the number of visits depended on the release density ($p < 0.0001$) and the section of the panicle visited ($p < 0.0015$). Floral visits were significantly more frequent as the release

density increased, with the highest number of visits being observed in HD (12 visits/panicle/5 min) and minimum number of visits in LD (2 visits /panicle/5 min). There was a significant effect on the panicle part visited, with the highest number of visits being recorded in the apical and middle sections. This pattern was equally observed across all treatments (Table 1).

The duration of the visits was mainly determined by the panicle section visited ($p = 0.0025$). In contrast, there was no significant effect of the release density ($p = 0.1893$). The minimum duration of the floral visits was 3 s, while the maximum exceeded the observation time of 300 s. This maximum was reached in all treatments and panicle sections, with the only exception of the basal region in the LD treatment, where the longest duration was 261 s (Table 2).

3.2. Pollen transport

In 2020, 135 hoverflies were collected and the pollen load on their bodies estimated. The number of mango pollen grains carried on the hoverflies body did not depend on the density at which they were released ($p = 0.1542$), but was strongly dependent on the phenological stage of flowering of the plants ($p < 0.0001$). Pollen load was significantly higher during full and late flowering for all treatments (Table 3). Mango pollen grains were observed on all fixed specimens, but the presence of other types of pollen (mostly *Oxalis* sp. and *Taraxacum* sp.) was also detected in some cases. *Oxalis* and *Taraxacum* pollen grains came from the floral visits performed by the hoverflies on weeds that appeared spontaneously under mango trees.

3.3. Pollen-pistil interaction - 2019 trial

In 2019, significant differences in pollen adhesion were found depending on pollinator density. In HD and MD, the proportion of flowers with adhered pollen grains was 96% and 95%, respectively, but it was lower in the LD treatment with just 76%. In control plants, without hoverflies present, the percentage of pollinated flowers was drastically reduced to 17%. Significant differences were also detected in the number of pollen grains attached to the stigma of the flowers according to the treatments ($F_{(3,8)} = 11.87$, $p = 0.003$). Treatments HD and MD showed the highest number of pollen grains adhered (approximately 9 grains/flower), followed by LD with an average of 4 pollen grains per flower, and the control which did not reach the minimum value of 1 pollen grain per stigma, given the high percentage of virgin, unvisited flowers (Table 4).

On the other hand, the percentage of pollen germination did not show significant differences between treatments ($F_{(3,8)} = 0.81$, $p = 0.5236$), with values between 71% and 87%. The number of pollen tubes reaching the base of the style and entering the ovary was higher in HD and lowest in C ($F_{(3,8)} = 9.60$, $p = 0.005$). Moreover, the proportion of pollinated pistils that had pollen tubes at the end of the style, at its junction with the ovary, was highest in HD and MD (85%), followed by LD (78%) and C (69%). Fertilization was significantly higher too in treatments HD and MD ($F_{(3,11)} = 28.89$; $p = 0.0001$), with percentages

Table 1

Hoverfly visits (mean \pm SE) in the different sections of the panicle at each release density. HD: high density (45 individuals/m²); MD: medium density (30 individuals/m²); LD: Low density (15 individuals/m²).

	Apical	Middle	Basal	Mean
HD	3.47 \pm 0.30	3.39 \pm 0.25	2.69 \pm 0.26	9.56 \pm 0.18a
MD	2.61 \pm 0.18	2.83 \pm 0.22	1.94 \pm 0.25	7.39 \pm 0.23b
LD	1.22 \pm 0.16	1.33 \pm 0.16	1.08 \pm 0.17	3.64 \pm 0.14c
Mean	2.44 \pm 0.16A	2.52 \pm 0.15A	1.91 \pm 0.15B	

* In the last column, different lower-case letters indicate significant differences between release densities. In the last row, different capital letters indicate significant differences between panicle portions (Tukey's test $p < 0.05$).

Table 2

Duration in seconds (mean \pm SE) of floral visits for different hoverflies release densities and different panicle visitation sections.

	Apical	Middle	Basal	Mean
HD	93.16 \pm 6.82	103.92 \pm 6.06	122.08 \pm 6.85	105.1 \pm 3.85a
MD	98.86 \pm 8.43	103.93 \pm 7.69	105.42 \pm 9.23	102.54 \pm 4.83a
LD	115.64 \pm 13.53	103.10 \pm 12.70	92.08 \pm 11.37	104.03 \pm 7.32a
Mean	98.96 \pm 4.97A	103.78 \pm 4.53A	110.66 \pm 5.04A	

* In the last column, different lower-case letters indicate significant differences between release densities. In the last row, different capital letters indicate significant differences between panicle portions (Tukey's test $p < 0.05$).

Table 3

Mango pollen grains load (mean \pm SE) on the insect body for the different release treatments throughout the flowering period.

	Early	Full	Late	Mean
HD	369.33 \pm 31.08	823.00 \pm 48.86	1070.33 \pm 67.74	754.22 \pm 52.24a
MD	282.33 \pm 44.10	1085.33 \pm 99.74	937.33 \pm 53.93	768.33 \pm 65.85a
LD	224.67 \pm 61.66	982.00 \pm 62.37	1108.00 \pm 89.21	771.56 \pm 71.54a
Mean	292.11 \pm 28.15B	963.44 \pm 44.56A	1038.56 \pm 41.62A	

* In the last column, different lower-case letters indicate significant differences among release densities. In the same last row, different capital letters indicate significant differences between panicle portions (Tukey's test $p < 0.05$).

of flowers of 56% and 53%, respectively. Conversely, treatment LD and C showed much lower values, with 23 and 8% of flowers, respectively (Table 4).

3.4. Pollen-pistil interaction - 2020 trial

In 2020, both the release density and flowering stage had significant effects on the percentage of pollinated flowers ($p < 0.0001$). HD and MD showed again higher percentages of pollinated flowers, with 77% and 63%, respectively. Considering phenological stages, important for programming hoverfly release, early flowering showed the lowest percentage of flowers with pollen grains attached to the stigma (38%), followed by full and late flowering (47% and 54%, respectively). Similarly, HD had more pollen grains per flower adhered to the stigma at all flowering stages, with an average of 5 pollen grains per flower, just twice as many as MD and 5 times more than LD (Tables 4 and 5).

Regarding pollen germination, the results did not show any significant differences between treatments, although there were significant differences among the flowering periods ($F_{(2,31)} = 5.52$; $p = 0.008$). Indeed, pollen germination appeared to increase as flowering progressed towards the full and later periods, and temperatures rose. No significant differences in the number of pollen tubes entering the ovary were observed, either for the different flowering stages or among treatments (Tables 4 and 5).

Considering ovule fertilization, both the flowering period and the density of release had significant effects on ovule fertilization ($p = 0.0016$ and $p = 0.0018$, respectively). The release of hoverflies increased the percentage of fertilized flowers, reaching significant differences with respect to the control. The percentage of fertilized flowers was also found to be significantly higher as the flowering progressed (Tables 4 and 5).

In general, positive effects on pollen-pistil features were observed after the introduction of hoverflies both in 2019 and 2020 (Table 4). These positive effects were, at least to some extent, driven by the higher pollinator activity in the HD and MD treatments. Thus, higher pollen adhesion had a positive effect on fertilization levels in both years, which means that the flowers that received more pollen had a higher

Table 4Pollen adhesion, pollen germination, pollen tube growth, and flower fertilization (Mean \pm SE) for the different pollination treatments in 2019 and 2020.

	2019				2020			
	Pollen adhesion (grains/flower)	Pollen germination (%)	Pollen tube growth	Fertilization (%)	Pollen adhesion (grains/flower)	Pollen germination (%)	Pollen tube growth	Fertilization (%)
HD	8.85 \pm 0.32ab	74.39 \pm 11.16a	2.02 \pm 0.24a	56.00 \pm 6.11a	5.22 \pm 0.82a	85.97 \pm 2.58a	1.04 \pm 0.10a	28.44 \pm 5.79a
MD	9.55 \pm 1.53a	70.91 \pm 6.97a	1.93 \pm 0.26ab	53.33 \pm 2.67a	2.57 \pm 0.59b	88.18 \pm 2.19a	0.87 \pm 0.09a	19.11 \pm 4.75ab
LD	3.81 \pm 1.97bc	83.61 \pm 6.36a	1.38 \pm 0.29ab	22.67 \pm 2.67b	0.87 \pm 0.20c	86.55 \pm 1.27a	0.85 \pm 0.05a	10.22 \pm 2.32ab
C	0.39 \pm 0.21c	86.87 \pm 6.72a	0.82 \pm 0.24b	8.00 \pm 4.00c	0.16 \pm 0.03c	86.00 \pm 5.50a	0.92 \pm 0.05a	4.00 \pm 1.15b

* In the same column, different letters indicate significant differences between treatments (Tukey's test $p < 0.05$).**Table 5**Pollen adhesion, pollen germination, pollen tube growth, and flower fertilization (Mean \pm SE) for the different flowering periods in 2020.

	Pollen adhesion (grains/flower)	Pollen germination (%)	Pollen tube growth (tubes/flower)	Fertilization (%)
Early	1.38 \pm 0.59b	78.27 \pm 2.67b	0.79 \pm 0.07a	8.67 \pm 4.77b
Full	2.19 \pm 0.78ab	87.01 \pm 2.70ab	1.02 \pm 0.04a	17.33 \pm 3.46a
Late	3.05 \pm 0.73a	90.47 \pm 1.76a	0.93 \pm 0.07a	20.33 \pm 4.01a

* In the same column, different letters indicate significant differences between treatments (Tukey's test $p < 0.05$).

probability of being successfully fertilized ($\chi^2 = 79.624$, $df = 2$, $p < 0.0001$). The range of 1–5 pollen grains attached showed the lowest percentage of flower fertilization (25%), followed by the range of 6–10 pollen grains (51%) and, finally, by flowers with more than 10 pollen grains which exhibited a 71% probability of fertilization.

3.5. Fruit set, harvest and components

During blooming, the average number of flowered panicles per tree was 22.21 ± 12.54 in 2019, and 26.45 ± 10.69 in 2020. The introduction of *E. aeneus* increased yield and fruit quality and significant differences were observed depending on the release density of this pollinator. In both years, 2019 and 2020, the percentage of panicles that developed commercial fruits, the number of fruits per plant and fruit weight, as well as initial and final fruit set, were higher in HD and MD than in LD and C treatments (Table 6). The percentage of fruitful inflorescences at harvest was positively influenced by the introduction of hoverflies in both years (Fig. 3). On the contrary, hoverfly density had no effect on the number of fruits per panicle which was strongly determined by heavy fruitlet competition within the panicle. However, a certain positive effect of density release persisted in this parameter, with HD = 1.43 ± 0.11 vs C = 1.06 ± 0.06 fruits/panicle in 2019, and HD = 1.50 ± 0.04 vs C = 1.42 ± 0.12 fruits/panicle in 2020.

Regarding commercial classification of the fruit, a higher percentage of fruits in the 500–700 g range was harvested in trees pollinated by hoverflies both seasons, although no differences were found among

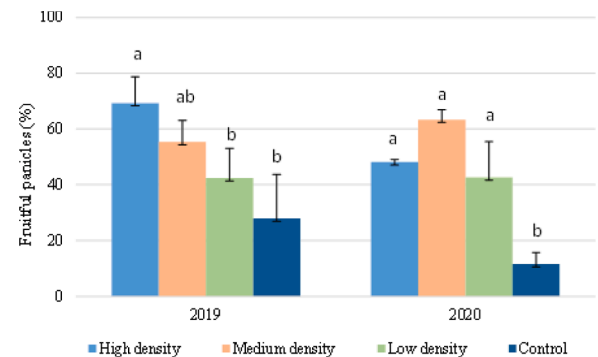


Fig. 3. Panicles with commercial fruit (%) at 2019 and 2020 harvest. In the same year, different letters indicate significant differences among treatments (Tukey's test $p < 0.05$).

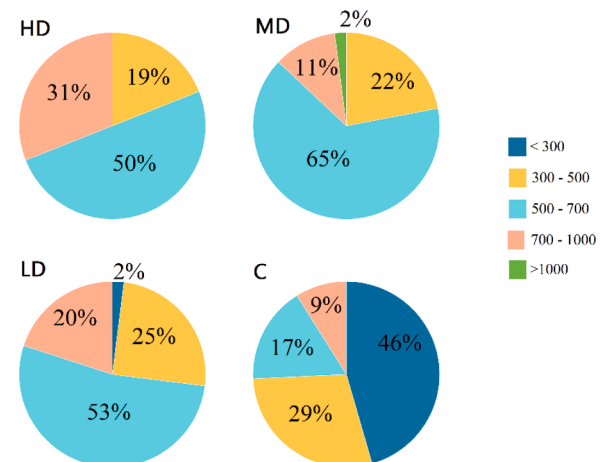


Fig. 4. Classification of fruits by commercial categories based on fruit weight (g) in 2019 and 2020 for the different treatments.

Table 6Initial fruit set (IFS), final fruit set (FFS) (fruits/panicle), fruit production per plant, and fruit weight (g) (Mean \pm SE) during 2019 and 2020 in the different release density treatments.

	2019				2020			
	IFS	FFS	Fruits per plant	Fruit weight (g)	IFS	FFS	Fruits per plant	Fruit weight (g)
HD	19.57 \pm 1.77a	1.67 \pm 0.09a	14.56 \pm 1.49a	605.48 \pm 58.17a	11.91 \pm 0.64a	2.36 \pm 0.42a	18.67 \pm 2.69a	683.33 \pm 32.55a
MD	23.73 \pm 2.71a	1.67 \pm 0.12a	14.67 \pm 6.18a	573.33 \pm 26.21a	9.31 \pm 0.56b	2.23 \pm 0.19a	17.89 \pm 2.21ab	633.33 \pm 47.25ab
LD	10.78 \pm 0.86b	0.99 \pm 0.05b	12.33 \pm 1.53ab	633.33 \pm 21.20a	5.85 \pm 0.41c	0.76 \pm 0.13b	12.99 \pm 0.97ab	540.74 \pm 43.55bc
C	5.06 \pm 0.91b	0.06 \pm 0.04c	4.33 \pm 1.89b	301.22 \pm 78.27b	3.58 \pm 0.28d	0.1 \pm 0.05b	6.33 \pm 1.92b	411.11 \pm 45.73c

* In the same column, different letters indicate significant differences between treatments (Tukey's test $p < 0.05$).

release densities. In control trees, almost half of the fruits (46%) weighed less than 300 g and were, therefore, considered non-commercial (Fig. 4).

Production improved due to the introduction of *E. aeneus*, in terms of crop yield (t/ha) and value (€/ha). In both years, higher yields and profits were obtained in HD treatment, and to a lesser extent in MD and LD. The lowest yields and profits were obtained in C treatment without insects introduced inside the cage (Table 7).

The production obtained with conventional pollination in the rest of the greenhouse, outside *E. aeneus* cages, was lower than that obtained by pollination with syrphids. In 2019, the production of the trees outside the enclosures was 13.67 ± 2.02 fruits/tree, with an average fruit weight of 489.33 ± 13.01 g, thereby supposing a yield of 13.48 t/ha. In 2020 ('off year'), the production of those trees dropped to 5.89 ± 1.52 fruits/tree, with an average fruit weight of 386.21 ± 30.48 g resulting in a yield of 4.55 t/ha.

4. Discussion

The global decline of pollinators, their delicate management and recurrent crop pollination deficits make it necessary to look for alternative pollination solutions for protected crops, including mango (Amano, 2004; Dag, 2008; Descamps et al., 2021; Evans et al., 2019; Garibaldi et al., 2018; Heinrich, 1979; Kendall et al., 2021; Kwon and Saeed, 2003; Mdutshwa et al., 2019; Occhiuzzi, 1999; Osterman et al., 2021; Reilly et al., 2020). This study provides, for the first time, detailed information confirming that *E. aeneus* is an effective pollinator of mango under protected cultivation. Furthermore, our results show that the release density of this pollinator positively affects yield and quality (size) of mango fruits.

The efficacy of *E. aeneus* lies in its abundance, foraging behavior and ability to transport pollen grains on its body. We found that the rate of inflorescence visits made by *E. aeneus* under the three densities tested was higher than those of other pollinators observed in open fields by Munj et al. (2017), such as *Chrysomya* sp., *Apis cerana indica* and *Tetragonula* sp. (5.9, 6.4 and 11.5 visits/h, respectively). Moreover, the highest number of floral visits at the three assessed densities were recorded in the apical and middle sections of the inflorescences, where a greater proportion of hermaphrodite flowers is present (Chaikiattiyos et al., 1997; Golec, 2019; Sánchez et al., 2022; Sukhvibul et al., 1999). It is well-known that pollinator behavior can affect pollination efficiency (Ne'eman et al., 2010) and, in this case, we found that a higher number of floral visits increased the amount of pollen grains deposited on the stigma of recipient flowers and the number of fertilized flowers, thus, resulting in a higher pollination efficacy. Our results showed a relationship between the number of pollen grains deposited and the success in flower fertilization, achieving higher fruit set (over 70%) in flowers with more than 10 pollen grains adhered to the stigma. A greater number of deposited pollen grains in the stigma can lead to better pollen germination and fertilization, as the competition between pollen tubes influences their growth speed through the style (Dutta et al., 2013; Hormaza and Herrero, 1992). This may affect seed quality, fruit set and, fruit size and characteristics (Delph and Havens, 1998; Mulcahy and Mulcahy, 1987). Our data suggest that optimum yield and economic return may be achieved via the implementation of high and medium

densities of *E. aeneus* (Table 7). These benefits come from higher fertilization levels, which are likely due to a better distribution of more readily available pollen grains among the flowers of the mango panicle. Strikingly, a higher number of fruits per tree under HD, and to a lesser extent in MD, was compatible with heavier fruits (Table 6), possibly owing to larger seed size and quality as reported by Lucas-García et al. (2021). This phenomenon is often observed in unisced drupes such as peach, olive, jujube and mango, where seed and fruit size are often related (Aráoz et al., 2004; Cuevas and Oller, 2002; Lucas-García et al., 2021; Rodríguez-Pleguezuelo, et al., 2012; Souza et al., 2016).

Regarding pollen transport, several previous studies have highlighted the capacity of dipterans to transfer mango pollen, with hoverflies typically being the group that shows greater pollen loads on their body (Huda et al., 2015; Jiron and Hedstrom, 1985; Usha and Srivastava, 2018). This was confirmed in our trial as *E. aeneus* showed a significant pollen transport capacity. Since no differences in pollen load and similar duration of floral visits were found in these three treatments, the higher pollination efficiency observed in the HD and MD treatments is explained by the greater number of floral visits occurring when more hoverflies are released. In addition, a higher number of individuals visiting the flowers did not affect pollen germination (Table 4).

Mango yield may range from 5 to 30 t/ha, depending on several factors such as year (given mango alternate bearing), variety, management, environmental conditions, tree age and size as well as planting density (Bally, 2006). In greenhouses, the production of well-developed monovarietal adult plantations is similar to that obtained in open-air fields, about 20 t/ha, but it can be increased in multivarietal plantations up to 37.5 t/ha (Galán-Saúco, 2002, 2014, 2015). These protected crops are generally pollinated by bees and bumblebees with the help of wild pollinators. The yield obtained with a single release of hoverflies was similar and even higher than that obtained in open-air or protected adult 'Osteen' plantations, but lower than expected likely due to young age of the trees and the monovarietal condition of this plantation (Table 7). Fruit weight and commercial classification were also found to be within the established ranges for this crop when grown outdoors (Galán-Saúco, 2014; Junta de Andalucía, 2020). The number of fruits per panicle and the number of panicles with fruit affect crop yield. The main differences observed here are the percentages of panicles that developed commercial fruits, as they reached values of 69.32% in HD in 2019, and 63.37% in MD in 2020 (Fig. 3). Indeed, these percentages are higher than those reported for 'Osteen' in open field (47%) (Pérez, 2013). This confirms the excellent efficacy of *E. aeneus* when released at a density greater than or equal to 30 individuals/m². A higher number of fruits per panicle would favor competition among them, but the development of only one or two fruits in the inflorescence increases the total number of fruits, the weight and the yield per tree at harvest (Yeshitela et al., 2004). Nevertheless, our results showed no differences in the number of fruits per inflorescence according to the density of release, as it was between 1.25 and 1.50 fruits per panicle, consistent with the number of fruits per panicle usually obtained in this crop (Bally, 2006; Pérez, 2013).

Our data suggest that optimum yield and economic return may be achieved via the implementation of high and medium densities of *E. aeneus* (Table 7). The comparison with yields obtained in similar 'Osteen' trees grown inside the same greenhouse but outside of the cages shows that the yields achieved thanks to the hoverflies exceeded those reached performing conventional pollination management with commercial bumblebees and wild insect visitors. Moreover, in both years, the production per tree and yield obtained where hoverflies were released (Tables 6 and 7) was found to be higher than in the C treatment (without insects). This increase in yield equates to an economic gain ranging between 16 and 29% in 2019, and between 212 and 456% in 2020, depending on the insect density released in the greenhouse. Nevertheless, due to some trial limitations, such as the low number of replicates or the young age of the trees, future trials on a larger scale and on cultivars with different characteristics are required.

Table 7

Crop yield (t/ha) and economic return (€/ha) under three hoverfly densities and control without insect release.

	2019		2020	
	t/ha	€/ha	t/ha	€/ha
HD	17.33	26,693.59	25.29	43,998.02
MD	16.56	25,495.31	22.38	38,946.19
LD	15.57	23,978.38	14.20	24,704.17
Control	2.78	4271.46	4.98	8661.30

In conclusion, this trial shows the pollination ability of *E. aeneus* for mango crops grown under greenhouses in southeastern Spain. Hoverfly pollination in larger protected areas with other or a combination of mango varieties should be also studied, since cross-pollination between different mango varieties typically improves yields (Dag et al., 1998; Dutta et al., 2013; El-Habashy et al., 2016; Galán-Saúco et al., 1997; Lucas-García et al., 2021; Pérez et al., 2016). If this is the case, the release of hoverflies at low densities should be further investigated, because of the possible positive effects of pollen diversity and pollinator mobility between different trees. Moreover, it is known that functional complementarity in plant-pollinator interactions favors more effective pollination. Therefore, a combination of different species of pollinators should be assessed under greenhouse conditions (Blüthgen and Klein, 2011). *E. aeneus* was used in this trial because it is a common visitor of mango flowers and it is abundant in the Mediterranean basin, but other species belonging to the cristalline syrphid group can be tested in other locations around the world. Finally, due to the high importance of open-air mango cultivation, the potential use of hoverflies as alternative or complementary pollinators in these production systems should also be investigated.

CRediT authorship contribution statement

M. Sánchez: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Y. Velásquez:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. **M. González:** Conceptualization, Methodology, Writing – original draft. **J. Cuevas:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

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.

Data availability

Data will be made available on request.

Acknowledgments

We are very grateful to Grupo La Caña for allowing us to carry out this study in their greenhouses. This study is part of a Ph.D. project supported by the Ministry of Innovation and Science of Spain that co-financed the contract to M. Sánchez (DI-17-09575: Predoctoral grant for research training in companies “Industrial Doctorates”).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.scienta.2022.111320](https://doi.org/10.1016/j.scienta.2022.111320).

References

- Akinaga, T., Hasbullah, R., 2002. Mango production using plastic greenhouse in Okinawa. *Acta Hortic.* 575, 745–749. <https://doi.org/10.17660/ActaHortic.2002.575.87>.
- Alqarni, A., Ahmed, K., Hannan, M., Ghose, G., Munshi, J., 2017. Flies for the pollination of greenhouse mango (*Mangifera indica* L., Anacardiaceae) in the Subtropical Iriomote Island, Japan. *J. Asiat. Soc. Banglad. Sci* 43, 135–141. <https://doi.org/10.3329/jasbs.v43i1.46252>.
- Amano, K., 2004. Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. Food and Fertilizer Technology Center. <http://www.ffc.agnet.org/library/article/tb167.html> (accessed 1.31.2022).
- Anderson, D., Sedgley, M., Short, J., Allwood, A., 1982. Insect pollination of mango in Northern Australia. *Aust. J. Agric. Res.* 33, 541–548. <https://doi.org/10.1071/AR9820541>.
- Aráoz, S., Longo, O.D., Karlin, O., 2004. Germinación de semillas de *Ziziphus mistol* Grisebach III. Correlaciones paramétricas del tamaño y peso de drupas, endocarpos y semillas con la germinación y el vigor. *Multequina* 13, 51–56. <https://www.redalyc.org/articulo.oa?id=42801306>.
- Aubert, B., Lossois, P., 1972. Considerations sur la phenologie des especes fruitieres arbustive. 2^e partie. *Fruits* 27, 269–286.
- Bally, I.S.E., 2006. *Mangifera indica* (mango). In: Elevitch, C.R. (Ed.), *Traditional trees of Pacific Islands: their culture, environment and use*. Permanent Agriculture Resources. Holualoa, Hawaii, pp. 441–464.
- Blüthgen, N., Klein, A., 2011. Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic Appl. Ecol.* 12, 282–291. <https://doi.org/10.1016/j.baec.2010.11.001>.
- Calatrava, J., Sayadi, S., Sarmiento, D., Guirado, E., 2013. Return analysis of ‘Osteen’ and ‘Kent’ mango orchards in South-Eastern Mainland Spain. *Acta Hortic.* 992, 87–93. <https://doi.org/10.17660/ActaHortic.2013.992.9>.
- Chaikiattiyos, S., Pongsomboon, W., Dasanonda, M., Anupunt, P., 1997. Floral sex expression of ‘Khieo Sawoei’ and ‘Nam Dok Mai’ mangoes grown in tropical and subtropical climates in Thailand. *Acta Hortic.* 455, 202–208. <https://doi.org/10.17660/ActaHortic.1997.455.27>.
- Cuevas, J., Oller, R., 2002. Olive seed set and its impact on seed and fruit weight. *Acta Hortic.* 586, 485–488. <https://doi.org/10.17660/ActaHortic.2002.586.100>.
- Dafni, A., Kevan, P., Husband, B., 2005. *Practical Pollination Biology*. Enviroquest, Ontario, Canada.
- Dag, A., 2008. Bee pollination of crop plants under environmental conditions unique to enclosures. *J. Apic. Res.* 47, 162–165. <https://doi.org/10.1080/00218839.2008.11101444>.
- Dag, A., Degani, C., Gazit, S., 2001. In-hive pollen transfer in mango. *Acta Hortic.* 561, 61–65. <https://doi.org/10.17660/ActaHortic.2001.561.8>.
- Dag, A., Eisenstein, D., Gazit, S., El-Batsri, R., Degani, C., 1998. Effect of pollinizer distance and selective fruitlet abscission on outcrossing rate and yield in ‘Tommy Atkins’ mango. *J. Am. Soc. Hortic. Sci.* 123, 618–622. <https://doi.org/10.21273/JASHS.123.4.618>.
- Dag, A., Gazit, S., 2000. Mango pollinators in Israel. *J. Appl. Hortic.* 2, 39–43. <https://doi.org/10.37855/jah.2000.v02i01.12>.
- Delph, L., Havens, K., 1998. Pollen competition in flowering plants. In: Birkhead, T.R., Møller, A.P. (Eds.), *Sperm Competition and Sexual Selection*. Academic Press, San Diego, pp. 149–173. <https://doi.org/10.1016/B978-012100543-6/50030-1>.
- Descamps, C., Jambre, A., Quinet, M., Jacquemart, A.-L., 2021. Warm temperatures reduce flower attractiveness and Bumblebee foraging. *Insects* 12, 493. <https://doi.org/10.3390/insects12060493>.
- Du Toit, A.P., Swart, D.J., 1993. Pollination of mango in the Letsitele Valley during the 1992 flowering season: first report. *Yearbook S. Afr. Mango Growers' Assoc.* 13, 129–130.
- Dutta, S.K., Srivastav, M., Rymbai, H., Chaudhary, R., Singh, A.K., Dubey, A.K., Lal, K., 2013. Pollen–pistil interaction studies in mango (*Mangifera indica* L.) cultivars. *Sci. Hortic.* 160, 213–221. <https://doi.org/10.1016/j.scienta.2013.05.012>.
- El-Habashy, S., Maklad, M.F., Ahmed, E.A.H., 2016. Effect of self and cross-pollination in some local mango cultivars on fruit set and retained fruits. *Am.-Euras. J. Agric. Environ. Sci.* 16, 1776–1783. <https://doi.org/10.5829/idosi.aejas.2016.1776.1783>.
- 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>.
- Fajardo, A., Medina, J., Opina, O., Cervancia, C., 2008. Insect pollinators and floral visitors of mango (*Mangifera indica* L. cv. Carabao). *Philipp. Agric. Sci.* 91, 372–382.
- FAO, 2021. Major Tropical fruits: Market review 2020. Food and Agriculture Organization of the United Nations, Rome. <https://www.fao.org/3/cb6196en/cb6196en.pdf>.
- FAOSTAT, 2021. Organización de las Naciones Unidas para la Alimentación y la Agricultura. FAOSTAT Statical Database. <https://www.fao.org/faostat/es/#data>.
- Free, J.B., Williams, I.H., 1976. Insect pollination of *Anacardium occidentale* L., *Mangifera indica* L., *Blighia sapida* Koenig and *Persea americana* Mill. *Trop. Agric.* 53, 125–139.
- Gajendra Singh, D., 1997. Pollination, pollinators and fruit setting in mango. *Acta Hortic.* 455, 116–123. <https://doi.org/10.17660/ActaHortic.1997.455.16>.
- Galán-Saúco, V., 2002. Greenhouse cultivation of tropical fruits. *Acta Hortic.* 575, 727–735. <https://doi.org/10.17660/ActaHortic.2002.575.85>.
- Galán-Saúco, V., 2014. Mango, litchi y pitaya. In: Hueso, J.J., Cuevas, J. (Eds.), *La fruticultura del siglo XXI en España. Serie Agricultura*. Cajamar Caja Rural, España, pp. 360–367.
- Galán-Saúco, V., 2015. Ventajas y desventajas del cultivo del mango (*Mangifera indica* L.) en zonas subtropicales y potencial del cultivo bajo invernadero. *Acta Hortic.* 1075, 167–177. <https://doi.org/10.17660/ActaHortic.2015.1075.19>.
- Galán-Saúco, V., Galván, D., Hernández Conde, J.C., Navarro, A., 1997. Preliminary studies on fruit-set of mango cultivar Tommy Atkins under greenhouse cultivation in the Canary Islands. *Acta Hortic.* 455, 530–537. <https://doi.org/10.17660/ActaHortic.1997.455.69>.
- Garibaldi, L.A., Cunningham, S.A., Aizen, M.A., Packer, L., Harder, L.D., 2018. Sustainable yields, sustainable growth or neither?, in: The potential for insect pollinators to alleviate global pollination deficits and enhance yield of fruit and seed crops. Food and Agriculture Organization of the United Nations, Italia, pp. 35–53.

- Geetha, G.A., Shivashankara, K.S., Reddy, Y.T.N., 2016. Varietal variations in temperature response for hermaphrodite flower production and fruit set in mango (*Mangifera indica* L.). S. Afr. J. Bot. 106, 196–203. <https://doi.org/10.1016/j.sajb.2016.07.011>.
- Golec, A.F.C., 2019. El estudio floral de mango (*Mangifera indica* var. Tommy Atkins), limón sutil (*Citrus aurantifolia*) y lúculo (*Pouteria lucuma*) en el departamento de Lima en Perú. Anales Científicos 80, 253–258.
- Heinrich, B., 1979. “Majoring” and “minoring” by foraging bumblebees, *Bombus vagans*: an experimental analysis. Ecology 60, 245–255. <https://doi.org/10.2307/1937652>.
- Hermoso, J.M., Guirado Sánchez, E., Farré Massip, J.M., 2018. Introducción al cultivo del mango en el sur peninsular. Caja Rural Granada, Granada.
- Hernández-Delgado, P.M., Aranguren, M., Reig, C., Fernández-Galván, D., Mesejo, C., Martínez-Fuentes, A., Galán-Saúco, V., Agustí, M., 2011. Phenological growth stages of mango (*Mangifera indica* L.) according to the BBCH scale. Sci. Hortic. 130, 536–540. <https://doi.org/10.1016/j.scienta.2011.07.027>.
- Hormaza, J.I., Herrero, M., 1992. Pollen selection. Theor. Appl. Genet. 83, 663–672. <https://doi.org/10.1007/BF00226682>.
- Huda, A.N., Salmah, M.R.C., Hassan, A.A., Hamdan, A., Razak, M.N.A., 2015. Pollination services of mango flower pollinators. J. Insect Sci. 15, 113. <https://doi.org/10.1093/jisesa/iev090>.
- Jiron, L.F., Hedstrom, I., 1985. Pollination ecology of mango (*Mangifera indica* L.) (Anacardiaceae) in the neotropical region. Turrialba 35, 269–277.
- Junta de Andalucía, 2020. Datos básicos de mango. Campaña 2020. Observatorio de precios y mercados. Consejería de Agricultura, Pesca y Desarrollo Rural. Junta de Andalucía. URL: <https://www.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/RecordContent?table=11114&element=3659697&subsector=&>.
- Junta de Andalucía, 2021. Observatorio de precios y mercados. Consejería de Agricultura, Pesca y Desarrollo Rural. Junta de Andalucía. URL: <https://www.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/RecordContent?table=11114&element=3659697&subsector=&>.
- Kendall, L.K., Evans, L.J., Gee, M., Smith, T.J., Gagic, V., Lobaton, J.D., Hall, M.A., Jones, J., Kirkland, L., Saunders, M.E., Sonter, C., Cutting, B.T., Parks, S., Hogendoorn, K., Spurr, C., Gracie, A., Simpson, M., Rader, R., 2021. The effect of protective covers on pollinator health and pollination service delivery. Agric. Ecosyst. Environ. 319, 107556. <https://doi.org/10.1016/j.agee.2021.107556>.
- Kumar, S., Joshi, P.C., Nath, P., Singh, V.K., Mansotra, D.K., 2016. Role of insects in pollination of mango trees. Int. Res. J. Biolog. Sci. 5, 64–67.
- Kwon, Y.J., Saeed, S., 2003. Effect of temperature on the foraging activity of *Bombus terrestris* L. (Hymenoptera: apidae) on greenhouse hot pepper (*Capsicum annuum* L.). Appl. Entomol. Zool. 38, 275–280. <https://doi.org/10.1303/aez.2003.275>.
- Lionakis, S.M., Loxou, B.K., 1997. Behaviour of some mango cultivars in the greenhouse, under net an outdoors in the area of Chania Crete. Acta Hortic. 455, 401–406. <https://doi.org/10.17660/ActaHortic.1997.455.51>.
- Lucas-García, R., Rosas-Guerrero, V., Alemán-Figueroa, L., Almazán-Núñez, R.C., Violante-González, J., Kuk-Dzul, J.G., 2021. Spatial proximity of ‘Ataulfo’ to ‘Haden’ cultivar increases mango yield and decreases incidence of nubbins. Agronomy 11, 450. <https://doi.org/10.3390/agronomy11030450>.
- Martin, F.W., 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol. 34, 125–128. <https://doi.org/10.3109/10520295909114663>.
- Mditshwa, A., Magwaza, L.S., Tesfay, S.Z., 2019. Shade netting on subtropical fruit: effect on environmental conditions, tree physiology and fruit quality. Sci. Hortic. 256, 108556. <https://doi.org/10.1016/j.scienta.2019.108556>.
- Medany, M., Abdrabbo, M., Farag, A., Hassanein, M., Abou Hadid, A., 2009. Growth and productivity of mango grown under greenhouse conditions. Egypt. J. Hort. 36, 373–382.
- Mizuno, S., Yoshida, T., Kiyokawa, K., Sasaki, M., 2007. Pattern of visiting flowers and pollination efficiency of three kinds of bees on “Irwin” mango fruits grown in plastic greenhouses. Jpn. J. Trop. Agric. 51, 116–122. <https://doi.org/10.11248/jsta1957.51.116>.
- Mohsen, A.M.A., 2019. Survey of insect mango pollinators and the pollination occurrence of mango trees in relations to fruit yields. Curr. Sci. Int. 8, 245–251.
- Mukherjee, S.K., 1997. Introduction: botany and importance. In: Litz, R.E. (Ed.), The Mango. Botany, Production and Uses. CAB International, Wallingford, pp. 1–19.
- Mulcahy, D.L., Mulcahy, G.B., 1987. The effects of pollen competition. Am. Sci. 75, 44–50. <http://www.jstor.org/stable/27854449>.
- Munj, A., Zote, V., Raut, R., Salvi, B., 2017. Survey and surveillance of pollinators of mango in South Konkan coastal region of Maharashtra. J. Entomol. Zool. Stud. 5, 190–192.
- Ne’eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S.G., Dafni, A., 2010. A framework for comparing pollinator performance: effectiveness and efficiency. Biol. Rev. Camb. Philos. Soc. 85, 435–451. <https://doi.org/10.1111/j.1469-185X.2009.00108.x>.
- Ochiazzi, P., 1999. Stingless bees thrive in glasshouse trial. Aussie Bee 12, 8–11.
- Osterman, J., Aizen, M.A., Biesmeijer, J.C., Bosch, J., Howlett, B.G., Inouye, D.W., Jung, C., Martins, D.J., Medel, R., Pauw, A., Seymour, C.L., Paxton, R.J., 2021. Global trends in the number and diversity of managed pollinator species. Agric. Ecosyst. Environ. 322, 107653. <https://doi.org/10.1016/j.agee.2021.107653>.
- Pérez, V., 2013. Biología reproductiva del mango (*Mangifera indica* L.) (Tesis Doctoral). Universidad de Málaga, Málaga.
- Pérez, V., Herrero, M., Hormaza, J.I., 2016. Self-fertility and preferential cross-fertilization in mango (*Mangifera indica*). Sci. Hortic. 213, 373–378. <https://doi.org/10.1016/j.scienta.2016.10.034>.
- Popenoe, W., 1917. The Pollination of the Mango. Bulletin of the U.S. Department of Agriculture 542, 1–20. <https://doi.org/10.5962/bhl.title.107940>.
- Rajan, V.V., Reddy, P.R., 2019. A dead heat in pollination race: a comparative evaluation of the efficiency of a fly (*Chrysomya megacephala*) and a bee (*Apis florea*) in mango pollination. J. Entomol. Zool. Stud. 7, 1087–1091.
- Ramírez, F., Davenport, T.L., 2016. Mango (*Mangifera indica* L.) pollination: a review. Sci. Hortic. 203, 158–168. <https://doi.org/10.1016/j.scienta.2016.03.011>.
- Ramírez, F., Davenport, T.L., 2012. Reproductive biology (physiology)- The case of mango. In: Valavi, S.G., Rajmohan, K., Govil, J.N., Peter, K.V., Thottappilly, G. (Eds.), Mango: production and processing technology. Studium Press, Houston, Texas, pp. 56–81.
- Reddy, P.V.R., Sreedevi, K., 2016. Arthropod communities associated with mango (*Mangifera indica* L.): diversity and interactions. In: Chakravarthy, A.K., Sridhara, S. (Eds.), Economic and ecological significance of arthropods in diversified ecosystems: sustaining regulatory mechanisms. Springer, Singapore. https://doi.org/10.1007/978-981-10-1524-3_14.
- Reilly, J.R., Artz, D.R., Biddinger, D., Bobiwash, K., Boyle, N.K., Brittain, C., Brokaw, J., Campbell, J.W., Daniels, J., Elle, E., Ellis, J.D., Fleischer, S.J., Gibbs, J., Gillespie, R. L., Gundersen, K.B., Gut, L., Hoffman, G., Joshi, N., Lundin, O., Mason, K., McGrady, C.M., Peterson, S.S., Pitts-Singer, T.L., Rao, S., Rothwell, N., Rowe, L., Ward, K.L., Williams, N.M., Wilson, J.K., Isaacs, R., Winfree, R., 2020. Crop production in the USA is frequently limited by a lack of pollinators. Proc. Royal Soc. B. 287, 20200922. <https://doi.org/10.1098/rspb.2020.0922>.
- Rodríguez-Pleguezuelo, C.R., Duran-Zuazo, V.H., Muriel-Fernández, J.L., Franco-Tarifa, D., 2012. Physico-chemical quality parameters of mango (*Mangifera indica* L.) fruits grown in a Mediterranean subtropical climate (SE Spain). J. Agric. Sci. Technol. 14, 365–374.
- Saeed, S., Naqqash, M.N., Jaleel, W., Saeed, Q., Ghouri, F., 2016. The effect of blow flies (Diptera: Calliphoridae) on the size and weight of mangos (*Mangifera indica* L.). PeerJ 4, e2076. <https://doi.org/10.7717/peerj.2076>.
- Sánchez, M., Velásquez, Y., González, M., Cuevas, J., 2022. Activity and foraging behaviour of the hoverfly *Eristalis aeneus* (Scopoli, 1763) in protected cultivation of mango (*Mangifera indica* L.). Bull. Entomol. Res. 112 (1), 101–109. <https://doi.org/10.1017/S0007485321000717>.
- Sharma, S., Abbas, S.R., Shukla, R.P., Sharma, S., 1998. An easy and quick method of breeding flies for pollination of mango blossoms. Insect Environ. 4, 76–77.
- Shi, Z.H., 1999. Effect of temperature on the flowering biology and fertilization of mangoes (*Mangifera indica* L.). J. Appl. Hortic. 01, 79–83. <https://doi.org/10.37855/jah.1999.v01i02.03>.
- Singh, R.N., 1954. Studies on floral biology and subsequent production of fruits in the mango (*Mangifera indica* L.) varieties Dushehari and Langra. Indian J. Hort. 11, 69–88.
- Souza, A.G., Smiderle, O.J., Spinelli, V.M., Souza, R.O.de, Bianchi, V.J., 2016. Correlation of biometrical characteristics of fruit and seed with twinning and vigor of *Prunus persica* rootstocks. J. Seed Sci. 38, 322–328. <https://doi.org/10.1590/2317-1545v38n4164650>.
- Sukhviyal, N., Whitley, A.W., Smith, M.K., Hetherington, S.E., Vithanage, V., 1999. Effect of temperature on inflorescence development and sex expression of mono- and polyembryonic mango (*Mangifera indica* L.) cultivars. J. Hortic. Sci. Biotechnol. 74, 64–68. <https://doi.org/10.1080/14620316.1999.11511073>.
- Sung, I.-H., Lin, M.-Y., Chang, C.-H., Cheng, A.-S., Chen, W.-S., 2006. Pollinators and their behaviors on mango flowers in Southern Taiwan. Formosan Entomol. 26, 161–170.
- Usha, U., Srivastava, P., 2018. Foraging reward and foraging behavior of insect visitors on mango flowers during blooming period. Int. J. Pure Appl. Biosci. 6, 437–440.
- Usha, U., Srivastava, P., Goswami, V., 2014. Diversity of floral insect visitors of mango during blooming period at Pantnagar. Indian J. Agric. Sci. 84, 363–364.
- Usman, M., Bilquees, F., Jaskani, M., 2001. Breeding in mango. Int. J. Agric. Biol. 8530 (3–4), 522–526.
- Vishwakarma, R., Singh, R., 2017. Foraging behaviour of insect visitors and their effect on yield of mango var. Amrapali. Ind. Jour. of Entomol. 79, 72. <https://doi.org/10.5958/0974-8172.2017.00016.5>.
- Yeshitela, T., Robbertse, P.J., Fivas, J., 2004. Effects of fruit thinning on “Sensation” mango (*Mangifera indica*) trees with respect to fruit quantity, quality and tree phenology. Exp. Agric. 40, 433–444. <https://doi.org/10.1017/S0014479704002091>.
- Yonemoto, J.Y., 2018. Mango cultivation in greenhouses. In: Galán-Saúco, V., Lu, P. (Eds.), Achieving sustainable cultivation of mangoes. Burleigh Dodds Science Publishing, London, UK, pp. 185–205.