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Pollination of Granadilla (*Passiflora ligularis*) Benefits From Large Wild Insects

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Abstract

The contribution of wild pollinators to food production has recently been assessed for many crops, although it remains unclear for several tropical crops. Granadilla (*Passiflora ligularis* Juss), a crop native to the tropical Andes, is one such crop where a gap exists regarding comprehensive knowledge about its pollination system. In a field experiment in the Colombian Andes, we 1) describe flower visitors in terms of visit quantity (visitation rate) and quality (touches of flower-reproductive structures), 2) assess the pollination system by comparing fruit set and fruit weight per flower in three pollination treatments: pollinator exclusion, open pollination, and supplementary pollination, and 3) evaluate pollination deficits (difference between open and supplementary pollination) in relation to pollinator density. We observed 12 bee species visiting granadilla flowers, with *Apis mellifera* Linnaeus being the most frequent species. However, large bees such as *Xylocopa lachnea* Moure and *Epicharis rustica* Olivier touched stigmata and anthers more often. Fruit set and fruit weight per flower were significantly lower in the pollinator exclusion treatment compared to open and supplementary pollination, while the latter treatments showed nonsignificant differences. Pollination deficit significantly decreased with the increasing density of large bees and wasps. Our results illustrate the high dependency of granadilla on wild pollinating insects and highlight the crucial role of large insects to granadilla production. This stresses the need to maintain or increase the density of large pollinators in granadilla production areas, which in turn will necessitate better knowledge on their ecological requirements to inform landscape planning and population-management programs.

Resumen

La contribución de polinizadores silvestres a la producción de alimento ha sido evaluada recientemente para muchos cultivos, aunque aún se desconoce para varios cultivos tropicales. La granadilla (*Passiflora ligularis* Juss) es un cultivo nativo de los Andes tropicales que carece de un conocimiento exhaustivo sobre su sistema de polinización. En un experimento de campo en los Andes colombianos, 1) describimos las especies de visitantes florales en términos de la cantidad (frecuencia) y calidad (contacto con las partes reproductivas de la flor) de las visitas, 2) evaluamos el sistema de polinización comparando el porcentaje de cuajamiento de frutos y el peso del fruto por flor entre tres tratamientos de polinización: exclusión de polinizadores, polinización abierta y polinización suplementaria, y 3) evaluamos los déficits de polinización (diferencia entre la polinización abierta y suplementaria) con relación a la densidad de polinizadores. Registramos 12 especies de abejas en flores de granadilla, siendo *Apis mellifera* Linnaeus la más frecuente. Sin embargo, las abejas grandes como *Xylocopa lachnea* Moure y *Epicharis rustica* Olivier hicieron contacto con los estigmas y las anteras con mayor frecuencia. El cuajamiento de frutos y el peso del fruto por flor fueron significativamente menores en el tratamiento de exclusión de polinizadores, en comparación con la polinización abierta y suplementaria, mientras que estos dos últimos tratamientos no mostraron diferencias significativas. El déficit de polinización disminuyó significativamente al aumentar la densidad de abejas y avispas grandes. Nuestros resultados demuestran la alta dependencia de la granadilla a la polinización por insectos silvestres, y resaltan el papel crucial de los insectos de cuerpo grande a la producción. Esto enfatiza

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la necesidad de mantener o aumentar la abundancia de polinizadores de gran tamaño en áreas de producción de granadilla, lo que a su vez requerirá un mejor conocimiento de sus necesidades ecológicas para informar la planificación del paisaje y los programas de manejo poblacionales.

Fruits are essential to human nutrition as they provide the majority of micronutrients, vitamins, and antioxidants, together with vegetables and nuts (Eilers et al. 2011, da Silva et al. 2014). The demand for tropical fruits, in particular, has increased rapidly in the past decade (da Silva et al. 2014, FAO 2016). This has significantly contributed to the economy of developing countries, which produce 99% of tropical fruits (25% in Latin America), mainly cultivated by small-holder farmers (FAO 2011). Yield of many tropical fruits traded at global markets fully or partially depends on pollination mediated by animals (Klein et al. 2007, Garibaldi et al. 2011). However, the pollination requirements of several crop species and most varieties of potential economic importance are still unknown, and there is a need for comprehensive studies across different ecological conditions to evaluate their pollination systems, including the dependency on managed and wild pollinating insects.

The genus *Passifora* includes several cultivated species of economic importance, with Colombia (170 species) and Brazil (150 species) as centers of diversification (Cerqueira-Silva et al. 2016). *Passiflora* flowers have sticky and heavy pollen, which make wind pollination usually ineffective (Souza and Pereira 2000, Aguiar-Menezes et al. 2002). Due to the relative large size of the flowers, legitimate pollinators are mainly large bees, for instance, of the genus *Xylocopa*, which make contact with the stigma during nectar collection (Akamine and Girolami 1959, Ángel-Coca et al. 2011, Yamamoto et al. 2012). Similar to other crops that rely on a narrow range of pollinating species, the production of *Passiflora* fruits has been considered severely threatened by the loss of pollinators (Klein et al. 2007). This particularly concerns the most economically important species *Passiflora edulis* (Malpighiales: Passifloraceae), and its two forms, the 'yellow passion fruit' (*P. edulis* f. *flavicarpa* Degener [Malpighiales: Passifloraceae]) and the 'purple passion fruit' (*P. edulis* f. *edulis* Sims). However, *Passiflora* has more than 50 edible species (Ocampo Pérez et al. 2010), which show a high within- and between-species variation in their dependency on animal pollination to fruit set (Knight and Sauls 1994, Kishore et al. 2010, Shivanna 2012).

Granadilla (*Passiflora ligularis* Juss [Malpighiales: Passifloraceae]) is a species native to the tropical Andes and cultivated from Argentina to Florida and in other countries such as New Zealand, South Africa, Australia, and Kenya (Lim 2012). Major producing countries are Colombia and Peru for local consumption and global markets, with a combined crop area in 2014 exceeding 10,000 ha and 100,000 tons of fruit production (Agronet 2017, SIEA 2017). In granadilla, although pollen transfer by bees among flowers of different plants (cross-pollination) has been long recognized (Rivera et al. 2002), pollination studies show a high variation in fruit set when flowers are naturally exposed to pollinators (Franco et al. 2007, Pinilla-Gallego and Nates-Parra 2015, Arias-Suárez et al. 2016). Moreover, the few studies that evaluate fruit set after autonomous self-pollination (flowers not exposed to pollinators) have found contradictory results (0–34%) (Arias-Suárez et al. 2016, R. Ospina-Torres, unpublished data). High variation in fruit set from both cross-pollination and autonomous self-pollination are limiting conclusions on the relative contribution of insects to the production of granadilla, specifically because robust estimations of density and visitation rate of pollinators are mostly lacking (but see Pinilla-Gallego and Nates-Parra 2015).

In addition to autonomous self-pollination, determining the full extent to which insects contribute to crop production requires information on the maximum sexual reproductive output of the plant given the available resources (i.e., optimum pollination, Vaissière et al. 2011). When optimum pollination is higher than open pollination (e.g., achieved by insects), a pollination deficit exists (Vaissière et al. 2011). A pollination deficit can be determined by comparing fruit set or any other parameter related to crop yield (e.g., seed number or fruit weight) from flowers that are hand-pollinated (as a measure of optimum pollination) with those that are open pollinated (Garratt et al. 2014b, Petersen and Nault 2014). Comparison of pollination deficits across and within cropping systems, together with information on pollinator density and performance (behavior on the flowers), can lead to a better understanding of the pollination system and services provided by insects (Morandin and Winston 2005).

We conducted a field experiment in the Andean mountains of Colombia to describe the insect community visiting flowers of granadilla and evaluate their contribution to pollination. We aim to answer the following questions: 1) which pollinators provide the most frequent visits and which make contact with flower reproductive parts more often?, 2) how much do insects contribute to the initial fruit set and the fruit weight per flower?, and 3) how are pollination deficits related to flower-visitor density?

Materials and Methods

Study Area and Experiment Setup

The field experiment was conducted between January 2015 and February 2016 in the Central Andes of Colombia (4° 40′45″N; –75°38′4″W), in the municipality of Filandia (Department of Quindío), between 1,800 and 2,100 m a.s.l. Mean annual rainfall is 2,817 mm and mean monthly temperature ranges between 16 and 24°C (Fagua et al. 2013), matching the optimal growing conditions of *P. ligularis* (Fischer et al. 2009). The landscape is predominantly a mosaic of pasture for cattle grazing (62%) and subandean forest (31%). Other land-use types represent less than 8%, and include human settlements and small crop areas. Flower-visiting insects, including the European honeybee (*Apis mellifera* Linnaeus (Hymenoptera: Apidae)), are not managed in our study area.

We established ten experimental plots, each located on cattle grazing pastures at 20 m distance to the forest and without other crops in the surroundings. The minimum distance between plots was 1 km. Four out of the ten plots were discarded from the analyses because most of their plants did not survive, grew slowly or were attacked by pathogens, problems mainly associated with the intensive drought caused by the ENSO phenomenon (El Nino-Southern Oscillation) in 2015. In each plot, 16 granadilla plants were planted in two 24-m-rows, with 3 m between plants, and 3 m between rows, covering a total area of approximately 144 m². The number of surviving plants varied between 9 and 14 across plots, for a minimum of three and a maximum five plants (replicates) per each of the three pollination treatments (see Pollination Treatments and Supp. Table S1). Seedlings of granadilla, obtained from a commercial plant nursery, were 2 mo old with an average height of 20 cm. A wire

trellis system 1.9 m in height was set to support granadilla vines. Plots were fenced to prevent damage by cattle. Plants were managed following guidelines from agronomists and technical manuals for producers (Rivera et al. 2002).

Flower-Visiting Insects of Granadilla

The flower-visiting community of granadilla was evaluated during the first flowering period, which occurred 10 mo after transplanting the seedlings into the plots. Observations were carried out between 0700 and 1300 hours, which is the period of maximum receptivity of the stigmas (Rivera et al. 2002), nectar and pollen presentation, and bee activity (R. Ospina-Torres, unpublished data). This period was divided into three subperiods: 1) 0700 to 0859 hours; 2) 0900 to 1059 hours, and 3) 1100 to 1300 hours, to survey at least three plots in the same day, maximizing the number of samples per plot and subperiod during the flowering peak.

We measured the behavior of each flower-visitor species in terms of 1) visitation rate, which is indicative of the animal contribution to plant pollination (Vázquez et al. 2005), and 2) visit quality, as the percentage of visits at which a visitor touched the reproductive floral parts, i.e., anthers and/or stigma. Visitation rate was estimated as the average visits per flower in a 10-min interval, across 30 flowers in each plot (10 flowers per subperiod). For visit quality, although contact with stigma is frequently used to assess pollination success (Monzón et al. 2004, Ne'eman et al. 2010, Woodcock et al. 2013), contact with anthers is directly related to pollen removal and hence a prerequisite of pollination (Harder and Barrett 1993). Observations were performed under good weather conditions with temperatures ≥15°C, low wind, and no rain (Westphal et al. 2008). We observed flower visitors for a total of 30 h per plot.

Besides the performance of individual species described by visitation rate, we further assessed flower-visitors density at the plot scale, which has been found to be an important predictor of crop yield across different crop systems (Garibaldi et al. 2016). Flower-visitor density serves as a proxy for the level of pollination services (i.e., potential number of compatible and viable pollen grains that reach a stigma during the effective pollination period) (Vaissière et al. 2011) and more likely includes rare but highly functional species such as *Xylocopa*, which could be overlooked during visitation rate assessments. Following the protocol from Vaissière et al. (2011), we sampled 100 open flowers during maximum 15 min (scanning round). Unambiguous flower-visitor species were identified, while simultaneously counting flowers with a hand counter. In each plot, we carried out three scanning rounds per subperiod on four different days (in total 36 scanning rounds per plot). Thysanoptera (thrips) and Nitidulidae (sap beetles) were excluded from the analyses to avoid bias as they can be easily overlooked due to their small size (Chrobock et al. 2013). We calculated the density of overall flower visitors, as well as the density by category of body size (i.e., small, medium and large), dividing the number of observed flower visitors by the number of open flowers in the respective plot and sampling day.

We finally categorized flower visitors (specifically Hymenoptera) according to their body size. For this, we measured the inter-tegular distance (ITD), which is the distance between the two insertion points of the wings. Species were considered small when ITD < 2.25 mm, medium for 2.26 ≤ ITD ≥ 4.5 mm and large for ITD ≥ 4.6 mm, modified from Benjamin et al., (2014) to discriminate medium bees such as *A. mellifera* and large bees such as *Xylocopa* spp. The ITD was averaged from a minimum of three and maximum ten pinned specimens of each species.

Pollination Treatments

We applied three pollination treatments to randomly selected plants to evaluate the pollination system of granadilla and detect pollination deficits. For each treatment, we selected three to five plants (the number varied according to the number of surviving plants in each plot). For each plant, we randomly selected a maximum of six flowers per sampling day to apply the respective pollination treatment; although a plant can produce more than six flowers per day, we distributed samples across the flowering season. Each flower was tagged with plastic bands indicating the pollination treatment and the flowering date. On average, 30 (±5) flowers per plant per treatment were tagged (Supp. Table S1). The three pollination treatments were as follows: 1) pollinator exclusion: flowers were bagged with tulle net bags (1 × 1 mm mesh size) the day before the flower opening and removed 48 h later; this was a measure of autonomous self-pollination (likely achieved by the close position of anthers and stigma and not by wind, as it hardly transfers the sticky pollen); 2) open pollination: flowers accessible to flower visitors to assess the contribution of insects, and 3) supplementary pollination: flowers accessible to insect pollination and additionally, hand-pollinated with fresh pollen collected in several flowers from different plant individuals; this was considered the optimum pollination level. Similar to *P. edulis*, we observed flowers differing in the maximum curvature reached by the styles, although no specific information for granadilla was available. According to the style curvature, three types of flowers are known in the yellow and purple passion fruits: totally curved, partially curved, and up-right (Ruggiero et al. 1976, Ishihata 1991). Since up-right style flowers act likely as male flowers and do not set fruit (Aguiar-Menezes et al. 2002), we excluded this flower type.

Fruit Set and Fruit Weight per Flower

We assessed initial fruit set (from now on 'fruit set') by inspecting the ovary of each tagged flower 5 to 7 d after pollination, as ovaries start swelling within the first 2 d after fertilization (Hammer 1987). Fruit set was assessed for each plant and calculated by dividing the number of fertilized ovules by the total number of tagged flowers per plant. Initial fruit set is considered a suitable index of pollination success and is independent of crop management (Zhang et al. 2015).

To include a variable relevant for producers, we calculated fruit weight per flower, representing the yield expected from each flower. For this, the weight of all ripe fruits (75–80 d after fertilization) resulting from the tagged flowers was divided by the number of tagged flowers. Fruit weight can increase with pollination quality and may differ from fruit set due to management or abortion (Bos et al. 2007). To show that fruit weight is dependent on pollination quality in granadilla, we measured seed set, which is closely related to the amount of deposited pollen grains and fertilized ovules (Weiss et al. 1994, Silveira et al. 2012). We counted seeds for all ripe fruits and calculated the number of seeds per flower.

The total number of flowers used to calculate fruit set in each pollination treatment was 663 (pollinator exclusion), 783 (open pollination), and 761 (supplementary pollination), across all plots. Fruit weight and seed number were obtained for all fruits produced in each pollination treatments: 19 fruits in the pollinator exclusion treatment, 228 for open-pollination and 320 in the supplementary-pollination treatment.

Finally, we estimated yield per hectare to facilitate comparisons with national reports. For this, we counted the number of open flowers in three plots every day during the flowering period, and estimated the number of flowers produced by a single plant across the whole season. This number was multiplied by the mean fruit-grams

per flower from plants within a pollination treatment, obtaining yield (kg) per plant per treatment. This value was then multiplied by 400, which is a recommended number of plants per hectare (Restrepo et al. 2011).

Pollination Deficits

We calculated pollination deficits in terms of fruit set (from now on called 'fruit set deficit') and fruit weight per flower (from now on called 'fruit weight deficit'). These deficits were calculated by subtracting values of fruit set and fruit weight per flower of plants in the open-pollination treatment, from the respective values of plants in the supplementary-pollination treatment. Since plants in both treatments were not paired within each plot, we calculated a reference value (average) across plants of the supplementary-pollination treatment of each plot, and then subtracted values of each plant that was open pollinated. Thus, for each plot, we obtained three to five values of fruit set and fruit weight deficits (variation due to different number of surviving plants).

Statistical Analyses

We compared fruit set and fruit weight per flower across pollination treatments to determine the contribution of insects to the pollination of granadilla. Comparisons were performed by fitting linear mixed models with random intercept (plot) and random slope (treatment), and pollination treatment as fixed factor, hence accounting for the variance induced by uncontrolled factors between plots. Fruit set was logit transformed to meet normal distribution of residuals. The goodness of fit (homoscedasticity, normality of errors and independence) was checked through visual inspection of residuals. Tukey's post-hoc test was applied to compare mean differences between pollination treatments using the glht function in the multcomp package in R (Hothorn et al. 2008). The relation between fruit set deficit and fruit weight deficit (response variables) and density of flower visitors across plots (explanatory variable) were assessed with linear mixed models. We fitted two models for each response variable, one with the overall density of flower visitors (including all body-size categories) as fixed factor, and the other with the density of medium visitors plus the density of large visitors as fixed factors (giving the quality of their flower visits, see Flower Visitation Rate and Quality). Plot was included as random effect in all models. Explanatory variables were centered and scaled allowing effect sizes to be more easily compared (Rhodes et al. 2009). All analyses were performed in R 3.3.2 (R Core Team 2016).

Results

Flower Visitation Rate and Quality

The flower-visiting community of *P. ligularis* included 12 species of bees (Hymenoptera: Apoidea), one species of scoliid wasp (Hymenoptera: Scoliidae), and other less abundant insects which make no contact with floral reproductive structures such as wasps of the genus *Synoeca*, butterflies of the genus *Heliconius* and leaf beetles of the family Chrysomelidae (grouped as 'Others'). Despite the low frequency of the scoliid wasp, this species was highly effective in touching simultaneously the floral reproductive parts and therefore was not included in 'Others' (Fig. 1) (for a species list see Table 1).

The honeybee (*A. mellifera*) was the most frequent flower visitor, performing, on average, one visit every 2.5 min across all plots (Table 1). *Xylocopa lachnea* Moure (Hymenoptera: Apidae) was observed in all plots, but performed, on average, only one visit every 43 min. The species *Epicharis rustica* Olivier (Hymenoptera: Apidae)
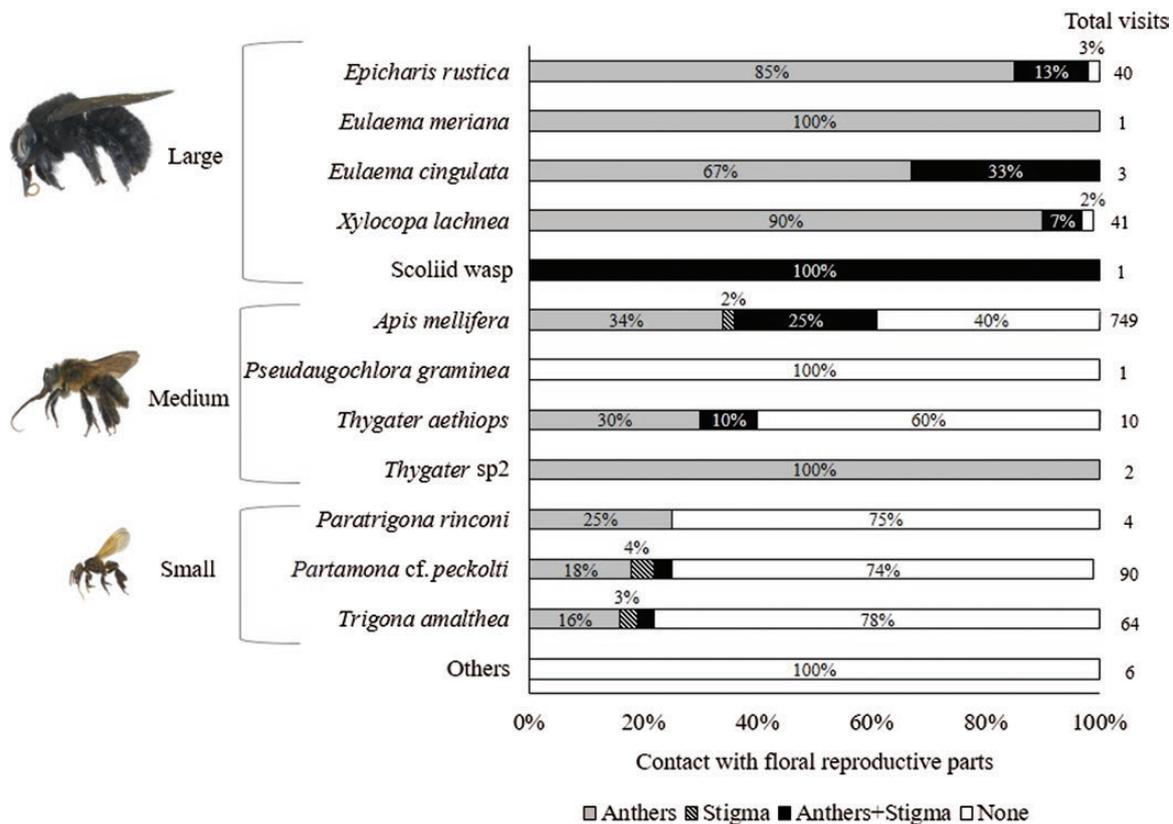

Fig. 1. Percentage of contacts with floral reproductive structures by each species of flower visitor. Grey: only anthers; hachured: only stigma; black: anthers and stigma; white: none.

Table 1. Number of flower visits performed by each bee species within a 10-min interval

Species	Body size	Plot A	Plot C	Plot G	Plot J	Plot L	Plot R	Mean
Apis mellifera	Medium	1.07	4.17	2.87	6.50	3.57	6.80	4.16 ± 2.19
Partamona cf. *peckolti*	Small	0.20	0.80	1.17	0.10	0.70	0.03	0.50 ± 0.46
Trigona amalthea	Small	2.13	0.00	0.00	0.00	0.00	0.00	0.36 ± 0.87
Xylocopa lachnea	Large	0.23	0.03	0.40	0.17	0.33	0.20	0.23 ± 0.13
Epicharis rustica	Large	0.33	0.03	0.73	0.00	0.13	0.10	0.22 ± 0.28
Thygater aethiops	Medium	0.00	0.00	0.00	0.03	0.03	0.27	0.06 ± 0.10
Paratrigona rinconi	Small	0.00	0.00	0.00	0.10	0.03	0.00	0.02 ± 0.04
Eulaema cingulata	Large	0.00	0.00	0.00	0.03	0.07	0.00	0.02 ± 0.03
Thygater sp2	Medium	0.00	0.00	0.07	0.00	0.00	0.00	0.01 ± 0.03
Eulaema meriana	Large	0.00	0.00	0.00	0.03	0.00	0.00	0.01 ± 0.01
Pseudaugochlora graminea	Medium	0.03	0.00	0.00	0.00	0.00	0.00	0.01 ± 0.01
Scoliid wasp	Large	0.00	0.00	0.00	0.00	0.00	0.03	0.01 ± 0.01
Others[a]		0.00	0.07	0.00	0.07	0.03	0.03	0.03 ± 0.03

Mean values (per 30 flowers) are shown for each plot (A, C, G, J, L, R) as well as the average across plots (pooled plots).

[a]Others include *Synoeca* wasps, *Heliconius* butterflies and leaf beetles (Chrysomelidae).

Table 2. Results of the post-hoc Tukey tests for differences among pollination treatments

Response variable	Between-pollination treatment comparison	Estimate ± SD	*P*-value
Fruit set	Pollinator exclusion vs Open	2.91 ± 0.18	<0.01
	Pollinator exclusion vs Supplementary	3.57 ± 0.39	<0.01
	Open vs Supplementary	0.66 ± 0.30	0.06
Fruit weight per flower	Pollinator exclusion vs Open	30.65 ± 5.67	<0.01
	Pollinator exclusion vs Supplementary	44.52 ± 5.00	<0.01
	Open vs Supplementary	13.88 ± 6.13	0.06

and *Partamona* cf. *peckolti* Friese (Hymenoptera: Apidae) were also common visitors to most plots, while species such as *Eulaema meriana* Olivier (Hymenoptera: Apidae) and *Trigona amalthea* Olivier (Hymenoptera: Apidae) were observed in only one plot.

On average, large Hymenoptera (bees and scoliid wasps) made contact with floral reproductive parts in 99% of their visits, medium bees in 50% and small bees in 24% (Fig. 1). Overall, contacts with the anthers were more frequent than contacts with stigma or the simultaneous touch of anther and stigma in a single visit.

Pollination System

Fruit set in open-pollinated flowers (mean ± SD 49% ± 11) was higher than in flowers where visitors were excluded (mean ± SD 4% ± 5); hence insect contribution to fruit set was around 45%. Fruit set in supplementary-pollinated flowers (mean ± SD 65% ± 14) was higher in comparison to open-pollinated flowers, but the difference was not statistically significant (Table 2, Fig. 2a). Similarly, fruit weight per flower was substantially higher in the open-pollination treatment compared to the pollinator exclusion treatment, and comparable to the supplementary-pollination treatment (Table 2, Fig. 2b). Fruit weight and seed number were significantly correlated (r_s (565) = 0.56, $P < .001$).

Based on our daily counts of open flowers, we estimated that one plant can produce 300–500 flowers in a flowering season. Based on this number, a total production of 9.6–16 kg of fruit per plant can be expected, 3.8–6.4 ton/ha for 400 plants/ha. Total yields without pollinators can be expected to be 0.3–0.6 ton/ha.

Pollination Deficits in Relation to Flower-Visitor Density

Fruit set and fruit weight were, on average, higher for supplementary-pollinated flowers than for open-pollinated flowers. Therefore, we found fruit set and fruit weight deficits (mean ± SD, 0.16 ± 0.18 and 14.1 ± 15.1, respectively), across plots. However, the deficits showed high variation between plots (e.g., J had even negative deficits). Mean density of overall, medium and large flower visitors across plots was 1.26 (SD = 0.25), 1.03 (SD = 0.36), and 0.03 (SD = 0.01), respectively. Fruit set deficit was neither related to the overall density of flower visitors nor to the density of medium and large visitors (Table 3). Similarly, fruit weight deficit was not related to the density of overall and medium visitors, but significantly decreased with increasing density of large-bodied visitors (−13.40 ± 3.94, $P = 0.04$, Fig. 3, Table 3).

Discussion

We found that insects are essential for the pollination of granadilla, as autonomous self-pollination only contributed to 4% of the fruit set. Large bees (in the genera *Epicharis*, *Eulaema,* and *Xylocopa*) and a scoliid wasp made contact with floral reproductive structures more often compared to medium and small insects, suggesting their efficacy in pollen transfer in a single visit (Freitas 2013). However, pollination effectiveness (total contribution to the plant reproductive success) of large Hymenoptera can be limited by their low visitation rate, as pollination effectiveness is a combination of pollinator efficacy and visitation rate (Rader et al. 2009, Freitas 2013). In previous studies, *Xylocopa* bees were not considered the main pollinators of granadilla as their densities and visitation rates were very low, and still fruit set was highly likely due to *A. mellifera,* which were commonly observed in flowers (Franco et al. 2007, Pinilla-Gallego and Nates-Parra 2015). Although *A. mellifera* has been suggested to be a pollen thief in other *Passiflora* species due to its small body size and quick depletion of available pollen (Yamamoto et al. 2012), a lower efficacy of a pollinator in transferring pollen can be compensated by a high visitation rate (Vázquez et al. 2005). This compensatory effect has already been suggested for *A. mellifera* in granadilla (Pinilla-Gallego and Nates-Parra 2015). Furthermore, due to the proximity of stigma and anthers in *Passiflora* flowers during one of the floral phases, medium bees such as *A. mellifera* could facilitate self-pollination (Akamine and Girolami 1959), as has been
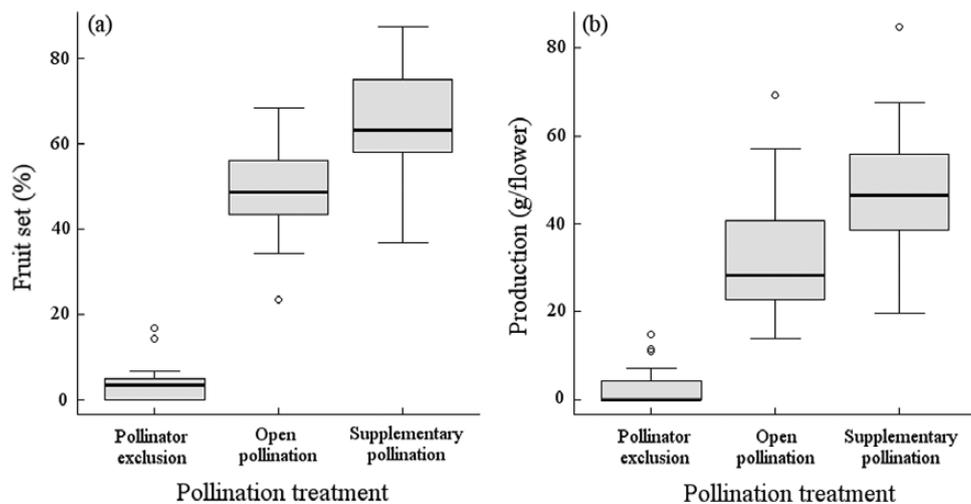
Fig. 2. Boxplots comparing fruit set (a) and fruit weight per flower (b) across pollination treatments. Box indicates quartiles with median marked as a horizontal line; points are outliers.

Table 3. Results of the linear mixed models for the effects of density of flower visitors on fruit set and fruit weight deficit

| Response variable	Explanatory variable	Estimate ± SE	df	*t*-value	*P*-value
Fruit set deficit	Overall density of flower visitors	0.00 ± 0.08	3.92	−0.02	0.99
	Density of medium flower visitors	0.018 ± 0.06	3.05	0.32	0.77
	Density of large flower visitors	−0.12 ± 0.07	3.02	−1.82	0.17
Fruit weight deficit	Overall density of flower visitors	16.14 ± 28.8	3.98	0.56	0.61
	Density of medium flower visitors	−2.50 ± 3.93	3.05	−0.06	0.57
	Density of large flower visitors	−13.40 ± 3.94	3.00	−3.41	0.04

observed in other commercial and wild *Passiflora* species (Kishore et al. 2010). Hence, our results suggest that a combined effect of large Hymenoptera, highly efficient in transferring pollen, and the frequent visitor *A. mellifera*, can help ensure fruit set in granadilla, a phenomenon also known for squash (Xie and An 2014) and apples (Thomson and Goodell 2001).

The fruit set found in open-pollinated flowers (42–55%), along with previous findings both in experimental (32–70%) (Arias-Suárez et al. 2016) and commercial fields (38–84% and 61–77%) (Franco et al. 2007, Pinilla-Gallego and Nates-Parra 2015), suggest that granadilla is an insect-pollinated species with intermediate rate of cross-pollination (i.e., 20–80%) (Goodwillie et al. 2005). A substantial proportion of plant species with intermediate cross-pollination rates are partially capable of self-pollination, as a mechanism of reproductive assurance when pollinators are scarce (Goodwillie et al. 2005, Shivanna 2012). This was also confirmed by our results, despite the fact that we found a very low fruit set when insects were excluded (4%). Our lower self-pollination rate in comparison to the previously reported rate by Arias-Suárez et al. (2016) (34.3%), could be explained by a higher proportion in our pollinator exclusion treatment of flowers with partially curved styles, which have shown a lower fruit set capacity (13%) as compared to flowers with totally curved style (45%) in *P. edulis* (Ruggiero et al. 1976). Still, our results confirm that insect pollination is crucial for profitable granadilla production.

Differences in fruit weight per flower between open- and supplementary-pollinated plants were not significant, suggesting that wild bees in our study region are providing sufficient pollination services required to maximize production. Large Hymenoptera, in particular, appears to contribute highly to this outcome since their increasing density was related to a decreasing fruit weight deficit. As large pollinators are likely to transfer many pollen grains at once, they have higher chances to maximize seed number per fruit in a single visit. Although the amount of pollen grains required for maximum seed set in granadilla is unknown, information from the edible species *P. vitifolia* Kunth (Malpighiales: Passifloracea) indicates that around 450 pollen grains maximize seed numbers per fruit, whereas only 25–50 grains are required for fruit set (Snow 1982). We found that fruit weight increased with seed number in *P. ligularis*, which confirms the similar observation in yellow passion fruit (Akamine and Girolami 1959). Thus, large pollinators could help to decrease fruit weight deficit in granadilla by maximizing seed set, which has also been suggested for apples (Garratt et al. 2014a), while they may not be related to the fruit set due to their low density. Although *Xylocopa* bees have been associated with higher production in passion fruits in Brazil (Junqueira and Augusto 2017), *E. rustica* appears to be an important pollinator of granadilla in our study region. Indeed, *Epicharis* bees have been found to be effective pollinators of the purple passion fruit in Colombia (Ángel-Coca et al. 2011).

Instead of pollination limitation, fruit abortion rates in open-pollinated and supplementary hand-pollinated plants (39 and 37%, respectively) suggest that plants were resource limited. This is also supported by the lower abortion rate found in plants with bagged flowers (26%), where the number of fruits per plant was very low. Extreme drought, windy conditions and high temperatures due to the El Niño event in 2015 (Northon 2017) were likely the causes of plant physiological stress and increased pest pressure (e.g., thrips benefit from high temperatures). These stressors likely decreased productivity in our study (4–6 ton/ha) compared to the mean national average (9–11 ton/ha) in Colombia (Agronet 2017). As granadilla is a species that can be both pollen and resource limited, further studies should attempt to investigate the interactive effect of these limitations.
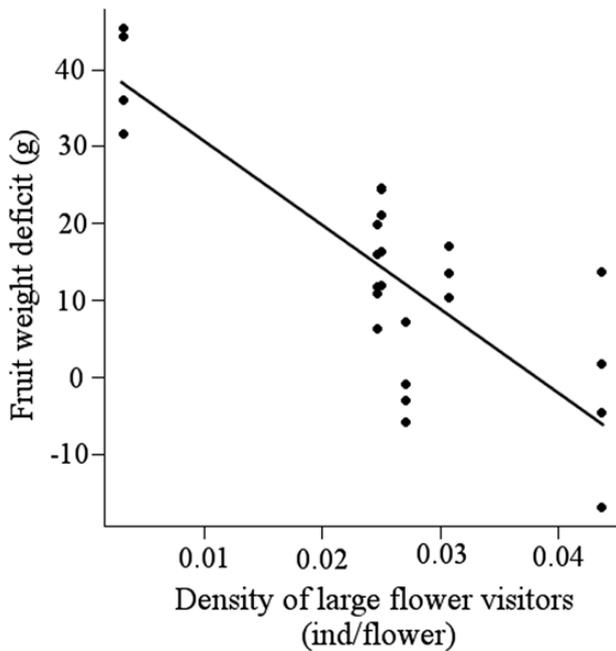
Fig. 3. Relationship between fruit weight deficit (estimated as the difference in fruit weight per flower between open-pollinated and supplementary-pollinated plants) and density of large flower visitors (ind/flower) across the six experimental plots.

Conclusions

Insects, in particular bees, can maximize crop production in granadilla by providing cross-pollination and sufficiently high pollen grain deposition. This was observed in our study area where forest represented 31% of the land-use types, and flower-visitor density was sufficient for crop yields not being limited by pollination (but likely by resource availability). Our study area contrast with some passion fruit growing areas in Colombia where natural habitats are less represented and producers rely on manual pollination given the low pollinator density (Calle et al. 2010). Enhancing natural pollination by insects, besides avoiding extra costs, can further prevent risks associated with flower manipulations such as stigma damage and pollen grain crowding (Young and Young 1992), and deleterious effects in the long term due to overproduction in one season (Ehrlen and Eriksson 1995). However, increasing density of effective *Passiflora* pollinators is a challenge, especially in extensive growing areas (Calle et al. 2010). Management implications may include the conservation of natural and semi-natural areas that sustain wild pollinators by providing permanent feeding and nesting resources, and direct population management programs within crop fields. However, knowledge on the management of large pollinators such as *Xylocopa* and *Epicharis* bees is limited (Junqueira et al. 2013, Pinilla-Gallego and Nates-Parra 2015) and awaits further research.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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