



## Crop yield mediated by honeybees in a star fruit orchard exhibiting atypical distyly

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### ABSTRACT

The dependence of commercial crops on pollinators is closely related to their reproductive strategies and has direct impacts on production yields. Fruit production of *Averrhoa carambola*, the star fruit, is highly dependent on bee pollination. This distylous species may exhibit monomorphism associated with self-compatibility, a condition that may influence its degree of dependence on pollinators for fruit set. We evaluated the floral morphology and mating system of *A. carambola* and their relationships with pollinator dependence in an apparently pin-monomorphic orchard to address the following questions: Is the orchard indeed pin-monomorphic? Is the orchard self-compatible? Are pollination services sufficient to reach maximum productivity? Is fruit set related to the number of visits? We investigated floral morphometrics, mating system, and pollination following standard methods of plant reproduction research. The orchard exhibited pin-monomorphism and self-compatibility. Herkogamy strongly impaired spontaneous self-pollination, emphasizing the relevance of pollinators to crop yield. Considering that *Apis mellifera* was the only pollinator observed, and that the orchard showed pollen limitation, we recommend the implementation of pollinator management strategies. Additional studies will be needed to assess if the atypical distyly observed here occurs in other situations.

**Keywords:** crop pollination, distyly, ecosystem services, fruit yields, self-compatibility, pollen limitation

The dependence of plant species on pollination services largely depends on their mating and pollination systems (Klein *et al.* 2007), so that crops with restrictive mating systems, and/or highly specialized pollination

mechanisms, tend to be more dependent on pollinators than those whose reproduction is more easily achieved (Garibaldi *et al.* 2011). It is therefore reasonable to expect that production among crops highly dependent on insect

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pollinators will be more impacted by their global declines (Potts *et al.* 2010; Lautenbach *et al.* 2012). Crop pollination, currently considered a threatened ecosystem service, is globally valued at approximately US \$ 235-577 billion annually (Giannini *et al.* 2015a; Potts *et al.* 2016), and is an important component of global food security (Smith *et al.* 2015). Studies focusing on pollination dependence and management of more vulnerable crops could therefore contribute to the mitigation of socioeconomic impacts caused by pollinator crisis (IPBES 2016; Potts *et al.* 2016).

Although more than 75 % of all crops depend on pollinators for maximum yields (Klein *et al.* 2007), pollination dependence data is missing for many species, such as the star fruit (*Averrhoa carambola* L., Oxalidaceae), which originated in Tropical Asia (Núñez-Elisea & Crane 2000), is widely cultivated in Brazil, where it yields ca. US\$ 97,000 (Bastos 2004). Star fruit plants exhibit distyly (Crane 1994), a floral dimorphism in which individuals produce flowers with short styles and long stamens (thrum morphs), or the inverse (pin morphs). Stigma in one morph is placed at similar position of anthers in the opposite morph, what is known as reciprocal herkogamy (Barrett & Shore 2008). Populations of typically distylous species, however, may exhibit deviations such as monomorphism (the presence of only one floral morph), which is associated with self-compatibility (Sakai & Wright 2007, but see Knight Jr 1965). *A. carambola* is commonly cited as a self- and intramorph-incompatible species (Knight Jr 1965; Saúco *et al.* 1993; Wong *et al.* 1994), although intramorph-compatibility has been observed in some varieties (Crane 1994; Fushimi 1998).

As distylous plants can set fruits only after intermorph (interplant) crosses, pollinators are determinants for fruit production (Jacquemyn *et al.* 2018). Star fruit trees are highly dependent on bees for their pollination (Giannini *et al.* 2015b), mainly *Apis mellifera* L (Ray 2002), although the degree of their dependence on pollinators and pollination value are unknown in Brazil (Giannini *et al.* 2015a). Pollination in Brazilian orchards is mainly performed by alien *A. mellifera*, as well as some native bee species (Silva-Junior 2016; Wolowski *et al.* 2019), but the effectiveness of those pollinators has not yet been determined. Pollinator efficiency can be estimated by relating the numbers of visits to fruit set (Klein *et al.* 2003, Goodwin 2011; Rollin & Garibaldi 2019).

The present study evaluated the floral morphology, mating system, and dependence on pollinators, of an apparently pin-monomorphic orchard of star fruit trees in northeastern Brazil, addressing the following questions: 1) Is the orchard pin-monomorphic? 2) Is the orchard self-compatible? 3) Are pollination services enough to reach maximum productivity? 4) How efficient are the pollinators?

Field data were collected at the experimental station of the Pernambuco Agronomy Institute (IPA) in Ibirimir (8°32'15" S, 37°41'30" W), Pernambuco State, in the

semiarid region of northeastern Brazil, between Dec/2012 and Dec/2013. The periods of anthesis, pollen availability, and stigmatic receptivity (detected using 10 % hydrogen peroxide; Dafni *et al.* 2005) were investigated on 30 bagged flowers distributed among 10 individuals.

The stigma and anther heights of five flowers collected from 20 individuals and preserved in 70 % alcohol were measured using a digital caliper. Stamen and stigma heights were checked for normality using the Shapiro-Wilk test, and differences were assessed using the Wilcoxon test (R Development Core Team 2015). If there was a statistical difference between anthers and stigmas, the presence of homostyly (an atypical condition, where anthers and stigmas have similar heights; Ganders 1979) would be discarded. Corolla length, total diameter, and tube diameter were also recorded in order to evaluate the accessibility of floral rewards and floral sexual elements by insect visitors.

The orchard mating system was evaluated using controlled crosses (spontaneous self-pollination – SS, self-pollination – SP, cross-pollination – CP, and natural pollination – NP), following standard methods for heterostylous species (Castro *et al.* 2004; see samples sizes in Tab. 1). We used around five individuals as pollen donors for CP. The fruits produced became totally developed between 60 and 75 days after anthesis, and were then counted. Fruit sets resulting from the controlled crosses were compared using Chi-square test, in Bioestat 5.3 software (Ayres *et al.* 2007). Comparisons of fruit sets among the CP, SP and SS crosses yielded information on the magnitudes of the differences between fruit formation under 'ideal' pollen deposition (CP) conditions, and after self-pollination performed by pollinators and/or gravity. Comparisons between CP, NP and SS were necessary to estimate the influence of pollinators on fruit set. The pollen limitation index – PLI (*sensu* Freitas *et al.* 2010) and the degree of dependence on pollinators – DDP (*sensu* Lázaro *et al.* 2014) were calculated. For PLI, negative values until 0.2 indicate the absence of PL, and values between 0.2 and 0.8 indicate low PL; values higher than 0.8 are interpreted as indicating extreme PL.

**Table 1.** Fruit set (%) resulted from controlled crosses in *Averrhoa carambola* in an experimental orchard in NE Brazil. Number of flowers used in each experiment are in given parenthesis. Different superscript letters indicate statistically different values (Chi-square test).

Treatment	Fruit set
Manual self-pollination	29 % (51) <sup>a</sup>
Spontaneous self-pollination	4 % (80) <sup>b</sup>
Natural pollination	40 % (75) <sup>c</sup>
Cross-pollination	52 % (58) <sup>c</sup>

Floral visitors were surveyed during focal observations of ten randomly selected individuals that were followed for one hour three times a day (at 07:00, 11:00, and 16:00) for five days (total of 15 hours). Floral visitor morphospecies,



numbers of flowers visited, and visitor behavior (resource collection and contact with sexual elements) were recorded. Those visitors were collected for identification by specialists. In order to evaluate the efficiency of visitors as pollinators, three inflorescences were randomly selected on ten different trees, and their pre-anthesis floral buds were isolated with mesh bags. Soon after flower opening the bags were removed, and as soon as the flowers were visited by an insect, they were re-bagged. The same procedure was performed (on different inflorescences) after two insect visits. The experiments were carried out during periods of high visitation frequency, and comparisons of fruit set after one and two visits were compared using the Fisher test, using Biostat 5.3 software (Ayres *et al.* 2007).

*A. carambola* flowers were small, pink (Fig. 1), with the stigma above the anthers ( $W=104$ ,  $p<0.05$ ; Tab. 2). They were diurnal, and lasted for approximately 12h (from 05:00 to 17:30), when pollen was available and the stigma receptive. Fruit set after CP was higher than after SP ( $\chi^2=5.57$ ,  $p<0.05$ ), or after SS ( $\chi^2=45.53$ ,  $p<0.001$ ), but did not differ from NP ( $\chi^2=1.81$ ,  $p>0.05$ ). Fruit set after NP was higher than after SS ( $\chi^2=30.35$ ,  $p<0.001$ , Tab. 1). The pollen limitation index was 0.23, and the DDP was 0.9. The flowers were visited exclusively by *Apis mellifera* L, which landed on the corolla lobes and inserted their heads into the flower to collect nectar and pollen, and thus came into contact with the anthers and stigma (Fig. 1). Visits were more frequent early in the morning, declining towards the middle of the day, and then increasing again near 16:00 (Fig. 2). Fruit set after a single visit ( $20.00 \pm 14.14\%$ ) and after two visits ( $20.00 \pm 0.00\%$ ) did not differ ( $p > 0.05$ ).



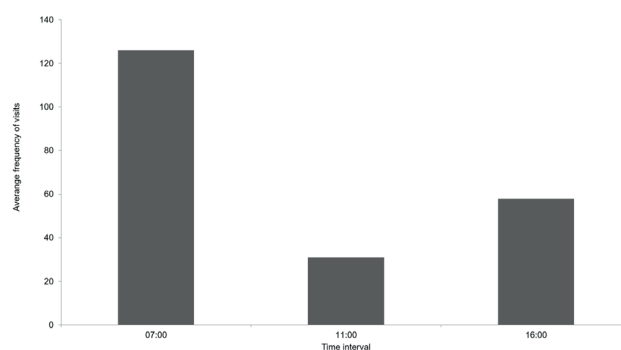
**Figure 1.** (A) Flower and (B) *Apis mellifera* pollinating star fruit flower (*Averrhoa carambola*, Oxalidaceae) in an experimental orchard of NE Brazil.

Because the orchard was pin-monomorphic and self-compatible, it did not fit the conditions necessary to be classified as typically distylous (*i.e.*, two floral morphs and self-incompatibility; Hamilton 1990). Atypical distyly has been recorded in the same family for *Oxalis* species (Arroyo *et al.* 2012; Weller *et al.* 2016; Baena-Díaz *et al.* 2012; Costa *et al.* 2017). Although atypical distyly is related to the collapse of mating systems in natural populations (Weber *et al.*

2013), here it may reflect the selection of genotypes with commercially valuable features.

**Table 2.** Floral morphometrics of *Averrhoa carambola* in an experimental orchard of NE Brazil. SD: standard deviation. Different superscript letters indicate statistically different values (Wilcoxon rank sum test).

Floral trait	Mean $\pm$ SD (mm)
Corolla length	4.4 $\pm$ 1.05
Corolla diameter	9.85 $\pm$ 1.61
Corolla tube diameter	2.42 $\pm$ 0.50
Stigma height	4.67 $\pm$ 0.58a
Anther height	3.16 $\pm$ 0.58b
Stigma-anther separation	1.51 $\pm$ 0.59



**Figure 2.** Frequency of floral visits observed in *Averrhoa carambola* in an experimental orchard of NE Brazil.

Self-compatibility is thought to contribute to reproductive assurance (Cruzan & Barrett 2016), although the herkogamy observed here appears to drastically reduce selfing, as fruit set after SS was very low (4%). In addition to reducing self-fertilization, herkogamy is also thought to reduce interference between male and female functions within the flower and maximize pollen receipt and dispatch – thus enhancing cross pollination rates (Lloyd & Webb 1986; Chen 2012). That assumption was confirmed, as fruit set after NP was 10 times higher than after SS (DDP = 0.9), reinforcing the presumed dependence of star fruit yields on pollinators (Giannini *et al.* 2015a). Therefore, despite being self-compatible, cross pollination has a great influence on fruit production, as fruit set after CP was 13-fold higher than after SS. The PLI calculations revealed that the orchard experiences a low degree of pollen limitation, even though the plants could set more fruits if more pollen was deposited onto the stigmas.

The orchard was pollinated exclusively by *A. mellifera*, which is the most widely used pollinator of commercial crops (Garibaldi *et al.* 2013), and those bees have been recommended to improve star fruit production (Ray 2002). Other studies have recorded bees of the genera *Trigona* as efficient pollinators of star fruits (Phoon 1985; Cabreira *et al.* 2011; Silva-Junior 2016). *Apis mellifera* and *Trigona* are both well-adapted to small, actinomorphic flowers with



short corolla tubes, such as those of *A. carambola*. Those same floral features are commonly observed in distylous species and appear to favor pollen transfer, as they allow easy access to both floral resources and sexual elements (Barrett & Shore 2008). The pollination efficiency *A. mellifera* was high, as a single visit was enough to assure half of the fruit set observed in NP.

Although *A. mellifera* was an efficient pollinator, the PLI results suggest the necessity of pollinator management in order to improve crop yields. As such, we encourage the experimental introduction of native bee species in the place of *A. mellifera*, as the former have been recorded as efficient pollinators of star fruit flowers and play important roles in crop production worldwide (Garibaldi *et al.* 2013). Although *Apis mellifera* was an efficient pollinator of star fruit flowers, that species is known to have negative impacts on several plant-pollinator relationships (Valido *et al.* 2019). Native bee species would improve fruit production by performing cross pollinations, as the orchard is pin-monomorphic, and fruit set after cross-pollination was found to be much higher than by spontaneous self-pollination.

In face of the heterogeneous visitation distribution rate during the day, we recommend the use of pesticides (definitely an unfriendly pollinator practice, IPBES 2016) only when absolutely necessary, and spraying should not occur during periods of high visitation (near 07:00 and 16:00).

Additional studies will be needed to determine if the atypical condition encountered here (*i.e.*, pin-monomorphism associated with self-compatibility) is only a local phenomenon or if it occurs in other orchards, as it is directly related to pollinator dependence. It would be informative to compare crop yields between monomorphic, dimorphic, and isoplethic (*i.e.*, with similar proportion of pin and thrum individuals) orchards, as the latter condition is known to favor fruit set in distylous species (Barrett & Shore 2008). The presence of thrum individuals in the orchard may enhance selfing, as their stamens are longer than their stigmas, and pollen can fall by gravity or easily deposited onto the stigmas by bees when foraging on the flower (Ree 1997). It would therefore also be interesting to determine to what extent the relative abundances of pin and thrum morphs affect crop yields, as, despite the fact that thrum individuals may enhance selfing, they are essential for the isoplethy condition that increases fruit set.

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