Acta Botanica Brasilica 29(2): 278-281. 2015.
doi: 10.1590/0102-33062014abb0006

Short Communication

Fruit set of distylous *Psychotria carthagenensis* Jacq. (Rubiaceae) mediated by *Apis mellifera* (Apidae) and species of *Augochloropsis* (Halictidae)

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Received: January 12, 2015 Accepted: February 22, 2015

ABSTRACT

Heterostyly is a floral polymorphism consisting in the presence of two morphs in the population that differ reciprocally in the position of their sexual organs. Heterostylous species depend on visitors to produce fruits, but the efficiency of insect species as pollinators greatly varies and depends on the morph visited. The aim of the present study was to determine the effect of a single visit by the bees *Apis mellifera* and species of *Augochloropsis* on the fruit set of the distylous species *Psychotria carthagenensis*. After a single visit from each bee species, flowers were bagged to monitor the fruit set. Pollination effectiveness between pollinators and morphs was compared. The results of the experiments were compared with data from manual intermorph cross-pollination using the G test. There were no significant differences in the fruit set between treatments (insect visit and cross-pollination), and between flowers visited by *Augochloropsis* spp. and flowers visited by *A. mellifera*. Our results suggest that pollination effectiveness of the studied bees was not related to floral morph, and that both exotic and native bees showed similar performances on the fruit set of *P. carthagenensis*.

Keywords: Bee pollination, heterostyly, honeybees, pollination effectiveness, reproductive success

Distyly is a floral dimorphism characterized by the presence of two floral morphs that differ reciprocally in the heights of stigmas and anthers in flowers. Distyly is usually accompanied by a heteromorphic incompatibility system and a set of morphological characters that differ between the two morphs, called ancillary characters (Ganders 1979; Barrett 1992; Dulberger 1992). The position of sex organs in flowers pollinated by animals plays an important role in the dispersion of pollen to co-specific stigmas (Thompson *et al.* 2003). Among heterostylous species that lack a heteromorphic incompatibility system, morphological differences between morphs particularly exert a strong influence over breeding patterns between individuals (Barrett *et al.* 2004). In heterostylous species, reciprocal herkogamy is expected to promote intermorph pollination through deposition of pollen from different morphs in separate parts of the pollinators' body (Lloyd & Webb 1992; Ornelas *et al.* 2004; Massinga *et al.* 2005).

The effectiveness of a pollinator is determined by its contribution to the fitness of a plant in terms of both qualitative and quantitative components (Herrera 1987, 1989; Moragues & Traveset 2005). Methods of effectiveness measurement include the analysis of its visiting behavior and frequency, the amount of pollen transported, pollen deposition rate on the stigma of the conspecific flower, the growth success of the pollen tube, the fertilization success of the ovules in the ovary (Gross 2005), and finally, the fruit set, which is the success of fruit formation (Ivey *et al.* 2003). Data on different species of pollinator are important to analyze the degree of specialization of the plant and to infer the role of each visitor on the reproductive success of a given plant species (Stebbins 1970; Fumero-Cabán & Meléndez-Ackerman 2007).

The role of each floral visitor is defined by the result of its interaction with a given plant species (Freitas 2014). The interactions, including those of exotic visitors, can become even more complex, and depending on the situation, consequences could be as follows: (1) negative, if resulting in competition with native pollinators (see Paini 2004), or in failure to deposit pollen on the anthers of native plant species (see Huryn 1997), and if changing the structure of mutualistic networks (e.g., Santos *et al.* 2012; Aizen *et al.* 2014); (2) positive, through effective pollination (see Huryn 1997) and if resulting in the establishment of new effective interactions (Olesen *et al.* 2002); and (3) neutral

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(Paini 2004). Therefore, to better understand these plant–pollinator interactions in a broader view, it is important to investigate them in a more specific manner.

Previous work in the studied populations showed that *Psychotria carthagenensis* Jacq. (Rubiaceae) is self-compatible, has a high degree of reciprocity between morphs, and an equal morph ratio in the populations (Faria *et al.* 2012). According to results of controlled hand pollinations in these populations, fruit set ranged from 5 to 35% in self-pollinations for short-styled (S) flowers and from 20 to 45% in long-styled (L) flowers. For intermorph crosses, it varied between 15% and 20% in S-flowers and 15% and 25% in L-flowers (Faria *et al.* 2012). This sub-forest shrub is two to three meters tall and is distributed from Costa Rica to Argentina (Delpetre *et al.* 2005). In Brazil, these plants are commonly found in areas of moist soil in the proximity of water bodies, both in the Pantanal wetland and savanna regions (Pott & Pott 1994; Felfili *et al.* 2001).

The objective of this study was to evaluate the efficiency of the two more common pollinators of *P. carthagenensis* on the plant's female reproductive success. For this, we allowed one single visit of *Apis mellifera* Linnaeus, 1758 or *Augochloropsis* Cockerell, 1897 spp. to L-flowers and S-flowers in three different populations of *P. carthagenensis* and then compared the fruit set.

The three populations of *P. carthagenensis* analyzed in this study occur in savanna fragments in protected areas located within an urban matrix in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil. These three areas are separated from each other by a mean distance of 12.45 km: *Prosa State Park* (PSP 135 ha, 20°27'00" S, 54°33'46" W), Reserve of the *Universidade Federal de Mato Grosso do Sul* (UFMS 35 ha, 20°29'58" S, 54°36'50" W), and Reserve of the *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA 175 ha, 20°25'41" S, 54°43'03" W). In all three areas, *P. carthagenensis* occurs on moist soils. The climate in the region is tropical savanna (Aw cf. Köppen 1948), characterized by a pronounced dry season from May to September and a rainy season from October to April. Annual accumulated rainfall is approximately 1532 mm and mean annual temperature is 20–22° C (EMBRAPA-CNPGC 1985). This experiment was conducted during the blooming season of 2009 (November and December) when, in the study areas, *P. carthagenensis* has a strong floral display in comparison with other low-stratum species (Faria RR, "personal obs.").

Flowers of both morphs of *P. carthagenensis* open between 0500 h and 0600 h. At this time, the lobes of the corolla are completely separated and perpendicularly positioned in relation to the floral axis, with the reproductive structures exposed. Floral senescence occurs between 1700 h and 1800 h on the same day (see Faria *et al.* 2012 for more details on the floral biology of the species).

Fruit production was calculated after one single visit of different pollinators, including *Apis mellifera* and several species of *Augochloropsis*. *Augochloropsis* spp. is considered herein as a single treatment, but may actually be more than one species, as the identification of this group on the species level through visual observation alone is difficult. However, all individuals of this bee species exhibited the same behavior during all visits. In the three populations, 10 plants of each floral morph were selected, and in each one, we marked three buds for the experiments (totaling 30 flowers). The plants and buds were chosen at random in all populations, according to their availability. Emasculated *P. carthagenensis* buds were isolated with nylon bags in the pre-anthesis phase and marked with a black cotton line. Emasculation of buds is necessary because studied populations are self-compatible and agamospermy was found in L-morphs (Faria *et al.* 2012). The desired number of flowers ($N = 30$) was reached for *Augochloropsis* spp. only at EMBRAPA. For this reason, in the UFMS and PSP populations, the experiment was only conducted with *A. mellifera*. We monitored 20 plants in UFMS, 20 plants in PSP, and 40 plants in EMBRAPA. During early anthesis, when the flowers were open and receptive, the bags were removed to await the visit from the pollinator of interest (*Augochloropsis* spp. or *Apis mellifera*). After the visit and departure of the pollinator, the flower was bagged again for the subsequent determination of fruit production.

Fruit set (number of fruits produced divided per number of flowers treated) after one visit of the two species of pollinators was compared with that obtained for flowers manually cross-pollinated in the same populations. We calculated the average fruit set for each plant. In the xenogamy treatment, the manual deposition of pollen (from the flower of a different morph) was performed on the receptive stigma of virgin, emasculated flowers. After handling, the flowers were bagged again for the subsequent determination of fruit production. Twenty flowers per morph, each from a different individual, were treated, totaling forty replicates for each population (Faria *et al.* 2012). The comparison between the fruit set following the visit from the bees and that obtained with xenogamy was performed on the basis of the premise that xenogamy would represent the maximal pollination limit (Sobrevilla & Arroyo 1982). To test for differences among treatments a G-test with an alpha level of 0.05 was performed (Sokal & Rolf 1995).

When visiting the flowers of *P. carthagenensis*, both *Apis mellifera* and *Augochloropsis* spp. introduce the anterior part of the body into the corolla. The former introduces its head and the latter introduces its head and thorax.

Fruit set from xenogamy treatment at PSP was 20% in both floral morphs; in the UFMS population it was 20% in S-flowers and 15% in L-flowers and at EMBRAPA it was 15% in S-flowers and 25% in L-flowers (Faria *et al.* 2012). After a single visit from *Apis mellifera*, the fruit set ranged from 10 to 33%, and after a single visit from *Augochloropsis* spp., the fruit set was 27% in S-flowers and 30% in L-flowers (Fig. 1).

In both morphs, no significant differences in the fruit set were observed in any of the populations when comparing flowers from the xenogamy treatment and those visited by either *Apis mellifera* or *Augochloropsis* spp. (Tab. 1). Moreover, no significant differences in the fruit set were observed in the comparison between the flowers visited by *Augochloropsis* spp. and flowers visited by *A. mellifera* in the EMBRAPA population (G = 1.71; df = 1; P = 0.191).

On the basis of the results of the present study, a single visit from *Apis mellifera* to *P. carthagenensis* flowers under natural conditions is sufficient in promoting fruit set in both floral morphs. In addition, in populations where both *Apis mellifera* and *Augochloropsis* spp. were analyzed, they achieved similar effects on the pollination of *P. carthagenensis.*

In all experiments, fruit set was never higher than 33%, which agrees with the results obtained in another study in the same areas and probably indicates resource limitation, abortion, or inbreeding depression (Faria *et al.* 2012). *Apis mellifera* was effective in promoting the fruit set in all populations of *P. carthagenensis*. *Augochloropsis* spp. presented similar results in the EMBRAPA population. Because of adaptive features, which lead to differences in the quality and quantity of pollen deposited on the stigmas of native plant species, one may expect differences in pollination effectiveness between exotic and native pollinators (Goulson 2003; Kenta *et al.* 2007; Madjidian *et al.* 2008), which supposedly evolved together with the plant species of a given site. Results presented here should be considered with caution, because fruit set was the only parameter evaluated as a measure of pollination effectiveness. In addition, comparisons of pollination efficiencies between both native and exotic pollinator species were only possible for one of the studied populations. Further studies are needed to clarify these issues by addressing other components of pollination effectiveness, such as the population dynamics of the pollinating species as well as differences in pollinator species on plant male function.

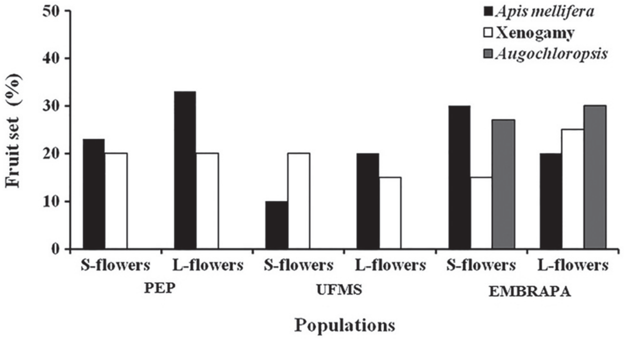

Figure 1. Fruit set (%) after single visits of *Apis mellifera* and *Augochloropsis* spp. to flowers of *Psychotria carthagenensis* and after xenogamy pollination treatment for the studied populations (PSP = Prosa State Park, UFMS = Reserve of the Universidade Federal de Mato Grosso do Sul, EMBRAPA = Reserve of the Empresa Brasileira de Pesquisa Agropecuária) at Campo Grande, MS, Brazil. (S-flowers = short-styled flowers, L-flowers = long-styled flowers).

Apis mellifera is reported to be an important pollinator of a number of native species (Dick 2001; Nadia *et al.* 2007), although some studies have demonstrated that this bee can reduce the reproductive success of the plants it visits (Gross & Mackay 1998; Carmo *et al.* 2004). It is difficult to draw conclusions regarding the impact of this exotic species on communities of native bees, as there are no previous studies on the interaction between native bees and plants prior to the introduction of *Apis mellifera* in Brazil (Wilms *et al.* 1996).

On the basis of the comparison with results from the xenogamy experiment, a single visit from the bees was sufficient to promote fruit set in the studied populations. Since *P. carthagenensis* flowers last only one day, the effectiveness of visitors in one visit is extremely important, ensuring its pollination.

In the population where both *Apis mellifera* and *Augochloropsis* spp. where analyzed, they achieved similar results in the pollination of *P. carthagenensis*. It is therefore possible that the origin of the pollen grains deposited on the stigmas by *Apis mellifera* and *Augochloropsis* spp. is a combination of autogamous, intramorph, and intermorph pollinations.

Our results suggest that pollination effectiveness of the studied bees was not related to floral morph, and that both exotic and native bees showed similar performances on the fruit set of *P. carthagenensis*. However, these findings must be viewed with caution because of the small number of studied populations, and because we have focused on only one measure of pollination effectiveness. Since several components are involved in the performance of different floral visitors in the pollination of a given plant species (Freitas 2014), additional studies investigating the relative

Table 1. Results for the statistical analysis comparing fruit set between treatments (bee species and xenogamy) for the populations of *Psychotria carthagenensis* studied at Campo Grande, MS, Brazil.

		***A. mellifera* vs. Xenogamy**			***Augochloropsis* spp *vs.* Xenogamy**		
Population[1]	**Morph**	**G-value**	**Df**	**P**	**G-value**	**Df**	**P**
PSP	L	1.088	1	0.30	-	-	-
	S	0.078	1	0.78	-	-	-
UFMS	L	0.207	1	0.65	-	-	-
	S	0.975	1	0.32	-	-	-
EMBRAPA	L	0.173	1	0.68	0.150	1	0.70
	S	1.548	1	0.21	0.988	1	0.32

[1] PSP = Prosa State Park, UFMS = Reserve of the Universidade Federal de Mato Grosso do Sul , EMBRAPA = Reserve of the Empresa Brasileira de Pesquisa Agropecuária
Morph: L = long-styled, S = short-styled
Df: degrees of freedom
P: significance value

roles of different visitors, more specifically comparing efficiencies of exotic and native pollen vectors, are necessary for better understanding the reproductive ecology of *P. carthagenensis*.

Acknowledgments

The authors are grateful to V.J. Pott for plant identification; to P. Menezes and V.A. Laura for logistical support in the PSP and EMBRAPA reserves, respectively; to C.C. Castro, M.R. Sigrist, A.P.L. Lemke and two anonymous referees for helpful comments; to L. Navarro for comments on prior drafts of the manuscript; and to the Brazilian fostering agencies FUNDECT/CAPES for the doctoral grant awarded to R.R. Faria (41/100.271/2006) and to FUNDECT for financial support (23/200.288/2008).

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