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A scientific note on new insights in the pollination of guarana (*Paullinia cupana* var. *sorbilis*)

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Guarana (*Paullinia cupana* var. *sorbilis* (Mart.) Ducke, Sapindaceae family) is an important and traditional crop of significant economic and social importance in the Amazon region. It is a Brazilian native plant, cultivated by large- and small-scale producers (Embrapa 2005). Its fruits are dehiscent capsules that contain one or three dark brown seeds, with 2.3 to 5 % caffeine. The seeds need to be processed to obtain the powder, syrup, or extract (Angelo et al. 2008), which is used in food, beverage, and pharmaceutical products worldwide.

The guarana plant is a monoecious species, with male and female flowers opening at different times (dichogamy) on the same plant (see Supplementary Material ESM1). Several studies have been conducted since the 1970s on the pollinators and floral biology of the guarana plant (Schultz and Valois 1974; Gondim 1978; Carranza et al. 1981; Erickson et al. 1984; Escobar et al. 1984; Ferreira 2003). The flowering period of *P. cupana* var. *sorbilis* varies yearly and appears to be related to rainfall patterns (Erickson et al. 1984; Escobar et al. 1984; Escobar et al. 1984). Generally, the flowering period in the Amazon region begins in late July and ends in November when there is less rainfall.

There is a gap in observations on floral visitors between 2 a.m. (at the beginning of anthesis) and 6 a.m., the time when observations began in previous

Corresponding author: C. Krug, cristiane.krug@embrapa.br Manuscript editor: Klaus Hartfelder studies by Schultz and Valois (1974), Gondim (1978), Escobar et al. (1984), and Ferreira (2003). According to these authors, the most effective pollinator species, based on flower observations, are the stingless bees of the genus *Melipona*. This preliminary study aims to answer two fundamental questions regarding the pollination ecology of *P. cupana* var. *sorbilis* as follows: (1) are there nocturnal/crepuscular flower visitors? and, if there are, (2) who are these visitors/pollinators?

To evaluate the occurrence of floral visitors from the beginning of anthesis, which begins at 2 a.m., surveys were conducted between 2 and 9 a.m. for 8 days, totaling 64 h of sampling. All the censuses/surveys were conducted on clear nights during all moon phases, on nonconsecutive days. Preliminary surveys to collect and identify nocturnal and crepuscular species were made in experimental and commercial fields of flowering guarana in the counties of Manaus (21 and 29/08/2011) and Maués (28 and 29/10/2011), respectively. Additional samples, to quantify (census) the nocturnal, crepuscular, and diurnal visitors, were collected in Manaus (04, 12, 19, and 26/09/2012). Sunrise occurred from 5:37 to 6:07 a.m. during the study.

Surveys were made every 30 min and each lasted 10 min. During each survey, the researcher walked slowly along the rows of guarana plants, counting the number of each type of bee (nocturnal, crepuscular, and diurnal) on the flowers in an area of 1 ha, which was surrounded by native vegetation. Due to easy recognition, the bee types were only counted as nocturnal (*Megalopta* spp.), crepuscular (*Ptiloglossa* sp.), or diurnal (all the other bees). Sampling was done using entomological nets. The flower visitors were observed and voucher specimens of the insects/bees were collected (authorization number 32357-1 MMA/ICMBio) and

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deposited in the Invertebrate Collection of the National Institute for Amazonian Research (INPA). The specimens were identified using the available literature and with the assistance of bee taxonomists.

At Manaus and Maués in 2011, a large number of nocturnal and crepuscular bees, belonging to the genera *Megalopta* spp. (Halictinae) and *Ptiloglossa* sp. (Colletinae), were observed visiting guarana flowers (see Supplementary Material ESM2 and ESM3). Bats did not visit the flowers during anthesis but some moths were seen resting on flowers and the vegetative parts of the plant, but did not collect nectar.

At Manaus, in 2012, bees were recorded as nocturnal, crepuscular, and diurnal visitors. Of the 1,333 bees recorded, 4 % were nocturnal (observed between 3 and 5 a.m.), 8 % were crepuscular (4 to 6 a.m.), and 88 % were diurnal visitors (after 5.30 a.m.). The temperature was almost constant until six in the morning, which is when diurnal bees begin to actively fly; between five and six, the three types of bees were active: nocturnal (Megalopta spp.), crepuscular (Ptiloglossa sp.), and diurnal (Figure 1). The nocturnal bee visitors comprised four species: Megalopta aeneicollis Friese, 1926, Megalopta amoena (Spinola, 1853), Megalopta sodalis (Vachal, 1904), and Megalopta sp. The crepuscular visitors consisted of only one bee species, Ptiloglossa aff. lucernarum, whereas there were more than 20 species of diurnal bees, almost all of them stingless bees. In the phases of the full moon and the waning moon, the nocturnal and crepuscular bees were active an hour earlier, whereas diurnal bees did not change the starting time of their activities.

Megalopta and Ptiloglossa were observed visiting guarana flowers from 3.30 to 5.30 a.m. and from 04.30 to 6.30 a.m., respectively. Both species actively collected nectar, but Megalopta showed a different behavior, moving slowly than Ptiloglossa in the flowers. Due to the behavior of the bees, pollen collection was not observed but this possibility was not discarded. Mating behavior was noted in P. aff. lucernarum during its activity on guarana flowers. Nests of these bees were not found inside or near the guarana fields and almost certainly these bees were nesting in the forest. Despite indications that both nocturnal and crepuscular bees can pollinate P. cupana, additional experiments are needed to clarify the role of these bees as effective pollinators.

Megalopta bees are strictly time constrained in their foraging behavior, flying only during two short periods, before sunrise and after sunset, possibly to explore rewarding pollen sources without competition and to minimize predation and nest parasites (Wcislo et al. 2004; Kelber et al. 2005). According to Smith et al. (2012), *Megalopta* bees in the tropical forest of Panama collected pollen from at least 64 plant species. Up to now, *Megalopta* and *Ptiloglossa* bees have not been mentioned as potential crop pollinators.

Garibaldi et al. (2013) described increased fruit set with flower-visitor richness, especially by wild insects,



Figure 1. Number of bees visiting guarana (*Paullinia cupana* var. *sorbilis*) flowers per hour, mean temperature per hour during the survey days.

Bees visiting guarana flowers

from the results for multiple crop systems. This was also reported by Yamamoto et al. (2012) for passion fruit. The relationship of nocturnal and crepuscular bees with guarana flowers may be closer than simple flowervisitor richness, but this demonstrates the use of floral resource in different niches, where competition is low or absent.

More research is needed to clarify the pollination biology of the guarana plant, including identification of the effective pollinators and the contribution of bees to fruit set, specially nocturnal and crepuscular species. The guarana crop is a potential alternative for increasing the income of family agriculture and can be viewed as an opportunity to develop the Amazon region by associating the ecosystem service of pollination provided by the bee diversity and the forest with good farming practices.

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Note scientifique: nouvelles perspectives dans la pollinisation du guarana (*Paullinia cupana* var. sorbilis)

Eine wissenschaftliche Notiz über neue Erkenntnisse bei der Bestäubung von Guarana (*Paullinia cupana* var. *sorbilis*)

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