

Visitation of bees to flowers of yellow passion fruit in Northern Paraná State, Brazil.

Humberto Godoy Androcioli^{1,*}, Ayres de Oliveira Menezes Junior², Ana Odete Santos Vieira², Dayanne Fabrício Bressan², Adriano Thibes Hoshino¹ and Camilla de Andrade Pacheco²

¹Instituto Agronômico do Paraná, Área de Proteção de Plantas, Rodovia Celso Garcia Cid, Km 375, CP 481, CEP 86047-902, Londrina, Paraná, Brazil. ²Universidade Estadual de Londrina, Centro de Ciências Agrárias e Centro de Ciências Biológicas, Departamento de Agronomia e Departamento de Biologia Animal e Vegetal, Rodovia Celso Garcia Cid, Pr 445 Km 380, CP 6001, CEP 86051-990, Londrina, Paraná, Brazil. *Corresponding author, E-mail: handrocioli@iapar.br

ABSTRACT

The yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) needs pollinators for fructification due to the existence of mechanisms that impede self-pollinating. Pollinators of great efficiency for the yellow passion fruit in Brazil are the bumblebee species (*Xylocopa frontalis, X. grisescens* and *X. Suspecta*). Deforestation by agricultural expansion in the region has a negative impact on these pollinators' populations, leaving space for the action of cleptobiotic species with further damage to fruit production. The objective of this study was to evaluate the frequency of pollinating bees and floral visitors during yellow passion fruit culture's post anthesis period in Northern Paraná and its fructification effects. Three passion fruit plantations in Northern Paraná were evaluated in the counties of Londrina and Assaí. Transect census were realized in each area, delimiting two 50 meter-paths, where the presence of bee in the open flowers was registered. Transects were run through for five minutes, every 15 minutes, starting at 1 pm and finishing at 8 pm. Results showed that the bumblebees present in the Northern Paraná region were the *Xylocopa frontalis* and *X. suspecta*, with the predominance of the first. Highest fructification rates occurred in the areas with more frequency of bumblebees and low frequency of cleptobiotic bees. Lowest fructification rates were due to the absence of bumblebees or high frequency of cleptobiotic bees.

Key words: Passiflora edulis Sims f. flavicarpa Deg., pollination, Xylocopa spp.

INTRODUCTION

Brazil is the largest producer of passion fruit in the world, with approximately 57 thousand ha of planted area, with 838 tons produced and a yield of 15 t/ha (Instituto Brasileiro de Geográfia e Estatística 2013). In the country, fruit production stands out in the Northeast, Southeast and Northern regions, with great growth potential in the South, more precisely in the State of Paraná. Passion fruit production in Paraná is based on the *Passiflora alata* Curtis (wingstem passion fruit) *and P. edulis* Sims. f. *flavicarpa* Deg. (purple granadilla) species, with a planted area of approximately 840 ha, 11 thousand tons produced and a yield of 13 t/ha (Instituto Brasileiro de Geográfia e Estatística 2013; Meletti 2011). Passion fruit culture is considered an important agricultural diversification option for small growers in the state of Paraná (Mazur et al., 2013).

The yellow passion fruit needs cross pollination for fructification due to the presence of mechanisms that impede self-pollination, a mandatory cross-pollination. The flower from the plant rejects its own pollen and therefore must exchange pollen with different plants for flower fecundation to occur, resulting in fruits. Passion fruit trees flowers pollination may be natural, realized by bumblebees or artificially, done manually (Meletti and Pacheco 2008).

Passion fruit tree fecundation is strongly influenced by pollination carried out by pollinating agents or manually; thus, it is extremely important to know its flower blooming periods, which normally last less than eight hours (Ataide et al., 2012; Bruckner et al., 1995).

Some bees collect the pollen present in passion fruit flowers and the nectar constitutes the most sought out floral resource for effective pollinators used as food and provisions for their nests as well as food for larvae. Despite the great diversity of visitors, only bees with body weight over 15 mm can act as effective pollinators of yellow passion fruit flowers (Varassim and Silva 1998; Martins et al., 2014), i.e., those from the *Xylocopa* (bumblebees) genus that nests in dead, dry or slightly rotten wood (Carvalho and Teófilo Sobrinho 1973).

The economic importance of bumblebees resides in the fact that they are the only efficient pollinators for most passion fruit species, especially the yellow passion fruit, being of special relevance in Brazil the *Xylocopa frontalis* (Oliver in 1789), *X. grisescens Lepeleiter*, 1841 and *X. suspecta* (Hymenoptera: Apidae) (Camargo and Moure 1988). Due to its morphological and behavioral characteristics, the *Xylocopa* spp. can increase passion fruit plantations up to 25% in number of fruit, when they are in adequate number in the orchard (Santos and Costa Neto 2012; Bruckner et al., 2000).

A series of factors such as the reduction of native forest area close to the orchards and the use of agrochemicals have reduced the number of bumblebees in passion fruit orchards and, consequently, a reduction in culture productivity. Another aggravating problem is the presence of bees from the *Apis* and *Trigona* genres, which, by being small, they touch the reproductive parts of the flower during their visits without transferring the pollen between different flowers stamens and the stigmas, making them harmful to orchards, since they compete with pollinators for resources

(Faleiro et al., 2011; Leone 1990). Consequently, to make the culture economically viable, manual pollination is being used around the country, increasing production costs considerably and spending with labor 15% of this cost (Cobra et al., 2015; Meletti and Pacheco 2008; Junqueira et al., 2006; Manica 1981).

The objective of the present study was to assess the frequency of pollinating bees and flower visitors during the yellow passion fruit post anthesis culture period in the Northern Paraná region and their effects on fructification rate.

MATERIAL AND METHODS

The study was carried out in three passion fruit plantations in Northern Paraná, Brazil, being two in the county of Londrina, one at the IAPAR – Agronomic Institute of Paraná experimental area and the other in the district of São Luiz, and one in the county of Assaí. Plantations were assessed four times in each property between the months of January and May of 2005. The Londrina/IAPAR in Feb 19/05, April 01/05, April 14/05 and April 15/05; the Londrina/ São Luís District in Jan 26/05, Feb 12/05, Feb 17/05 and Feb 24/05 and; the Assaí in Jan 02/05, Feb 10/05, Feb 15/05 and Feb 22/05. The IAPAR and Assaí orchards were in their second production year while the São Luiz was in its third production year.

Bees observation was carried out by transect census with two 50-meter paths each, corresponding to the two sides of the planting lines, under the trellis system, in the passion fruit orchard. Path walks started at 1 pm and were done for 5 minutes with intervals of 15 minutes until 8 pm, totaling 21 paths/day. Before going down each path, climate conditions, temperature and air relative humidity were registered. At the end of each observation day, all flowers were bagged in netted fabric (2 x 2 mm thin mesh) to assess fructification percentage.

Some samples of the bees species observed were captured and sent to Doctor Gabriel Mello from Paraná Federal University Department of Entomology for identification. *Xylocopa frontalis* and *X. suspecta* bees were grouped as bumblebees, and *Trigona spineps* and *T. hialinata* as *Trigona* in the figures and tables presented. Bees visitation frequency was calculated by the bees mean by transect (100 meters in 10 minutes) and multiplied by three, therefore adjusting the frequency to the half-hour period, with posterior division by the average number of open flowers in the same period.

Data for time, number of open flowers at each half-hour, total number of flowers open in each period and number of visitation by bumble bees (*Apis mellifera* and *Trigona*) were analyzed by Spearman correlation method with Prob $|\mathbf{r}|$ under H0: Rho=0, using the SAS System statistical package (SAS Institute 1999).

RESULTS AND DISCUSSION

The period of the day in which the yellow passion fruit (*Passiflora edulis f. flavicarpa* Deg.) flowers started to open varied according to the location. Under the IAPAR orchard conditions, flower opening/blooming period was from 1 pm to 6 pm, while in the other two locations, São Luiz and Assaí, they began to open around 40 minutes after the flower openings at the IAPAR orchard. Closing of the anthesis occurred at 6 pm. Flowering/blooming duration in the three locations was greater than the 12 to 4:30 pm hours observed by Benevides et al. (2009) in their studies with sour passion fruit in the state of Rio de Janeiro. However, they were closer to the 1 pm to 6 pm period reported by Montero et al. (2013) when analyzing the anthesis of the sour passion fruit in the state of São Paulo. Flowers anthesis peak showed little variability and clear concentration in the period from 2 pm to 2:20 pm at the IAPAR orchard in Londrina (Figure 1).

In the other locations, however, flowers peak was late and lasted for longer periods, especially in Assaí, with the predominance of the 3 to 3:45 pm hours (Figure 1), possibly due to the locations' edaphoclimatic conditions. Cobra et al. (2015) report on a flowering peak at 3:30 pm, when eight cultivars analyzed showed 100% of open flowers, a result similar to that found in this study with trials conducted in the district of São Luiz and Assaí.

Flowers with early anthesis and late senescence (IAPAR, District of São Luiz e Assaí) were most favorable since they gave more time for the grower to realize manual pollination and to make the passion fruit available to natural pollinators for a longer period. Corroborating with the results found by Cobra et al. (2015), Harder and Johnson (2005) reported on flower longevity as an influencing factor on the number of the pollinators visitations to the orchard, the quantity and quality of the pollen received and disseminated as well as on the possibility of greater fruit production.

Xylocopa frontalis and *Xylocopa suspecta* bees, also known as bumblebees, were considered yellow passion fruit pollinators in the orchards studied, being the *X. frontalis* species predominant and responsible for around 80% of bumblebees' visitations (Figure 2).

Higher bumblebee visitation frequency (above 0.4 visits/30 min.) occurred at the IAPAR orchard in Londrina from 1 to 1:30 pm, reducing gradually by sunset time (Figure 3). Peak time was anticipated a little, 2 and 3 pm in relation to that reported by Souza et al. (2002) who observed greater visitation of *X. frontalis* at 3 pm and within the time described by Cobra et al. (2015), between 1 to 3:30 pm.



Figure 1. Average number (n=4) of flowers in anthesis during the afternoon period at three localities in Northern Paraná: IAPAR-Londrina (A), Assaí (B) and São Luiz-Londrina (C).



Figure 2. Bumblebees (Xylocopa frontalis) pollinating passionflower flowers in Londrina, PR.

The highest fructification rates (57%) (Table 1) and the only significant (positive) correlation between the number of open flowers and bumblebees visitations (Tables 2, 3 and 4) were found at the IAPAR orchard in Londrina/PR. This can be explained by the fact that the IAPAR area has a permanent preservation forest next to the orchard, which, probably contributed to the maintenance of a larger bumblebee population through diversity of alternative foods, shelters and locations for insects nesting. This is in agreement with the observations made by Silva et al. (2013) who reported that several factors such as local temperature, nectar production and the amount of flowers available can have a positive influence on the presence of bees in the culture of passion fruit. It is also in agreement with studies conducted by Yamamoto et al. (2010) who assessed large bees from *Xylocopa* and *Bombus* genus, classifying them as the most effective pollinators of the sour passion fruit.

At the São Luís orchard, bumblebees visitation frequency was higher (0.6 bumblebee/ 30 min.) from 1:30 to 2 pm, when only a small number of flowers were open (Table 4). However, from 2:30 to 3 pm, time of greater flowers anthesis (Figure 1), visitation frequency was of only 0.2 bumblebees/30 min, resulting in low fruit ripening (Table 1).

Location	Amount Flowers	Amount Fruit	Fructification (%)	T° Max	T° Min.	UR Max	UR Min.	
Assaí	187	9	5	34.07	26.12	53.22	36.92	
São Luís	358	31	12	35.63	27.95	53.35	39.86	
Londrina	273	157	57	34.61	29.63	54.97	36.57	

Table 1. Total number of flowers and fruit; fructification percentage; maximum, minimum, temperature (T°) and relative humidity (UR) mean for the four assessed in the three orchards in Northern Paraná.

Table 2. Correlation between the parameters evaluated during four assessments realized at the county of Assaí, analyzed by the Spearman Correlation method with Prob > |r| under H0: Rho=0.

			Amount			
		Amount	Flower	Xylocopa	Apis	Trigona
(n=84)	Time	Flower	opened	spp.	melifera	spp.
N		249	249	10	88	113
Time		-0.34336	0.7787	0.09551	0.09184	0.08160
Amount Flower	0.0014		(-)	0.12436	0.27894	0.36262
Amount Flower opened	<.0001	(-)		0.15519	0.09074	0.11463
<i>Xylocopa</i> spp.	0.3875	0.2597	0.1586		0.0939	0.21453
Apis melifera	0.4060	0.0102	0.4117	0.3955		0.39907
<i>Trigona</i> spp.	0.4606	0.0007	0.2991	0.0500	0.0002	

Table 3. Correlation between parameters evaluated during four assessments realized in the county of Londrina (IAPAR), analyzed by the Spearmen Correlation method with Prob > $|\mathbf{r}|$ under H0: Rho=0.

			Amount			
		Amount	Flower	Xylocopa	Apis	Trigona
(n=71)	Time	Flower	opened	spp.	melifera	spp.
N		281	281	70	35	106
Time		-0.48089	0.5032	-0.09928	-0.02035	-0.29987
Amount Flower	<.0001		(-)	0.57092	0.22478	0.50058
Amount Flower opened	<.0001	(-)		0.04988	0.02657	0.19052
<i>Xylocopa</i> spp.	0.4101	<.0001	0.6796		0.44262	0.28306
Apis melifera	0.8663	0.0595	0.8259	0.0001		0.20718
Trigona spp.	0.0111	<.0001	0.1115	0.0168	0.0830	

Table 4. Correlation between parameters evaluated in four visitations at the São Luiz district (Londrina – PR), analyzed by the Spearman correlation method with Prob > $|\mathbf{r}|$ under H0: Rho=0.

			Amount			
		Amount	Flower	Xylocopa	Apis	Trigona
(n=84)	Time	Flower	opened	spp.	melifera	spp.
N		358	358	20	575	101
Time		-0.38420	0.7385	0.08226	-0.06488	-0.09428
Amount Flower	0.0003		(-)	0.08353	0.71083	0.51255
Amount Flower opened	<.0001	(-)		0.13146	0.39989	0.14218
<i>Xylocopa</i> spp.	0.4569	0.4500	0.2333		0.13370	0.02070
Apis melifera	0.5576	<.0001	0.0002	0.2253		0.53906
Trigona spp.	0.3936	<.0001	0.197	0.8518	<.0001	

While in Assai (Table 2), bumblebees frequency was very low, being inefficient as pollinators, resulting in a 5% fructification rate (Table 1). This may be the result of insufficient bumblebee populations to supply pollination needs

due to the phytosanitary management previously adopted by the owner such as the application of insecticides during the bumblebee flowers visitation period. Aizen et al. (2008) reported that agricultural practices with low anthropic action favor diversity and pollinators abundance.

Apis mellifera, Trigona spineps and Trigona hialinata bees were present in the three locations In the Assaí and São Luiz orchards the frequency of these bees was superior to that of bumblebees. However, at the IAPAR orchard, the frequency of these bees was inferior to that of bumblebees (Figure 3). Apis mellifera and Trigona, bees, sometimes occurring in great number, were used in the experiment only as nectar and pollen cleptobiotic bees, sometimes competing for resources with bumblebees at the flowers anthesis peak times. This can also explain the low fructification rate at the São Luiz location. Siqueira et al. (2009) related the presence of *A. mellifera* to the reduction in pollen availability and large bees' activity, thus compromising orchard fructification.

Correlation analysis points out that, in general, there was positive correlation between the visitation of cleptobiotic bees and bumblebees with the number of open flowers (Table 5), which is explained by the greater availability of resources for these insects. In addition, many authors have reported on the harmful actions of cleptobiotic bees on yellow passion fruit. The A. mellifera bee can remove all the pollen from the semi-open flower buds anthers before the arrival of the bumblebees without, however, realizing the pollination. (Yamamoto et al. 2012; Faria and Stehmann 2010; Gross and Mackay 1998; Carvalho and Teófilo Sobrinho 1973). The *Trigona* bee may pierce the flowers' nectar chamber to remove the nectar, making them less attractive to the *Xylocopa*, reducing visitation time and frequency of this pollinator. Agonistic interactions were also observed between these bees, in which *Xylocopa* spp. started to avoid flowers in which the Trigona was present thus reducing the number of visitations to passion fruit flowers (Cobra et al. 2015; Sázima and Sázima 1989).

Table 5. Correlation between evaluated parameters considering data from all visitations, in all properties, analyzed by the Spearman Correlation method with Prob > |r| under H0: Rho=0.

(n=84)	Time	Amount Flower	Amount Flower opened	<i>Xylocopa</i> spp.	Apis melifera	<i>Trigona</i> spp.	
N		888	888	100	698	320	
Time		-0.38683	0.6473	-0.00784	0.07808	-0.08865	
Amount Flower	<.0001		(-)	0.26490	0.41502	0.44634	
Amount Flower opened	<.0001	(-)		0.14590	0.27594	0.19505	
<i>Xylocopa</i> spp.	0.9040	<.0001	0.0241		0.11648	0.18693	
Apis melifera	0.2291	<.0001	<.0001	0.0723		0.32444	
<i>Trigona</i> spp.	0.1719	<.0001	0.0025	0.0037	<.0001		

CONCLUSIONS

The most frequently found bees in the Northern Paraná region were the *Xylocopa frontalis* and X. *suspecta*, with the predominance of the *Xylocopa frontalis* bumblebee.

The highest fructification rate occurred in areas with great bumblebee frequency and low frequency of cleptobiotic bees.

Lowest fructification rates occurred due to lack of bumblebees or high frequency of cleptobiotic bees.

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