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Fruit flies and parasitoids associated with guava in Barbalha, Ceará, Brazil

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ABSTRACT: Guava's fruits are usually infested by fruit flies. There is little information regarding the native parasitoids associated with fruit flies, which may vary according to the local conditions. A study was made from September, 2013, to August, 2014, in a family orchard, in Barbalha. The fruit flies and their parasitoids were correlated to climatic variables besides their infestation, frequency, and parasitism rates. The fruits were collected weekly and taken to a laboratory, where they were counted, weighted, and stored on trays with vermiculite, and kept under non-controlled conditions, and covered with organza until pupation. The pupae were collected between seven to ten days later and kept until their emergence as adults. The variables fruit infestation, parasitism, and frequency rates were calculated, and in April, we registered the greatest values of fruit flies/parasitoids abundance and diversity. *Anastrepha sororcula* and *Anastrepha zenildae* were the most abundant and their occurrence and parasitism was greater during April due to favorable climatic conditions. The fruit fly infestation was more common during the rainy season, from January to June, and the parasitism occurred with more intensity in April, and the parasitoid *Doryctobracon areolatus* was the most frequent.

Key words: Braconidae; Myrtaceae; natural biological control; Tephritidae

Moscas-das-frutas e parasitoides associados a goiaba em Barbalha, Ceará, Brasil

RESUMO: A goiaba é infestada por moscas-das-frutas com pouca informação sobre parasitoides nativos associados que podem variar em função das condições locais. Um estudo foi realizado no período de setembro de 2013 a agosto de 2014, em pomar familiar de Barbalha. Moscas-das-frutas e seus parasitoides foram correlacionados com variáveis climáticas, além do índice de infestação, frequência e taxas de parasitismo. Os frutos foram coletados semanalmente e levados ao laboratório, onde foram contados, pesados e armazenados em bandejas contendo vermiculita e mantidos em condições não controladas, cobertas com organza até a pupação. As pupas foram coletadas após sete a dez dias e mantidas até a emergência do adulto e as taxas de infestação de frutos, parasitismo e as frequências dos parasitoides foram calculados e em abril foram observados os maiores valores de abundância e diversidade de moscas/parasitoides. *Anastrepha sororcula* e *Anastrepha zenildae* são as mais abundantes e o parasitismo observado aumentou neste mês devido a condições climáticas favoráveis. A infestação foi mais comum na estação chuvosa de janeiro a junho e o parasitismo ocorreu com maior intensidade em abril e *Doryctobracon areolatus* foi o mais frequente.

Palavras-chave: Braconidae; Myrtaceae; controle biológico natural; Tephritidae

Introduction

The northeastern Brazil's semiarid climate offers favorable weather conditions for fruit production with proper irrigation techniques. Several counties in the region of Cariri, such as Crato, Barbalha, Santana do Cariri, and Missão Velha, located on the southern state of Ceará, where the semiarid climate prevails, have experienced expansion of guava orchards due to favorable climatic conditions and irrigation techniques (Azevedo et al., 2010).

The occurrence of fruit flies (Diptera: Tephritidae) is a limiting factor for guava (*Psidium guajava* L.) production because these tephritids' larvae consume the fruits' pulp, making them unfit for *in natura* consumption and for industrial fruit processing. In the region of Cariri, fruit flies are economically worrisome because they cause serious losses on *in nature* fruits, resulting in and increasing losses of commercial plantations, and increasing the production costs due to the application of chemical insecticides (Azevedo et al., 2010).

The main control of tephritids in orchards is made through the application of synthetic insecticides. But biological control has been playing a prominent role in integrated management tactics employed for these pests (Sarwar, 2015). Information on the population dynamics of fruit flies and their relation to biotic and abiotic factors must be gathered and properly interpreted, enabling the effectiveness of each region's control programs for regional control (Aluja et al., 2012). For instance, in the semiarid region of Ceará, braconids of the genera *Doryctobracon* (Szépligeti, 1911), *Utetes* (Viereck, 1913), and *Opius* (Lima, 1938) are common natural fruit fly parasitoids (Jahnke et al., 2014; Araujo et al., 2015).

Therefore, being aware of aspects related to these beneficial species' ecology in the guava agroecosystem as potential biocontrol fruit fly agents is of paramount importance to adopt measures in an integrated pest management program that fosters these agents' conservation and number increase in the region of interest. The improvement of the pest's natural mortality would result in a subsequent reduction of chemical insecticide needs, minimizing the environmental impact and health risks for guava producers and consumers.

With these considerations, the main purposes hereof were: 1) to correlate climatic factors (specify rainfall, temperature, and relative humidity) with the incidence of fruit flies and their parasitoids under the conditions of the region Cariri, Ceará; 2) to estimate the fruit fly species infestation rates in guavas, and 3) to determine the natural parasitism rates and the frequency of native parasitoids.

Material and Methods

The fruits were collected in a 10-year-old commercial guava orchard, of the Paluma variety, located in the city of Barbalha (07º 17 '18 "S 39º 20 '57 "W, at 459.2 m high), region of Cariri, State of Ceará, Brazil. The study encompassed the fruit development and maturation stages. The plants were cultivated on a 6 x 5 m orchard, totalizing nearly 0.5 ha of effective area. The area was maintained through family labor, in which all routine cultural practices were adopted, such as fertilization, weeding, and irrigation. However, defense chemical insecticides were not applied to control pests and guava diseases.

The guava sampling was performed every week, randomly, and according to the fruit development period, from September, 2013, to August, 2014, collecting every fruit of the trees, totalizing 167 trees in the whole orchard. Mature fruits were collected from the trees and from ground, if they presented a good condition for fly larva development. The sample size varied depending on the availability of fruits on the collection day. The collected samples were properly labeled (fruit, date, and place) and taken to the Agricultural Entomology Laboratory of the Federal University of Cariri (UFCA), Crato (CE).

At the laboratory, the fruits were counted and deposited on a 5-centimeter layer of vermiculite, moistened with distilled water, and which was kept wet during the entire observation period with plastic trays (42 x 27 cm). The trays were covered with white organza fabric, fastened with elastic bands. Then, the trays were placed on steel shelves in a room under controlled conditions (temperature ±26ºC, relative humidity ±60%, and 12-hour photophase).

After a period of seven to ten days, the vermiculite was sieved to obtain the puparia. As for the fruits, after opened, they were replaced in vermiculite if they still had larvae. The puparia were counted and placed on Petri dishes with 10 cm of diameter and coated with moistened filter paper, where they remained until the emergence of the adults (fruit flies and/or parasitoids). The emerged adults were stored in plastic containers with 70% alcohol diluted in water, where they remained until the species identification process.

The identification of fruit flies and parasitoids was carried out at the Laboratory of Applied Entomology of the Federal Rural University of the Semi-Arid (UFERSA), in Mossoró (RN), by an expert, according to the taxonomic keys elaborated by Zucchi (2000) and Canal & Zucchi (2000), respectively. The fruit flies were identified by their wing banding pattern, thoracic pattern, and mainly by their female aculeous morphometry. The parasitoids were identified by the characteristics of their mouth parts (mandible and clipeo), wings, and propodeum base.

The rates of fruit fly infestation (I), natural parasitism (P), frequency of parasitoid species individuals (F), and pupal viability (V) were calculated through the following formulas:

$$I = \frac{\text{Number of pupae obtained}}{\text{Number of collected fruits}}$$

$$P = \frac{\text{Number of emerged parasitoids}}{\text{Emerged flies + number of emerged parasitoids}} \times 100$$

$$F = \frac{\text{Number of individuals of a given species}}{\text{Total number of emerged parasitoids}} \times 100$$

$$V = \frac{\text{Number of emerged flies + parasitoids}}{\text{Total pupae}} \times 100$$

Results and Discussion

During the study period, 1184 fruit fly pupae were collected from 667 fruits gathered on the orchard located in the city of Barbalha (CE). Five fruit fly species were obtained: *Anastrepha zenildae* (Zucchi, 1979) (62.7%), *Anastrepha sororcula* (Zucchi, 1979) (27.3%), *Anastrepha obliqua* (Macquart, 1933) (19%), *Anastrepha antunesi* (Lima, 1938) (0.6%), and *Ceratitis capitata* (Wiedemann, 1824) (26.2%). These data show that *Anastrepha zenildae* was highly; in opposition the specie *Anastrepha antunesi*. According to Araujo & Zucchi (2003), *Anastrepha zenildae* is the fly species that initiates the infestation on the orchard and is adapted to guavas cultivated in semi-arid regions. Similar results were found by Querino et al. (2014) in state of Minas Gerais.

During the driest months, the surrounding vegetation remains completely dry and, therefore, is unproper for the development of fruit flies. Consequently, the flies collected in this study were virtually guava-restricted, and, among other factors, must have been influenced by the availability of guavas in the orchard. However, Dutra et al. (2009) noticed a low occurrence of *Anastrepha zenildae* (1.8%) in an organic guava orchard in Una, using McPhail traps, southern Bahia. This result suggests that this species' adults were not from the guava orchard, but they came from other host plants near the orchard and/or surrounding forest area, probably of totally different conditions from the orchard where the present research was carried out.

In our study in Barbalha, *Anastrepha zenildae* was the most frequent species, but it did not come from other host plants near the orchard and/or surrounding forest area. Differently from the data obtained by Jesus-Barros et al. (2012), whose flies came from native fruits collected subspontaneous or naturalized and exotic plant species in urban and rural areas. In the same study, the authors concluded that *Anastrepha zenildae*, *Anastrepha sororcula*, and *Anastrepha obliqua* are dominant and constant species in the studied region.

April was the month in which the greatest fruit fly abundance and diversity occurred, and the most abundant and frequent fruit fly species throughout the study year were *Anastrepha zenildae*, *Anastrepha oblique*, and *Ceratitis capitata* (Table 1). Similar results were found by Araujo et al. (2008), who noticed, with the assistance of McPhail traps, that the fruit fly population increased from April onwards in a guava orchard in Russas (CE), with a population peak in May, from which *Anastrepha zenildae* was the predominant species. Already Marsaro Jr. et al. (2013), in Boa Vista, State of Roraima, noticed that besides these three species found in the present research, in their study, the most frequent and dominant species in the guava orchards were *Anastrepha striata* (Schiner, 1868), *Anastrepha sororcula*, and *Anastrepha fraterculus* (Wiedemann, 1830), with the greatest peak in the month of June.

These species are also frequently found infesting guavas in other orchards located in the Brazilian semi-arid region (Alvarenga et al., 2009). Additionally, Moura & Moura (2006) reported that *Ceratitis capitata* infests guavas with great intensity in the state of Ceará. *Anastrepha obligua* and *Anastrepha antunesi* were the least abundant and frequent in the evaluated guava orchard, possibly because guava was not the preferred host fruits thereof. The month of October, 2013, was the only one that did not present any fruit fly or parasitoid emergence (Table 1).

Besides fruit flies, four parasitoids species were found: *Doryctobracon areolatus* (Szépligeti, 1911) (75%), *Asobara anastrephae* (Muesebeck, 1958) (18.4%), *Utetes anastrephae* (Viereck, 1913) (5.3%), and *Opius bellus* (Gahan, 1930) (0.6%), all belonging to the Family Braconidae. Similar to results found by Jesus-Barros et al. (2012). The month of greatest abundance and richness of these parasitoids was also in

Table 1. Monthly amount of fruit fly and parasitoids species in guavas during the period of September, 2013, to August, 2014, in the city of Barbalha (CE).

	Months												Total
	Sep	Oct	Nov	Dec	Jan	Fev	Mar	Apr	May	Jun	Jul	Aug	
Fruit flies													
Ceratitis capitata	4	-	6	4	7	5	3	11	1	-	3	1	45
Anastrepha obliqua	-	-	-	-	4	13	-	12	-	-	3	-	32
Anastrepha sororcula	4	-	5	11	13	-	-	2	9	2	1	-	47
Anastrepha zenildae	4	-	-	-	4	2	2	11	23	1	-	-	47
Anastrepha antunesi	-	-	-	-	-	-	-	1	-	-	-	-	1
Total	12	0	11	15	28	20	5	37	33	3	7	1	
Parasitoids													
Doryctobracon areolatus	-	-	1	-	1	13	-	40	2	-	-	-	57
Opius bellus	-	-	-	-	-	-	-	1	-	-	-	-	1
Asobara anastrephae	-	-	-	-	-	-	-	13	1	-	-	-	14
Utetes anastrephae	-	-	-	-	-	-	-	4	-	-	-	-	4
Total	0	0	1	0	1	13	0	58	3	0	0	0	-

April, possibly due to a greater abundance and diversity of fruit flies then (Table 1). The species *Doryctobracon areolatus*, *Opius bellus*, and *Utetes anastrephae* are also common in other Brazilian regions, parasitizing guava fruit fly larvae (Araujo et al., 2015).

These species were already recorded in the region of Cariri in guavas (Azevedo et al., 2013). Furthermore, *Doryctobracon areolatus* is the species of widest geographic distribution in Brazil (Taira et al., 2013).

The fruits were collected in 2013 and 2014, with fruit collection peaks in the months of March and April, 2014, months in which, consequently, were registered the greatest amounts of pupae (Figure 1). In the semi-arid regions, rainfalls, in combination with host availability, are the predominant factors that influence the population dynamics of fruit flies and their parasitoids. In the guava orchard in Barbalha, the greatest rainfall volume and guava fruit development were registered precisely in the months of greatest population peaks. Veloso et al. (2012) reported that the occurrence of fruit flies commonly coincides with the fruit development periods of host plants. However, studies performed by Araujo & Zucchi (2003) in a semi-arid guava orchard proved that the orchard fruit availability is essential to increase the fruit fly population. Despite that, other factors may interfere with this pest's infestation level in orchards, such as planted varieties and their proximity to other orchards.

The climatic data show that the temperature generally remained constant throughout the study period, varying from 24 to 27 ºC; the rainfall was concentrated in the months of February, March, and April, 2014; the relative air humidity varied according to the rainfall, with the greatest rate (86%) registered in April and the lowest one (49%) in October, 2013 (Figure 2).

The greatest guava infestation rates were noticed in the period from January to June, 2014 (Table 2), which

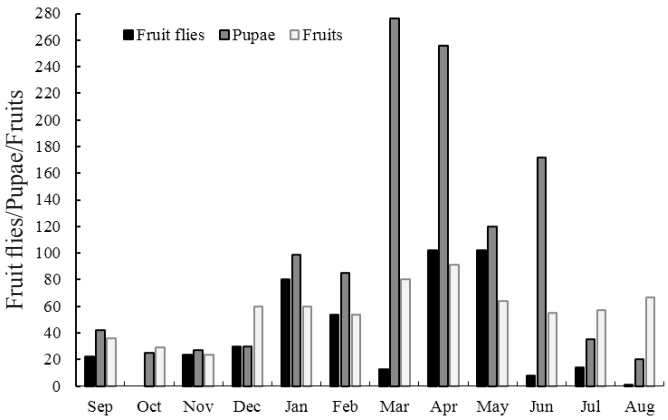

Figure 1. Ratio between guava availability and the fruit fly (*Anastrepha* spp. and *Ceratitis capitata*) population fluctuation, collected from guava orchard puparia from September, 2013, to August, 2014, in the city of Barbalha (CE).

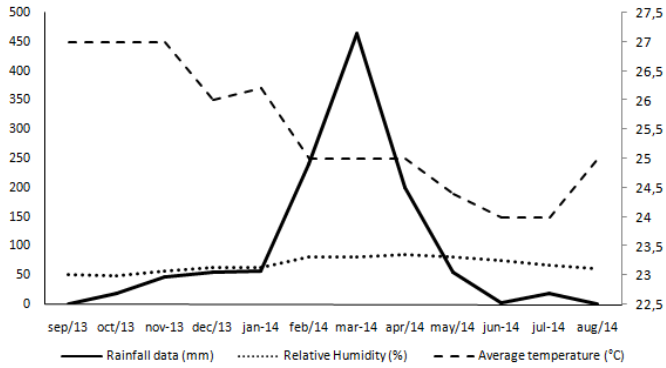

Figure 2. Climatic factors from September, 2013, to August, 2014, in the city of Barbalha (CE).

corresponds to the rainy season of the year in the region of Cariri. The lowest infestation was seen in August of the same year, the first month of the dry season in the region. In that month, it were registered a very little rainfall level (0.4 mm), an elevated temperature, and low air humidity. All these climatic factors may have contributed to reduce the infestation because the adult do not emerge from the puparia in dry soils.

Corroborating with the present research, Calore et al. (2013) also noticed an increase in the population density of *Anastrepha* spp. in a guava orchard with increasing temperatures and its population peaks were registered from January to March, coinciding with the availability period of ripe guavas in the orchard, results that are very similar to those presented here. Duarte et al. (2012) also noticed that the abundance of guavas in Jaboticabal (SP) increases these tephritids' population density, although this information did not present significant correlation with the meteorological data.

The monthly mean of the fruit infestation rate (I) was of 1.58 puparia/fruit, with March as the month of greatest infestation with 3.45 puparia/fruit because it was the wettest month (Figure 1) and August as the month of lowest infestation, with 0.3 puparia/fruit because there was practically no rain (0.4 mm). In a study made by Araujo & Zucchi (2003) in the city of Mossoró (RN), it was noticed that one of the months with the greatest infestation was April, and there was a mean of 6 puparia/fruit.

The climate of the region of Mossoró is very hot, dry, and its rainy season occurs in the summer and autumn. The research was conducted in a guava orchard with other host plants in its environment. Therefore, its fruit infestation was greater than hereof and its peaks also occurred in different moments in comparison with the conditions of the Cearense Cariri. However, Araujo et al. (2005) noticed, in the region of Mossoró and Assu (RN), a mean infestation of *Anastrepha* spp. in guava of 2.67 puparia/fruit, a very similar result to the one obtained hereby.

Silva & Silva (2007) reported a guava natural infestation rate of 0.24 puparia/fruit in the city of Ferreira Gomes (AP), which is much less than the results hereof. However, Leal

Table 2. Guava infestation rates I = No of puparia obtained / no of collected fruits, natural parasitism rate P = No of emerged parasitoids / emerged flies + no of emerged parasitoids x 100 and parasitoid frequency F = No of individuals of a species / total number of emerged parasitoids x 100 and Pupal viability V = No of emerged flies + parasitoids x 100 / total of pupae from September, 2013, to August, 2014, in the city of Barbalha (CE).

Month	(I)	(P)	(F%)	(V%)
September	1.17	0	0	52.38
October	0.86	0	0	0.00
November	1.13	5	*Doryctobracon areolatus* (100)	92.59
December	0.45	0	0	100.00
January	1.65	1.67	*Doryctobracon areolatus* (100)	81.82
February	1.57	25	*Doryctobracon areolatus* (100)	78.82
March	3.45	0	0	4.71
April	2.81	43.28	*Doryctobracon areolatus* (68.97) *Asobara anastrephae* (22.41) *Utetes anastrephae* (6.9) *Opius bellus* (1.72)	62.50
May	1.88	4.11	*Doryctobracon areolatus* (66.67) *Asobara anastrephae* (33.33)	87.50
June	3.13	0	0	4.65
July	0.61	0	0	40.00
August	0.30	0	0	5.00
Monthly average	1.58	6.59	*Doryctobracon areolatus* (75) *Asobara anastrephae* (18.42) *Utetes anastrephae* (5.26) *Opius bellus* (1.32)	

et al. (2009) found mean fruit fly infestation rates in guavas of the Paluma variety, in the state of Rio de Janeiro, of 26.5 puparia/fruit, which is much greater than our results.

The greatest monthly parasitism rate (P) for guavas was also registered in April, with 43.28%, and a mean parasitism rate of 6.59% (Table 2). In the semi-arid region of Bahia, Cova & Bittencourt (2003) noticed that the natural parasitism rate in guavas had a mean of 11%. However, in Bolivia, Ovruski et al. (2009) obtained lesser natural parasitism rates, ranging from 0.43 to 8.40% for guava and peach (*Prunus persica* L.), respectively, and more recently, Jahnke et al. (2014) got parasitism rates varying from 8.3% to 25.5% for *Anastrepha fraterculus* and *Doryctobracon areolatus* in two guava species in State of Rio Grande do Sul, Brazil. In guava orchards and dry forests in the State of Minas Gerais, Brazil, *Doryctobracon areolatus* was the most abundant parasitoid and it was associated with *Anastrepha obliqua*, *Anastrepha sororcula*, and *Anastrepha zenildae* (De Souza et al., 2012).

We know that parasitism varies a lot in natural environments and that is affected by several factors, such as fruit fly species, distribution and abundance of host fruits, collection site, and environment (Nicácio et al., 2011). The frequency of individuals per species (E) was analyzed every month, and the parasitoid *Doryctobracon areolatus* presented a frequency of 100% in the months of November, 2013, and January and February, 2014, with a mean of 75% in the twelve evaluated months (Table 2). Araujo & Zucchi (2003), in a study carried out in the semi-arid region of Rio Grande do Norte, found that *Doryctobracon areolatus* was the most frequent parasitoid, representing 96.6% of the total sample. In the semi-arid region of Bahia, among the three families of recorded parasitoids, the *Doryctobracon areolatus* braconid was the most frequent species (Cova & Bittencourt, 2003).

Araujo et al. (2015) in a study of fruit fly parasitoids in the region of Baixo Jaguaribe (CE), found the occurrence of four parasitoids species: *Doryctobracon areolatus*, *Opius bellus*, *Utetes anastrephae*, and *Tetrastichus giffardianus* (Silvestri, 1915) (Hymenoptera: Eulophidae), from which *Doryctobracon areolatus* was the most frequent and with greater geographical distribution one in the region.

The fruit fly natural parasitism in Brazilian fruit producing regions varies a lot because it is affected by host fruit, host fly, local, and collection season (Leal et al., 2009). Studies of fruit fly parasitoid species show that, in Brazil, *Doryctobracon areolatus* is the most frequent parasitizing species of *Anastrepha* spp. and *Ceratitis capitata* larvae (Nunes et al., 2012; Taira et al., 2013; Araujo et al., 2015). In most studies on the semi-arid region, *Doryctobracon areolatus* is the most abundant species, probably due to its proper adaptation to the environmental conditions and to the most common fruit species in this region such as: guava, juá (*Ziziphus joazeiro*), yellow monbin (*Spondias mombin*), cajarana (*Spondias* sp.), and acerola (*Malpighia glabra*). Generally, the studies carried out in the semi-arid region, about 80% of the parasitoids are associated with tephritid larvae (*Anastrepha obliqua*, *Anastrepha zenildae*, and *Anastrepha sororcula*), mainly in Myrtaceae and Anacardiaceae (Alvarenga et al., 2009; Souza et al., 2012; Araujo et al., 2015; Sousa et al., 2017).

Parasitism success is probably linked to its life history and co-evolution with fruit flies of the *Anastrepha* genus (both from the Neotropical region). Consequently, *Doryctobracon areolatus* is distinguished from other native parasitoid species because it parasitizes several fruit flies. Additionally,

its longer ovipositor (3.8 mm) helps it to parasitize fruit fly larvae on different-sized fruits, which makes it successful in comparison with other parasitoids (Aluja et al., 2013).

Doryctobracon areolatus stands out from other parasitoid species in sympatric conditions because it is the only native parasitoid capable of attacking early stages of fruit fly larvae, which enables it to arrive earlier and excel in an intrinsic competition with other parasitoid species (Murillo et al., 2015). This parasitoid's early action on fruit flies may represent an ecological advantage that prevents its displacement or local extinction due to other competitors such as *Utetes anastrephae* and *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae), since these latter species will invariably attack mature larva stages that might have been already parasitized by *Doryctobracon areolatus* (Murillo et al., 2015).

Pupal viability was elevated in most studied months in 2013 and 2014, with greatest values in the months of November and December, 2013, and January and February, 2014 (Table 2). This period corresponds to the beginning of the rainy season in the Cearense Cariri, in which the temperature drops (Figure 2).

The month of October was the only one that did not present fruit fly. It is one of the hottest months of Cariri, Ceará, and although the plastic trays with vermiculite were moistened every day in the isolated room in which they remained, the vermiculite layer may have heated too fast, which might have caused the zero pupal viability since the average temperature was 26 °C, which implies that very high temperatures might have been registered in this month. Santos et al. (2012) registered a quarterly viability, from August to October, 2010, of 59.60% of pupal viability in organic guava in Maceió, (AL), a very similar value to the one hereof in September and also under northeastern semi-arid conditions. On the other hand, the pupal viability was similar to the one hereof, with 52.5% of the fruits obtained from three cities of Piauí (Araújo et al., 2014). In Bahia, the pupae viability was of 71.2% in cajá orchard (Silva et al., 2008) because, in *Spondias*, flies usually present a good development.

Conclusions

April is the month of greatest abundance and diversity of fruit fly species and their parasitoids; *Anastrepha sororcula* and *Anastrepha zenildae* are the most abundant throughout the year, as well as the native parasitoid *Doryctobracon areolatus*.

The guavas are more infested by fruit flies from January to June, 2014, corresponding to the rainy season in Cariri, Ceará.

At studied location, the natural parasitism of the native parasites from the region of Cariri with greater intensity in the month of April due to its climatic conditions of rainfall and relative air humidity, which were favorable for these insects' development. *Doryctobracon areolatus* is most frequent native parasitoid in the guava orchard of Barbalha for the conditions presented by Cariri, state of Ceará.

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