Fruit flies (Diptera: Tephritidae) and their parasitoids associated with acerola, mango, and guava in the municipality of Brasil Novo, Pará

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10.1590/0034-737X202168060010

ABSTRACT

Fruit flies are phytophagous insects that are important because of the damages caused to fruits, mainly by larvae that feed on the pulp. Surveys of the diversity of these tefritids are still scarce in Brazil, especially in the Amazon region, the objective of this study was to establish the tritrophic relationship existing between species of *Anastrepha*, their parasitoids, and the fruits acerola (*Malpighia emarginata* DC.), mango (Mangifera indica L.), and guava (Psidium guajava L.) in the municipality of Brasil Novo, Pará. Freshly fallen fruits were collected weekly from January to December 2018, in three farms. Throughout the survey, 4,324 puparium were collected in guavas, 2,682 in mangoes, and 644 in acerolas. The species *Anastrepha obliqua* (Macquart, 1835) were identified in mango (90.3%), acerola (7.7%) and guava (2.1%), and *Anastrepha striata* Schiner, 1868 were identified in guava (98.8%) and acerola (1.2%). Five species of parasitoids were identified in association with *A. obliqua* and six species of parasitoids were identified in association with *A. striata*. The specie *Doryctobracon areolatus* (Szépligeti, 1911) was the most frequent among the species of parasitoids recorded.

Keywords: Amazon; *Anastrepha* sp.; biodiversity; tritrophic relationship.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are considered insect pests of great importance in world fruit production, causing significant economic losses in production and/or leading to increased costs and management practices of orchards (Zucchi *et al*., 2011). The economic losses caused by fruit fly infestation reach approximately US $ 1 billion per year worldwide and US$ 242 million per year in Brazil (Oliveira *et al*., 2013).

Damage is caused to the fruits by the females at egg laying and by the larvae feeding on the fruit pulp (Silva *et al*., 2013), and losses can reach 100% in some untreated orchards depending on the cultivated species (Hernandes *et al.*, 2013).

The economically important species of Tephritidae in Brazil are separated into four genera: *Anastrepha* Schiner, 1968; *Rhagoletis* Loew, 1862; *Ceratitis* MacLeay, 1829; and *Bactrocera* Macquart, 1835. The last two are represented by a single species each, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824), and the carambola fruit fly, *Bactrocera carambolae* (Drew & Hancock, 1994). The genus *Anastrepha* is to date represented by 121 species identified throughout the Brazilian territory and infest several native and/or exotic fruits (Zucchi & Moraes, 2008).

Knowledge about the diversity of fruit flies, host plants, and infestation rates is fundamental to define management practices for this insect pest (Silva *et al*., 2011). However, according to Zucchi & Moraes (2008), only 51% of fruit flies recorded in Brazil have at least one known host.

Of the 28 *Anastrepha* species recorded in the state of Pará, only 11 have at least one known host plant (Adaime *et al*., 2016). Twenty-two host plant species were described

Submitted on September 21th, 2020 and accepted on February 17th, 2021.

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in association with *Anastrepha* species, in addition to *C. capitata* which is associated with four plant species (Adaime *et al.*, 2016; Araújo *et al*., 2016).

Therefore, our objectives were study the tritrophic relationship between species of *Anastrepha*, their parasitoids, and the three commercial fruits acerola (*Malpighia emargin*ata DC.), mango (*Mangifera indica* L.), and guava (*Psidium guajava* L.) in the municipality of Brasil Novo, state of Pará, and to determine the infestation rates and percentage of parasitism.

MATERIAL AND METHODS

The study was carried out in three farms (Santa Rita: 03°18'7.03"S 052°28'57.98"W; Pouso Alegre: 03°18'18.55"S 052°28'28.15"W; Boa Vista: 03°17'37.07"S 052°29'3.80"W) in the Municipality of Brasil Novo, Meso-Southwestern Pará (IBGE, 2017), from January to December 2018. According to the Köppen classification, the climate of the region is classified as Am - humid tropical, with average total annual rainfall ranging between 1,500 mm and 2,000 mm, with the lowest rainfall rates between June and November (National Institute of Meteorology/ Ministry of Agriculture, Livestock and Supply - INMET/ MAPA, 2019).

Freshly fallen fruits from acerola, mango, and guava trees, which were not in an advanced stage of decay, hollow inside and/or half-eaten by birds and other animals, were collected weekly. The sample size varied according to fruit availability in the field, as recommended by Silva *et al*. (2011).

After collection, the fruits were packed in trays or plastic bags and taken to the Agricultural Entomology Laboratory (LEA) at the Federal University of Pará - UFPA, campus Altamira – PA. In the laboratory, the fruits were counted, separated, and weighed. The fruits were placed in plastic containers with the bottom covered with a layer of sterilized and moistened sand, covered with voile fabric, tightened with an elastic band or a holed lid, and kept in a protected and ventilated area.

The fruit samples were examined every five days to keep moisture and remove the puparium. The puparium were placed in a new container with a thin layer of moistened sand, covered with voile fabric tightened with an elastic band or a holed lid, and monitored daily for emergence of fruit flies and/or their parasitoids. After emergence, the insects were kept alive for 48 hours, so that their morphological structures acquired a peculiar color, which is important for taxonomic identification. Then, the insects were sorted by sex, counted, and stored in 70% alcohol until species identification, as recommended de Silva *et al*. (2011).

The insects were identified at the Agriculture Insect Rearing Laboratory of the National Research Institute of the Amazon (INPA). The adult females collected from the fruits were analyzed according to the wing and thoracic patterns and morphometric measurements of the aculeus apex, according to the dichotomous keys by Zucchi (2000) and Zucchi *et al*. (2011).

The parasitoids of the Braconidae family were identified based on the shape of the mandible and the clypeus, structure and color of the wing and the propodeum (Marinho *et al*., 2011). The individuals of the Figitidae family were analyzed based on the characteristics of the antenna, thorax, and anterior wing venation (Guimarães & Zucchi, 2011). The individuals of the Pteromalidae family were identified based on the legs, wings and antennae (Wharton & Yoder, 2019). The voucher specimens of fruit flies and parasitoids sampled were deposited in the Biological Scientific Collections at INPA.

The parameters evaluated were the indices of infestation per kilogram of fruit, pupal viability (PV), parasitism rate (PT), and frequency of parasitoids per species (F). Calculations were made according to Sá *et al.* (2008) (infestation rates); Souza *et al*. (2005) (pupal viability); Araújo *et al.* (2015) (parasitism rate); Araújo *et al.* (2014) (frequency of parasitoids per species).

RESULTS AND DISCUSSION

A total of 4,103 fruits of acerola, mango, and guava were collected, corresponding to 122.3 kg, from which 7,650 puparium of fruit flies were obtained, the majority from samples of guava with 4,324 puparium, followed by mango with 2,682 puparium, and acerola with 644 puparium.

Two species of fruit flies were identified infesting the fruits sampled: 1,339 individuals of *Anastrepha obliqua* (Macquart, 1835) and 438 individuals of *Anastrepha striata* Schiner, 1868. *A. obliqua* infested the samples of all fruit species, with frequencies of 90.3% in mangoes, 7.7% in acerolas and 2.1% in guavas, while *A. striata* infested guavas and acerolas, with frequencies of 98.8% and 1.2%, respectively.

The species *A. striata* is an important native agricultural pest that occurs in the Amazon region and has guava as its main host (Jesus-Barros *et al*., 2012). This is the first record of *A. striata* infesting acerola fruits in the state of Pará, and there is only one record of infestation of this fruit in the Amazon region, in Ilha de Santana, AP, by Almeida *et al*. (2016).

The species *A. obliqua* is predominant in the Amazon region, since the species has a polyphagous feeding habit and infest several host plants (Zucchi & Moraes, 2008). However, it preferentially attacks plants in the family Anacardiaceae (Ferreira *et al*., 2003). In the Amazon region, this species is described as infesting 33 species of host plants belonging to eight botanical families - Anacardiaceae, Apocynaceae, Chrysobalanaceae,

Combretaceae, Malpighiaceae, Myrtaceae, Oxalidaceae and Sapotaceae. (Adaime *et al*., 2016).

The average fruit-fly infestation rates in the guava samples were 3.3 puparium/fruit and 110.8 puparium/kg (Table 1). The average infestation rate/kg was higher than those reported by other studies such as the survey carried out by Santos *et al*. (2012), in which they found infestation of 99.3 puparium/kg in collections carried out in organic guava crops in the municipality of Maceió, Alagoas. Similarly, Moura & Moura (2011) found infestation levels lower than the findings of the present study, reporting 30.3 puparium/kg in samples collected in a guava orchard in Fortaleza, Ceará.

The average pupal viability (PV) in the guava samples was 63.2% (Table 1). This result is lower than the PV found by Dias *et al*. (2013) in a survey carried out in municipalities along the border area of Rio Grande do Sul, Argentina and Uruguay, where, in guava fruits, the PV of *A. fraterculus* was 76% and *C. capitata* was 85%. However, the result of this study was higher than that reported by Santos *et al*. (2012) for organic guava crops in the municipality of Maceió, Alagoas, where they found VP of 59.6%.

In this study, the PV found for guava fruits was higher than the other fruits. According to Sá *et al*. (2008), a high pupal viability is not desirable in pest management, because hosts that allow good larval performance contribute to the maintenance and increase of the fruit fly population; therefore, as it is a potential host for fruit fly, the guava crop should have special attention.

The average rates of infestation by fruit flies in the mango samples were 4.8 puparium/fruit and 34.9 puparium/kg (Table 2). These rates were higher than the reports of Sousa *et al*. (2019) for mango of Tommy Atkins variety, with infestation rates of 4.1 puparium/kg. However, they were lower than the infestation rates Raga *et al*. (2011) found in a survey carried out in 67 municipalities in the state of São Paulo, 59 puparium/fruit and 283.3 puparium/kg.

Acerola fruits showed the lowest infestation per fruit, with mean of 0.3 puparium/fruit, which is probably due to their smaller size (Table 3). Leite *et al*. (2017) report infestation rate of 0.04 puparium/fruit in Nossa Senhora do Livramento, BA, while Araújo *et al*. (2011) describe infestation rates of 0.01 to 0.91 puparium/fruit in Mossoró, RN.

The average PV obtained in the present study in the acerola samples was 21.8% (Table 3), which was close to that observed by Lemos *et al*. (2017) in surveys carried out in commercial orchards in the state of Amapá, where PV was 20%. However, our results were inferior to the reports of other studies. Almeida *et al*. (2016) carried out a survey in three municipalities in the state of Amapá and found PV of 58%; Marsaro Júnior *et al*. (2011) carried out a survey five municipalities in the state of Roraima and found PV of 71%.

The lowest PV was recorded in the samples of acerola, which may indicate a low preference for this fruit by the *Anastrepha* species compared with the other fruits studied in this work. Another reason that may have influenced VP and If is the high level of parasitism in relation to other fruit.

Of the 599 parasitoids that emerged in the collected samples, 292 were found in guavas, 205 in mangoes, and 102 in acerolas. The species associated with *A. striata* in guava were the family Figitidae (*Aganaspis pelleranoi* (Brèthes, 1924)); the family Braconidae (*Doryctobracon areolatus* (Szépligeti, 1911), *Odontosema albinerve*

Table 1: Infestation rates by *Anastrepha* species in commercial crop of guava (*Psidium guajava*, Myrtaceae) in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018. If: Infestation per fruit; Ifk: Infestation per kilogram of fruit; PV (%): pupal viability

Farm	Nº Fruits	Weight (kg)	Pupae	Males	*A. striata*	*A. obliqua*	If	Ifk	PV (%)
Santa Rita	310	7.4	961	201	250	1	3.1	129	55.8
Pouso Alegre	598	17.5	2.366	774	783	0	4	135.4	68.8
Boa Vista	336	14.7	997	323	290	9	3	68	64.9
Total	1,244	39.6	4,324	1,298	1,323	10	3.3	110.8	63.2

Table 2: Infestation rates by *Anastrepha* species in commercial crop of mango (*Mangifera indica*, Anacardiaceae) in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018. If: Infestation per fruit; Ifk: Infestation per kilogram of fruit; PV (%): pupal viability

Farm	Nº Fruits	Weight (kg)	Pupae	Males	*A. striata*	*A. obliqua*	If	Ifk	PV (%)
Santa Rita	237	29.4	810	116	0	89	3.4	27.6	26
Pouso Alegre	21	3.7	67	3	0	17	3.2	17.9	30.3
Boa Vista	230	30.6	1.805	339	0	330	7.8	59.1	41.3
Total	488	63.7	2,682	458	0	436	4.8	34.9	32.5

Table 3: Infestation rates by *Anastrepha* species in commercial crop of acerola (*Malpighia emarginata*, Malpighiaceae) in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018. If: Infestation per fruit; Ifk: Infestation per kilogram of fruit; PV (%): pupal viability

Farm	Nº Fruits	Weight (kg)	Pupae	Males	*A. striata*	*A. obliqua*	If	Ifk	PV (%)
Santa Rita	539	1.7	163	11	0	7	0.3	94.6	13.1
Pouso Alegre	598	5.1	387	52	4	26	0.3	75.4	25.4
Boa Vista	336	2.2	94	6	12	4	0.2	43.1	26.8
Total	1,473	9	644	69	16	37	0.3	71	21.8

Kieffer, 1909, *Opius bellus* (Gahan, 1930), *Utetes anastrephae* (Viereck, 1913)); and the family Pteromalidae (*Pachycrepoideus vindemmiae* (Rondani, 1875)) (Table 4). The results found in this study agree with those of Jesus-Barros *et al*. (2012), who found that these species were associated with *A. striata* in surveys carried out in five municipalities in the state of Amapá and surveys carried out by Dutra *et al*. (2013) in the state of Amazonas.

The parasitoid species *A. pelleranoi*, *D. areolatus*, *O. albinerve*, *O. bellus*, and *U. anastrephae* associated with *A. obliqua* were found in mango fruits. The two species of parasitoids *D. areolatus* and *U. anastrephae* associated with *A. obliqua* were recorded in acerola fruits (Table 4). These species were also found associated with *A. obliqua* in the survey carried out by Marsaro Júnior *et al*. (2011) in the state of Roraima and by Sousa *et al*. (2016) in three municipalities in the state of Amapá.

Table 4: Tritrophic relationships observed between species of parasitoids, fruit flies and host fruits collected in three properties in the municipality of Brasil Novo, state of Pará, Brazil, January-December / 2018

Fruit Fly	Fruit	Species parasitoids
Anastrepha striata	Guava	*Doryctobracon areolatus* *Aganaspis pelleranoi* *Utetes anastrephae* *Pachycrepoideus vindemmiae* *Odontosema albinerve* *Opius bellus*
Anastrepha obliqua	Mango	*Doryctobracon areolatus* *Utetes anastrephae* *Odontosema albinerve* *Aganaspis pelleranoi* *Opius bellus*
Acerola		*Doryctobracon areolatus* *Utetes anastrephae*

The average parasitism rate in guava samples was 8% (Table 5). The results were close to those found by Leal *et al*. (2009), who recorded levels of parasitism from 1.5 to 11.5% in surveys carried out in four municipalities in the state of Rio de Janeiro. Further, the results of the present study were higher than that reported by Bittencourt *et al*. (2012) for surveys in garden orchards in the Southcoast of Bahia, in which the authors observed parasitism rate of 1.61 in guava samples.

The average parasitism rate in mango fruits was 4.8% (Table 6), the lowest in relation to the other fruits studied, a result close to that found by Marinho *et al*. (2009). The factors that can initially interfere in parasitism are the volatiles of infested fruits (Eitam *et al*., 2003), the removal of fruits from the field to the laboratory, but a characteristic that can directly affect the parasitism index is the morphology of the fruit because in smaller fruits with shallow pulp the indexes are higher in relation to large fruits (Hickel, 2002).

The average parasitism rate in acerola was 15.1% (Table 7), which was higher than the rates recorded in the other fruit species sampled, probably due to the small size of the fruit. According to Nascimento *et al*. (2015), parasitism is influenced by the physical characteristics of the fruit, with the highest rates occurring in small-sized fruits as in the case of *Spondias mombin* L.

In general, parasitism rates are low, but varies according to location and host species in the area (Carvalho *et al*., 2010), therefore, the selection of plant hosts with high rates of parasitism should be considered for planting in fruit growing areas, aiming to increase natural parasitism of fruit flies (Silva *et al*., 2013).

Table 5: Parasitism indexes of *Anastrepha* species in guava fruits *Psidium guajava* (Myrtaceae) in three properties in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018

Farm	N° Pupae	Nº Parasitoids	Parasitism (%)
Santa Rita	961	151	15.7
Pouso Alegre	2,366	103	4.4
Boa Vista	997	38	3.8
Total	4,324	292	8

Table 6: Parasitism indexes of *Anastrepha* species in mango fruits *Mangifera indica* (Anacardiaceae) in three properties in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018

Farm	N° Pupae	Nº Parasitoids	Parasitism (%)
Santa Rita	810	23	2.8
Pouso Alegre	67	1	1.5
Boa Vista	1,805	181	10
Total	2,682	205	4.8

Table 7: Parasitism indexes of *Anastrepha* species in acerola fruits *Malpighia emarginata* (Malpighiaceae) in three properties in the municipality of Brasil Novo, state of Pará, Brazil, January to December 2018

Farm	N° Pupae	Nº Parasitoids	Parasitism (%)
Santa Rita	163	26	16
Pouso Alegre	387	64	16.5
Boa Vista	94	12	12.8
Total	644	102	15.1

Table 8: Frequency of parasitoids (%) in guava, mango and acerola fruits collected in three properties in the municipality of Brasil Novo, state of Pará, Brazil, January-December / 2018

Frequency of parasitoids (%)					
Fruit	Species	Farm			Total
		Santa Rita	Pouso Alegre	Boa Vista	
Guava	*Doryctobracon areolatus*	27.2	76.7	68.4	57.4
	Aganaspis pelleranoi	66.2	19.4	18.4	34.7
	Utetes anastrephae	-	1.9	10.5	4.2
	Odontosema albinerve	2.0	1.9	2.6	2.2
	Pachycrepoideus vindemmiae	4.0	-	-	1.3
	Opius bellus	0.7	-	-	0.2
Mango	*Doryctobracon areolatus*	95.7	-	79.6	58.4
	Aganaspis pelleranoi	-	100	11.6	37.2
	Utetes anastrephae	4.3	-	6.1	3.5
	Odontosema albinerve	-	-	2.2	0.7
	Opius bellus	-	-	0.6	0.2
Acerola	*Doryctobracon areolatus*	42.3	73.4	66.7	60.8
	Aganaspis pelleranoi	34.6	3.1	16.7	18.1
	Utetes anastrephae	11.5	20.3	16.7	16.2
	Pachycrepoideus vindemmiae	7.7	-	-	2.6
	Opius bellus	3.8	3.1	-	2.3

Among the parasitoids observed, *D. areolatus* presented the highest frequency in all the fruits studied, varying between 57.4 to 60.8%, followed by *A. pelleranoi*, varying from 18.1 to 58.4%, and *U. anastrephae*, varying from 3.5 to 16.2% (Table 8). Several surveys conducted in Brazil showed that *D. areolatus* is the most frequent species in collections of fruit flies, which is due to the size of its ovipositor and egg-laying performance at different stages from immature eggs to third-instar larvae (Marinho *et al*., 2011; Nunes *et al.*, 2011).

CONCLUSIONS

Two species of *Anastrepha* were identified infesting the collected fruit samples, *A. obliqua* in fruits of mango (90.3%), acerola (7.7%) and guava (2.1%), and *A. striata* in guava (98.8%) and acerola (1.2%).

Guava was the most susceptible crop to fruit fly infestation among the three fruit species studied.

This is the first record of *A. striata* infesting acerola (*Malpighia emarginata*) fruits in the state of Pará and

second record in Brazil, adding to the existing list of hosts of this insect pest.

Doryctobracon areolatus was the most frequent parasitoid species in all samples of the fruits collected, providing important information for the management of fruit flies.

ACKNOWLEDGEMENTS

The authors thank the Instituto Nacional de Pesquisas da Amazônia (INPA) and the Universidade Federal do Pará (UFPA) for the support given to the research and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the master's degree scholarship. We also thank the anonymous reviewers and the academic editor for improving the manuscript. There was no conflict of interests in carrying the research and the paper is available for full disclosure.

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