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ORIGINAL ARTICLE

Tritrophic interactions between fruit flies, their host plants and their parasitoids in the municipality of Tomé-Açu, Pará, Brazil

Interações tritróficas entre moscas-das-frutas, suas plantas hospedeiras e seus parasitoides no município de Tomé-Açu, Pará, Brasil

Natália Silva de Souza[1]*

[1] Universidade Federal do Pará, Instituto Amazônico de Agriculturas Familiares, Programa de Pós-graduação em Agriculturas Amazônicas, Rua Augusto Correa, 01, Cep: 66075-110, Belém, Pará, Brasil.

*** Corresponding Author:**
E-mail: ss.natalia08@gmail.com

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ABSTRACT: Fruit flies (Diptera, Tephritidae) stand out as the most harmful pests to fruit crops in different parts of the planet. Biological pest control is one of the alternatives used in integrated management programs for fruit flies to help reduce the population density of these insects. This work aims to contribute to the expansion of scientific knowledge about the distribution of fruit flies in the territory of Pará, identifying species of occurrence, their host plants and their parasitoids. The study was conducted in a representative municipality of Nordeste Paraense, particularly in the municipality of Tomé-açu. The fruit samples were randomly collected. The emerged parasitoids were identified in the Entomology Laboratory of Embrapa Eastern Amazon and Embrapa Amapá. In the municipality of Tomé-Açu, acerola (*Malpighia punicifolia*), taperebá (*Spondias mombin*), guava (*Psidium guajava*), ingá (*Inga edulis*) and mango (*Mangifera indica*) are important population repositories of fruit fly species. Only Anastrepha species occur and infest fruit trees in the studied region. We report the first associations for the State of Pará: A. sororcula and A. fraterculus in carambola (Averrhoa carambola); *A. obliqua* in camu camu (*Myrciaria dubia*), cashew (*Anacardium occidentale*), abiu (*Pouteria caimito*) and mango. And yet, the first association of *A. distincta* in mango for Brazil.

RESUMO: *As moscas-das-frutas (Diptera, Tephritidae) se destacam como as pragas mais prejudiciais a cultivos de frutas em diferentes partes do planeta. O controle biológico de pragas é uma das alternativas utilizadas em programas de manejo integrado de moscas-das-frutas por auxiliar a redução da densidade populacional desses insetos. Este trabalho tem como objetivo contribuir para a ampliação do conhecimento científico acerca da distribuição de moscas-das-frutas no território paraense, identificando espécies de ocorrência, suas plantas hospedeiras e seus parasitoides. O estudo foi conduzido em município representativo do Nordeste Paraense, particularmente no município de Tomé-açu. As amostras de frutos foram coletadas ao acaso. Os parasitoides emergidos foram identificados no Laboratório de Entomologia da Embrapa Amazônia Oriental e da Embrapa Amapá. No município de Tomé-Açu, acerola (Malpighia punicifolia), taperebá (Spondias mombin), goiaba (Psidium guajava), ingá (Inga edulis) e manga (Mangifera indica) constituem importantes repositórios populacionais de espécies de moscas-das-frutas. Somente espécies de Anastrepha ocorrem e infestam fruteiras na região estudada. Reportamos as primeiras associações para o Estado do Pará: A. sororcula e A. fraterculus em carambola (Averrhoa carambola); A. obliqua em camu camu (Myrciaria dubia), caju (Anacardium occidentale), abiu (Pouteria caimito) e manga. E ainda, a primeira associação de A. distincta em manga para o Brasil.*

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1 Introduction

Brazil ranks among the three largest fruit producers worldwide, after China and India. Fruit growing in Brazil is a strategic segment with added value for social inclusion, as it generates jobs and income and also complies with the environmental requirements for sustainability.

In the state of Pará, fruit growing is socioeconomically important as the large variety of Amazon fruit and their derived products have a special place in the domestic and international markets. It is an important source of income for local populations and has also been developed in agroforestry systems (AFS), which promotes sustainable production (Lemos *et al.*, 2011a).

The genus *Anastrepha* Schiner is endemic to tropical and subtropical regions in the Americas and comprises species of economic importance. In Brazil, a total of 128 species has been reported so far (Zucchi & Moraes, 2008). The Brazilian Amazon harbors 60 species of *Anastrepha* (Zucchi *et al.*, 2011) and *Anastrepha obliqua* Macquart is the most frequent species in the region infesting fruits of 25 hosts (Silva & Ronchi-Teles, 2000; Zucchi *et al.*, 2011).

Knowledge on host/fruit fly/parasitoid associations in the Amazon has increased in the past few years especially in the states of Pará, Amapá, and Rondônia (Lemos *et al.*, 2011; Jesus-Barros *et al.*, 2012; Adaime *et al.*, 2018). These studies are paramount as they provide important information on native parasitoids, which play an important role as biological control agents of fruit flies considered pests (Ovruski *et al.*, 2000; Adaime *et al.*, 2018).

Therefore, this work aims to contribute to the expansion of scientific knowledge about the distribution of fruit flies in the territory of Pará, identifying species of occurrence, their host plants and their parasitoides in the municipality of Tomé-açu located in the State of Pará, eastern Amazon.

2 Material and methods

Three expeditions were carried out to collect host fruit in the microregion of Tomé-Açu, Pará, Brazil, in April and November of 2008 and March of 2009 according to the phenology of host plants and the anual rainfall of the area. The collections were carried out in orchards and fruit agroforestry systems (AFS) surrounded by a Eastern Amazonian secondary growth forest. Sampling sites were GPS recorded.

Ripe or ripening fruit were collected randomly from trees and also from the ground among fruit that had recently fallen. Variation in sample sizes was a result of fruit availability throughout the collection period, fruit characteristics, and plant density in the study areas. Samples were classified as compounded or simple following Silva *et al.* (2011). The collected fruit were placed in coolers in the field and covered with organza cloth mesh. The coolers were placed in the shade when transported to the Laboratório de Entomologia, Embrapa Amazônia Oriental, Belém, Pará.

Sample processing in the laboratory followed he procedure developed in previous studies (Silva *et al.*, 2011).

Adult fruit fly females were identified at Embrapa Amazônia Oriental, Embrapa Amapá and Instituto Biológico (IB-SP) following Zucchi (2000). Parasitoids were identified at Embrapa Amazônia Oriental and Embrapa Amapá following Zucchi *et al.*, (2011).

Infestation indices calculated as the average number of larvae per fruit (Imf) (larvae/fruit) and number of larvae per kg of fresh fruit (larvae/kg). Percentage of parasitismo was calculated dividing the number of emerged parasitoids by the total number of pupae (*Ip*).

3 Results and Discussion

A total of 131 fruit samples was collected, of these 50 samples (12 plant species in 7 families) were infested whereas 81 (25 plant species in 13 families) yielded no fruit fly pupae. A total of 2,957 (1,557♀) *Anastrepha* specimens and 245 braconid parasitoids was recovered (Tables 2 & 3). Eight species of *Anastrepha* were recovered from the collected fruit: *Anastrepha obliqua* (Macquart) (n = 1,176♀), *Anastrepha distincta* Greene (n = 173♀), *Anastrepha striata* (n = 108♀), *Anastrepha serpentina* (Wiedemann) (n = 85♀), *Anastrepha leptozona* Hendel (n = 02♀), *Anastrepha antunesi* Lima (n = 01♀), *Anastrepha sororcula* Zucchi (n = 01♀), and *Anastrepha fraterculus* (Wiedemann) (n = 01) (Table 1).

Anastrepha obliqua was the most abundant and polyphagous species and was found infesting eight plant species in the families Anacardiaceae, Malpighiaceae, Myrtaceae, Oxalidaceae and Sapotaceae. This species was more abundant in acerola (n= 686♀), yellow mombim (n= 281♀), carambola (n= 140♀), and mango (n= 45♀) (Table 1). *Anastrepha serpentina* infested four plant species in the families Rutaceae and Sapotaceae (Table 1). *Anastrepha distincta* was found in two species in the families Fabaceae and Anacardiaceae. The remaining species infested a single plant species: *A. antunesi* in one species of Sapotaceae; *A. fraterculus* and *A. sororcula* in one species of Oxalidaceae; *A. leptozona* in one species of Sapotacaeae and *A. striata* in a single species of Myrtaceae (Table 1).

Here we report for the first time field infestation by *A. distincta* on mango (*Mangifera indica* L.) for Brazil. We also report for the first time for the state of Pará field infestation by *A. fraterculus* and *A. sororcula* on carambola (*Averrhoa carambola* L.) and by *A. obliqua* on camu-camu (*Myrciaria dubia*: Myrtaceae), cashew (*Anacardium occidentale* L.: Anacardiaceae), abiu (*Pouteria caimito* Radlk: Anacardiaceae), and mango.

Our results revealed simultaneous infestation by two or three Anastrepha species as A. obliqua, A. serpentina, and A. leptozona infesting abiu (Table 2). A. obliqua and A. distincta infesting mango, A. obliqua and A. antunesi infesting yellow mombim, A. obliqua and A. striata in guava, A. obliqua and A. fraterculus in carambola, and A.

Table 1. Main fruit trees host fruit flies in the municipality of Tomé-Açu, Pará, Brazil.
Tabela 1. Principais fruteiras hospedeiras de moscas-das-frutas no município de Tomé-Açu, Pará, Brazil.

Fruit host	Species of fruit flies							
Botanical Family / Species	*A. antunesi*	*A. distincta*	*A. fraterculus*	*A. leptozona*	*A. obliqua*	*A. serpentina*	*A. sororcula*	*A. striata*
Anacardiaceae								
Anacardium occidentale L. (cashew)					xx			
Mangifera indica L. (mango)		xx			xx			
Spondias mombin L. (cajá / taperebá)	xx				xx			
Fabaceae								
Inga edulis Mart. (ingá)		xx						
Malpighiaceae								
Malpighia puncifolia L. (acerola)					xx			
Myrtaceae								
Myrciaria dubia (camu camu)					xx			
Psidium guajava L. (guava)					xx			xx
Oxalidaceae								
Averrhoa carambola L. (carambola / star fruit)			xx		xx		xx	
Rutaceae								
Citrus reticulata Blanco (tangerine)						xx		
C. x sinensis L. (orange)						xx		
Sapotaceae								
Pouteria caimito Radlk. (abiu)				xx	xx	xx		
Manilkara zapota (L.) P. Royen (sapoti)						xx		

obliqua and A. sororcula in carambola (Table 2). These results reinforce the hypothesis that there seem to be niche overlaps among the species of fruit flies that occur in the Brazilian Amazon.

The highest infestation levels by *Anastrepha* were recorded for acerola (565.37 pupae/kg of fruit), yellow mombim (405.34 pupae/kg of fruit), and guava (302.63 pupae/kg of fruit) whereas oranges had the lowest infestation level (0.25 pupae/kg of fruit). On the other hand, the highest infestation levels per fruit were recorded for ingá (44.2 pupae/fruit), mango (19.14 pupae/fruit), and guava (9.0 pupae/fruit) and murici had the lowest infestation level (0.04 pupae/fruit).

A total of 245 specimens of braconid parasitoids (Hymenoptera: Braconidae) within four species were found parasitizing larvae of *Anastrepha: Opius bellus* (Gahan) (n = 116), *Doryctobracon areolatus* (Szépligeti) (n = 111), *Utetes anastrephae* (Viereck) (n = 16), and *Asobara anastrephae* (Muesebeck) (n = 02) (Table 3). Braconids were found associated with *Anastrepha* larvae infesting yellow mombim, acerola, guava, carambola, and sapoti. The highest parasitization rates were recorded for *Anastrepha* larvae in fruits of yellow mombim (31.67%), acerola (15.15%), and guava (12.17%). Up to four species of braconid parasitoids were found infesting *Anastrepha obliqua* larvae in acerola (*A. anastrephae*, *D. areolatus*, *O. bellus*, and *U. anastrephae*), three species in larvae infesting yellow mombim (*D. areolatus*, *O. bellus*, and *U. anastrephae*), and carambola (*A. anastrephae* and *O. bellus*) (Table 3). Only *D. areolatus* was found parasitizing *A. striata* and *A. obliqua* larvae in guava and *A. serpentina* larvae in sapoti (Table 3).

A total of eight species was recorded in Tomé- Açu, which represents 38% of the total number of species reported for the state of Pará (21 species) (Zucchi & Moraes, 2008) and is probably due to the high diversity of host plants grown locally. The eight species recorded belong to four species groups. The *fraterculus* group was the largest species group recorded in our study with five species followed by the *leptozona*, *serpentina*, and striata groups, each of the three with a single species. Previous studies have indicated that the most widely distributed species in the Amazon belong to the *fraterculus* group (Zucchi *et al.*, 2011).

Table 2. Species of fruit flies and their hosts in the municipality of Tomé-Açu, Pará.
Tabela 2. Espécies de moscas-das-frutas e seus hospedeiros no município de Tomé-Açu, Pará.

Fruit flies	Nº pupae	Nº adults (Nº ♀)	Hosts
A. obliqua	21	13 (7)	Caju (Anacardiaceae)
A. obliqua e *A. distincta*	24	8 (5 e 1)	Manga (Anacardiaceae)
A. obliqua	589	195 (41)	
A. obliqua e *A. antunesi*	895	362 (189 e 1)	Taperebá (Anacardiaceae)
A. obliqua	592	208 (92)	
A. distincta	537	218 (172)	Inga (Fabaceae)
A. obliqua	2.373	1.296 (686)	Acerola (Malpighiaceae)
A. obliqua	11	11 (10)	Camu (Myrtaceae)
A. striata	223	146 (97)	Goiaba (Myrtaceae)
A. obliqua e *A. striata*	71	45 (5 e 11)	
A. obliqua e *A. fraterculus*	180	56 (33 e 1)	
A. obliqua	618	211 (103)	Carambola (Oxalidaceae)
A. obliqua e *A. sororcula*	22	07 (4 e 1)	
A. serpentina	04	01 (1)	Tangerina (Rutaceae)
A. serpentina	13	01 (1)	Laranja (Rutaceae)
A. serpentina, *A. leptozona* e *A. obliqua*	61	23 (6, 2 e 1)	Abiu (Sapotaceae)
A. serpentina	254	156 (77)	Sapoti (Sapotaceae)
Total	**6.488**	**2.957 (1.557)**	-

Table 3. Parasitoid species (Braconidae) associated with fruit flies and fruit trees in the municipality of Tomé-Açu, Pará, Brazil.
Tabela 3. Espécies de parasitoides (Braconidae) associadas a moscas-das-frutas no município de Tomé-Açu, Pará, Brazil.

Hosts/ Family botany	Fruit flies	Parasitoids	Total (nº)
Spondias mombin L. (Taperebá)/ Anacardiaceae	*Anastrepha obliqua*	*Doryctobracon areolatus*	58
		Opius bellus	67
		Utetes anastrephae	9
Malpighia puncifolia L. (Acerola)/ Malpighiaceae	*A. obliqua*	*Asobara anastrephae*	01
		D. areolatus	36
		O. bellus	36
		U. anastrephae	07
Psidium guajava L. (Goiaba)/ Myrtaceae	*A. obliqua* e *A. striata*	*D. areolatus*	16
Averrhoa carambola L. (Carambola)/ Oxalidaceae	*A. obliqua*	*A. anastrephae*	01
		O. bellus	13
Manilkara zapota (Sapoti)/ Sapotaceae	*A. serpentina*	*D. areolatus*	01
Total			**245**

Anastrepha obliqua infested eight hosts out of a total of 13 in this study. Our findings revealed four new hosts (camu-camu, caju, abiu, and mango) for *A. obliqua* in the state of Pará and corroborate previous studies showing its preference for hosts in the Anacardiadeae family in the Amazon (Malavasi & Zucchi, 2000; Lima Jr *et al.*, 2007; Carvalho *et al.*, 2010; Zucchi *et al.*, 2011). These new host records show the importance of fruit fly surveys in the Amazon region, particularly in Pará, which is the main fruit producing state in the region.

In our study, *Anastrepha serpentina* was found infesting hosts in the Rutaceae (oranges and tangerines) and Sapotaceae (abiu and sapodilla). This species has long been reported as preferentially infesting sapotaceous fruit, however, new records of infestation in other host families have been reported in Brazil, especially in the Amazon (Zucchi & Moraes, 2008). In the state of Pará, studies revealed that sapoti and abiu fruits are attacked preferably by *A. serpentine* (Lemos *et al.*, 2011a).

Anastrepha distincta was found infesting mango (1♀) for the first time in Brazil. Although Feitosa *et al.* (2008) had reported *A. distincta* in traps on a mango orchard [var. Tommy Atkins, Keitt and Kent] in the state of Piauí in the Brazilian Northeast, their results did not allow the confirmation of mango as a host. However, in this study the preferred host for *A. distincta* was inga (172♀). The association between *A. distincta* and inga had previously been reported for other states in the Brazilian Amazon such as Amapá (Silva *et al.*, 2011; Jesus-Barros *et al.*, 2012), Roraima (Marsaro Jr. *et al.*, 2011), Rondônia (Pereira *et al.*, 2010), Amazonas, and Pará (Zucchi *et al.*, 2011).

The remaining *Anastrepha* species were found infesting only one host in the municipality of Tomé-Açu. *A. antunesi* infested yellow mombim, *A. leptozona* was found infesting abiu, and *A. striata* infesting guava, which confirmed these plant species as the main hosts for these tephritids in the Brazilian Amazon (Silva & Ronchi-Teles, 2000). *A. fraterculus* and *A. sororcula* were found infesting only carambola in distinctive samples.

Interestingly, simultaneous infestations of the same fruit by two or three *Anastrepha* species were observed in at least five host species (abiu, mango, yellow mombim, guava, and carambola). This is the first time that a triple simultaneous infestation by *A. obliqua*, *A. serpentina* and *A. leptozona* was reported for abiu since previous studies have reported only double simultaneous infestations by *A. serpentina* and *A. leptozona* (Jesus-Barros *et al.*, 2012). Its is noteworthy that *A. obliqua* has been recorded in all simultaneous infestations found in this study, which is probably due to the fact that this is the predominant and most polyphagous Anastrepha species in the Amazon region in Brazil.

We detected parasitism in larvae of *A. obliqua*, *A. striata*, and *A. serpentina* reared from five host species by five parasitoid species, *Asobara anastrephae*, *Doryctobracon areolatus*, *Opius bellus*, and *Utetes anastrephae*.

Asobara anastrephae had been recorded in seven Brazilian states, including the states of Amazonas, Amapá, and Tocantins in the Amazon region this parasitoid species had already been associated to larvae of seven tephritid species including *A. obliqua* and *A. fraterculus* (Zucchi & Moraes, 2008; Marinho *et al.*, 2011), as reported in this study. Our study also reports for the first time the association between *D. areolatus* and *A. serpentina* for the state of Pará.

In this study, *D. areolatus* was the only parasitoid species on larvae of *A. serpentina* in abiu. This association had already been observed in the state of Roraima (Marsaro Jr., 2011).

O. bellus and *D. areolatus* were the most abundant parasitoid species in Tomé-Açú and also associated to larvae in a larger number of plant hosts, which indicate their importante role as biological control agents of fruit flies in the region. The highest parasitism rates were observed in *Anastrepha* larvae infesting yellow mombim (31.67%), acerola (15.15%), and guava (12.17%), which indicate that these plant hosts are important parasitoid reservoirs in the region.

It is noteworthy the parasitism rates were also higher in *Anastrepha* larvae infesting yellow mombim in previous studies, including simultaneous infestation by two *Anastrepha* species and up to three parasitoids from the same fruit in the states of Acre, Amapá, and Roraima also in the Amazon region in Brazil (Thomazini & Albuquerque, 2009; Silva *et al.*, 2010, Marsaro Jr. *et al.*, 2011; Jesus-Barros *et al.*, 2012). Our results corroborate previous results that showed a pattern of high parasitsm rates in small and soft-skinned fruit, similarly to what was found by Carvalho *et al.* (2010) and Silva *et al.* (2010) in Brazil.

4 Conclusion

Only *Anastrepha* species occur and infest fruit trees in the studied region. Eight species of fruit flies (*Anastrepha obliqua, A. distincta, A. striata, A. serpentine, A. leptozona, A. antunesi, A. sororcula* and *A. fraterculus*) occur in the municipality of Tomé-Açu, Pará. Only parasitoids of the family Braconidae (*Doryctobracon areolatus, Opius bellus, Asobara anastrephae* and *Utetes anastrephae*) are registered in the municipality of Tomé-Açu (PA).

This research reports the first associations of: *A. fraterculus* and *A. sororcula* with carambola for the State of Pará; *A. obliqua* with camu camu, cashew, abiu fruits for the State of Pará; *A. obliqua* and *A. distincta* in mango for the State of Pará and the first association *A. distincta* with mango in Brazil.

Our study revealed several *Anastrepha*-host-parasitoid associations in Tomé-Açu, the main fruit producing region in the state of Pará, Eastern Amazon. This region has several fruit crops with an overlapping availability of host fruits throughout the year, which allows the continuous maintenance of fruit fly populations. Thus, our study provide data that can be potentially used to address integrated pest management in this region.

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