Research Article

Frugivorous flies (Diptera: Tephritidae; Lonchaeidae) associated with guava tree: species diversity, parasitoids and population fluctuation in the Espírito Santo state, Brazil[1]

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ABSTRACT

The commercial cultivation of guava tree (*Psidium guajava* L.) occurs in all Brazilian regions, where fruit flies cause direct losses to production and affect fresh fruit exports due to quarantine restrictions. This study aimed to determine the incidence, diversity and population fluctuations of frugivorous fly species that infest guava trees, as well as to survey their associated parasitoids, in the Espírito santo state, Brazil. Fruits and flies captured in traps were sampled in three guava-producing regions. *Anastrepha fraterculus* (Wied.) was the main species associated with guava trees, particularly in the coastal region. *Anastrepha chiclayae* was recorded for the first time associated with guava trees in Brazil. Medfly was not considered an important species. *Neosilba zadolicha* was the most common Lonchaeidae species collected, but with low importance. Higher populations of frugivorous flies were found in hot and humid areas. Two population peaks of *A. fraterculus* were observed during October (spring) and from March to April (summer-autumn). Tephritid flies occurred in all surveyed regions, with higher infestations in the coastal area, followed by the northern and mountain regions, respectively. *Doryctobracon areolatus* and *Aganaspis pelleranoi* were the most common species of parasitoids; however, the natural biological control of frugivorous flies in guava trees was very low and had little impact on fruit fly populations.

KEYWORDS: *Psidium guajava* L., *Anastrepha fraterculus*, *Neosilba zadolicha*, *Doryctobracon areolatus*, *Aganaspis pelleranoi*.

RESUMO

Moscas frugívoras (Diptera: Tephritidae; Lonchaeidae) associadas a goiabeira: diversidade de espécies, parasitoides e flutuação populacional no Espírito Santo, Brasil

O cultivo comercial de goiabeira (*Psidium guajava* L.) ocorre em todas as regiões brasileiras, onde moscas-das-frutas causam perdas diretas na produção e afetam as exportações de frutas frescas devido a restrições quarentenárias. Objetivou-se determinar a incidência, diversidade e flutuações populacionais de espécies de moscas frugívoras que infestam goiabeira e levantar seus parasitoides associados, no estado do Espírito Santo, Brasil. Frutos e moscas capturadas por armadilhas foram amostrados em três regiões produtoras de goiaba. *Anastrepha fraterculus* (Wied.) foi a principal espécie associada a goiabeiras, principalmente na região litorânea. *Anastrepha chiclayae* foi registrada pela primeira vez associada a goiabeiras no Brasil. Medfly não foi uma espécie importante. *Neosilba zadolicha* foi a espécie de Lonchaeidae mais comum, mas com baixa importância. Maiores populações de moscas frugívoras foram encontradas em regiões quentes e úmidas. Dois picos populacionais de *A. fraterculus* foram observados em outubro (primavera) e de março a abril (verão-outono). Tefritídeos ocorreram em todas as regiões pesquisadas, com maiores infestações na área litorânea, seguida da norte e serrana, respectivamente. *Doryctobracon areolatus* e *Aganaspis pelleranoi* foram as espécies de parasitoides mais comuns; no entanto, o controle biológico natural de moscas frugívoras em goiabeira foi muito baixo e apresentou controle populacional de baixo impacto.

PALAVRAS-CHAVE: *Psidium guajava* L., *Anastrepha fraterculus*, *Neosilba zadolicha*, *Doryctobracon areolatus*, *Aganaspis pelleranoi*.

INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical tree of the Myrtaceae family, with a probable center of origin in southern Mexico or northern South America (Medina 1988). Guava fruits are widely cultivated in tropical and subtropical regions of the world (Risterucci et al. 2005), and Brazil is one

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of the world's largest producers of this fruit, with commercial cultivation in many Brazilian regions (IBGE 2021, Tridge 2022). In the Brazilian state of Espírito Santo, guava trees are commonly present in commercial and domestic orchards, and are also common in farms as native plants.

The guava fruit production is intended for fresh consumption or processed for juice, candy, jam, frozen pulp, nectar, ice cream and other uses (Incaper 2022). Frugivorous flies, mainly from the Tephritidae and Lonchaeidae families, are the main threat to guava cultivation: flies lay their eggs in guava fruits, which become larvae that consume the fruit pulp, making it unsuitable for fresh consumption and industrial processing. The openings made by the female fly during oviposition harm the appearance of the fruits and may facilitate contamination by opportunistic pathogens (fungi or bacteria), which cause necrosis and rot in the fruits (Fanton & Martins 2003, Souza-Filho et al. 2009, Borges 2022).

Tephritids are an important group of pests in the world's fruit production, causing significant economic impact on the production of many fruits. Tephritids are also one of the main factors preventing export of fresh fruits due to quarantine restrictions imposed by importing countries (Godoy et al. 2011, Vargas et al. 2014). Worldwide, losses caused by fruit flies in various fruit trees may reach 100 % (Carey & Dowell 1989). In guava trees, fruit flies can cause the loss of a large number of fruits, reaching more than 64 % (Barbosa et al. 2001, Araujo & Zucchi 2003).

Species of the *Anastrepha* Schiner and *Ceratitis capitata* (Wied.) (medfly) genus are the most important tephritids in Brazil, due to their wide distribution and large number of host plants (Zucchi & Moraes 2022a, Zucchi & Moraes 2022b).

The *Anastrepha* genus includes almost 300 species (Norrbom et al. 2018). Most *Anastrepha* occur in the neotropical region, and are native to Central and South America (Hernández-Ortiz 2007). Brazil is considered to have the greatest diversity of *Anastrepha* in the world, with 128 known species (Zucchi & Moraes 2022a). *Ceratitis capitata*, the only species of *Ceratitis* in Brazil, is distributed worldwide in all tropical and temperate areas, and is considered the most cosmopolitan and invasive species among tephritids, and the most harmful to fruit production (Zucchi 2015). *Anastrepha fraterculus* (Wied.) (South American fruit fly), *A. obliqua* (Macquart) and medfly are the tephritids with the largest number of hosts, as well as the largest geographic distribution in Brazil, and these species have been considered the main pests of Brazilian fruit production (Zucchi & Moraes 2022a, Zucchi & Moraes 2022b).

Neosilba McAlpine is the most important Lonchaeidae genus in Brazil, with 40 known species, and its distribution is limited to the neotropical region, Florida and southern United States, causing damage to many fruit species (Pinto 2017, Sousa et al. 2021). Twenty-five *Neosilba* species have been recorded in Brazil, with *Neosilba zadolicha* McAlpine & Steyskal and *Neosilba pendula* (Bezzi) being the most common species, with the largest number of known host fruit species (Aguiar-Menezes et al. 2004, Raga et al. 2015, Pinto 2017, Sousa et al. 2021).

Sixteen species of *Anastrepha* and ten species of *Neosilba* have been previously associated with guava fruits in Brazil, and the importance of each of these species as pests of guava fruits varies depending on the region where the guava grows (Araujo et al. 2013, Lemos et al. 2015b, Raga et al. 2015, Maciel et al. 2017, Pinto 2017, Sousa et al. 2021, Zucchi & Moraes 2022a). The diversity and abundance of fruit fly species in orchards is influenced by climate, altitude, hosts and adjacent orchards (Silva et al. 2010).

Several species of parasitoids have been used for biological control of fruit flies worldwide (Clausen 1978). Fruit fly parasitoids include species in the hymenopteran families Braconidae, Diapriidae, Figitidae, Eulophidae and Pteromalidae (Hymenoptera), with Braconidae having the greatest number of known fruit fly parasitoid species (Wharton & Gilstrap 1983, Wharton 1989). Among the species used for biocontrol are the braconids *Diachasmimorpha longicaudata* (Ashmead), *D. kraussii* (Fulaway), *D. tryoni* (Cameron) and *Fopius arisanus* (Sonan) (Zamec et al. 2012). *Diachasmimorpha longicaudata* and *F. arisanus* were introduced into Brazil in 1994 and 2012, respectively (Paranhos et al. 2013). Few studies associating fruit flies and their host fruit trees and parasitoids have been conducted in the Espírito Santo state, of which just one reported the braconid *Doryctobracon areolatus* (Szepligeti, 1911) associated with guava (Madalon et al. 2017).

To address such gaps, this study was carried out to determine the diversity of frugivorous flies species, highlighting the main species and their parasitoids associated with guava growing in three geographic

regions (coastal, mountain and northern) and two times of the year (spring/summer; autumn/winter), in the Espírito Santo state (southeastern Brazil). In addition, seasonal population fluctuations of adult frugivorous flies in a commercial guava orchard in the coastal region were determined.

MATERIAL AND METHODS

Two studies were carried out to verify the association of frugivorous flies with guava crops. The first (fruit study) was based on fruit collection with direct sampling and the second one (trapping study) was based on indirect sampling, using trapping to monitor the adult fruit fly populations in areas of guava production.

Samples of ripe guava fruits were randomly collected directly from guava plants in mixed domestic fruit orchards or from isolated guava plants located in farms of 13 municipalities of the Espírito Santo state, from September 1997 to August 2009. The varieties and age of the guava trees from which the fruits were collected were not determined, since the vast majority were guava trees spread by various forms, such as people, animals and birds. The sampled municipalities are located in the coastal, mountain and northern regions, which differ in altitude and climatic characteristics (Incaper 2021).

The coastal region comprises the municipalities of Alfredo Chaves, Guarapari, Serra, Viana, Vila Velha and Vitória, with altitude of 0-200 m, annual rainfall (RF) = 1,300-1,700 mm, relative humidity (RH) = 53-95 % and monthly average temperature (T) = 22-24 ºC. In the mountain region, samples were taken from Domingos Martins, Santa Teresa and Venda Nova do Imigrante (altitude of 600-1,100 m; RF = 1,300-1,500 mm; RH = 47-99 %; T = 20-22 ºC). The northern region is characterized by altitudes ≤ 100 m, RF = 1,000-1,300 mm, RH = 45-95 %, T = 24-26 ºC and comprises the municipalities of Linhares, Pedro Canário, Sooretama and Vila Valério (Figure 1).

A total of 198 guava samples were collected, consisting of 3,593 fruits (302 kg). The samples were placed in paper bags and transported to laboratories. Each fruit sample was weighed and standardized to 1.5 kg. The number of fruits in each sample was determined and the fruits placed in screened plastic boxes on a layer of ~2 cm of moistened washed sand to obtain all the fruit fly puparia that developed. The sand substrate was sieved twice to separate and count

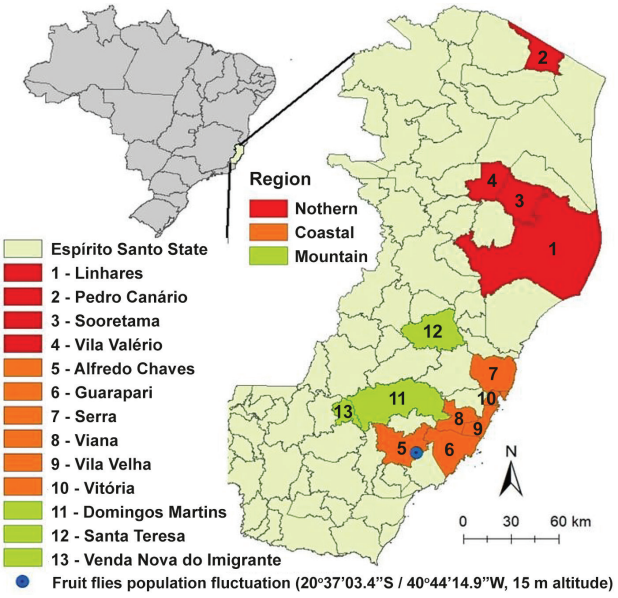

Figure 1. Map of the Espírito Santo state, Brazil, indicating the municipalities and geographical regions sampled in the study.

pupae, and the pupae were transferred to screened cages to enable development and emergence of adult frugivorous flies and their associated parasitoids, if any. The adult flies and parasitoids that completed the development stage were sexed and quantified, and *Anastrepha* females, lonchaeids and parasitoids placed in 70 % ethanol for identification.

Frugivorous fly infestation rates were expressed in number of pupae per kilogram of fruits and number of pupae per fruit (Malavasi & Morgante 1980). Pupal viability (PV) was calculated by the formula: PV (%) = [(number of emerged parasitoids + number of emerged frugivorous flies)/total number of obtained frugivorous fly pupae] x 100. The parasitism index was calculated by the formula: parasitism (%) = [number of emerged parasitoids/(number of emerged flies + number of emerged parasitoids)] x 100 (Madalon et al. 2017).

Concerning the faunistic insect analysis, constancy indicates the distribution of each species throughout the collections, showing the percentage of times that a given species is present, when compared to the total number of collections carried out; and frequency indicates the number of individuals of a given species, if compared to the total number of individuals captured from the analyzed group, being expressed in percentage (%) (Thomazini & Thomazini 2002, Muller 2008, Soares et al. 2018).

Tephritid population monitoring was carried out in a 1 hectare (ha) area of a 7-year-old commercial guava orchard, Paluma cultivar, with 6 x 4 m spacing, from June 2001 to December 2008, in the municipality of Alfredo Chaves (coastal region) (20º37'03.4''S, 40º44'14.9''W and 15 m of altitude) (Figure 1), and a total of 331 samples were collected. Two McPhail traps spaced 30 m apart were placed in the central planting line of the area, installed on tree branches at 1.8 m above the ground, and baited with 5 % hydrolyzed protein. The traps were examined weekly, with captured specimens collected and preserved in ethanol, traps washed and bait replaced. The collected specimens were sorted and quantified, with *Anastrepha* females fixed in 70 % ethanol for identification.

Meteorological data for the three sampled regions and for the municipality of Alfredo Chaves were obtained from the meteorology sector of Incaper (Incaper 2021).

Tephritidae specimens of the *Anastrepha* genus were identified by Dr. Keiko Uramoto, from the Universidade de São Paulo (USP), São Paulo, Brazil, whilhe Lonchaeidae specimens were identified by Dr. Pedro Carlos Strikis (São Paulo, Brazil) and parasitoids by Dr. Jorge Anderson Guimarães (Embrapa Hortaliças, Brasília, Brazil).

For the statistical analysis, the data of the variables number of pupae and tephritid specimens obtained in guava fruits were transformed by the function $y = \sqrt{x + 0.5}$ and the means compared by the t-test ($p < 0.05$), using the R software (R Core Team 2021).

RESULTS AND DISCUSSION

In the present study, 88.9 % of the sampled guava fruits were infested by frugivorous flies (Table 1), and 25,300 fruit fly pupae were obtained. The observed pupal viability of tephritids was 54.7 %, and the infestation rates were 83.8 pupae kg^{-1} and 7.0 pupae $fruit^{-1}$. The majority of the frugivorous fly adults belong to the Tephritidae family (13,416 specimens = 98.3 % of the total emerged adults), and 110 specimens belong to the Lonchaeidae family (0.8 %). A total of 122 parasitoid specimens were obtained (equal to 0.9 % of the total number of adult frugivorous flies that emerged from frugivorous fly pupae).

Seven Tephritidae species emerged from the guava fruits: *Anastrepha chiclayae* (Greene), *A. distincta* Greene, *A. fraterculus*, *A. obliqua*, *A. sororcula* Zucchi, *A. zenildae* Zucchi and *Ceratitis capitata*. The South American fruit fly was the most frequent species in the three studied regions, representing 93.7 % of the specimens that emerged, and this species was found in 71.2 % of the evaluated samples (Table 2).

All the specimens of the six Lonchaeidae species obtained from guava fruits in the present study belong to the *Neosilba* genus: *N. bella* Strikis & Prado, *N. certa* (Walker), *N. glaberrima* (Wied.), *N. inesperata* Strikis & Prado, *N. pendula* and *N. zadolicha*. *Neosilba pendula* and *N. zadolicha* were the most numerous lonchaeids obtained, constituting 15.6 and 46.7 %, respectively, of the specimens that emerged (Table 2).

The overall parasitism index of frugivorous flies in guava was 0.88 %. The parasitism index in the coastal and northern regions were 0.33 % and 1.63 %, respectively, and no parasitism was observed in the mountain region. Five species of parasitoids belonging to two Hymenoptera families were collected: *Asobara anastrephae* (Muesebeck), *Diachasmimorpha longicaudata* (Ashmead), *Doryctobracon areolatus* (Szepligeti), *Utetes anastrephae* (Viereck) (all Braconidae) and *Aganaspis pelleranoi* (Brèthidas) (Figitidae). *Doryctobracon areolatus* was the most frequent parasitoid species (54 %), followed by *A. pelleranoi* (24 %) (Table 2).

Tritrophic interactions were verified between the South American fruit fly and the braconid

Table 1. Number of collected samples and percentage of samples infested per month, from September 1997 to August 2009.

Number of collected samples	Spring/summer[1]						X	Autumn/winter[2]						X
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.		Apr.	May	June	July	Aug.	Sep.	
Total/average (X)	10.0	11.0	6.0	23.0	32.0	32.0	19.0	15.0	11.0	16.0	13.0	15.0	14.0	14.0
Infested (nº)	8.0	9.0	6.0	21.0	28.0	31.0	17.2	14.0	10.0	15.0	8.0	14.0	12.0	12.2
Infested (%)	80.0	81.8	100.0	913.0	87.5	96.9	90.4	93.3	90.9	93.8	61.5	93.3	92.9	86.9

[1] September 23 to March 20; [2] March 21 to September 22.

Table 2. Species of frugivorous flies (Diptera: Tephritidae and Lonchaeidae) and their parasitoids (Hymenoptera: Braconidae and Figitidae) associated with guava fruits (*Psidium guajava* L.) collected from September 1997 to August 2009, in the Espírito Santo state, Brazil.

Species	Number of evaluated samples[1]		Emerged specimens		Municipality
	With the species	Constancy (%)	Number	Frequency (%)	
DIPTERA					
Tephritidae (♀)					
Anastrepha chiclayae	1	0.51	1	0.02	Viana
Anastrepha distincta	1	0.51	3	0.05	Viana
Anastrepha fraterculus	141	71.21	5.560	93.71	Alfredo Chaves, Domingos Martins, Guarapari, Linhares, Santa Teresa, Serra, Sooretama, Venda Nova do Imigrante, Viana, Vitória
Anastrepha obliqua	21	10.61	57	0.96	Domingos Martins, Guarapari, Linhares, Viana
Anastrepha sororcula	25	12.63	87	1.47	Linhares
Anastrepha zenildae	17	8.59	119	2.01	Guarapari, Linhares, Sooretama, Viana
Ceratitis capitata	11	5.56	106	1.79	Guarapari, Linhares, Santa Teresa, Viana
Lonchaeidae (♂)					
Neosilba bella	4	2.02	4	8.89	Linhares, Sooretama, Viana
Neosilba certa	2	1.01	6	13.33	Viana
Neosilba glaberrima	4	2.02	6	13.33	Alfredo Chaves, Sooretama, Viana
Neosilba inesperata	1	0.51	1	2.22	Linhares
Neosilba pendula	5	2.53	7	15.56	Viana
Neosilba zadolicha	5	2.53	21	46.67	Domingos Martins, Sooretama, Viana
HYMENOPTERA					
Braconidae (♂ e ♀)					
Asobara anastrephae	5	2.53	13	13.00	Guarapari, Viana
Doryctobracon areolatus	12	6.06	54	54.00	Guarapari, Linhares, Serra, Sooretama, Viana
Diachasmimorpha longicaudata	1	0.51	1	1.00	Viana
Utetes anastrephae	4	2.02	8	8.00	Viana
Figitidae (♂ and ♀)					
Aganaspis pelleranoi	7	3.54	24	24.00	Linhares, Sooretama, Viana

[1] Number of samples in which the respective species occurred from a total of 198 fruit samples (= total of 3,593 guavas and 302 kg of fruits).

parasitoids *A. anastrephae*, *D. areolatus* and *U. anastrephae*, and for the figitid parasitoid *A. pelleranoi* in guava fruits under field conditions. Due to the fact that there was an overlap of frugivorous fly species in the samples for the other species of parasitoids that completed the development stage, it was not possible to confirm the specific tritrophic relationship (Leonel Junior et al. 1995). Verification of tritrophic interactions among parasitoids, fruit flies and host plants provides a better understanding of bioecological relationships and the knowledge of plant species that may serve as reservoirs for hosts of beneficial parasitoids, which can maintain and increase parasitoid populations to act in the natural or conservative control of target pests.

The coastal region was the most frugivorous fly infested region, with rates of 96.1 pupae kg^{-1} of guavas and 8.8 pupae $fruit^{-1}$, followed by the northern (47.7 pupae kg^{-1} and 3.2 pupae $fruit^{-1}$) and mountain (45.3 pupae kg^{-1} and 3.1 pupae $fruit^{-1}$) regions (Table 3).

The number of frugivorous fly pupae obtained from fruits was higher in the autumn-winter than in the spring-summer period in the northern region, but no significant difference in the number of obtained frugivorous fly pupae was observed between the two evaluated periods in the coastal and mountain regions. The emergence of *Anastrepha* specimens was greater in the autumn-winter in the coastal and northern regions, and, in the mountain region, the *Anastrepha* emergence was equal for both periods (Table 4).

Except for *A. sororcula*, the *Anastrepha* and *C. capitata* species observed in the present study have been previously recorded in the northern region

Table 3. *Anastrepha* infestation rates (mean ± standard error) in the three evaluated regions of the Espírito Santo state, Brazil, from September 1997 to August 2009.

Total number of pupae kg^{-1}			Total number of pupae fruit^{-1}		
Coastal	Mountain	Northern	Coastal	Mountain	Northern
96.1 ± 0.61 a*	45.3 ± 1.18 b	-	8.8 ± 0.06 a	3.1 ± 0.75 b	-
96.1 ± 0.61 a	-	47.7 ± 0.89 b	8.8 ± 0.59 a	-	3.2 ± 0.51 b
-	45.3 ± 1.18 a	47.7 ± 0.89 a	-	3.1 ± 0.75 a	3.2 ± 0.51 a

* Means followed by the same letter in each row, for each evaluated variable, do not differ by the t-test ($p < 0.05$).

Table 4. Mean (± standard error) number of pupae and *Anastrepha* infestation rates (number kg^{-1} of fruits) in three regions of the Espírito Santo state, Brazil, in two periods of the year, from September 1997 to August 2009.

Period of the year	Number of pupae			Number of *Anastrepha* specimens kg^{-1} of fruit		
	Coastal	Mountain	Northern	Coastal	Mountain	Northern
Spring/summer	70.3 ± 0.57 a*	47.5 ± 1.58 a	-	33.8 ± 0.43 a	24.4 ± 1.33 a	-
	70.3 ± 0.57 a	-	20.2 ± 0.87 b	33.8 ± 0.43 a	-	10.9 ± 0.6 b
	-	47.5 ± 1.58 a	20.2 ± 0.87 a	-	24.4 ± 1.33 a	10.9 ± 0.6 a
Autumn/winter	95.8 ± 0.67 a	16.9 ± 1.21 b	-	54.1 ± 0.54 a	5.2 ± 0.74 b	-
	95.8 ± 0.67 a	-	63.2 ± 0.91 a	54.1 ± 0.54 a	-	27.7 ± 0.6 b
	-	16.9 ± 1.21 b	63.2 ± 0.91 a	-	5.2 ± 0.74 b	27.7 ± 0.6 a

* Means followed by the same letter horizontally, within each evaluated variable, do not differ from each other by the t-test ($p < 0.05$).

(Linhares) associated with other fruit hosts (Uramoto et al. 2008).

Sixteen tephritid species have been associated with guava in Brazil: *Anastrepha antunesi* Lima, *A. atrigona* Hendel, *A. bahiensis* Lima, *A. bistrigata* Bezzi, *A. coronilli* Carrejo & González, *A. distincta* Greene, *A. fraterculus*, *A. leptozona* Hendel, *A. obliqua*, *A. parishi* Stone, *A. pseudoparallela* (Loew), *A. sororcula* Zucchi, *A. striata* Schiner, *A. turpiniae* Stone, *A. zenildae* Zucchi and *C. capitata* (Zucchi et al. 2022a, Zucchi et al. 2022b). However, *Anastrepha atrigona*, *A. bistrigata*, *A. coronilli*, *A. parishi*, *A. striata* and *A. turpiniae* have not been recorded in Espírito Santo yet (Uramoto et al. 2008, Uramoto & Zucchi 2010, Martins 2011, Zucchi & Moraes 2022a).

The South American fruit fly is the species with the widest range in Brazil. It is found in all regions of the country and occurs in 24 of the 26 Brazilian states (Zucchi & Moraes 2022a). It is also the fruit fly species with the highest number of associated host plant species (159), belonging to 34 botanical families, including Myrtaceae (54), Rosaceae (17) and Rutaceae (15) (Zucchi & Moraes 2022a). As observed in this study, the species is considered the main tephritid that causes damage to guava crops in the Brazilian states of Bahia, Santa Catarina, São Paulo and northwestern Espírito Santo (Raga et al. 2006, Silva et al. 2010, Garcia & Norrbom 2011, Silva et al. 2011, Madalon et al. 2017, Sousa et al. 2021). However, this species has less importance for this crop in other states: *Anastrepha zenildae* is the predominant fruit fly species in the northern Minas Gerais state (Alvarenga et al. 2009); *A. striata* in Acre, Amapá, Maranhão, Rondônia and Roraima (Silva et al. 2007, Pereira et al. 2010, Marsaro Júnior et al. 2011, Adaime et al. 2017a, Adaime et al. 2017b, Maciel et al. 2017); and *C. capitata* in Ceará and Mato Grosso do Sul (Souza et al. 2008, Nicácio & Uchôa 2011).

The South American fruit fly has also been found in all regions of the Espírito Santo state and associated with crops of great economic importance (Martins 2011). Four tephritid species were found in the northwest region of this state in commercial guava cultivation of the Paluma cultivar. The South American fruit fly, *A. obliqua*, *A. zenildae* and *C. capitata* were also the most frequent species. In contrast, the low importance of the medfly as a pest of guava crops observed in the present study is similar to the results of Madalon et al. (2017). The medfly was first noted in Brazil at the beginning of the last century, and it is now distributed in all regions of the country. With a high degree of polyphagy, the medfly has been found in a large number of host plant species (115) from 31 families, including species of economic

importance (Zucchi & Moraes 2022b). Medfly and the South American fruit fly are the most important fruit fly species for Brazilian fruit production, due to the large number of hosts they attack (Zucchi & Moraes 2022a, Zucchi & Moraes 2022b).

The occurrence of *Anastrepha chiclayae* Greene in guava fruits in Brazil was verified for the first time in this study. *Anastrepha chiclayae* had its identity recently clarified in Brazil: morphological, morphometric and molecular studies (integrative analysis) have shown that specimens previously misidentified as *A. dissimilis* in Brazil are *A. chiclayae* (Araújo et al. 2023). This species is distributed in all the Brazilian geographic regions, with plant hosts including *Passiflora caerulea* L., *Passiflora edulis* Sims and *Passiflora elegans* Mart. (Passifloraceae), *Pouteria caimito* (Ruiz & Pav.) Radlk. (Sapotaceae) and *Sarcomphalus joazeiro* (Mart.) Hauenschild (Rhamnaceae) (Zucchi & Moraes 2022a, Araújo et al. 2023). *Anastrepha dissimilis* is not known to occur in Brazil (Araújo et al. 2023).

Ten Lonchaeidae species of the *Neosilba* genus have been previously associated with guava in Brazil, including *N. bella* Strikis & Prado, *N. bifida* Strikis & Prado, *N. certa* (Walker), *N. cornuphallus* Strikis, *N. dimidiata* (Curran), *N. glaberrima* (Wied.), *N. inesperata* Strikis & Prado, *N. pendula*, *N. pradoi* Strikis & Lerena and *N. zadolicha* (Strikis & Lerena 2009, Raga et al. 2015, Gisloti et al. 2017, Pinto 2017, Sousa et al. 2021). The six species of lonchaeids obtained from guava fruits in the present study belong to the *Neosilba* genus, with *N. pendula*, and *N. zadolicha* being the most frequent collected species, corresponding to 15.6 and 46.7 % of the *Neosilba* collected species, respectively (Table 2). These two species are the most generalized ones of this genus in Brazil, with the highest number of host fruits (Raga et al. 2015, Pinto 2017, Sousa et al. 2021). All of the *Neosilba* species collected in the present study have been previously recorded associated with guava in Brazil, and *N. zadolicha* has been the lonchaeid species most frequently recorded from fruits of guava in the Brazilian states of Alagoas, Amapá and São Paulo (Lemos et al. 2015a, Raga et al. 2015, Gisloti et al. 2017, Santos et al. 2017). *Neosilba bella* was previously recorded in the Espírito Santo state, in the coastal region (municipality of Viana), associated with *Eugenia uniflora* L. and *Inga laurina* Willd., and was also reported in the northern region (municipality of Linhares), associated with *E. gemminiflora* Berg. and *Talisia esculenta* (Cambess.) Radlk. (Strikis & Prado 2006). The latter has been reported as a host plant, native to the Amazon region, and widespread in the Brazilian biomes of Caatinga, Cerrado and Atlantic Forest (Acevedo-Rodríguez 2015). *Neosilba certa*, *N. glaberrima*, *N. inesperata*, *N. pendula* and *N. zadolicha* were recorded for first time in the Espírito Santo state. With these new records, a total of eight *Neosilba* species are now known from this Brazilian state.

Doryctobracon areolatus was the parasitoid species most frequently associated with frugivorous flies in guava (54 %), followed by *A. pelleranoi* (24 %). Previous studies indicate that *D. areolatu* occurs in the tropics, and has been the most frequent fruit fly parasitoid, with the widest geographic distribution in several Brazilian regions, parasitizing several species of fruit flies associated with different fruit species (Souza-Filho et al. 2007, Silva et al. 2010). Previous studies indicate that this native parasitoid has a high potential for use in fruit fly biological control programs (Nunes et al. 2011, Nava et al. 2019, Rabelo et al. 2020). In Espírito Santo, *D. areolatus* was previously recorded in the coastal and northwest regions, respectively in the municipalities of Alfredo Chaves and São Roque do Canaã (Leonel Junior et al. 1995, Madalon et al. 2017). The dominance of this parasitoid over related species is possibly due to the length of its ovipositor, which allows this species to reach fruit fly larvae of different host plant species. Parasitoids with long ovipositors parasitize larvae in large and small fruits, but those with short ovipositors are limited to parasitizing larvae in small fruits (Taira et al. 2013).

Diachasmimorpha longicaudata is a parasitoid species of Indo-Pacific origin introduced to Brazil in 1994, in Janaúba, Minas Gerais state, and Conceição do Almeida, Bahia state (Walder et al. 1995). In the present study, *D. longicaudata* was recovered in the municipality of Viana, located approximately 1,300 km from where inundative releases of this parasitoid were first performed in Brazil. It was noted that no inundative release of this parasitoid was done in Espírito Santo. Results of the present study may be an indication of the ability of this parasitoid to survive and disperse under field conditions, and potential as a biological control agent. The fruit fly parasitoids *Asobara anastrephae*,

D. longicaudata, *U. anastrephae* and *A. pelleranoi* were also found for the first time associated with guava in Espírito Santo.

The natural parasitism of tephritids and lance flies noted in the present study was very low. Low levels of natural parasitism of fruit flies were also noted in cultivated guava in other Brazilian regions, including the northwest Espírito Santo (Madalon et al. 2017), northern Minas Gerais (Alvarenga et al. 2009) and the Cerrado-Pantanal biome of Mato Grosso do Sul (Taira et al. 2013). Low levels of natural parasitism may indicate that the natural occurrence of parasitoids associated with guavas is not enough to reduce fruit fly populations in Brazil. Therefore, sustainable forms of management of the insect population to prevent the increase or reduce the natural population of fruit flies should be implemented within an integrated pest management program in Brazilian guava crops.

Sixteen Tephritidae species were collected from the traps installed in the guava trees. There were 32 specimens of medfly (7 ♂ and 25 ♀) and 4,600 specimens of *Anastrepha* spp. (27.1 ♂ and 72.9 % ♀), belonging to 15 species (Table 5). There are previous records of *A. bahiensis* and *A. serpentina* in Espírito Santo, in the municipality of Linhares (Uramoto et al. 2008). The South American fruit fly was the most numerous species, comprising 89.6 % of the collected specimens. Six of the species collected in the traps were also reared from guava fruits: *A. distincta*, *A. fraterculus*, *A. obliqua*, *A. sororcula*, *A. zenildae* and *C. capitata*.

Table 5. *Anastrepha* species collected with McPhail traps in a commercial guava orchard, in the municipality of Alfredo Chaves, Espírito Santo state, Brazil, from June 2001 to December 2008.

Anastrepha species	Number of specimens (♀)	Frequency (%)
Anastrepha amita Zucchi	2	0.06
Anastrepha bahiensis Lima	7	0.21
Anastrepha barbiellinii Lima	4	0.12
Anastrepha consobrina (Loew)	1	0.03
Anastrepha distincta Greene	146	4.36
Anastrepha fraterculus (Wied.)	3,003	89.62
Anastrepha grandis (Macquart)	15	0.45
Anastrepha leptozona Hendel	65	1.94
Anastrepha montei Lima	1	0.03
Anastrepha obliqua (Macquart)	61	1.82
Anastrepha pickeli Lima	4	0.12
Anastrepha pseudoparallela (Loew)	5	0.15
Anastrepha serpentina (Wied.)	34	1.01
Anastrepha sororcula Zucchi	1	0.03
Anastrepha zenildae Zucchi	2	0.06
Total	3,351	100.00

Population fluctuations of the South American fruit fly exhibited a great variation during the evaluated period (June 2001 to December 2008) (Figure 2). The highest population peaks of the South American fruit fly occurred in October 2001 and October 2004, with averages of 165.6 and 90.5 specimens, respectively. No population peaks were apparent for the same periods in other years of observation, with no evident pattern of occurrence over the years (Figure 2). There was also an upward trend in the population in March-April, but much less than that seen in October (Figure 3).

Climatic factors had a low influence on the population fluctuations of *A. fraterculus* observed in the present study. The climate in the municipality of Alfredo Chaves is mild, with T = 22 ºC, and a 5.8 ºC variation in the average temperatures throughout the year (24.9 and 19.1 ºC); RF = 1,652 mm, and well distributed; RH = 77.40-83.75 % during the year (Incaper 2021). This may be because the lower developmental threshold for this species is 10.3 ºC, and the optimal development temperature is 15-27 ºC (Machado et al. 1995). Therefore, the coastal region has optimal conditions for the development of fruit flies throughout the year. Thus, fruit fly population fluctuations during the year, and between years, may be related to biotic factors such as fruits that remain on trees or on the ground in orchards, and/or fruits of other host plants in the surroundings. The population fluctuations observed in this study also may be related to crop management factors, including the possible need to use pesticides for pest management throughout the sampling period.

The temporal fluctuation of fruit fly populations in tropical areas has been observed to be mainly related to the availability of host plant fruits, and not to the climatic variables; thus, the availability of ripe fruits has been the factor responsible for determining the population size of the most abundant species of fruit flies, and, consequently, the largest infestations (Corsato 2004, Lemos et al. 2015b). However, Madalon et al. (2017) found a strong correlation between fruit fly populations and temperature in northwest Espírito Santo, indicating that pest population growth is favored at higher temperatures.

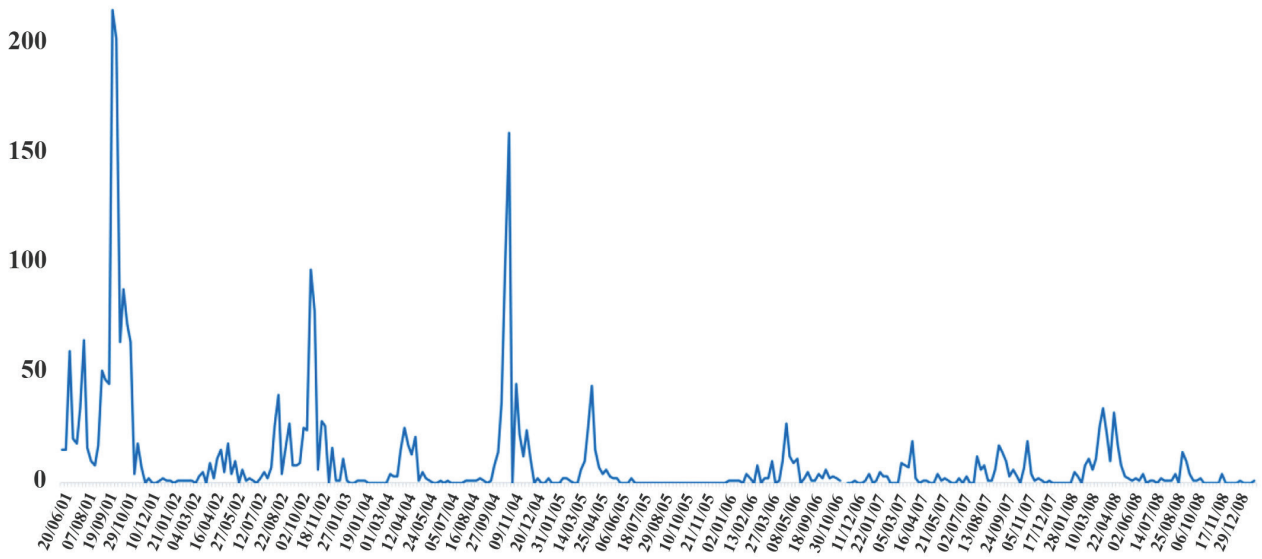

Figure 2. Weekly monitoring of the *Anastrepha fraterculus* (Wied.) population, from June 2001 to December 2008, using McPhail traps baited with 5 % hydrolyzed protein, in the municipality of Alfredo Chaves, Espírito Santo state, Brazil.

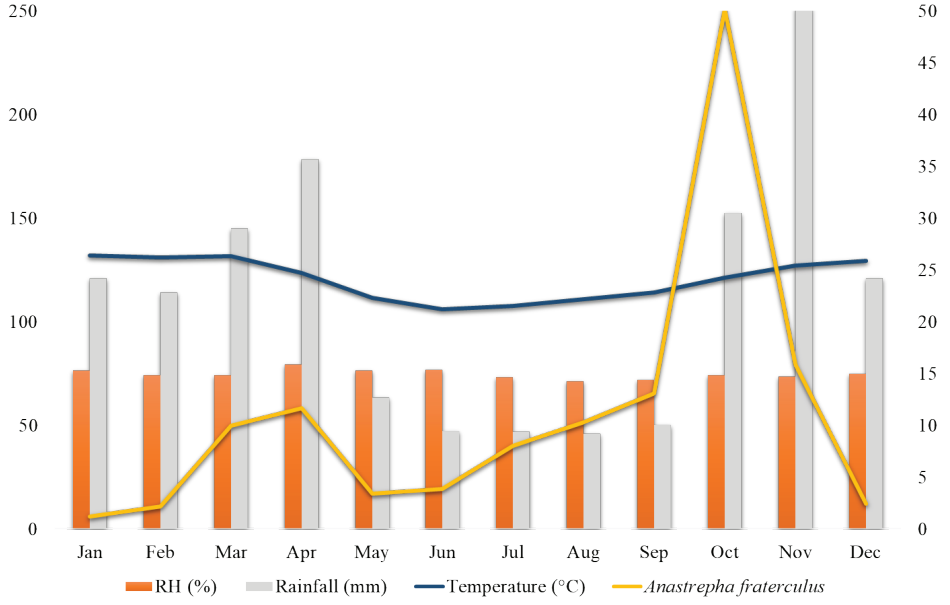

Figure 3. Monthly average number of *Anastrepha fraterculus* (Wied.) and climatic variables (relative humidity, temperature and rainfall), from June 2001 to December 2008, in the municipality of Alfredo Chaves, Espírito Santo state, Brazil.

CONCLUSIONS

1. The South American fruit fly (*Anastrepha fraterculus*) is the main species associated with guava in the Espírito Santo state, Brazil, occurring throughout the year, particularly in the coastal region;
2. Higher populations of frugivorous flies were found in hot and humid regions of the state;
3. Medfly and lonchaeids were not important pests associated with guava fruits in Espírito Santo;
4. Two population peaks of the South American fruit fly occur in October (spring) and from March to April (summer-autumn);
5. *Neosilba zadolicha* was the most common species of Lonchaeidae found in guava fruits in the state;
6. Four native and one exotic species of hymenopteran parasitoids were associated with *Anastrepha* fruit

flies infesting guava, being *Doryctobracon areolatus* (Braconidae) and *Aganaspis pelleranoi* (Figitidae) the most common parasitoid species; however, the parasitism rate was very low (less than 2 %).

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