

Article

Tritrophic Interactions Among Fruit Flies (Diptera: Tephritidae), Its Parasitoids and Cultivated and Wild Hosts in the Pampa Biome, Rio Grande do Sul, Brazil

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Abstract

Fruit fly (Diptera: Tephritidae) species are a serious threat for fruit-growers worldwide. The parasitoids (Hymenoptera) are natural enemies of these flies. In this context, the aim of this work was to assess fruit infestation by tephritid flies, both in native and exotic fruit trees, in the Southern region of Rio Grande do Sul (Brazil). Moreover, the incidence of native parasitoids on fly larvae was estimated. Fruits with signals of attack by fruit flies were collected randomly both in the trees and on the ground. From 2013 to 2015, a total of 5729 fruits (194.48 kg) were collected, corresponding to 34 tree species from 16 botanical families. Fruits were taken to the laboratory, individualized, weighted and kept in vermiculite for pupae emergence. Pupae were counted and emerged adults were counted and identified. The association between fruit flies, hosts and parasitoids was determined when only a given species of tephritid emerged. Half of the sampled fruit tree species presented infestation by flies. The main species of tephritid fly was *Anastrepha fraterculus*. This study showed that natural parasitism rates of fruit flies were low; however, several parasitoid species from the Figitidae and Braconidae families were detected, including *Aganaspis pelleranoi*, *Doryctobracon areolatus*, *Doryctobracon brasiliensis*, *Opius bellus*, *Utetes anastrephae*, and *Cerchysiella insularis*.

Keywords: *Anastrepha fraterculus*; ecosystem services; Hymenoptera; natural parasitism



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1. Introduction

Fruit flies (Tephritidae) are one of the main pests attacking temperate fruit trees worldwide, remaining an important challenge to plant health as they limit fruit production [1]. In the Neotropical region, from the south of the United States to the north of Argentina, the genus *Anastrepha* Schiner is the most abundant among the family Tephritidae [2]. Brazil has more than 120 species of *Anastrepha*, 10 of which cause economic losses [1,3].

The state of Rio Grande do Sul (RS) is the main producer of temperate fruits in Brazil, as in 2023 it accounted for 65.2%, 47.5% and 46.8% of total peach, pear and apple production in the country [4]. Most of the fruit tree orchards in this state are located within the Pampa Biome, an ecosystem that extends over an area of approximately 700,000 km² of mainly plain lowlands, shared between Argentina, Brazil and Uruguay [5,6]. In Brazil, it covers the southernmost end of the country, representing about 176,000 km² in RS, approximately 63% of the area of the state [6–8].

In RS, there are 17 registered species of the genus *Anastrepha* and the species *Ceratitis capitata* [9–15]. The South American fruit fly *Anastrepha fraterculus* (Wiedemann, 1830) is the most frequent and abundant pest in the orchards of the south of Brazil, especially in the southern half of RS [16,17]. Hymenoptera are the most important natural enemies in the control of larvae of frugivorous dipterans, especially the parasitoids of the subfamilies Eucilinae (Figitidae) and Opiinae (Braconidae) [13,18,19]. There are seven species of parasitoid hymenoptera associated with fruit flies in RS [20–22]. However, the populations of flies and their associated natural enemies are affected by habitat structure [23,24]. The distribution and abundance of plant hosts, landscape around the orchards and essential resources strongly influence the behavior, population dynamics and abundance of these insects [25].

In Brazil, several studies of Tephritidae fruit flies, hosts and parasitoids have been carried out in different locations under diverse habitat and climate conditions [26–30], showing that specimens of Braconidae and Figitidae have potential for being used as biological control agents of fruit flies [13,19,31–33]. Therefore, understanding the abundance and parasitism level of these species is essential to design efficient biocontrol programs.

Considering that all the main temperate fruits are susceptible to the damages caused by these frugivorous tephritids [34] and that changes in the exploitation of ecological niches may occur, the objective of the current study was to assess the degree of infestation and natural parasitism in wild and cultivated fruits commonly attacked by fruit flies, as well as to provide more detailed information on the diversity and abundance of parasitoids in peach-growing areas in the Rio Grande do Sul State (Brazil). In this work, we documented (i) tritrophic interactions among hosts, fruit flies and their parasitoids and (ii) infestation rates by systematically collecting fruits over three growing seasons (2013–2015) in peach orchards adjacent to patches of native vegetation.

2. Materials and Methods

2.1. Study Sites

This survey was conducted in the South of the Rio Grande do Sul State (Brazil), specifically in the localities of Pelotas, Capão do Leão and Morro Redondo (Figure 1). These areas are located within the Pampa Biome [5,6].

According to the Köppen classification system [35], the study area is within the ‘Cfa’ (without dry season and hot summer) climate region, where average temperatures in the coldest months, July and August, are approximately 11.4 °C. During the warmest months, December and January, the average temperature is around 22.6 °C [36].

Specifically, we conducted the surveys in four commercial farms (Figure 1 and Table 1). Two of these orchards were located in Pelotas (Orchard F1: 31°25.931′ S and 52°32.870′ W; Orchard F2: 31°29.807′ S and 52°32.573′ W) and two in Morro Redondo (Orchard F3: 31°36.636′ S and 52°40.383′ W; Orchard F4: 31°33′57″ S and 52°38′41.93″ W). Each farm had early-, average- and late-maturing peach cultivars (Figure 1). F1 comprised 28.2 ha devoted to peach production, consisting of early- (Precocinho), average- (Maciel) and late-maturing (Eldorado) cultivars (Table 1). F2 comprised 15.92 ha allocated to peach production, with cultivars Bonão (early), Esmeralda (average) and Jubileu (late-maturing). The third area (F3)

was 12.73 ha in surface and included early- (cv. Precocinho), average- (cv. Granada) and late-maturing (cv. Maciel) cultivars. Finally, F4, with 6 ha, had early- (cv. Libra), average- (cv. Sensação) and late-maturing (cv. Santa Aurea) cultivars. In these farms, phytosanitary management was carried out using a conventional protection strategy to control tephritid fruit flies, and the trees were subjected to 4–5 broad-spectrum insecticide treatments against fruit flies per growing season. In addition, protection was complemented by toxic baits containing hydrolyzed protein (3%) and an organophosphate insecticide (Malathion 1000 CE, Sifatec, Tlalnepantla, Mexico, 200 mL in 100 L) set on the orchard edges.

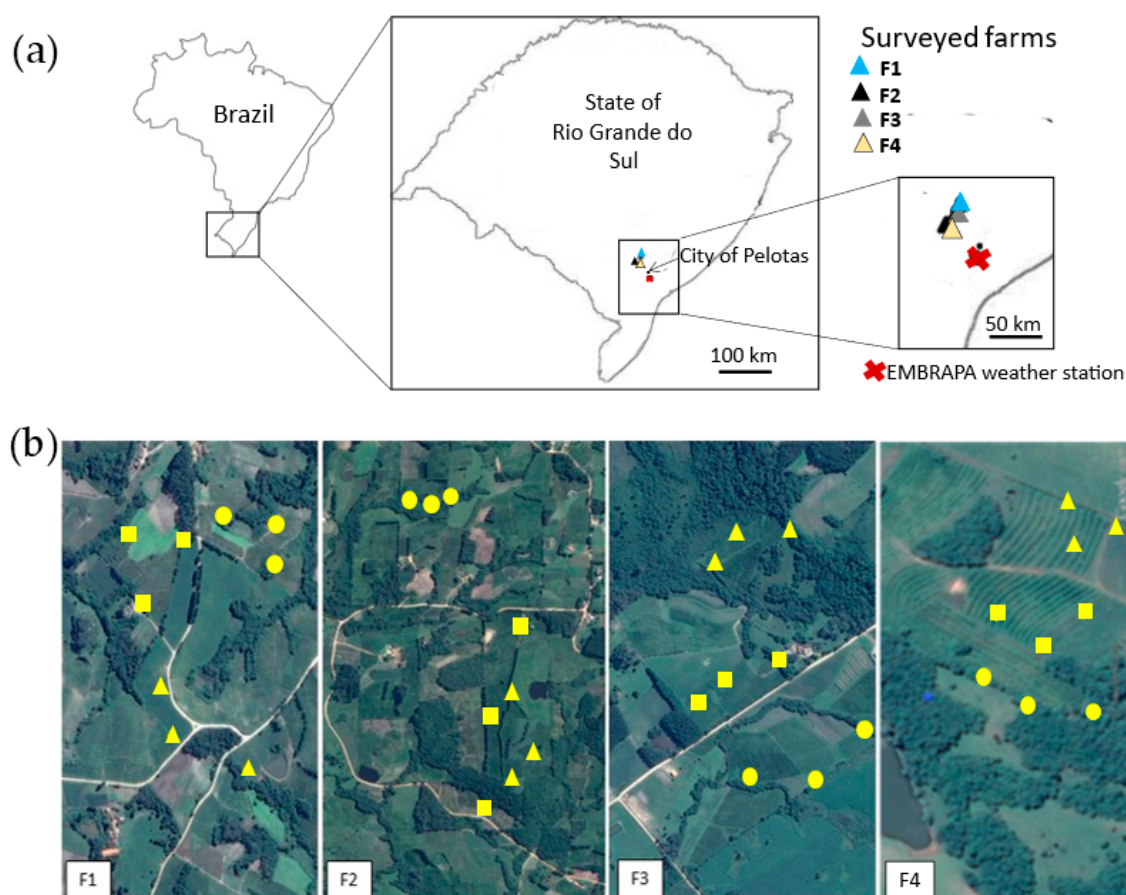


Figure 1. Location of the four study farms (F1 to F4) and the closest EMBRAPA weather station within the Rio Grande do Sul State in Brazil (a). Aerial views of the study farms: yellow squares, triangles and circles indicate the location of the traps for the early-, medium- and late-maturing peach cultivars, respectively (b).

Table 1. Distance (mean $\pm$ standard error) between traps (m) and area (ha) of orchards in Pelotas (F1 and F2) and Morro Redondo (F3 and F4) as a function of the duration of the peach-growing cycle.

Farm	Distance Between Traps (m)			Area of Orchard Devoted to Peach Production (ha)		
	Early	Average	Late	Early	Average	Late
F1	331 $\pm$ 58.1	661 $\pm$ 124	143 $\pm$ 28.0	14.18	12.68	1.34
F2	236 $\pm$ 43.2	188 $\pm$ 5.3	160 $\pm$ 19.5	6.24	4.48	5.20
F3	204 $\pm$ 1.1	187 $\pm$ 36.2	174 $\pm$ 29.6	3.25	3.72	5.76
F4	70 $\pm$ 1.3	100 $\pm$ 2.0	50 $\pm$ 5.7	1.7	2.5	1.8

In total, 36 McPhail traps were installed (9 per orchard), each with 400 mL of food bait, containing 5% hydrolyzed corn protein (Bionastrepha®). The traps were located at the edges of the peach orchards in each municipality. The distance between traps depended on both the surface of the orchard and the location of the different cultivars within each orchard (Table 1).

The captured insects were stored in labeled (place and data of collection) containers with 70% ethanol and were transported to the Laboratory of Ecology of Insects (LABELI), located at the Institute of Biology, Zoology and Genetics of the Federal University of Pelotas, RS (Brazil). The fruit fly specimens of the genus *Anastrepha* Schiner were sexed and identified using the keys of Zucchi [3]. Characteristics of the females, primarily of the aculeus, body and wing markings, were considered. For males, just the genus was confirmed because they do not present morphological characteristics for their specific recognition [3].

Fresh fruits (either peaches or non-cultivated fruits) were collected fortnightly from September 2013 to December 2015, according to the fruiting season of each plant species (Table 2). Peach trees were the only cultivated species in the study area; the remainder of the species surveyed were wild trees. Fruits from each species were collected according to their availability in each area.

Table 2. Fruiting season of primary and secondary vegetal hosts of fruit flies in the Pelotas region in Rio Grande do Sul (Brazil). Samplings were conducted between 2013 and 2015. Primary and secondary hosts were defined according to Aluja et al. [37].

[illegible]

Table 2. Cont.

Family/Species	Fruiting Season ¹											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rutaceae												
<i>Citrus aurantium</i> subsp.				S	S	S	S	S	S	S	S	S
<i>Bergamia</i>												
<i>Citrus sinensis</i>				S	S	S	S	S	S	S	S	S
Solanaceae												
<i>Ciphomandra betacea</i>												S
<i>Vassobia breviflora</i>					S							

¹ Primary vegetal host (P) and secondary host (S).

At each sampling date, ripe fruits were randomly collected directly from the trees (canopy samples). Moreover, recently fallen fruits that were in good condition (whole fruits, with no signs of rotting) were collected directly from the soil (ground samples) [38]. Canopy and ground samples were handled separately to determine if they differed in parasitoid species [39]. Peach fruits were collected within orchards while those fruits from non-cultivated hosts were collected in non-cropped areas.

The fruits were labeled, packed in plastic bags and sent immediately to the Insect Ecology Laboratory at the Federal University of Pelotas, RS, for observing possible infestation by fruit flies and their consequent species identification. Additionally, leaf and flower samplings of the fruit trees were collected for their botanical identification at the Botany Department of the Federal University of Pelotas.

In the laboratory, fruit samples were counted, weighed individually, and arranged individually on 500 mL plastic pots containing vermiculite, which served as a pupation substrate. After this, the plastic pots were covered with voile fixed with elastic tape and were maintained at 25 °C. Each fruit remained in the pot until the larvae left them (approximately 25 days) to recover pupae, which were kept in plastic containers (8 cm diameter) with vermiculite and a voile cover until the emergence of adults [36]. Every three days, the vermiculite was examined, and the puparia were removed using spatulas. The samples were discarded after 30 days. The flasks containing the puparia were kept in chambers under controlled conditions: temperature (26.5 ± 0.5 °C), relative humidity ($70 \pm 5\%$) and photophase (12 h). Flasks were checked and moistened daily with distilled water. After the adult insects emerged, they were stored in labeled flasks containing 70% ethanol for subsequent identification [29].

2.4. Identification of Parasitoids

The parasitoids were identified according to Noyes [40] for the determination of the genus, Guimarães et al. [41] for Eucilinae, and Wharton and Yoder [42] for Braconidae. The associations among fruit fly/host plant/parasitoid were only recorded if the parasitoid emerging from a fruit was only one species of Tephritidae [43]. The parasitoids collected directly from the traps were not considered in this work.

2.5. Infestation and Parasitism Rates, Data Analysis

Fruit infestation indexes were calculated according to Marsaro Júnior et al. [44]: (1) by dividing the total number of puparia obtained by the number of fruits in the sample (puparia/fruit) and (2) by dividing the total number of puparia by the total mass (kg) of fruits in the sample (puparia/kg of fruit).

The pupae viability in each host was calculated as the total number of fruit flies plus the total number of parasitoids divided by the number of pupae and was expressed in percentage.

The total parasitism index was expressed as follows: $\text{total parasitoid number} \times 100 / \text{number of flies} + \text{total parasitoid number}$ [45]. The specific parasitism index in each vegetal host was calculated as follows: $\text{number of parasitoids of a given species} \times 100 / \text{total number of parasitoids}$ [46].

Data on the number of pupae per host was checked for normality using the Shapiro–Wilk test and q-q plots. The homoscedasticity assumption was checked using the Bartlett test. Data did not meet the normality and homoscedasticity assumptions. Therefore, non-transformed data were analyzed using Kruskal–Wallis, a non-parametric test, followed by Dunn’s post hoc test ($p < 0.05$) with Benjamini–Hochberg adjustment [47] (dunn.test package) to discern differences among plant hosts.

3. Results

3.1. Fruit Flies Captured in the Traps

Between August 2011 and March 2016, 1071 males of *Anastrepha* sp., 2091 females of *A. fraterculus*, 10 males and 20 females of *C. capitata*, 2 males and 2 females of *Anastrepha daci-formis* Bezzi, and 1909 males and 6 females of *Anastrepha luederwaldti* Lima were recovered from the traps.

3.2. Infestation Indices of Fruits Flies

A total of 5729 fruits were collected during the sampling campaigns (September 2013 to December 2015), amounting to 194.48 kg, most of them from *P. persica*, followed by *C. sinensis* and *P. guajava* (Table 3).

Table 3. Units and weight (kg) of vegetal host species collected from soil and from tree canopy in the Pampa Biome, Rio Grande do Sul (Brazil), between 2013 and 2015.

Fruit Tree Species	Fruits Collected from the Soil		Fruits Collected from the Tree Canopy		Total Weight (kg)	Total Units
	Units	Weight (kg)	Units	Weight (kg)		
Anarcadiaceae						
<i>Lithraea brasiliensis</i>	0	0	450	0.45	0.45	450
Arecaceae						
<i>Butia capitata</i>	99	1.19	0	0	1.19	99
Ebenaceae						
<i>Diospyros kaki</i>	0	0	36	4.3	4.3	36
Erythroxylaceae						
<i>Erythroxylum argentinum</i>	0	0	0	0	0	0
Euphorbiaceae						
<i>Ricinus communis</i>	0	0	108	0.22	0.22	108
Fabaceae						
<i>Sesbania punicea</i>	0	0	8	0.32	0.32	8
Melatomataceae						
<i>Leandra</i> sp.	0	0	200	0.2	0.2	200

Table 3. Cont.

Fruit Tree Species	Fruits Collected from the Soil		Fruits Collected from the Tree Canopy		Total Weight (kg)	Total Units
	Units	Weight (kg)	Units	Weight (kg)		
Moraceae						
<i>Ficus carica</i>	0	0	32	0.37	0.37	32
Myrtaceae						
<i>Acca sellowiana</i>	72	2.48	110	3.1	5.58	182
<i>Campomanesia xanthocarpa</i>	0	0	51	0.16	0.16	51
<i>Eugenia uniflora</i>	42	0.1	35	0.72	0.82	77
<i>Psidium cattleianum</i>	231	0.61	83	1.96	2.57	314
<i>Psidium guajava</i>	0	0	135	17.66	17.66	135
<i>Psidium longipetiolatum</i>	200	1.19	152	1.74	2.93	352
<i>Syzygium jambolanum</i>	10	0.003	185	0.26	0.263	195
Passifloraceae						
<i>Passiflora cincinnata</i>	0	0	114	1.39	1.39	114
Phytolaccaceae						
<i>Rivina humilis</i>	0	0	150	0.15	0.15	150
Rosaceae						
<i>Cydonia oblonga</i>	0	0	8	0.17	0.17	8
<i>Fragaria</i> × <i>ananassa</i>	0	0	132	1.40	1.40	132
<i>Eriobotrya japonica</i>	52	0.41	897	10.37	10.78	949
<i>Prunus persica</i>	453	41.67	633	72.83	114.5	1086
<i>Rubus</i> sp.	0	0	192	0.81	0.81	192
Rubiaceae						
<i>Coffea arabica</i>	0	0	137	0.17	0.17	137
Rutaceae						
<i>Citrus arantium</i> subsp. <i>bergamia</i>	0	0	24	2.45	2.45	24
<i>Citrus sinensis</i>	77	9.36	118	15.12	24.48	195
Sapindaceae						
<i>Alophylus edulis</i>	0	0	3	0.003	0.003	3
<i>Cupania vernalis</i>	0	0	340	0.66	0.66	340
<i>Matayba</i> sp.	0	0	0	0	0	0
Solanaceae						
<i>Cyphomandra betacea</i>	0	0	12	0.29	0.29	12
<i>Solanum americanum</i>	6	0.012	0	0	0.012	6
<i>Solanum</i> sp.	0	0	5	0.01	0.01	5
<i>Physalis</i> sp.	0	0	52	0.12	0.12	52
<i>Vassobia breviflora</i>	0	0	85	0.05	0.05	85
Total	1242	57.03	4487	137.45	194.48	5729

From the total of 7397 puparia recovered from these samples, 4270 specimens of Tephritidae flies were obtained (Table 4). Infestation was recorded in 37.5% of the 32 plant species sampled (Table 4). In Myrtaceae, the number of pupae per fruit varied from 0.03 in *C. xanthocarpa* to 11.2 in *P. guajava*, while in the Rosaceae family it was lowest in *Fragaria* × *ananassa* with 0.004 and highest in *E. japonica* with 1.40 (Table 4). The percentage

of pupae emergence varied among plant species, from 0% in *P. cincinnata*, *C. arantium*, and *V. breviflora*, to more than 90% in *E. uniflora* and *Fragaria × ananassa* (Table 4).

Table 4. Number of pupae, percentage of pupae emergence and ratios of pupae per fruit and per kg of fruit, as well as total number of flies and sex ratio in different plant host species in the Region of Pelotas, Rio Grande do Sul (Brazil), between 2013 and 2015.

Fruit Tree Species	Pupae	% Pupae Emergence	Pupae/Fruit	Pupae/kg of Fruit	Total Number of Flies	Sex Ratio
Moraceae						
<i>Ficus carica</i>	8	62.50	0.15	19.6	5	0.29
Myrtaceae						
<i>Acca sellowiana</i>	495	86.06	1.43	42.2	426	0.36
<i>Campomnesia xanthocarpa</i>	2	50.00	0.05	12.3	1	0.50
<i>Eugenia uniflora</i>	74	93.24	0.39	112.0	69	0.30
<i>Psidium cattleianum</i>	221	76.02	0.73	86.5	168	0.32
<i>Psidium guajava</i>	4341	57.80	11.20	115.0	2509	0.33
<i>Psidium longipetiolatum</i>	135	83.70	0.80	46.1	113	0.52
Passifloraceae						
<i>Passiflora cincinnata</i>	6	0	0.05	4.3	0	0
Rosaceae						
<i>Fragaria × ananassa</i>	1	100	0.004	0.5	1	0
<i>Eriobotrya japonica</i>	1201	52.54	1.40	139.0	631	0.44
<i>Prunus persica</i>	740	37.30	0.69	9.2	276	0.32
<i>Rubus</i> sp.	50	10	0.31	35.0	5	0.29
Rutaceae						
<i>Citrus arantium</i> subsp. <i>bergamia</i>	10	0	0.42	4.1	0	0
<i>Citrus sinensis</i>	105	61.90	0.34	3.2	65	0.34
Solanaceae						
<i>Cyphomandra betacea</i>	4	25	0	13.8	1	0
<i>Vassobia breviflora</i>	4	0	0.04	61.6	0	0

Although the average number of pupae per fruit was not significantly different among vegetal hosts (p -value > 0.05), the total number of pupae per fruit did significantly differ (p -value < 0.001) due to the large numbers found in *P. guajava* (Figure 2).

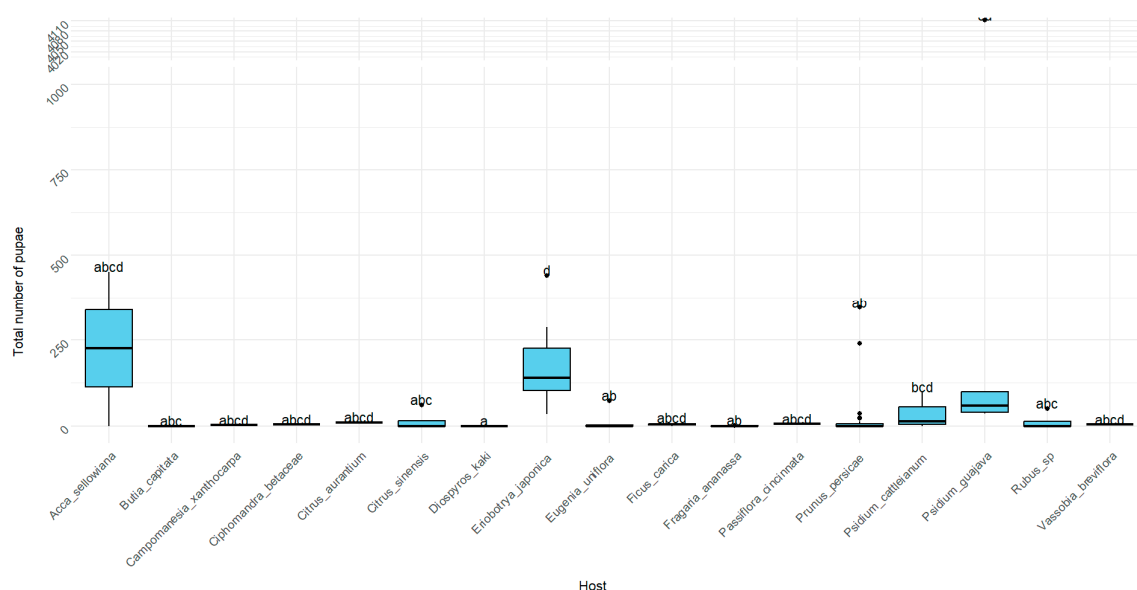


Figure 2. Boxplot of the total number of pupae in each vegetal host surveyed in the current study. Different letters on the boxes indicate significant differences among hosts according to Dunn's test ($p < 0.05$).

In this study, the fruit fly species recovered from fruits were *A. fraterculus* (in 99.6% of the infested fruits) and *C. capitata*, whose individuals were recovered in fig (*Ficus carica* L.) and orange (*Citrus sinensis* (L.) Osbeck). The highest number of adult fruit flies was recovered from fruits of *P. guajava* (2509 individuals), while no adults were recovered from fruits of *P. cincinnata*, *V. breviflora* and *C. aurantium* (Table 4). Sex ratio differed among fruit species (Table 4).

3.3. Parasitoids

Nine species of parasitoids were recovered from the samples (some of them depicted in Figure 3) and they were distributed in the following families: Figitidae—*Aganaspis pelleranoi* (Brèthes, 1924) and *Odontosema albinerve* (Kieffer, 1909); Braconidae—*Doryctobracon areolatus* (Szépligeti, 1911), *Opius bellus* (Gahan, 1930), *Utetes anastrephae* (Viereck, 1913), and *Doryctobracon brasiliensis* (Szépligeti, 1911); Encyrtidae—*Cerchysiella insularis* (Howard, 1897); and Pteromalidae—*Theocolax elegans* (Westwood, 1874) and *Anisopteromalus calandrae* (Howard, 1881). These parasitoids were recovered from the fruit fly pupae that emerged from the collected fruits.

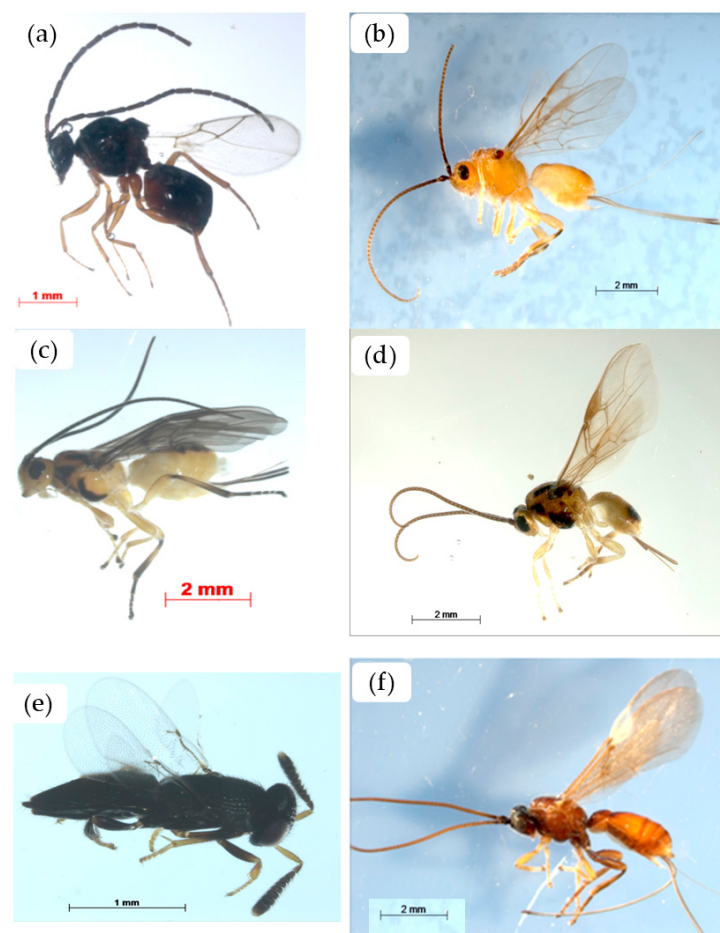


Figure 3. Parasitoid species identified in the South region of Rio Grande do Sul, RS, Brazil: (a) *Aganaspis pelleranoi* (Brèthes 1924), (b) *Doryctobracon areolatus* (Szépligeti, 1911), (c) *Opius* (*Bellopius*) *bellus* (Gahan, 1930), (d) *Utetes anastrephae* (Viereck, 1913), (e) *Cerchysiella insularis* (Howard, 1897), and (f) *Doryctobracon brasiliensis* (Szépligeti, 1911).

Despite the wide range of plant hosts surveyed in the current study, parasitoids were recovered only from five species of plants: *A. sellowiana*, *E. japonica*, *E. uniflora*, *P. cattleianum*, and *P. persica* (Table 5). The number of parasitoids recovered was low. An association between *A. fraterculus* and all the parasitoid species recovered in the current study was

confirmed (Table 5), except for the two Pteromalidae. The latter were only recovered from fruits of *E. uniflora*. In this study, we detected the association between *A. fraterculus* (fruit fly) and *C. insularis* (parasitoid) in *A. sellowiana* (vegetal host) for the first time. Moreover, *T. elegans* was detected for the first time outside the São Paulo state, with this being its first record in RS.

Table 5. Plant host species, site and parasitoid species associated with *Anastrepha fraterculus* recovered from fallen fruits and from fruits still on the tree in the Region of Pelotas, Rio Grande do Sul (Brazil), between 2013 and 2015.

Fruit Tree Species	Site	Parasitoid Species	Parasitoids Recovered from Fallen Fruits		Parasitoids Recovered from Fruits Still on the Tree	
			Total No.	kg Fruit	Total No.	kg Fruit
<i>Acca sellowiana</i>	Pelotas	<i>Doryctobracon areolatus</i>	2	2.4775	3	2.8718
	Pelotas	<i>Cerchysiella insularis</i>	5			
<i>Eugenia uniflora</i>	Morro Redondo	<i>Aganaspis pelleranoi</i>		0.0471	1	0.3615
	Pelotas	<i>Opius (Belopius) bellus</i>			1	0.11634
	Pelotas	<i>Odontosema albinerve</i>	4	0.01075	0	0.07462
<i>Psidium cattleianum</i>	Pelotas	<i>Aganaspis pelleranoi</i>	2	1.50363	0	1.62486
		<i>Doryctobracon areolatus</i>	2	0.35932	0	0.54765
<i>Eriobotrya japonica</i>	Pelotas	<i>Utetes anastrephae</i>	0	0.4507	5	9.5729
	Pelotas	<i>Doryctobracon brasiliensis</i>			1	1.1083
<i>Prunus persica</i>	Pelotas	<i>Aganaspis pelleranoi</i>	1	6.2314	0	0.3795

The total parasitism was 29.6% for *E. uniflora*, 2.38% for *P. persica*, 2.32% for *A. sellowiana*, 0.68% for *P. cattleianum*, and 0.31% for *E. japonica*. The specific parasitism differed among plant hosts (Figure 4). At least two parasitoid species were recovered from the plant hosts, except for *P. persica* in which only one parasitoid species was found.

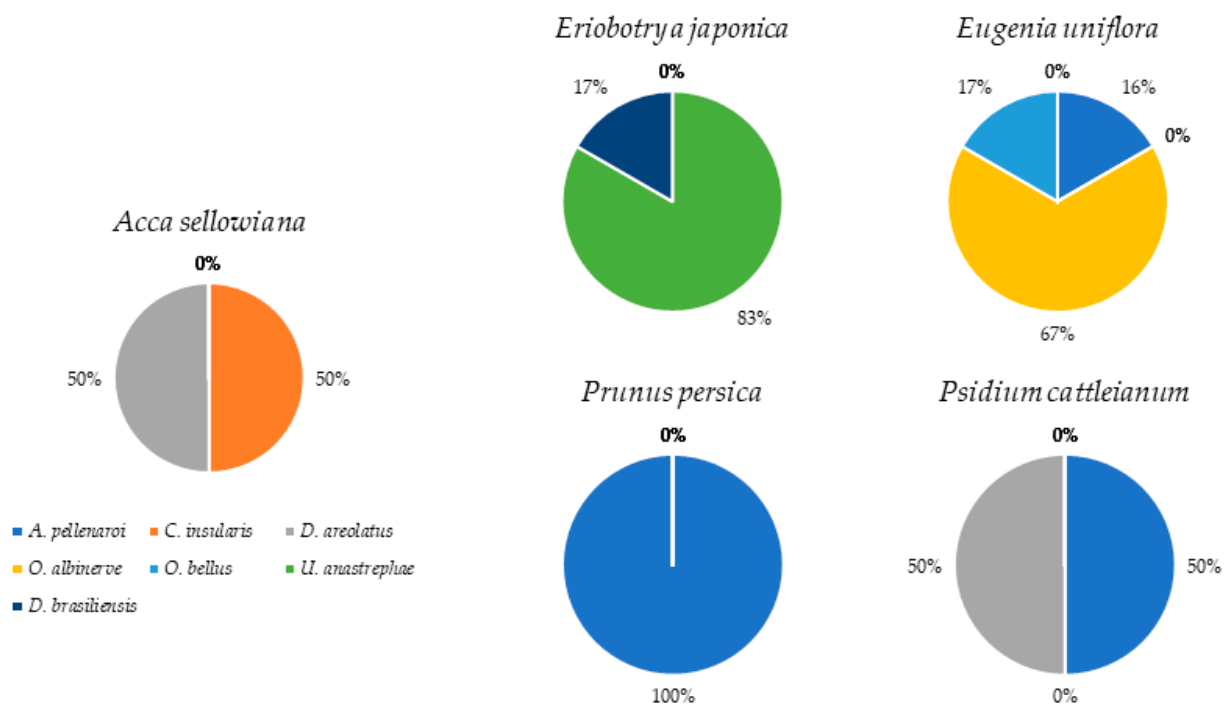


Figure 4. Specific parasitism in vegetal hosts of fruit flies in the Pampa Biome in Rio Grande do Sul, Brazil, between 2013 and 2015.

4. Discussion

4.1. Sampling of Fruit Flies with Traps

Anastrepha fraterculus was the main species of fruit fly detected in the current study, which is consistent with previous observations in this region that showed that *A. fraterculus* was responsible for 90.5% of all fruit fly infestations [13]. In the current survey, the fruit fly *C. capitata* was found in exotic hosts such as *Ficus carica* and *Citrus sinensis*. The infestation of *C. capitata* has been observed in the southern part of the RS State in exotic and native fruits [13,14]. Infestations of *C. capitata* in *Ficus carica* in this region were observed for the first time, although to a lower extent than infestations by *A. fraterculus*. Although *A. fraterculus* and *C. capitata* were frequently collected in monitoring traps, other flies were recovered rarely as *A. luederwaldti* and *A. deciformis*. These last two species have been previously reported in the southern part of RS [15,22]. However, the presence of the latter two species is residual, and they have no economic importance, as they do not constitute a pest due to their low population densities. The placement of the traps in the current study allowed us to verify the presence of these less abundant species, which make up part of the local biodiversity. However, it must be borne in mind that the intention of the traps was not for controlling pest populations.

4.2. Infestation Indices of Fruit Flies

The South American fruit fly and Mediterranean fly are polyphagous species, so they exploit a wide range of plant species [24,37]. Despite the diversity of hosts in which the fruit fly can develop, there is a proven preference among the infested fruits, with it being possible to classify them as primary and secondary [37]. In the current study, the highest infestation rates were observed in Myrtaceae and Rosaceae fruits, as previously reported for this region [13,48,49]. Among the species of the Rosaceae family, *Eriobotrya japonica* and *Prunus persica* presented the highest rates of infestation, similarly to those reported by Nunes et al. [13]. In addition, some infestation was observed in fruits of *Diospyros kaki* in the current study, contrasting with previous reports [13].

These results can be partially explained by the stimulating effect of Myrtaceae plants on fruit flies, which are guided to their hosts by chemical stimuli [24,37,50,51], as evidenced in laboratory studies in which volatile compounds from *E. uniflora*, *C. xanthocarpa* and *P. cattleianum* have caused the greatest electroantennographic responses in *A. fraterculus* females and males [52]. Studies in *P. guajava* and *P. cattleianum* extracts revealed the presence of eight bioactive substances in male antennae and seven in female antennae [53,54].

In the current work, high rates of infestation were recorded in *A. selloviana*, as previously reported [13,49]. The fruits of this plant remain green until harvest, but color is not a determinant factor for oviposition [52]. In contrast, a combination of physical and chemical factors, as well as fruit fly density per host, seems to be determinant at the time of oviposition. In a situation of high population density, or in the absence of a preferred host, there is an increase in the acceptance of hosts initially considered as secondary or even inadequate [37,55,56]. In this context, identifying secondary host plants is relevant for controlling fruit fly attacks in an efficient manner. The low rates of *A. fraterculus* infestation in *Rubus* sp. observed in this survey suggest that this plant species is a secondary host, matching results from laboratory studies in which the longest periods of oviposition, fecundity and longevity were observed for *A. fraterculus* fed on fruits of *E. uniflora* and *P. cattleianum* during the larval stage when compared to those fed on *Rubus* sp. [57]. This information suggests that the greater attractiveness of flies to hosts of the Myrtaceae family is related also to their suitability for fly development.

In the region where this survey was conducted, *C. capitata* occupies mainly niches of exotic hosts, such as orange and fig, and co-occurs with *A. fraterculus* to a lesser extent.

However, in other zones of the Rio Grande do Sul state (Brazil), *C. capitata* was found infesting more vegetal hosts than *A. fraterculus* [14]. The competitive interaction between two species can result in ecological displacements, with one or both species changing or reducing their niche until coexistence becomes possible [58]. Therefore, additional studies should be conducted to better understand the reasons that lead to the prevalence of *A. fraterculus* in relation to *C. capitata* or vice versa between geographically close regions.

4.3. Parasitism

Seven species of parasitoids were associated with *A. fraterculus* in this survey, but only five of them were previously recorded in Rio Grande do Sul [13,59], with *C. insularis* parasitizing *A. fraterculus* observed for the first time in this study. This species of parasitoid has been previously associated with *Anastrepha suspensa* [60]; however, it is mainly associated with Coleoptera (Nitidulidae) [61]. Another parasitoid species detected in the current work was *D. areolatus*, which was associated with *A. fraterculus* in *P. cattleianum* and *A. sellowiana*, as previously reported in this region [13,59] and other zones of Rio Grande do Sul state [49]. The braconid *D. areolatus* has an ovipositor longer than that of *O. bellus* [62], and has been cited by several authors as the most common parasite of tephritid flies in different plant species in Brazil [13,63–68]. For instance, Leonel Jr. et al. [69] found *D. areolatus* parasitizing fruit flies in 21 species of fruit trees in ten Brazilian states, including Rio Grande do Sul. Two species, *A. pelleranoi* and *O. bellus*, were associated with *A. fraterculus* in *E. uniflora*, corroborating previous reports [13,59]. In addition, *U. anastrephae* and *D. brasiliensis* were associated with *A. fraterculus* in *E. japonica*, and this association was not observed for the Pelotas region prior to the current study. In part, this association with *E. uniflora* and *E. japonica* may be related to the morphology of *U. anastrephae* and *O. bellus*, which have a small ovipositor and therefore prefer less thick fruits [46]. In *P. persica* and *P. cattleianum*, *A. pelleranoi* was the parasitoid species found, as previously observed by Nunes et al. [13]. No parasitoids were recovered from *P. guajava*, in contrast with previous reports [13,59]. This parasitoid, *A. pelleranoi*, locates its host by vibrotaxis, being more attracted by volatiles of fruits containing tephritid larvae, showing no preference between *A. fraterculus* or *C. capitata* [70]. These data are relevant, since both species of flies infest the peach tree [14,64].

Another parasitoid species found in the current survey, although not associated with *A. fraterculus*, was *T. elegans*, which is a Pteromalid ectoparasitoid used to suppress the larval stage of several stored-product insect pests [71], as a biological control agent [72,73]. It has also been reported to attack coleopteran and lepidopteran pests [74]. This species, *T. elegans*, has been previously recorded in São Paulo [75], but this study represents the first record of this species in RS. The other Pteromalidae species, *A. calandrae*, is also a biocontrol agent against stored-product pests [76].

The information presented in this survey reveals a pattern of association between species of parasitoids, flies and plant hosts in the municipalities of Pelotas, Morro Redondo and Capão do Leão (RS, Brazil). A very small number of parasitoids were obtained in this survey, which can be due to the fact that fruits were collected in the surroundings and/or directly in areas of conventional production. Previous studies have shown higher abundance of parasitoids, but they were conducted in non-commercial orchards or under organic management [13,49]. The level of conservation of a given site can have significant influence on both the quantity and diversity of parasitoid species, as in the least disturbed environment the tendency is to find more species [23]. Future studies in the Pelotas region may focus on the investigation of the effect of agricultural practices, landscape and climatic effects on the parasitoid community.

5. Conclusions

This study assessed fruit infestation by tephritid flies (*A. fraterculus* and *C. capitata*) in native and exotic fruit trees in the southern region of the state of Rio Grande do Sul (Brazil). Additionally, in this work, seven species of parasitoids were associated with *A. fraterculus*: *D. areolatus*, *D. brasiliensis*, *A. pellenaroi*, *O. bellus*, *U. anastrephae*, *C. insularis*, and *O. albinervae*. However, parasitism rates were low. In addition, a species of parasitoid was recorded for the first time in RS: *T. elegans*. The plant species considered as primary hosts of fruit flies were Myrtaceae (*P. guajaba*, *E. uniflora*, and *A. sellowiana*) and Rosaceae (*P. persica* and *E. japonica*). Secondary hosts include *Fragaria* × *ananassa*, *Rubus* sp., *C. aurantium*, *C. sinensis*, *F. carica*, *C. betacea*, and *V. breviflora*. Finally, the main host multipliers of parasitoids were *P. cattleianum*, *E. japonica*, *A. sellowiana*, *E. uniflora*, and *P. persica*.

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Abbreviations

The following abbreviations are used in this manuscript:

RS Rio Grande do Sul
F1 to F4 Farms 1 to 4

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