

Original Article

Natural parasitism in fruit fly (Diptera: Tephritidae) and interaction with wild hosts surrounding apple orchards adjacent to Atlantic Forest fragments in Paraná State, Brazil

Parasitismo natural de mosca-das-frutas (Diptera: Tephritidae) e interação com hospedeiros silvestres em pomares de macieira adjacentes a fragmentos de Mata Atlântica no Estado do Paraná, Brasil

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Abstract

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), is an important pest in the subtropical region of Brazil. This insect has tritrophic relation between wild fruits and parasitoids and is associated with apple (*Malus domestica* Borkh.) orchards adjacent to the Atlantic Forest in Paraná. We thus investigated the degree of infestation of the fruit fly and natural parasitism in wild and cultivated fruits surrounding apple orchards. For this purpose, we collected fruits of *Acca sellowiana* (Berg.) Burret, *Campomanesia xanthocarpa* (Mart), *Eugenia uniflora* L., *Eugenia pyriformis* Cambessèdes, *Psidium cattleianum* Sabine, *Psidium guajava* (L.), *Annona neosericea* Rainer and *Eriobotrya japonica* (Thumb) in apple orchards adjacent to the Atlantic Forest located in Campo do Tenente, Lapa and Porto Amazonas counties. In total, we collected 18,289 fruits during four growing years. The occurrence of *A. fraterculus* depends on the susceptible period of apple fruits. *A. sellowiana* and *P. cattleianum* were considered primary fruit fly multipliers and *P. guajava* was secondary, all occurring after the apple harvest (IS period). The group of parasitoids with *A. fraterculus* was *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), *Opius bellus* (Gahan, 1930), *Doryctobracon areolatus* (Szépligeti, 1911) and *Doryctobracon brasiliensis* (Szépligeti, 1911) (Hymenoptera: Braconidae) all of which are first records in the Atlantic Forest in Paraná. First record of *O. bellus* occurring in the State of Paraná, as well as, first record of the tritrophic association between host plant *A. neosericea*, parasitoids *D. areolatus* and *O. bellus* and fruit fly *A. fraterculus*. The host *P. cattleianum* stood out among the Myrtaceae species in regard to the high diversity of parasitoid species (81% of parasitoids). The total number of Figitidae species (76.5%) was higher than that of Braconidae species. The influence of climatic events in southern Brazil on wild fruit production should be further studied to understand the association of *A. fraterculus* with the tritrophic relationship.

Keywords: Atlantic Forest, landscape, fruit flies, parasitoid interaction, diversity.

Resumo

Mosca-das-frutas sul-americana, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), é uma importante praga da região subtropical do Brasil. Este inseto tem relação tritrófica entre frutos silvestres e parasitoides e está associado a pomares de macieiras (*Malus domestica* Borkh.) adjacentes à Mata Atlântica no Paraná. Assim, investigamos o grau de infestação da mosca-das-frutas e o parasitismo natural em frutas silvestres e cultivadas ao redor de pomares de maçã. Para tanto, foram coletados frutos de *Acca sellowiana* (Berg.) Burret, *Campomanesia xanthocarpa* (Mart), *Eugenia uniflora* L., *Eugenia pyriformis* Cambessèdes, *Psidium cattleianum* Sabine, *Psidium guajava* (L.), *Annona neosericea* Rainer e *Eriobotrya japonica* (Thumb) em pomares de maçã adjacentes à Mata Atlântica localizados nos municípios de Campo do Tenente, Lapa e Porto Amazonas. No total, coletamos 18.289 frutos durante quatro anos de cultivo. A ocorrência de *A. fraterculus* depende do período de suscetibilidade dos frutos da maçã. *A. sellowiana* e *P. cattleianum* foram considerados multiplicadores primários de mosca-das-frutas e *P. guajava* foi secundário, todos ocorrendo após a colheita da maçã (período IS). Os parasitoides associados a *A. fraterculus* foram *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae), *Opius bellus* (Gahan, 1930), *Doryctobracon areolatus* (Szépligeti, 1911) e *Doryctobracon brasiliensis* (Szépligeti, 1911) (Hymenoptera: Braconidae), todos os quais são primeiros registros na Mata Atlântica no Paraná. Primeiro registro de *O. bellus* ocorrendo no Estado do Paraná, assim como, primeiro registro da associação tritrófica entre o hospedeiro *A. neosericea*, parasitoides *D. areolatus* e *O. bellus* e mosca-das-frutas *A. fraterculus*. O hospedeiro *P. cattleianum* se destacou entre as espécies de Myrtaceae pela alta diversidade de parasitoides associados (81% dos parasitoides). O número total de espécies de Figitidae (76,5%) foi superior ao de espécies de Braconidae. A influência de eventos climáticos no sul do Brasil na produção de frutas silvestres deve ser mais estudada para entender a associação de *A. fraterculus* com a relação tritrófica.

Palavras-chave: Mata Atlântica, paisagem, mosca-das-frutas, parasitoide, diversidade.

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1. Introduction

The population dynamics of fruit flies and their associated natural enemies is strongly influenced by habitat structure (Aluja et al., 2014; Schliserman et al., 2014). Attacks on fruit flies remain an important phytosanitary challenge, limiting fruit production worldwide (Montoya et al., 2016) due to the damage to the fruit pulp caused by larvae (Bisognin et al., 2015).

The genus *Anastrepha* Schiner stands out among the family Tephritidae in the Neotropical region, which extends from the south of the United States to the north of Argentina (Norrbom et al., 1999). Today, there are 283 species within this genus (Norrbom and Korytkowski, 2009). Brazil has the largest number of *Anastrepha* species (121), ten of which cause economic losses (Zucchi, 2000). In Paraná State, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), is the main pest of apple (*Malus domestica* Borkh.) orchards, and the apple-growing areas in Paraná State are adjacent to patches of native Atlantic Forest (Monteiro et al., 2019). Worldwide, the Atlantic Forest biome is recognized as an important hotspot of biodiversity (Myers et al., 2000). The management capabilities of current agro-ecosystems are reduced, and this situation necessitates rethinking the management of fruit flies. Today, integrated pest management (IPM) programs against fruit flies focus on a more sustainable approach to mitigate the adverse effects commonly associated with the use of pesticides.

The majority of parasitoids associated with Tephritidae belong to the subfamilies Opiinae (Braconidae) and Eucoilinae (Figitidae) (Guimarães and Zucchi, 2004). In Brazil, several studies of Tephritidae fruit flies, hosts and parasitoids have been carried out in different locations with a diversity of habitat and climate conditions (Silva et al., 2010; Souza et al., 2012; Adaime et al., 2018). All these surveys showed that specimens of Braconidae and Figitidae have potential for use in biological control (Garcia and Corseuil, 2004; Nunes et al., 2012; Gonçalves et al., 2016). Understanding the abundance and parasitism level of

these species constitute essential information to design biological control programs (García-Medel et al., 2007).

Therefore, the aim of the current study was to assess the degree of infestation and natural parasitism in wild and cultivated fruit commonly attacked by fruit fly, as well as to provide more detailed information on the diversity and abundance of parasitoids in apple-growing areas in Paraná State. In this study, we documented: i) tritrophic interactions among hosts, fruit flies and their natural enemies; ii) infestation rates by systematically collecting wild and commercial fruits over four growing seasons (2013/14– 2016/17) in apple orchards adjacent to patches of native vegetation.

2. Materials and Methods

2.1. Study area

The study was conducted on six farms growing both ‘Gala’ and ‘Eva’ apple cultivars located in the counties of Campo do Tenente (CT), Lapa (LA) and Porto Amazonas (PA), Paraná State, which constituted 250, 110 and 130 hectares, respectively. The cultivar ‘Eva’ is early-maturing and has low chilling requirements, whereas the cultivar ‘Gala’ is mid-maturing and produces fruits later than ‘Eva’ (Hauagge and Tsuneta, 1999). Most of the apple orchards are cultivated with 35% ‘Eva’ and 55% ‘Gala’; each orchard contains 10% of pollinator apple cultivars.

2.2. Climate

The period of study comprised four growing seasons (August to July) from 2013/14 to 2016/17 (Y1–Y4). The climate in southern Brazil is humid-temperate with moderately hot summers and no dry season and is characterized by low temperatures between May and September, with a gradual increase of temperature to December (Aparecido et al., 2016). During the study period, the annual average temperature (Tave) was 17.6°C and varied slightly among years (from 17.3°C in Y1 to 18.0°C in Y3), but Y3 was the hottest year (Figure 1).

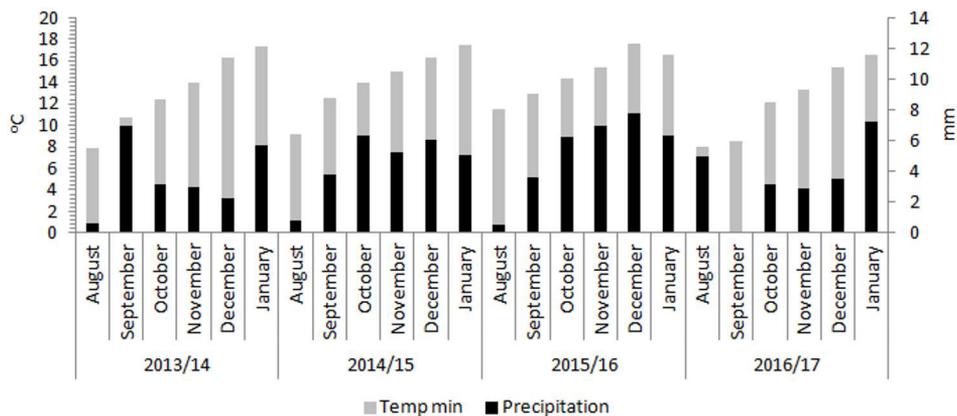


Figure 1. Minimum air temperature (°C) and rainfall (mm) monthly in Porto Amazonas, Brazil, from September to January over four growing seasons (Meteorological System of Paraná – SIMEPAR).

The minimum temperature (T_{min}) in August, September, October and November in (Y2) was 1.2°C, 1.9°C, 1.5°C and 1.0°C higher, respectively, than that the same period in Y1, as the T_{min} in Y3 was 2.3°C, 0.3°C, 0.5°C and 0.5°C higher than that in Y2, respectively. In the last year, the T_{min} was 3.4°, 4.4°, 2.3° and 2.2°C colder than that in Y3 and 1.1°, 4.0°, 1.8°, respectively, and 1.7°C colder than that in Y2 (Meteorological System of Paraná – SIMEPAR).

The total rainfall per season varied from 1,345.0 to 1,626.6, 1,901.1 and 1,202.4 mm from Y1 to Y4, respectively. The mean daily precipitation in October, November and December in Y2 was 3.1, 2.3 and 3.8 mm higher, respectively, than that in Y1. Overall, Y3 was characterized as a very strong El Niño according to the Oceanic Niño Index and was considered the most intense El Niño in the last 40 years (Ferreira et al., 2016; INPE, 2016), leading to 51 mm more rainfall each month. Both Y2 and Y3 were rainy years in the six and seven months, respectively, but the precipitation in Y2 was slightly more intense in September and October than that in Y3. In contrast, Y4 was a year of very little rainfall, with no rain in September and less daily precipitation, 3.1, 4.1 and 4.2 mm in October, November and December, respectively, compared to that in Y3 (SIMEPAR).

2.3. Landscape

The native vegetation in this area is a mixed-ombrophilous southern Atlantic Forest biome, which is recognized worldwide as a hotspot of biodiversity (Myers et al., 2000). To characterize the landscapes adjacent to commercial apple orchards, monitoring of native and exotic plant species that may have a relationship with fruit fly was carried out (Foelkel, 2015). This was done from the border of all the apple orchards up to 50 m within the forest, divided into sectors every 100 m, by walking along and within the forest during the year preceding the beginning of the experiment (Schliserman et al., 2014; Araujo et al., 2019).

The agricultural year was defined as beginning in August, with the breaking of dormancy. Two sampling periods were established based on the phenology of two apple cultivars: the susceptibility (S) and insusceptibility (IS) periods (Araujo et al., 2019). The susceptible period was subdivided into S1, which was composed of 30 days after full bloom, when the apples were at the “J” stage of development (i.e., fruits were between 20 to 25 mm in diameter), and characterized by a low fluctuation history of fruit flies in the region, and S2, covering 45 days before harvest, which coincides with a high fluctuation of fruit flies and their control (Araujo et al., 2019). The dates of S1 ranged from 1/9 to 20/10, and those of S2 ranged from 21/10 to 10/2. The IS period was the time from the postharvest of apples until stage J.

Five orchards used similar IPM, and only one orchard sprayed more insecticides than the average amount used by the other orchards and was denominated the conventional orchard (CO). The insecticides sprayed in S1 were almost exclusively for Lepidoptera (Tortricidae) pest control (mean 3.1 applications in IPM) in 95.5% of cases. In the CO, there was an average increase of 61.3% than the IPM used in the other orchards. In S2, the average amount of

insecticides used increased (mean 3.8 applications in IPM) with a higher occurrence of fruit fly. The total number of sprays in the CO was 75% higher than that in the IPM used in the other orchards.

2.4. Fruit sampling and insect emergence

Fruits were collected from nine hosts tree demarcated, packed in separate plastic boxes (from the tree or the soil), and sent to the IPM Laboratory at the Federal University of Paraná for insect identification. In the laboratory, fruits were counted from each sample, weighed and arranged in plastic boxes (30 cm length x 20 cm width x 15 cm height) with a 2 cm layer of vermiculite, which served as a pupation substrate. Each plastic box was closed with vented lids covered with organza. All samples were kept in climate-controlled chambers at $25 \pm 1^\circ\text{C}$ and 70% RH with a photoperiod of 16:8 (L:D) h. The vermiculite was examined weekly to remove pupae for rearing and discarded after 30 days from sampling.

To obtain parasitoids, one new procedure for the organization of pupae was developed using 48-well microplates (127.6 mm length, 85.5 mm width, and 20.2 mm depth) (Kasvi, China) adapted for daily observation over 60 days. The recovered pupae were transferred into transparent culture plates with a single pupa deposited in each well. The plates were covered with a filter and closed with a polystyrene lid. The filter of each plate was in contact with its pupa, and it was moistened with distilled water. The plates were kept in climate-controlled chambers at $25 \pm 1^\circ\text{C}$ and 70% RH for a photoperiod of 16:8 (L:D) h. The parasitoids and fruit flies that emerged were stored individually in vials with 92% ethanol for subsequent identification.

2.5. Identification of fruit flies and parasitoids

Fruit fly specimens of the genus *Anastrepha* were sexed and identified using Zucchi (2000). Female species were identified based primarily on the aculeus, body and wing markings. Braconidae parasitoids were identified based on Daza and Zucchi (2000).

2.6. Data summary

Fruit infestation was calculated either as the number of pupae per fruit and as the number of pupae per kg of fruit to account for differences in individual fruit weight among hosts (Marsaro Júnior et al., 2013). The pupae viability in each host was calculated by the number of adults+parasitoids/pupae*100. Hosts were classified into primary, secondary and tertiary multipliers by classes based on the quartiles and the number of pupae per kg of fruit, which was determined using the Excel program (Microsoft, San Francisco, USA). The first, second, third and fourth quartiles were defined as 0-10, 1-39, 40-132 and more than 133 pupae per kilo, respectively, using all data. Multiplier hosts were calculated using the formula fruit fly number in the three+four quartiles by total fruit fly number*100, so the primary host represented more than 65.0%, the secondary represented between 35.0% and 65.0%, and the tertiary represented less than 35.0%.

The percentage of parasitism was calculated by dividing the number of emerged adult parasitoids by the total number of pupae in all samples of the host*100 (Schliserman et al., 2010). The sample parasitism percentage was calculated by dividing the number of samples with parasitoids by the number of samples with fruit fly pupae*100. The percentage of parasitoid species in relationship to the parasitoid community was calculated as the number of parasitoids divided by the total number of parasitoids*100.

The ratio of parasitism was calculated as the number of parasitoids in each sample to the number of adult fruit flies plus the number of parasitoids. In this case, the rate was related to the fruit flies that emerged and excluded natural or methodological mortality.

2.7. Statistical analyses

The variation in the number of pupae per sample was analysed using a general linear model including the farm (6 levels), the sampling year (Y1-Y4), the host (*E. uniflora* was excluded due to its small sample size), the weight of the fruit sample (quantitative) and the interaction of the last two variables. We used a negative binomial distribution to account for overdispersion in the data. Model residuals were inspected visually (package DHARMA) with R.3.4.1 software (R Development Core Team, 2017). When a factor was significant, pairwise comparisons between factor levels were performed using post-hoc Tukey tests (package multcomp) with R.3.4.1 software. The same model was used to analyse apple infestations, although the host factor was removed from the model in this case.

To assess factors explaining variations in parasitism in fruit fly hosts, the proportion of parasitoids among recovered adults was analysed using a generalized linear model including the host plant (Levels) and the number of recovered adults as fixed factors. The observation identifier was included as a random factor to account for overdispersion of data. All the model residuals were inspected visually (package DHARMA). When a factor was significant, pairwise comparisons between factor levels were investigated using post-hoc Tukey tests (package multcomp).

3. Results

3.1. Host plants in the experimental area

Characterization of the surroundings of the apple orchards revealed eight species fruit fly host species: the Myrtaceae family - *Acca sellowiana* (Berg.) Burret, *Campomanesia xanthocarpa* (Mart.), *Eugenia uniflora* L., *Eugenia pyriformis* Cambessèdes, *Psidium cattleianum* Sabine and *Psidium guajava* L.; Annonaceae family - *Annona neosericea* Rainer; and Rosaceae family - *Eriobotrya japonica* (Thumb) (Table 1).

CT contained four fruit fly hosts, LA contained seven hosts and PA contained only one host (Table 2). *A. neosericea* was present in the three municipalities surveyed (PA, CT and LA); *C. xanthocarpa*, *E. uniflora* and *P. cattleianum* were found in two of the three municipalities surveyed (CT and LA); and finally, *A. sellowiana*, *P. guajava* and *E. japonica* were only present in LA. Fruits from *E. uniflora*, *E. pyriformis*

Table 1. Fruit fly hosts and samples of fruits in the Paraná Atlantic Forest Brazil (2013-17).

Plant family	Wild and commercial hosts	Common name	Status	Fruit (n)	Weight (kg)
Annonaceae	<i>Annona neosericea</i> Rainer	Araticum	Native/Wild	175	9.1
Myrtaceae	<i>Acca sellowiana</i> (Berg.) Burret	Feijoa	Native/Wild	584	15.2
	<i>Campomanesia xanthocarpa</i> (Mart.)	Guabiroba	Native/Wild	2501	9.5
	<i>Eugenia uniflora</i> Linnaeus	Pitanga	Native/Wild	86	0.2
	<i>Eugenia pyriformis</i> Cambessèdes	Uvaia	Native/Wild	13	0.2
	<i>Psidium cattleianum</i> Sabine	Araçá	Native/Wild	5664	46.4
	<i>Psidium guajava</i> Linnaeus	Guava	Native/Wild	488	14.4
	Rosaceae	<i>Eriobotrya japonica</i> (Thumb.)	Loquat	Exotic/Wild	595
	<i>Malus domestica</i> Bork.	Apples	Exotic/Cultivated	8183	627.1

Table 2. Sampling period in wild and exotic fruit fly hosts in three periods of susceptible apples during four growing years in three municipalities of Paraná, Brazil (2013-17).

Plant family	Fruit host	Area ¹	Susceptible period ²	Months														
				Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.			
Rosaceae	<i>E. japonica</i>	LA	S1 and IS	X	X												X	
	<i>M. domestica</i>	CT	S1 - S2	X	X	X	X	X	*	*	*	*						
	'Eva'	LA	S1 - S2	X	X	X	X	X	*	*								
		PA	S1 - S2	X	X	X	X	X	*	*	*	*	*	*	*	*		
	<i>M. domestica</i>	CT	S1 - S2		X	X	X	X	X	*	*	*						
	'Gala'	LA	S1 - S2		X	X	X	X	X									
		PA	S1 - S2		X	X	X	X	X									
	<i>M. domestica</i>	CT	IS		X	X	X	X	X	X	X	X	X	X	X	X	X	
	'pollinater'	PA	IS		X	X	X	X	X	X	X	X	X	X	X	X	X	
	Myrtaceae	<i>C. Xanthocarpa</i>	CT	S1 - S2				X	X									
		LA	S1 - S2		X	X	X											
<i>E. uniflora</i>		LA	S1 and IS				X	X				X						
<i>E. pyriformis</i>		CT	IS									X	X					
		LA	IS									X	X					
<i>A. sellowiana</i>		LA	IS							X	X							
<i>P. cattleianum</i>		CT	IS							X	X	X						
<i>P. guajava</i>		LA	IS									X	X					
Annonaceae		<i>A. neosericea</i>	CT	IS							X	X	X					
			LA	IS									X					
		PA	IS									X		X				

¹ CT = Campo do Tenente; LA = Lapa; PA = Porto Amazonas. ² Susceptibility (S1 and S2) and insusceptibility period (IS). ³Post-harvest fruits.

and *A. sellowiana* were sampled during Y3 and Y4, and fruits from *C. xanthocarpa* and *E. japonica* were sampled in Y2, Y3 and Y4 due to the heterogeneity in the fruit production of these trees.

3.2. Fruit samples

Fruit sampling in the S1 period occurred in four hosts: *M. domesticus*, *E. japonica*, *E. uniflora* and *C. xanthocarpa*; fruit sampling in the S2 period occurred in two hosts: *M. domesticus* and *C. xanthocarpa*. Most of the native hosts were sampled during IS: *A. neosericea*, *P. cattleianum*, *A. sellowiana*, *P. guajava*, *E. uniflora* and *E. pyriformis*.

A total of 18,289 fruits (731.66 kg) were sampled from 1,396 samples, of which 17.1% were native host fruits and the remainder were exotic hosts (Table 1 and 3). In addition, 60.8% (n= 829 samples) were collected during the S period and 41.4% (n= 567) in IS. In S1, there were 65 samples (7.8% samples of the S period; n₁= 1,098 fruits; n₂= 14.3% of all fruits in S), of which 85.0% were apple fruits, 11.0% were *E. japonica*, and the remainder were early *C. xanthocarpa*. In S2, there were 764 samples (n₁= 6,579 fruits; n₂= 85.7% of all fruits in S), of which 96.2% were apple and 3.3% were *C. xanthocarpa*. In IS, 10,612 fruits were collected, of which 62.1% were apple (n₁= 3,504 fruits; n₂= 33.0% of all fruits in

IS) and 21.7% were *P. cattleianum* (n₁= 5,664; n₂= 53.4.0%), while the other hosts had 6.0% each.

3.3. Index of pupae fruit flies

Fruit fly pupae were collected from 55.7% of the samples (n= 780 samples with pupae; n₁= 27,531 pupae) and 16.6% were only collected from wild hosts (n= 232; n₁= 23.037; n₂= 83.6% of all pupae). Only 27.0% of the pupae did not emerge. All the identified flies were *A. fraterculus*.

The number of pupae per fruit of all hosts varied by growing season; 6.6% of the total pupae were collected in Y2; 25.1% and 17.6% of the total pupae were collected in Y1 and Y3, respectively; and 50.7% of the total pupae, the highest number, were collected in Y4. The wild hosts in S had 0.5 pupae per fruit (S1= 1.0 pupa per fruit, n₁= 606 total pupae in period; S2= 0.3, n₁= 326) compared to 4.1 pupae per fruit in the IS period (n₁= 22,105). The number of pupae per fruit of all hosts in S was 0.3 (S1= 0.0 pupa per fruit, n₁= 4; S2= 0.3, n₁= 1,339), increasing by four times in IS (n₁= 3,504).

In the apple, the number of pupae increased with the weight of the samples (F= 35.4, p= <0.0001) and depended on the growing season (Chi²= 24.8, df= 3, p= <0.0001); the number of pupae was higher in Y2 than in Y1, and no

Table 3. Number of *Anastrepha fraterculus* and parasitism per host during four growing seasons in Paraná Atlantic Forest, Brazil (2013-2017).

Host plant	Growing season	Fruit sampled (n)	Fruit sampled (kg)	Pupae (n)	Pupae/kg	Pupae/fruit	Fruit fly adult (n)	Parasitoid (n)	Parasitism (%) ¹
<i>M. domestica</i>	2013/14	5164	414.8	2983	7.1	0.5	2384	4	0.4
	2014/15	1554	103.6	927	13.7	1.1	630	1	0.1
	2015/16	1410	105.3	579	9.1	0.6	373	0	0.0
	2016/17	25	3.4	5	1.5	0.2	2	0	0.0
<i>P. cattleianum</i>	2013/14	394	8.4	3621	457.3	10.5	2364	177	4.6
	2014/15	10	0.1	26	216.7	2.6	18	0	0.0
	2015/16	1464	14.4	1194	92.8	1.0	876	13	5.4
	2016/17	3796	23.5	9036	394.4	2.8	5795	226	4.8
<i>A. neosericea</i>	2013/14	77	5.8	152	24.7	1.9	103	0	0.0
	2014/15	47	1.5	148	102.4	3.3	140	0	0.0
	2015/16	38	1.6	162	88.2	4.8	120	3	1.0
	2016/17	13	0.2	2	9.1	0.3	0	0	0.0
<i>P. guayava</i>	2013/14	35	1.5	166	125.3	9.0	140	0	0.0
	2014/15	47	2.7	103	37.7	2.0	71	0	0.0
	2015/16	342	8.4	1655	194.7	4.8	1516	24	1.5
	2016/17	64	1.8	125	68.1	2.0	76	1	1.0
<i>C. xanthocarpa</i>	2014/15	20	0.1	18	180.0	0.9	5	0	0.0
	2015/16	937	3.3	239	90.9	0.4	172	17	9.6
	2016/17	1544	6.1	92	13.4	0.1	43	1	1.2
<i>E. japonica</i>	2014/15	489	6.6	594	81.9	1.3	536	0	0.0
	2015/16	79	2.3	9	2.9	0.1	9	0	0.0
	2016/17	27	0.8	5	6.5	0.2	2	0	0.0
<i>A. sellowiana</i>	2015/16	205	4.4	988	216.7	5.2	518	7	1.6
	2016/17	379	10.8	4668	438.9	12.6	3671	42	1.1
<i>E. uniflora</i>	2015/16	26	0.1	12	150.0	0.6	3	0	0.0
	2016/17	60	0.1	1	6.7	0.0	1	0	0.0
<i>E. pyriformis</i>	2016/17	13	0.2	21	194.4	2.1	9	0	0.0

¹ Number of parasitoid/number of pupa, calculated with all samples.

other pairwise difference was significant. The number of pupae per kg of fruit in wild hosts was 242.9 in IS against 59.1 pupae in S (S1= 70.3; S2= 55.1), and in apple hosts, it was 18.5 and 3.1 (S1= 0.5 and S2= 3.3), respectively. In native hosts, fruit infestation was high with a large variability among years (Table 4) and was higher in Y4 than in Y1 (F= 1.82, P= 0.003) and Y3 (F= 0.78, P= <0.001) and higher in *A. sellowiana* (364.9 pupae per kg), *P. cattleianum* (302.6), *E. pyriformis* (194.4) and *P. guajava* (132.9) than in the other species. Among Rosaceae, *E. japonica* and apple had 52.0 and 7.8 pupae per kg, respectively.

Using the quartile model, the *A. sellowiana* and *P. cattleianum* hosts were considered to be primary multipliers, with 87.5% and 67.5% of pupae in the 3+4 quartiles (from 292.8 to 1.730.4 pupae per kg), respectively. *P. guajava* was ranked as a secondary multiplier

Table 4. ANOVA of the number of *A. fraterculus* pupae in six native hosts¹ as a function of year, sample weight, host, and the interaction between the two latter variables.

	Chisq	Df	Pr (>Chisq)
Years	22.949	3	4.1 10 ⁻⁵ ***
Weight of fruits	13.86	1	1.9 10 ⁻⁴ ***
Host	74.386	6	5.1 10 ⁻¹⁴ ***
Host x weight	11.749	6	0.07

¹ *E. uniflora* and *E. pyriformis* were removed from the statistical analysis due to the small number of samples. *** 0.001.

with 44.4% of pupae, and the other hosts were defined as tertiary multipliers (below 35% of pupae).

Analysing adult emergence in wild hosts in the S period, there were 746 adults (S1= 547, n₁= 73.3% of S period;

S2= 199.0). During the IS period, 15,442 adults (95.4% of all adults) were recovered. The viability of pupae was high in *E. japonica* (87%) and *C. xanthocarpa* (90%) in S1, excluding pupae that were parasitized. In S2 there was a 34.4% reduction in pupae viability in *C. xanthocarpa* in relation to that in S1, while in *E. uniflora*, there were no pupae. Viability during IS in *A. neosericea*, *P. cattleianum*, *A. sellowiana*, *P. guajava* and *E. japonica* was 61.0%, 71.0%, 75.0%, 83.0% and 85%, respectively, but in *E. pyriformis* and *E. uniflora*, viability was lower (33.0% and 50%, respectively). The pupae collected from apple in S1 had lower viability (50.0%) than those from S2 (72.0%), while the viability of pupae collected in IS was 67.0% without insecticide pressure. In IS, the viability average percentage of pupae collected from Myrtaceae and Rosaceae was similar (71.0 and 68.0%, respectively).

3.4. Parasitoid species

The species identified in this study were *Doryctobracon areolatus* (Szèpligeti, 1911), *Doryctobracon brasiliensis* (Szèpligeti, 1911), *Opius bellus* (Gahan, 1930) (Braconidae: Opiinae), *Aganaspis pelleranoi* (Brèthes, 1924) and *Aganaspis* sp. (Figitidae: Eucoilinae) (Table 5). *Aganaspis* was the most abundant genus, totalling 76.6% of all parasitoids, with *A. pelleranoi* representing 16.7% and *Aganaspis* sp representing 59.9%. Among Braconidae (23.4%), *D. areolatus* was the most abundant (14.0%), followed by *D. brasiliensis* (8.5%) and *O. bellus* (0.9%). Almost the braconid and figitid species recovered in this study were collected from fallen fruits (91.7% and 87.3%, respectively).

The emergence of parasitoids occurred on six of the nine hosts (the exceptions were *E. japonica*, *E. uniflora* and *E. pyriformis*) (Table 5). Most parasitoids emerged from *P. cattleianum* (416 parasitoids), followed by *A. sellowiana* (49), *P. guajava* (25) and *C. xanthocarpa* (18) ($P < 0.001$). The largest diversity of parasitoids occurred in *P. cattleianum*, with five species present, followed by *C. xanthocarpa* and *A. sellowiana*, with four species each (Table 5). In contrast, a single species was recovered from apple. These data correlated well only the number of

pupae per kg of fruit of fruit fly with in *P. cattleianum* for Braconidae ($r^2 = 0.90$) and Figitidae ($r^2 = 0.75$).

We observed that 516 parasitoids emerged from 27,531 fruit fly pupae recovered from 88 samples with parasitoids (6.3%). The sample parasitism percentage was higher in Myrtaceae than Rosaceae species. In S2, *C. xanthocarpa* was the only host that had parasitism, with SPP= 28.6% of samples with pupae ($n_1 = 6$ samples with parasitoids, $n_2 = 21$ samples with pupae), and the sample parasitism percentage was 0.8% in apple ($n_1 = 2$, $n_2 = 243$). In the IS period, the sample parasitism percentage was 50.0% in *A. sellowiana* ($n_1 = 12$, $n_2 = 24$), 45.8% in *P. cattleianum* ($n_1 = 54$, $n_2 = 118$), 33.3% in *P. guajava* ($n_1 = 9$, $n_2 = 27$), 0.8% in *A. neosericea* ($n_1 = 2$, $n_2 = 21$) and 1.0% in apple ($n_1 = 3$, $n_2 = 301$).

The percentage of parasitism in the S1 period was null for two hosts, *E. japonica* and *E. uniflora* (Table 5). During the S2 period, the percentage of parasitism occurred only in *C. xanthocarpa* (percentage of parasitism = 6.9%, $n = 18$ parasitoids, $n_1 = 326$ pupae, $n_2 = 21$ samples with pupae) (Figure 2) because no parasitism occurred in *E. uniflora* (Figure 3). The percentage of parasitism in apple was 0.2% ($n = 2$, $n_1 = 1,339$, $n_2 = 243$) during S2. The average percentage of parasitism in the IS period in wild hosts was 3.2% ($n = 493$, $n_1 = 22,105$, $n_2 = 201$), while in *P. cattleianum*, it was the most important (5.0%, $n = 416$, $n_1 = 13,877$, $n_2 = 118$) (Figure 3), followed by *A. sellowiana* (1.3%, $n = 49$, $n_1 = 5,656$, $n_2 = 24$), *P. guajava* (percentage of parasitism= 0.8%, $n = 25$, $n_1 = 2,049$, $n_2 = 27$) and *A. neosericea* (0.4%, $n = 3$, $n_1 = 464$, $n_2 = 21$). The percentage of parasitism in apples in IS was low (0.1%, $n = 3$, $n_1 = 3,151$, $n_2 = 301$). The ratio of parasitism was high than the percentage of parasitism in *C. xanthocarpa* due to the lower mortality of pupae in this host (Figure 2).

The number of parasitoids in wild hosts varied among growing seasons. In Y2, there was no parasitism. In Y1, the percentage of parasitism was 3.0% ($n = 177$ parasitoids, $n_1 = 3,939$ pupae, $n_2 = 31$ samples with pupae), in Y3 the percentage of parasitism was 4.4% ($n = 64$, $n_1 = 4,259$, $n_2 = 90$) and in Y4 the percentage of parasitism was 3.3% ($n = 270$, $n_1 = 13,950$, $n_2 = 90$). In apple, there were few parasitoids in Y1 (0.4%, $n = 4$, $n_1 = 2,983$, $n_2 = 393$) and Y2 (0.1%, $n = 1$, $n_1 = 927$, $n_2 = 79$) and no parasitoids in Y3 and Y4.

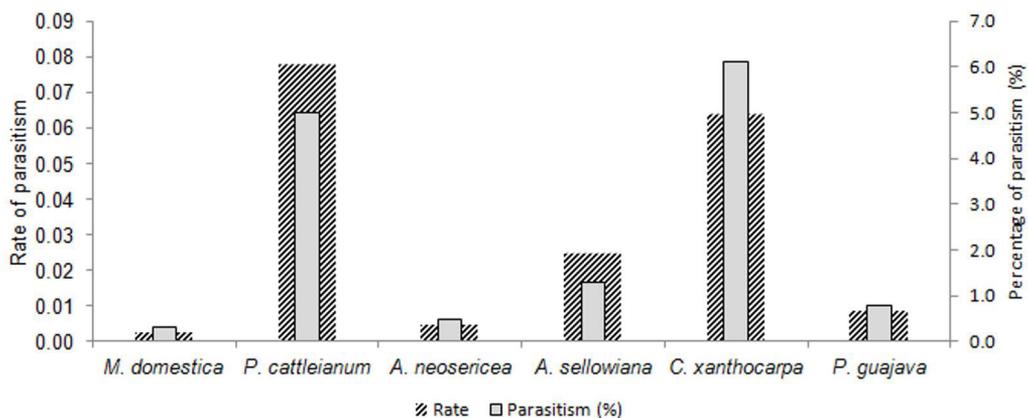


Figure 2. Relative abundance of *A. fraterculus* parasitoids recovered from six host plants from apple orchards adjacent to the Atlantic Forest of Paraná, Brazil (2013-17).

Table 5. Parasitoids per *A. fraterculus* wild hosts in four growing seasons collected in the Paraná Atlantic Forest, Brazil (2013-2017).

Parasitoid species	Wild host species	Growing season	Sampled with parasitoid (n)	Fruit sampled (kg)	Pupae (n)	Pupae/kg	Pupae/fruit	Fruit fly adults	Parasitoid (n)	Parasitism (%) ¹	Rate parasitism ²	
<i>Doryctobracon areolatus</i>	<i>A. sellowiana</i>	2015/16	1	0.34	34	101.5	14.8	31	1	2.9	0.03	
		2016/17	3	2.08	1338	644.6	16.6	1088	5	0.4	0.00	
	<i>P. cattleianum</i>	2013/14	9	4.13	1767	485.6	11.9	1206	53	3.0	0.04	
		2015/16	1	0.27	11	41.5	0.2	2	2	18.2	0.50	
		2016/17	6	2.40	795	318.9	2.2	360	9	1.1	0.02	
		2015/16	1	0.30	19	166.1	9.8	40	1	5.3	0.02	
<i>C. xanthocarpa</i>	2015/16	1	0.23	7	30.4	0.1	0	1	14.3	1.00		
<i>Doryctobracon brasiliensis</i>	<i>A. sellowiana</i>	2015/16	4	1.82	419	220.4	5.1	216	6	1.4	0.03	
		2016/17	8	4.84	2298	459.4	11.7	1636	2	0.1	0.00	
	<i>P. cattleianum</i>	2013/14	12	5.29	2031	422.6	10.1	1407	20	1.0	0.01	
		2015/16	3	0.99	18	17.7	0.2	8	6	33.3	0.43	
		2016/17	3	0.64	127	226.6	1.5	44	6	4.7	0.12	
		<i>P. guayava</i>	2016/17	4	2.55	688	261.0	6.9	590	4	0.6	0.01
<i>Opius bellus</i>	<i>P. cattleianum</i>	2013/14	2	0.71	263	366.5	8.5	142	2	0.8	0.01	
	<i>A. neosericea</i>	2015/16	2	0.44	68	150.9	6.1	56	2	2.9	0.03	
	<i>C. xanthocarpa</i>	2016/17	1	0.46	9	19.8	0.1	8	1	11.1	0.11	
<i>Aganaspis pelleranoi</i>	<i>A. sellowiana</i>	2016/17	5	3.10	1810	564.4	14.2	1428	18	1.0	0.01	
	<i>P. cattleianum</i>	2013/14	1	0.35	138	400.0	8.1	78	2	1.4	0.03	
2015/16		1	0.32	4	1270.0	0.1	1	1	25.0	0.50		
2016/17		15	6.04	2405	410.1	2.7	1406	49	2.0	0.03		
<i>P. guayava</i>		2015/16	5	3.52	965	268.4	6.7	870	9	0.9	0.01	
		2016/17	1	0.54	33	61.1	1.3	32	1	3.0	0.03	
<i>C. xanthocarpa</i>		2015/16	3	0.69	76	111.4	0.4	46	6	7.9	0.12	
<i>Aganaspis sp</i>	<i>A. sellowiana</i>	2016/17	8	4.84	2298	459.4	11.7	1636	17	0.7	0.01	
	<i>M. domestica</i>	2013/14	4	2.17	29	13.1	1.4	18	4	13.8	0.18	
		2014/15	1	0.35	18	51.4	3.0	15	1	5.6	0.06	
		2013/14	8	3.57	1414	443.9	11.2	970	100	7.1	0.09	
	<i>P. cattleianum</i>	2015/16	4	1.29	185	153.5	1.6	134	4	2.2	0.03	
		2016/17	30	12.07	5069	431.0	3.0	2798	162	3.2	0.05	
		<i>P. guayava</i>	2015/16	7	4.24	1039	237.7	5.8	901	11	1.1	0.01
			<i>C. xanthocarpa</i>	2015/16	5	1.14	122	107.6	0.4	88	10	8.2
	Average	2013/14	6.0	2.7	940.3	355.3	8.5	636.8	30.2	3.2	0.05	
			2014/15	1.0	0.4	18.0	51.4	3.0	15.0	1.0	5.6	0.06
2015/16		2.9	1.2	228.2	221.3	4.0	184.1	4.6	2.0	0.02		
2016/17		7.6	3.6	1533.6	350.6	6.5	1002.4	24.9	1.6	0.02		

¹ Number of parasitoids/number of pupae. ² Number of parasitoids/(fruit fly adults+parasitoids). ¹ and ² calculated with all samples.

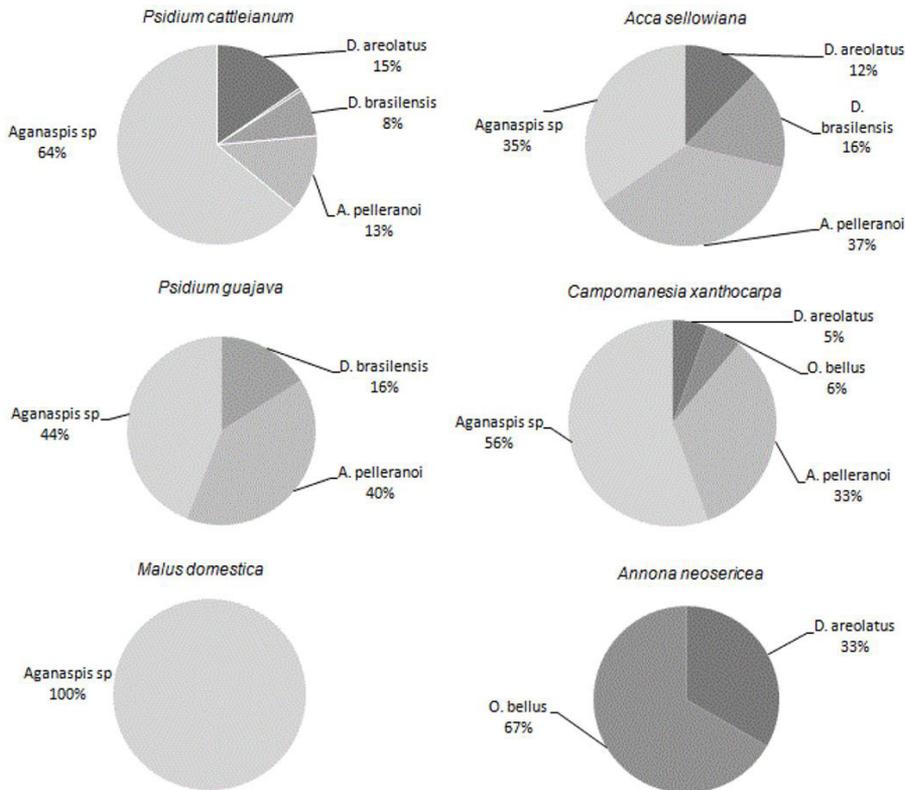


Figure 3. Percentage and rate of parasitism of fruit fly host plants in the Paraná Atlantic Forest, 2012-2017. The percentage of parasitism was calculated for total pupae; the rate of parasitism was calculated for adult emergence. Different letters indicate differences ($p < 0.05$) of the percentage of parasitism (Tukey 5%).

4. Discussion

The Atlantic Forest off the coast of Brazil has a rich native flora (Myers et al., 2000), and many species are described as fruit fly hosts (Zucchi, 2000); however, few species are considered to be multiplier hosts (Foelkel, 2015; Aluja et al., 2014; Araujo et al., 2019). As apple orchards are planted in areas adjacent to the Atlantic Forest, it is possible that native fruit trees can be sources of fruit fly for orchards, and their removal is often considered by farmers. However, the presence of Myrtaceae in the Atlantic Forest, on the edge of apple orchards, did not increase of fruit fly compared to forests without hosts (Araujo et al., 2019). We identified only six Myrtaceae, one Annonaceae and one exotic Rosaceae host as the most likely multipliers of fruit fly hosts of the Paraná Atlantic Forest corroborated by Foelkel (2015); in our research, five host cannot be considered to be fruit fly multipliers and may have been influenced by the climate and/or their location in the forest, for example, by the level of shading (Muniz, 2008). This was the case for *C. xanthocarpa* of which practically none fruited in Y1 and Y2. The same phenomenon occurred in *E. uniflora*, with no fruit in Y1 and Y2. In the IS period, in *A. neosericea* there were few fruits in Y4. *E. pyriformis* only produced fruits in the last year. Other factors may also be related to the abundance of fruits, such as the genetics of these hosts.

The separation of the agricultural cycle into the S and IS periods was relevant in this study. In the S, period the maturation of fruits of *E. uniflora* and *C. xanthocarpa* were early compared to the maturation of apple. There was an expectation that these hosts could produce populations of fruit fly that would be able to migrate to the apple plots, but *E. uniflora* had few fruits that did not produce pupae and *C. xanthocarpa* had almost the same number of pupae per kg of fruit (0.24, $n = 26$ sampling, $n_1 = 2,231$ fruits) compared to apple (0.30, $n = 736$, $n_1 = 4,319$). One of possible reasons for these findings was the poor quality of both fruits produced in the S period because these fruits lose their bark consistency in high humidity (Sacramento et al., 2007). A similar case was observed in *E. japonica*, where the number of pupae per fruit in Y2 decreased compared to that in Y3.

The samples with pupae were larger in the wild hosts (83.0% of samples) than the apple (47.9%). It is usually stated that the reduction in fruit fly in apple is due to the use of insecticides; however, in the IS period there were no phytosanitary treatments. Most pupae were collected in IS (85%) and may be associated with temperatures that are higher and favourable for the insect (Rosa et al., 2017). In agreement, the number of pupae per fruit in the IS period was 3.0, while in S, it was six times lower. Finally, it must be considered that apple is not a preferred host of fruit fly (Ovruski et al., 2010).

A. fraterculus was the only species of fruit fly found in native and exotic plants in the Atlantic Forest in Paraná. Myrtaceae hosts were the main hosts, with 78.0% of adult fruit fly found in all fruit trees. Considering only wild fruits, this percentage of Myrtaceae increased to 94.4%, similar to the report by Bisognin et al. (2015). However, when the potential of each host in the four growing seasons was analysed, only *A. sellowiana* and *P. cattleianum* were considered primary multipliers of SSF, and *P. guajava* was secondary, all occurring in IS. Primary multipliers annually produce large quantities of fruit fly, as observed by Bisognin et al. (2015) and Nunes et al. (2012). In our study, the pupae values for *P. cattleianum* were 12.3 times higher than those obtained by Bisognin et al. (2015). Among the tertiary hosts defined in this work, *E. japonica* also had a low number of *Anastrepha* flies according to Uramoto et al. (2004) and Souza-Filho et al. (2009); however, it was considered a fruit fly multiplier by Schliserman et al. (2010).

P. cattleianum stood out among the Myrtaceae species not only due to the high rate of fruit fly infestation but also to the diversity of parasitoid species (81% of parasitoids), in agreement with Raga et al. (2005, 2011) and Silva et al. (2010). The presence/absence of parasitoids can be tightly associated with the diversity and type of host fruit in a particular environment (Ovruski et al., 2000; Schliserman et al., 2010).

In this study, the total number of Figitidae species was higher than that of Braconidae species, unlike the findings of other authors (Ovruski et al., 2000; Garcia and Corseuil, 2004; Nicácio et al., 2011; Nunes et al., 2012). These results may be due to our pupae management methodology because some Figitidae took up to 60 days to emerge after the formation of fruit fly pupae. The paper filter humidification system allows pupae to be monitored for long periods of time. Most braconids emerged up to 15 days after the emergence of adults of *Anastrepha*.

Information on parasitoids with fruit fly is scarce for the Paraná State (Menezes Junior et al., 1997; Daza and Zucchi, 2000), and the presence of *A. pelleranoi*, *D. areolatus*, *O. bellus* and *D. brasiliensis* were the first records in Atlantic Forest in Paraná. *O. bellus* was the first register in Paraná State, as well as, first record of the tritrophic association between host plant *A. neosericea*, parasitoids *D. areolatus* and *O. bellus* and fruit fly *A. fraterculus*, as expected since all these species are native to the Neotropical region (Ovruski et al., 2000). *D. areolatus*, *O. bellus* and *A. pelleranoi* are widely distributed in Latin America; however, *D. brasiliensis* is known to occur in southern Brazil and northern Argentina (Ovruski et al., 2000; Schliserman et al., 2010).

The finding that *D. areolatus* was the most abundant among the Braconidae species registered agrees with previous surveys that also highlight this species as the most abundant in many agro-ecosystems (Ovruski et al., 2000; Garcia and Corseuil, 2004; Nunes et al., 2012). The parasitism of braconids on *C. xanthocarpa* in S was low (1.7%), while no parasitism occurred on *E. uniflora* and *E. japonica*, although Nunes et al. (2012) showed that *E. uniflora* is a species with good number of parasitoids. In the IS period, Braconidae were mostly found on *P. cattleianum* (81.0% of the total of braconids) and *A. sellowiana* (11.6%). The temperature in IS (17 to 27°C) may be favourable

for the development of parasitoids because 98.3% of all braconids were emerged in this period; it was corroborated by (Gonçalves et al., 2014).

Figitids were also more abundant on *P. cattleianum* (80.5% of figitids) than on other species, such as *A. sellowiana* (8.9%). Like braconids, figitids had a good correlation with the pupae abundance of fruit fly in *P. cattleianum* ($r^2=0.75$). The parasitism of figitids on the *C. xanthocarpa* host was low (4.1%), similar to that of braconids, and there was no parasitism on *E. uniflora* or *E. japonica*, as reported by Souza-Filho et al. (2009). Although *A. pelleranoi* responds positively to volatiles of Myrtaceae plants (Guimarães and Zucchi, 2004), this species has been considered to be more generalist than braconids, occurring in peach and apple (Ovruski et al., 2000; Nunes et al., 2012). Thus, *A. pelleranoi* may be present in the S and IS periods because the most suitable temperature for their development is from 18 to 25°C (Gonçalves et al., 2014); however, they were abundant only in IS (95.4% of all figitids) with increased Tmin.

The braconid *D. areolatus*, similar to *A. pelleranoi*, can develop in mild temperatures (17 to 25°C), but they can withstand a higher temperature than *A. pelleranoi* (Souza-Filho et al., 2009; Silva et al., 2010; Adaime et al., 2018). The low temperatures at the altitude of 900 m in Paraná orchards could be better for *A. pelleranoi*.

Parasitoids utilize a wide variety of fruit-associated chemicals in host location (Godfray, 1994; Eitam et al., 2003). Both of the most abundant species of our survey, *D. areolatus* and *A. pelleranoi*, seem to be mainly attracted by volatiles of fruits (Eitam et al., 2003; Guimarães and Zucchi, 2004), and the fruit fly larvae in the third instar to attract figitids, mainly in fallen fruits (Gonçalves et al., 2016). During our study, the majority of samples were from fallen fruits, in agreement with Schliserman et al. (2010) and Ovruski et al. (2000), who recovered the majority of Figitidae from the ground.

In our study, the highest level of fruit fly parasitism and the greatest diversity were detected in wild hosts than in agricultural areas, as observed by Aluja et al. (2014) and Souza et al. (2012). Many parasitoids have movement ranges that are substantially shorter than those of their of fruit fly hosts (Aluja et al., 2014); therefore, the absence of fruit in the S period or the disposition of the host has a greater effect on parasitoids than polyphagous fruit flies because there is evidence that parasitoids tend to follow their host and multiply into the same fragments as the host (Wajnberg et al., 2007; Aluja et al., 2014).

In general, the diversity and abundance of parasitoids species are very sensitive to ecosystem disturbances, such as climate and phytosanitary events (Aluja et al., 2014; Adaime et al., 2018). Native species of parasitoids are particularly abundant in forests and non-commercial landscapes (Sivinski et al., 2006), and natural suppression of Atlantic Forest adjacent areas could increase the number of adult fruit flies available to move into the orchards, as shown by Aluja et al. (2014) and Araujo et al. (2019). Our results reinforce the importance of tritrophic research among vegetal hosts, fruit flies, and their parasitoids.

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