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Faunistic analyses of fruit fly species (Diptera: Tephritidae) in orchards surrounded by Atlantic Forest fragments in the metropolitan region of Curitiba, Paraná state, Brazil

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Abstract

The objective of this trial was to characterize the assemblage structure of fruit flies (Diptera: Tephritidae) by determining the diversity and faunal indices. Fruit flies were collected for ten years between 2003 and 2015 in six municipalities of the Metropolitan Region of Curitiba, Paraná. The orchards were divided into three crop protection strategies: organic, conventional, and integrated pest management. The characteristic of each community was determined using the Shannon-Wiener, Margalef, and Pielou's indexes. The frequency, constancy, and dominance indexes were also determined. Biological material was analyzed using the explanatory variables: site, crop, plant protection strategy, and susceptibility period. A total of 8,089 fruit flies were collected, of which 4,681 were females. The species *Anastrepha daciformis* Bezzi, *A. dissimilis* Stone, *A. distincta* Greene and *A. pickeli* Lima were recorded for the first time in Paraná State, the occurrence of three other species was recorded for the first time in the Metropolitan Region of Curitiba (*A. montei* Lima, *A. obliqua* Macquart, and *A. sororcula* Zucchi), in addition *A. fraterculus* (Wiedemann), *A. grandis* (Macquart) and *Ceratitis capitata* (Wiedemann). *A. fraterculus* accounted for 98.5% of the collected species. Only *A. fraterculus* and *A. sororcula* were found in fruits. Fruit flies occurred predominantly in peach trees cultivated under organic conditions. The Shannon index indicated that the municipality of Cerro Azul had the highest diversity and richness whereas apple orchards had the lowest diversity. Crops cultivated under organic conditions exhibited the highest diversity whereas those cultivated under conventional conditions had the highest evenness index. The largest specimens number was collected during the period of crop susceptibility.

Keywords: Anastrepha, population dynamics, faunal indexes, Atlantic Forest biome.

Análise faunística de moscas-das-frutas (Diptera: Tephritidae) em pomares rodeados por fragmentos da Mata Atlântica na região metropolitana de Curitiba, Paraná, Brasil

Resumo

O objetivo deste estudo foi caracterizar agrupamento de espécies de moscas-das-frutas (Diptera: Tephritidae) pela determinação da diversidade e dos índices faunísticos. As moscas-das-frutas foram coletadas por dez anos entre 2003 e 2015 em seis municípios da Região Metropolitana de Curitiba, no Paraná. Os pomares foram divididos em três estratégias de proteção de culturas: orgânica, convencional e integrada. A característica de cada comunidade foi determinada usando os índices de Shannon-Wiener, Margalef e Pielou. Os índices de freqüência, constância e dominância também foram determinados. O material biológico foi analisado utilizando as variáveis explicativas: local, cultura, estratégia de proteção e período de susceptibilidade. Foram coletadas 8.089 moscas-das-frutas, sendo 4.681 fêmeas. As espécies *Anastrepha daciformis* Bezzi, *A. dissimilis* Stone, *A. distincta* Greene e *A. pickeli* Lima foram registradas pela primeira vez no Estado do Paraná, e pela primeira vez, na Região Metropolitana de Curitiba, as espécies *A. montei* Lima, *A. obliqua* Macquart e *A. sororcula* Zucchi), além de *A. fraterculus* (Wiedemann), *A. grandis* (Macquart) e *Ceratitis capitata* (Wiedemann). *A. fraterculus* foi responsável por 98,5% das espécies coletadas. Apenas *A. fraterculus* e *A. sororcula* foram encontrados em frutos. As moscas-das-frutas ocorreram predominantemente em pessegueiros cultivados sob condições orgânicas. O índice de Shannon indicou que o município de Cerro Azul teve a maior diversidade e riqueza, enquanto que a cultura macieira teve a menor diversidade. As culturas sob condição orgânica exibiram a maior diversidade, enquanto as cultivadas em condições convencionais apresentaram o maior índice de uniformidade. O maior número de espécimes foi coletado durante o período de suscetibilidade à cultura.

Palavras-chave: Anastrepha, dinâmica populacional, índices faunísticos, bioma Mata Atlântica.

1. Introduction

The Metropolitan Region of Curitiba (MRC) is composed of 37 municipalities in a radius of approximately 80 km. These municipalities have favorable soil and climate conditions for the growth of subtropical fruits and account for 50% of the apple production (*Malus domestica* Borkh) and 60% of the tangerine production (*Citrus reticulata* Blanco) in the state of Paraná, Brazil (Andrade, 2015). These cultures are cultivated not far away of Atlantic Forest fragment, which is considered one of the Brazilian biome on worldwide biodiversity hotspots (Myers et al., 2000).

Fruit flies are the main pests of orchards (Zucchi, 2000 a, b); however, occurrence records of this pest in the MRC are scarce. The occurrence of fruit flies in this region was initially registered in the 1940s by Vellozo et al. (2001), with the identification of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) in peach orchards in Curitiba, and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in citrus orchards in Cerro Azul. Fehn (1981) identified *C. capitata*, *A. fraterculus*, *A. grandis* (Macquart), *A. pseudoparallela* (Loew), and *A. serpentina* (Wiedemann) (Diptera: Tephritidae) in peach orchards located in four municipalities of the MRC.

Garcia (2003) listed 26 species of tephritids for the State of Paraná. In a study conducted in the central eastern region of Paraná (Ponta Grossa), Husch et al. (2012) found the species *Anastrepha montei* Lima (Diptera: Tephritidae) in peach orchards using traps.

Knowledge of fruit fly species and their commercial host plants in different geographical areas is essential to understand their bioecology. Several factors are related to species diversity and abundance. In general, the weather regulates the development of native hosts and natural antagonism (Aluja et al., 1997). The occurrence of different fruit fly species depends on their crops preference and is affected by the landscape, namely the crop-forest system. In the MRC, many orchards are near or surrounded by Atlantic Forest fragments, which leads to interspecific competition. Although many native and cultivated plant species are not considered as fruit flies prime hosts, these plants play a crucial role in the multiplication of flies and their natural enemies. The diversity of a species may be greater in stable ecosystems, however, the number of ecosystems that are disturbed by insecticides application may be higher than that of some balanced ecosystems because dominance and competitive exclusion are more intense in the former (Huston, 1979). A general trend is that the size of the sampling area and the sampling period have a direct influence on the number of species (Sanders, 1968).

This study was based on the hypothesis that several fruit fly species occur in the orchards in the MRC but that not all species interact with the host fruits because they are affected by local crop, plant protection strategies and period of commercial fruit plants susceptibility. This study aimed the assemblage structure of fruit flies (Diptera: Tephritidae) in Rosaceae and *Citrus* orchards surrounded by Atlantic Forest fragments in six municipalities of the MRC.

2. Material and Methods

2.1. Characterization of the study and collection sites

Fruit flies were collected from orchards in six municipalities of the MRC, Paraná, Brazil: I) Araucária (25°35'34" S, 49°24'36" W, mean height of 897 m), II) Campo do Tenente (25°58'40" S, 49°40'58" W, mean height of 802 m), III) Cerro Azul (24°49'25" S, 49°15'40" W, mean height of 318 m), IV) Lapa (25°46'1" S, 49°42'57" W, mean height of 908 m), V) Pinhais (25°26'41" S, 49°11'33" W, mean height of 893 m) and VI) Porto Amazonas (25°32'42" S, 49°53'24" W, mean height of 793 m). The orchards located in the first five municipalities have Atlantic Forest fragments, and preliminary studies conducted by (Foelkel, 2015) identified several fruit-fly crop hosts, including cherry (*Eugenia sulcata*, *E. uniflora*), araticum (*Annona coriacea*), guava (*Psidium littorale*), guabiroba (*Eugenia variabilis*), loquat (*Eriobotrya japonica*), and feijoa (*Feijoa sellwiana*). Although Porto Amazonas has fragments of the same forest, but native plant hosts were not identified in the fragments adjacent to the orchards.

Fruit flies were collected in the following crops and locations: 'Irati' plum cultivar (*Prunus domestica*) (in Araucária and Porto Amazonas), apple cultivars 'Condessa', 'Gala', 'Eva', and 'Granny Smith' (*M. domestica*) (in Campo do Tenente, Lapa, and Porto Amazonas), peach cultivars 'Charme', 'Chimarrita', 'Coral', and 'Ouro' (*Prunus persica*) (in Porto Amazonas, Araucária, Lapa, and Pinhais, respectively), pear (*Pyrus communis*) (Lapa and Porto Amazonas), and citrus [orange (*Citrus sinensis*) and ponkan (*Citrus reticulata*) (Porto Amazonas and Cerro Azul, respectively)].

The samples were collected between 2003 and 2015, except in 2007, 2008, and 2011, and were conducted to the Integrated Pest Management Laboratory (LAMIP) of the UFPR. Samples were collected using traps and fruits. Intact and mature fruits were sampled in the fruit trees canopy or on the ground under the canopy, according to their availability, in plum (2015), citrus (2013, 2014, 2015), apple (2013, 2014 and 2015), peach (2003, 2004, 2006, 2013 and 2015), and pear (2013) plants. The fruits were collected at nine-day intervals on average, distributed in trays containing vermiculite, covered with voile-type fabric, and maintained at 25 °C. Vermiculite was sifted to separate the pupae and stored in Petri dishes with moistened filter paper until the emergence of adults.

Fruit flies were collected using traps in plum (2004 and 2010), citrus (2015), apple (2009, 2010, 2012, 2013, 2014, and 2015), peach (2003, 2004, 2006, 2012, 2013, 2014, and 2015), and pear (2004 and 2010). The traps were of the McPhail type (Biocontrol, Araçatuba, São Paulo) with protein hydrolysate baits at 5% (Bio Anastrepha; Biocontrol, Araçatuba, São Paulo). Two traps were installed at a height of 1.8 m at every two hectares of apple orchard and every one hectare for other crops. Samples were collected weekly and annually, and the specimens were kept in 75% ethanol. The number of fruit flies collected in fruit and traps in each orchard was expressed by the ratio between the number of specimens collected and the number of samples collected per day (no flies/no samples collected per day).

2.2. Taxonomic Identification

The adults found in both fruits and traps were separated by sex. Females were identified to the species level. The flies collected in fruits were not killed immediately after emergence to allow better characterization of the color of the wings and the imago. The fruit fly specimens of the genus *Anastrepha* Schiner were sexed and identified using the identification keys of Zucchi (2000a). Characters of the females, primarily of the aculeus, body and wing were considered for identifying the species of fruit flies, except for specimens of *C. capitata*, which were identified by the characteristics of the wings and the postocular and scutellar bristles because it is the only *Ceratitis species* found in Brazil.

2.3. Analysis of the factors that interfere in the occurrence of fruit flies

The fluctuation in the number of these insects does not follow a defined pattern but is rather directly influenced by some factors (Salles, 1995). In this study, four interference factors were identified, and their effects on fruit flies occurrence were assessed:

a. Site: Araucária, Campo do Tenente, Cerro Azul, Lapa, Pinhais, and Porto Amazonas;

b. Crop: apple, citrus, peach, pear and plum;

c. Plant protection strategy: three strategies were identified in the orchards studied and were characterized as: i. Conventional: exclusive use of insecticides and acaricides for pest control. Spraying was conducted according to a predetermined schedule. Plum, apple, citrus, peach, and pear orchards were included in this system (Araucária, Lapa, and Porto Amazonas); ii. Integrated Pest Management (IPM): spraying of plant protection products for the monitoring of pests and use of techniques that reduce the application of insecticides, including applied biological control of mites and mating disruption of *Grapholita molesta* Busck (Lepidoptera: Tortricidae). Apple (Campo do Tenente, Lapa) and citrus (Cerro Azul) orchards were included in this category; and iii. Organic: No synthetic plant protection product was used for the management of pests and diseases. Peach (Pinhais) and citrus (Cerro Azul) orchards were included in this category;

d. Period of crop susceptibility in the MRC: this period was defined for each crop on the basis of information related to the vegetative and developmental stage of fruits (Sugayama and Malavasi, 2000), and their correlation with the damage caused by fruit flies. These data were obtained from observations of producers and collections of fruits in the orchards during the study period. The susceptible period was between October 1 and December 15 for plum and peach, between March 1 and July 31 for citrus, between November 1 and January 10 for 'Eva' apple, between December 1 and January 30 for 'Gala' apple, and between December 15 and March 31 for pear. The months outside this period were considered the non-susceptible period.

2.4. Statistical analyses

The comparative analysis of the means of the explanatory factors site, plant protection strategy and susceptibility period was performed using the Student-Newman-Keuls (SNK) test, considering the number of fruit flies collected in traps and fruits in all samples, and expressed as the number of flies per sample per day. Analysis of variance (ANOVA) was performed for the three crops whose sampling period was longer than three years: citrus, apple, and peach. The mean number of fruit flies collected per day in traps was compared with that collected in fruits (number of flies per sample per day) for citrus, apple, and peach during a three-year sampling period.

The characteristic of each fruit fly community collected in fruits and traps was determined using the Shannon Wiener diversity index (H'), the Margalef index (α), and the Pielou's evenness index (E), according to the equations: $H' = -\Sigma pi \ln pi$, $\alpha = S-1/\ln N$, and $E = H'/\ln S$, using Excel® software. The faunal indices of frequency (F), constancy (C), and dominance (D) (Southwood and Henderson, 2000; Garcia et al., 2003; Alberti et al., 2009) used the explanatory factors site, crop, and plant protection strategy. The H' index measures the degree of uncertainty in predicting which species a fruit fly collected randomly belongs to considering a population of 'n' species (S) and the total number of female fruit flies (N) collected. The greater the diversity, the lower the probability of identifying the species collected at random. The α index evaluates the relationship between the number of species and the number of specimens of each species and indicates the pattern of use of niches by each species. The E index evaluates the uniformity of the number of fruit flies of each species, where zero indicates the occurrence of a single species and is considered a high rate. Frequency index is the ratio between the number of fruit flies of each species and the total number of flies collected in each orchard and is calculated by $F = n/N100$, where n is the number of female fruit flies of a certain species, and N is the total number of female flies of all species collected. The C index is the percentage of occurrence of the species in a sample and may be constant (w) (more than 50% of the collections), accessory (y) (25% to 50% of the collections), and accidental (z) (less than 25% of the collections) (Silveira Neto et al., 1976). The D index is the ratio between the number of flies of the dominant species and the total number of collected flies, dominance being characterized by a percentage of fruit flies higher than 1/S, where S is the total number of species collected (Southwood, 1995).

3. Results

3.1. Fruit fly species

A total of 8,028 fruit flies was collected in the six municipalities of the MRC, including 4,681 females and 3,416 males (sex ratio = 0.58). Ten species of Tephritidae were collected: *A. fraterculus* (n = 4.612; 98.5% of the female flies collected), *A. grandis* (n = 22, 0.47%); *Anastrepha distincta* Greene (n = 21; 0.45%), *Anastrepha dissimilis* Stone (n = 9 0.19%), *A. montei* (n = 5, 0.11%), *C. capitata* (n = 5, 0.11%), *Anastrepha sororcula* (Zucchi) (n = 4; 0.09%), *Anastrepha obliqua* (Macquart) (n = 1, 0.02%), *Anastrepha pickeli* Lima (n = 1, 0.02%), and *Anastrepha daciformis* Bezzi (n = 1, 0.02%).

3.2. Analysis of fruit fly collection

The number of fruit flies collected in fruits and traps was significant ($F = 126.20$, $p \leq 0.0001$). The mean number of fruits per sample (n = 1,020) was 6.7. The mean number of specimens of *A. fraterculus* (both sexes) collected in fruits was 0.55 flies per sample per day. Among the identified species, only two occurred in fruits: *A. fraterculus* (n = 2,074) and *A. sororcula* (n = 2, in peach). The first accounted for more than 99.9% of the species collected in fruits. The average number of specimens of *A. fraterculus* collected in traps (n = 5,322 samples) was 0.10 flies per sample per day. Taking only into account the female flies of the ten identified species, a mean of 2.03 and 0.50 females was collected per fruit and per trap respectively. All species were collected in traps.

The number of fruit flies collected per sample per day was significant between treatments: crop ($F = 39.35$, $p < 0.0001$), site ($F = 114.73$, $p < 0.0001$), plant protection strategy ($F = 223.77$, $p < 0.0001$), and susceptible period ($F = 13.61$; $p < 0.0001$) (Table 1). The crop in which most flies were collected was peach ($F = 39.35$, $p \leq 0.0001$) whereas the number of flies collected in citrus and apple was not significantly different. The two damaged peach varieties were exclusively grown under the organic system, without fruit fly control management. The 'Granny Smith' apple is a pollinator of commercial cultivars ('Condessa', 'Eva', and 'Gala') and is a late crop, i.e., only part of the crop is collected, and this variety accounted for 18% of the fruit flies collected in apple orchards.

3.3. Environment analysis in the MRC municipalities

The orchard with the highest occurrence of fruit flies were located in Pinhais, with the collection of 5.12 flies per sample per day ($F = 181.55$, $p \leq 0.0001$). The number of flies collected in Araucária, Cerro Azul, and Porto Amazonas was not significantly different and corresponded to 0.59, 0.50, and 0.41 flies collected per sample per day, respectively. The number of flies collected in Lapa was significantly different from that collected in Campo do Tenente (Table 2). *A. fraterculus* was the most common species collected in all MRC orchards and the frequency of occurrence of most collected fruit fly species was low (<0.1%), with the exception of *A. grandis* and *A. distincta* in Cerro Azul (Table 2). *A. fraterculus* was also the only dominant specie and it was characterized as constant in pear and peach orchards (Table 3), i.e. the number of samples with *A. fraterculus* was greater than 50% of all samples evaluated, and was accessory in citrus. It remained constant in the municipalities where peach and pear were cultivated (except in Porto Amazonas) and accidental in the municipalities where apple was cultivated (Campo do Tenente, Lapa, and Porto Amazonas) (Tables 2 and 3).

The Shannon index indicated that the orchards evaluated in Cerro Azul were more diverse than the other orchards considering the factors site and crop. The diversity in Cerro Azul was not dependent on sample size (n = 90) but was dependent on a stable ecosystem (Odum, 1983). This result was not observed in other municipalities. Despite the higher number of samples collected in apple orchards (n = 5,124) (Campo do Tenente, Lapa, and Porto Amazonas), this crop had the lowest diversity indices because of the high frequency of *A. fraterculus* in the rural municipalities (Tables 2 and 3). Nevertheless, the results of the Margalef index indicated that Porto Amazonas had the largest species richness with eight species of Tephritidae captured, although the frequency of all these species was low.

Table 1. Mean number of fruit flies (flies per sample per day) collected in apple, citrus and peach with three pest control management in the six municipality of Metropolitan Region of Curitiba, Brazil.

Municipality	Mean fly	Crops[1]	Mean fly	Management	Mean fly
Pinhais	5.12a ± 2.33	Peach	0.49a ± 1.59	Organic	2.32a ± 2.01
Araucária	0.56b ± 1.04	Citrus	0.26b ± 0.72	Conventional	0.27b ± 1.24
Cerro Azul	0.41bc ± 0.77	Apple	0.12b ± 0.84	IPM[2]	0.05c ± 0.52
Lapa	0.16c ± 1.19				
Porto Amazonas	0.26bc ± 1.01				
Campo do Tenente	0.02d ± 0.27				

The means followed by the same letter in the column indicate the absence of significant differences using the SNK test at a level of significance of 5%; [1]Fruit flies were collected in apple cultivars 'Condessa', 'Gala', 'Eva', and 'Granny Smith' (*M. domestica*) (in Campo do Tenente, Lapa, and Porto Amazonas) (2013, 2014 and 2015), peach cultivars 'Charme', 'Chimarrita', 'Coral', and 'Ouro' (*P. persicae*) (in Porto Amazonas, Araucária, Lapa, and Pinhais, respectively) (2003, 2004, 2006, 2013 and 2015) and citrus [orange (*Citrus sinensis*) and ponkan (*Citrus reticulata*) (Porto Amazonas and Cerro Azul, respectively)] citrus (2013, 2014, 2015); [2]IPM – Integrated Pest Management.

Table 2. Faunal and diversity index for female fruit flies collected in Rosaceae and Citrus orchards in six municipalities in the Metropolitan Region of Curitiba, Paraná, Brazil, between 2003 and 2015.

Specie fruit fly	Araucária[1]				Campo do Tenente				Cerro Azul				Lapa				Pinhais				Porto Amazonas			
	N	F	C	D	N	F	C	D	N	F	C	D	N	F	C	D	N	F	C	D	N	F	C	D
A. fraterculus	253	0.988	w	d	276	1.000	z	d	137	0.761	y	d	1379	0.996	z	d	694	0.989	w	d	1873	0.995	z	d
A. dissimilis	0	-	z	-	0	-	z	-	0	-	z	-	3	0.002	z	n	4	0.006	z	n	2	-	z	-
A. grandis	0	-	z	-	0	-	z	-	19	0.106	z	n	1	0.001	z	n	1	0.001	z	n	1	0.001	z	n
A. sororcula	0	-	z	-	0	-	z	-	2	0.011	z	n	0	-	z	-	2	0.003	z	n	0	-	z	-
A. obliqua	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	1	0.001	z	n
A. distincta	3	0.012	z	n	0	-	z	-	17	0.094	z	n	0	-	z	-	0	-	z	-	1	0.001	z	n
A. pickeli	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	1	0.001	z	n
A. montei	0	-	z	-	0	-	z	-	5	0.028	z	n	0	-	z	-	0	-	z	-	0	-	z	-
A. daciformis	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-	1	0.001	z	n
C. capitata	0	-	z	-	0	-	z	-	0	-	z	-	2	0.001	z	n	1	0.001	z	n	2	0.001	z	n
Total	256				276				180				1385				702				1882			
S	2				1				5				4				5				8			
H'	0.064				0.000				0.793				0.032				0.076				0.032			
α	0.180				0.000				0.770				0.415				0.610				0.928			
E	0.092				0.000				0.492				0.023				0.047				0.015			

N = number of identified females flies; F = frequency; C = constancy (w = constant, y = accessory, z = accidental); D = dominance (d = dominant, n = non-dominant); S = number of species; H' = Shannon Wiener index; α = Margalef index; E = Pielou's evenness; [1]Fruit flies were collected in apple cultivars 'Condessa', 'Gala', 'Eva', and 'Granny Smith' (*M. domestica*) (in Campo do Tenente, Lapa, and Porto Amazonas) (2013, 2014 and 2015), peach cultivars 'Charme', 'Chimarrita', 'Coral', and 'Ouro' (*P. persicae*) (in Porto Amazonas, Araucária, Lapa, and Pinhais, respectively) (2003, 2004, 2006, 2013 and 2015) and citrus [orange (*Citrus sinensis*) and ponkan (*Citrus reticulata*) (Porto Amazonas and Cerro Azul, respectively)] citrus (2013, 2014, 2015).

Campo do Tenente had the highest evenness index for *A. fraterculus* (Table 2), i.e., a single species occurred in an apple cultivation area of 250 ha. Among the crops evaluated, apple orchards had the highest evenness index (Table 3).

The influence of plant protection strategy on the number of fruit flies collected was significant ($F = 223.77$, $p \leq 0.001$). The mean number of flies collected under the organic system (5.12 flies per sample per day) was significantly higher than that found in the conventional system (0.20 flies per sample per day), which in turn was greater than that found under the Integrated Pest Management system (0.13 flies per sample per day).

The analysis of the relationship between diversity and plant protection strategies showed that *A. fraterculus* was dominant in orchards cultivated under three plant protection strategies, whereas the frequency of the remaining species was lower than 0.02% (Table 4).

The number of flies per sample per day was significantly higher in the susceptible period (n = 0.27) ($F = 13.61$, $p = 0.0002$). The highest occurrence of *A. fraterculus* in

Table 3. Faunal and diversity index for female fruit flies found in different crops in the Metropolitan Region of Curitiba, Brazil, between 2003 and 2015.

Specie fruit fly	Plum				Citrus				Apple				Pear				Peach			
	N	F	C	D	N	F	C	D	N	F	C	D	N	F	C	D	N	F	C	D
A. fraterculus	99	0.971	z	d	139	0.764	y	d	2656	0.997	z	d	94	0.959	w	d	1618	0.993	w	d
A. dissimilis	0	-	z	-	0	-	z	-	3	0.001	z	n	0	-	z	-	6	0.004	z	n
A. grandis	0	-	z	-	19	0.104	z	n	1	0.000	z	n	1	0.010	z	n	1	0.001	z	n
A. sororcula	0	-	z	-	2	0.011	z	n	0	-	z	-	0	-	z	-	2	0.001	z	n
A. obliqua	0	-	z	-	0	-	z	-	1	0.000	z	n	0	-	z	-	0	-	z	-
A. distincta	2	0.020	z	n	17	0.093	z	n	0	-	z	-	1	0.010	z	n	1	0.001	z	n
A. pickeli	0	-	z	-	0	-	z	-	0	-	z	-	1	0.010	z	n	0	-	z	-
A. montei	0	-	z	-	5	0.027	z	n	0	-	z	-	0	-	z	-	0	-	z	-
A. daciformis	1	0.010	z	n	0	-	z	-	0	-	z	-	0	-	z	-	0	-	z	-
C. capitata	0	-	z	-	0	-	z	-	3	0.001	z	n	1	0.010	z	n	1	0.001	z	n
Total	102				182				2664				98				1629			
S	3				5				5				5				6			
H’	0.151				0.812				0.024				0.227				0.049			
α	0.432				0.769				0.507				0.872				0.676			
E	0.138				0.504				0.015				0.141				0.027			

N = number of identified females flies; F = frequency; C = constancy (w = constant, y = accessory, z = accidental); D = dominance (d = dominant, n = non-dominant); S = number of species; H’ = Shannon Wiener index; α = Margalef index; E = Pielou’s evenness.

Table 4. Faunal and diversity index for female fruit flies influenced by plant protection strategy in the Metropolitan Region of Curitiba, Brazil, between 2003 and 2015.

Specie fruit fly	Conventional				IPM[1]				Organic			
	N	F	C	D	N	F	C	D	N	F	C	D
A. fraterculus	2964	0.996	z	d	853	0.988	z	d	795	0.944	w	d
A. dissimilis	2	0.001	z	n	3	0.003	z	n	4	0.005	z	n
A. grandis	1	0.000	z	n	5	0.006	z	n	16	0.019	z	n
A. sororcula	1	0.000	z	n	0	0.000	z	n	4	0.005	z	n
A. obliqua	4	0.001	z	n	0	0.000	z	n	0	0.000	z	n
A. distincta	1	0.000	z	n	0	0.000	z	n	17	0.020	z	n
A. pickeli	0	0.000	z	n	0	0.000	z	n	0	0.000	z	n
A. montei	0	0.000	z	n	0	0.000	z	n	5	0.006	z	n
A. daciformis	2	0.001	z	n	0	0.000	z	n	0	0.000	z	n
C. capitata	1	0.000	z	n	2	0.002	z	n	1	0.001	z	n
Total	2976				863				842			
S	8				4				8			
H’	0.029				0.075				0.298			
α	0.875				0.444				0.891			
E	0.014				0.054				0.153			

N = number of identified females flies; F = frequency; C= constancy (w = constant, y = accessory, z = accidental); D = dominance (d = dominant, n = non-dominant); S = number of species; H’ = Shannon Wiener index; α = Margalef index; E = Pielou’s evenness.
[1]IPM – Integrated Pest Management.

orchards was during the period of fruit development and maturation.

4. Discussion

Among the ten Tephritidae species that were collected in MRC municipalities, the occurrence of four Anastrepha species was recorded for the first time in the Paraná State (*A. daciformis, A. dissimilis, A. distincta,* and *A. pickeli*), in addition, the occurrence of three other species was recorded for the first time in the MRC (*A. montei*, *A. obliqua*, and *A. sororcula*). With regard to the occurrence of fruit flies in each crop, all the fly species collected in apple orchards were reported for the first time in Paraná, as were *A. dissimilis*, *A. sororcula*, and *A. distincta* in peach orchards, *A. distincta*, *A. daciformis*, and *A. fraterculus* in plum orchards, *A. distincta*, *A. fraterculus*, *A. grandis*, *A. pickeli*, and *C. capitata* in pear orchards, and *A. distincta*, *A. fraterculus*, *A. grandis*, *A. montei*, and *A. sororcula* in ponkan orchards.

Studies conducted by Fehn (1981) in the MRC have shown the occurrence of *A. fraterculus* and *A. grandis* in peach orchards, whereas Husch et al. (2012) recorded the occurrence of *A. montei* in the same crop in Ponta Grossa. The absence of *C. capitata* in citrus crops is an important finding, particularly in Cerro Azul, because the weather is warmer and the conditions for the occurrence of this species in this site might be as favorable as those found in northern Paraná and southern São Paulo. Zucchi (2000b) reported that the collection of fruit flies using McPhail traps only provides information about the possible occurrence of fly species in the orchards; however, it does not prove that a crop hosts certain fruit fly species. This information was confirmed by collecting fruits and assessing the emergence of adults. However, the collection of flies in fruits may not be representative of the diversity of flies present in the region because it is almost impossible to identify all host plant species found in the Atlantic Forest (Uramoto et al., 2004).

The small number of fruit fly species collected in fruits corroborates the results of Uramoto et al. (2004) where in only a third of the species were collected in fruit samples. Therefore, although ten species occurred in orchards, only two species were associated with fruit damage.

The greater number of flies collected in Pinhais was related to the organic system used for the cultivation of peach orchards. The faunal index indicated that *A. fraterculus* was the most common species, thus corroborating the results of Fehn (1981) and Husch et al. (2012). The low fruit fly frequency of occurrence observed in the sampled crops (Table 2) may indicates that the species are not resident. All sampled orchards had Brazilian Atlantic forest fragments in their surroundings. This biome has one of the largest diversities of plant and animal species (Martini et al., 2007), and contains native and exotic host plants for fruit flies. Monteiro et al. (unpublished data) conducted preliminary studies on these fragments and identified crops of cherry (*E. sulcata*, *E. uniflora*), Araticum (*A. coriacea*), guava (*P. littorale*), guabiroba (*E. variabilis*), loquat (*E. japonica*), and feijoa (*F. sellwiana*) (except in Porto Amazonas). Therefore, part of the population collected in traps in the orchards was passing or had no significant interest in feeding on commercial fruit trees, and this is supported by the fact that only *A. fraterculus* and *A. sororcula* were found in fruits. It was expected to collect more species in fruit samples because the sampling was conducted in organic orchards and pollinators in the post-harvest period and without insecticides (Bomfim et al., 2007). The only dominant species in all crops and municipalities evaluated was *A. fraterculus*, which indicated the prevalence of this species compared with other species (Aguiar-Menezes et al., 2008; Dutra et al., 2009).

A stable ecossystem was observed in Cerro Azul and it could explain the fruit fly species diversity (Odum, 1983). The characteristic of the Atlantic Forest and plant protection under the organic system allowed the coexistence of *A. fraterculus*, *A. grandis*, and *A. distincta* in the same physical space. Apple orchards had the lowest diversity indices due the high *A. fraterculus* frequency in all municipalities. Diversity tends to be low in biotic communities that are under stress (Odum, 1983), whether by agents external to the community, as is the case of insecticides, or internal to the community, as is the case of biological agents (Aluja et al., 1997). The control of fruit flies in apple orchards using organophosphate insecticides may have contributed to the low Shannon index observed, associated with the absence of native hosts in the Atlantic Forest fragment surrounding the orchards, as observed by Uramoto et al. (2004), Ferrara et al. (2005), and Aguiar-Menezes et al. (2008). Porto Amazonas had the largest species richness and it was due to the diversity of crops present in the site (citrus, apple, peach, and pear) (Table 2). In contrast to the diversity index, the richness index is dependent on sample size, i.e., the number of species found will be high if the sample size was high (Melo, 2008). In Cerro Azul, species richness was positively affected by citrus crops and the diversity of native hosts in the Atlantic Forest. In Pinhais, the richness was influenced by organic cultivation of peaches and the hosts in the forest. The low richness observed in plum and peach orchards in Araucária was related to the conventional plant protection strategy, and the low richness in apple orchards in Campo do Tenente was related to the fact that a single species occurred in a six-year evaluation period. The highest *A. fraterculus* evenness index in Campo do Tenente could be also occurring because of the effect of the monoculture on the insect species of a community as reported by Bomfim et al. (2007). In the case of Cerro Azul, the probability of finding *A. fraterculus* among the other tephritid species was lower because of the frequency of *A. grandis* and *A. distincta*.

The three plant protection strategies also resulted in *A. fraterculus* domination in orchards. Other species were constant only in citrus and peach crops cultivated under the organic system (Table 3). Considering the dominance of *A. fraterculus*, the three plant protection strategies yielded a low diversity of tephritid flies, although the organic strategy led to the greatest diversity (Table 4) according to the Shannon index, and this result was related to the frequency of *A. distincta* and *A. grandis* in citrus. The Margalef index was similar for crops cultivated under the organic and conventional management. In the latter, the positive effect was due to the total number of female flies collected in all five crops (Table 3) and was observed in Porto Amazonas (Table 2). The evenness index indicated a higher probability of finding the same fruit fly species in orchards cultivated under the conventional system and was influenced by the high frequency of *A. fraterculus* and the evenness index in apple (Table 3).

A. fraterculus was most captured in orchards during fruit development and maturation. Sugayama and Malavasi (2000) reported that fruit growth causes the release of volatile compounds (kairomones) that stimulate the flight of females in search for hosts for oviposition. This stimulation occurs when the mean diameter of the fruits is between 20 and 35 mm (Sugayama and Malavasi, 2000). In the non-susceptible period, fruit flies were probably attracted to the orchards by food present in the traps, and this explains why the insects were collected during this period, with the exception of those in orchards in Porto Amazonas, which were probably attracted by 'Eva' apple three that flower after the harvest and develop fruits that persist throughout the winter (Foelkel, 2015). Furthermore, the 'Granny Smith' apple cultivar, which is a pollinator, is left in the orchard after harvest of the commercial variety. Both cultivars were responsible for 90% of the flies collected in the non-susceptible period. The elimination of fruits of pollinating apple plants and of fruits that matured out of season during thinning could contribute to the decreased number of fruit flies in the subsequent susceptible period.

As a conclusion, the species *Anastrepha daciformis*, *A. dissimilis*, *A. distincta*, and *A. pickeli* are recorded for the first time in the state of Paraná, Brazil. The mean number of collected specimens of *Anastrepha* sp. is higher in fruits than in traps, although 98.5% of the collected fruit flies are *A. fraterculus*. The crop with the highest number of specimens found in fruits and traps is peach. Campo do Tenente exhibits the lowest number of fruit flies species. *A. fraterculus* is the most common species in all cultures and municipalities. It is the dominant species in all municipalities, although it is constant only in pear and peach. The greatest insect diversity is found in citrus orchards cultivated in Cerro Azul under the organic system in the presence of an abundance of native hosts from the Atlantic Forest. The apple crops have the lowest diversity indexes.

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