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Population Dynamics and Infestation Rate of Fruit Flies in Stone Fruits in São Paulo State, Brazil

Adalton Raga^{1*}, Laís Ívina Silva de Paula¹, Miguel Francisco de Souza-Filho¹
and Jairo Lopes de Castro²

¹Laboratory of Economic Entomology, Instituto Biológico, Campinas, SP, Brazil.

²Pólo Regional, APTA, P.O.Box 62, 18300-970, Capão Bonito, SP, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author AR designed the study, wrote the protocol and wrote the first draft of the manuscript. Author LISP was responsible for the insect screening in the laboratory. Author MFSF identified the *Anastrepha* specimens and author JLC was responsible for field activities. All authors read and approved the final manuscript.

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ABSTRACT

Fruit fly species (Diptera: Tephritidae) are considered key pests in peach (*Prunus persica* L. Batsch) and plum (*Prunus salicina* Lindl.) crops in Brazil, causing both fruit losses and increased fruit costs due to greater use of spray insecticides. To measure the degree of infestation, for this study mature fruits were randomly collected over 2004 - 2006 period from a canopy of 34 varieties of peaches, nectarines and plums, as well as from ungrafted Mume and Okinawa rootstocks in the southwestern region of the São Paulo state, Brazil. Recovered fruit fly pupae were kept in a small cage until adult emergence. Except for "Harry Pickstone", all other stone-fruit varieties were infested

*Corresponding author: E-mail: adalton@biologico.sp.gov.br, adalton.1@ig.com.br;

by Tephritidae. From 1,454 Tephritidae pupae, 1,310 adults emerged. We found 669 *Ceratitis capitata* (Wied.) (361 females and 308 males) and 641 *Anastrepha fraterculus* (Wied.) (297 females and 344 males). The three varieties of nectarine (Josefina, Rosalina and Rubro-sol) studied were highly susceptible to *A. fraterculus*, having average rates of infestation above 1.60 adult per fruit. Peaks of both fruit flies species occurred between October and December 2004.

Keywords: *Anastrepha fraterculus*; *Ceratitis capitata*; peach; plum; nectarine.

1. INTRODUCTION

Global production of fruit has experienced a remarkable increase, with Latin America and the Caribbean accounting for over 13% of the growth. Output has been rising at an annual rate of approximately 3% over the last decade [1]. In 2011, almost 640 million tons of fruit, and more than 1 billion tons of vegetables, were produced in the world. Brazil is the largest fruit producer in Latin America, and the third-leading supplier in the world [1].

Although most commercial citrus production in Brazil (563,000 ha) is concentrated in São Paulo, there are locations where winter temperatures range from 0-150 cold hours [2], providing favorable conditions for stone-fruit crops.

In 2014, Brazil produced 211,109 tons of peaches and nectarines [3]. The economic potential of peach and nectarine crops in the state of São Paulo stems from the competitive advantage its earlier harvesting period, affords in relation compared not only to the main Brazilian producing regions, but also most of rival countries located in the Southern Hemisphere, such as Chile, Argentina, Uruguay, and South Africa [4]. In 2014, the state of São Paulo had approximately 495,000 fruit-producing peach trees [5]. Species of fruit flies (Diptera: Tephritidae) are insect pests that cause economic losses in stone-fruit production in Brazil.

In São Paulo state, located in the southeastern region, the medfly *Ceratitis capitata* (Wied.) was detected at the beginning of the 20th century, and coexists with at least 35 *Anastrepha* species. *Ceratitis capitata* and *A. fraterculus* (Wied.) are particularly noticeable due to the large number of reported hosts in São Paulo, and infest fruits from over 15 plant families [6,7,8]. The South American fruit fly *Anastrepha fraterculus* is especially abundant in species of *Citrus*, *Rosaceae* and *Myrtaceae* from the southern and southeastern Brazilian regions [6,9].

Among the tephritids reported for the state of São Paulo, *A. fraterculus*, *C. capitata*, *A. obliqua* (Macquart), *A. sororcula* Zucchi, and *A. turpiniae* Stone may infest peaches [6,10,11,12], while the incidence of *A. fraterculus* and *C. capitata* is recorded in plums [6,11]. To avoid or prevent crop losses, insecticides are used as cover sprays to control fruit flies in peach and plum crops in Brazil once the fructification period begins.

An accurately determining the status of a particular plant species as the host of a given fruit fly has become critical because of intensive international trade and the expansion of fruit-growing regions in many parts of the world [13]. Most of the pome fruits and stone-fruit cultivars grown commonly today, were chosen for their quality characteristics. Until recently, resistance to insect pests and diseases has generally not been the focus for selection [14].

Research on the incidence and the degree of infestation by fruit flies in different genetic materials of *Rosaceae* is rare in Brazil. By assessing population dynamics and undertaken a comparative evaluation of natural infestation by Tephritidae, this study aims to further the advancement of Integrated Pest Management and add the genetic improvement of stone-fruits (*Rosaceae*).

2. MATERIALS AND METHODS

2.1 Experimental Site

The assay was conducted during 2004-2005 and 2005-2006 seasons, on trees from the Germplasm Bank of Fruit Trees from Temperate and Subtropical Climates of the Agronomic Institute of Campinas. The trees are located on the APTA Regional farm (S24°02'28.2"; W48°23'02.4", 727 m above sea level), Capão Bonito, in the southwestern region of the state of São Paulo (Fig. 1), which is characterized by a humid, subtropical climate (defined as Cwa, i.e., a mild, temperate climate with a dry winter and a



Fig. 1. Map showing the Germplasm Bank of stone fruits. Capão Bonito, SP, Brazil

hot summer, according to the Köppen classification). Annually, the municipality of Capão Bonito has approximately 100 hours of cold (temperature below 7.2°C) and an average rainfall of 1,236 mm [2].

The experimental area measured 2,415 m², and peach, plum, and nectarine plants on an Okinawa rootstock, as well as ungrafted Okinawa and Mume rootstocks of were evaluated. The materials were eleven years old. Each material consisted of three trees. The spacing between trees was 5 m x 3 m. During the collections, there were no applications of insecticides.

2.2 Population Dynamics of Fruit Flies

The population dynamics of adults were assessed, from January 9, 2004 to March 27, 2006, using 10 yellow-based, Mc Phail traps baited with 400 ml of hydrolyzed protein solution (Bio Anastrepha) at 5% v/v. Traps were installed at an approximate height of 1.60 m from the canopy in a shaded area, and distanced at least 20 m apart.

Captured insects were collected, and the attractant renewed weekly. The tephritids collected were separated from the solution, transferred to labelled glass vials, containing 70% alcohol, and delivered to the Economic Entomology Laboratory of the Biological Institute in Campinas (SP) for screening, subsequent sexing, counting, and identification. Any fluctuation in population was demonstrated by the fly/trap/day (FTD) index.

2.3 Infestation Rate of Peaches, Plums, and Nectarines by Fruit Flies

A total, of 1,631 ripe, unbagged fruits from 34 genetic materials of Rosaceae was collected: 16 of peaches, 13 of plums, 3 of nectarines, and ungrafted specimens of Okinawa and Mume rootstocks (Table 1). The time of collection and the number of fruits collected varied according to the seasonal availability of the genetic material in each crop. Samples were gathered on 13/10/2004 (Aurora 1, Aurora 2, Douradão, Dourado 1, Dourado 2, Ouromel 2, Jóia 1, Jóia 2, Jóia 4, Regis, Ouromel 3, Centenária, Rubro-Sol, Okinawa), 05/11/2004 (Aurora 2, Diamante, Rosalina, Brasão, Josefina, Okinawa), 01/12/2004 (Reubennel - 1, Grancuore, Carmesin, Gema de Ouro), 13/01/2005 (Centenária, Januária), 24/10/2005 (Aurora 1, Flor da Prince, Douradão, IAC 680/13, Dourado 2, Ouromel 2, Jóia 1, Jóia 2, Jóia 4, Tropical), 01/12/2005 (FLA 87-1, FLA 87-7, Mume), and 18/01/2006 (Reubennel - 1, Januária, Grancuore, Gema de Ouro, Roxa de Itaquera, Centenária, Kelsey 31, Kelsey Paulista, Harry Pieckstone).

The fruit samples of each cultivar were transferred to the Economic Entomology Laboratory of the Biological Institute in Campinas (SP), where they were counted and weighed. The samples were individualized in 250 ml volume, lined with a bottom substrate layer made up of an equivalent mixture of sand and vermiculite. The containers were capped with voile bound with elastic. Approximately 20 days

after collection, when the egg-larval period is completed, the fruits were removed from the containers, and sieved to recover the pupae. These pupae were kept, in their respective containers, in an acclimatized room ($25 \pm 2^\circ\text{C}$ and $70 \pm 10\%$ relative humidity) for an additional period of 20 days to allow the emergence of adults. The emerged tephritids were counted and transferred to 50 ml glass containers containing 70% ethanol and top with rubber caps, for later sexing and identification.

2.4 Diversity of Fruit Flies Species

The identification of the *Anastrepha* specimens was based on the aculeus of females [15-17]. *Ceratitis* individuals were counted and sexed, because only *C. capitata* has been reported in Brazil [6].

2.5 Statistical Analysis

The population fluctuation results for Tephritidae adults were correlated with two climatic variables (temperature and rainfall volume), using the Statistical Analysis System (SAS) program, with significance level for Pearson's correlation coefficient measuring 5%. Rainfall and temperature data were obtained from a local meteorological station located approximately 250 m from the experimental area.

The fauna analysis was based on the Simpson index, frequency, dominance, constancy (Shanon-Wiener index), equitability (modified Hill index), and richness (Margalef index) of species obtained from fruits, by summation of the two crop seasons [18].

3. RESULTS AND DISCUSSION

3.1 Population Dynamics of Fruit Fly Species

A total of 2,533 adults of Tephritidae were captured in McPhail traps: 1,320 *C. capitata* (52.1%) and 1,213 *Anastrepha* spp. (47.9%). There was a predominance of females, with 996 specimens of *C. capitata* (75.4%) and 861 specimens of *Anastrepha* spp. (71.0%) recorded. Generally, traps baited with food attractants captured more females than males [10], because young fruit fly females need ingest sugar and/or protein to develop their immature ovaries.

From female specimens, the following species of *Anastrepha* were identified: *A. fraterculus*

(Wied.), *A. grandis* (Macquart), *A. pseudoparallela* (Loew), *A. barbiellinii* Lima, *A. daciformis* Bezzi, and *A. fischeri* Lima. Of the species mentioned, only *C. capitata* and *A. fraterculus* infest stone fruits in Brazil [6,10, 12,19,20]. Population peaks of both species were obtained in January 2004, and from September to December of 2004, a period that coincided with the development and ripening time of stone fruits in the southwestern area of the São Paulo state.

The highest tephritid population peak occurred on 16/01/2004, with a 3.80 FTD, coinciding with the highest *C. capitata* (2.34 FTD) and *A. fraterculus* (1.46 FTD) population peaks. There was no capture of tephritids in 34 of 117 (which is to say 29.1%) of the samples during the study period. *Ceratitis capitata* and *Anastrepha* spp. were absent in 54.7% and 30.8% of samples with traps, respectively. The great majority of samples with no captures of adults occurred when leaves and fruits were absents (Fig. 2).

Ceratitis capitata and *A. fraterculus* are polyphagous species, and the population peaks of these species are influenced by the availability of several host fruits [8,12] under favorable environmental conditions. In São Paulo state, the early emigrant populations of *C. capitata* in stone fruit orchards probably originate from citrus orchards, coffee plantations, or both [9,21], because the fructification of these stone fruits begins approximately two months after the coffee or mid-season citrus harvest. The medfly has many host plants also listed for *A. fraterculus* [6,13,22], a wide geographic distribution in Brazil, and a dominance in urban and peri-urban environments.

If two or more species coexist, competition for the host may occur [23]. The population peaks of *C. capitata* and *A. fraterculus* were very similar in stone fruits, proving that both species coexist and exploit the same resources. The population peak of fruit flies was obtained on 01/16/2004, with FTD values of 2.34 and 1.66 for *C. capitata* and *A. fraterculus*, respectively. During the period studied, due to vegetative rest and leaf fall of orchard plants, especially from March to August of 2004, and March to November of 2005, capture values were very low. This demonstrated that, after the fruiting period, populations of the fruit fly move to other host plants to forage [24], as they can explore different hosts throughout the year [13]. The population peaks of fruit flies coincided with the reproductive phenology and ripening of host

fruits. During periods when the preferred host fruit is in short supply, fruit fly populations may remain at significantly low level, or even undetectable numbers for as long as seven

months, a phenomenon which has important implications for pest management, because the initial populations of flies during fructification are immigrants.

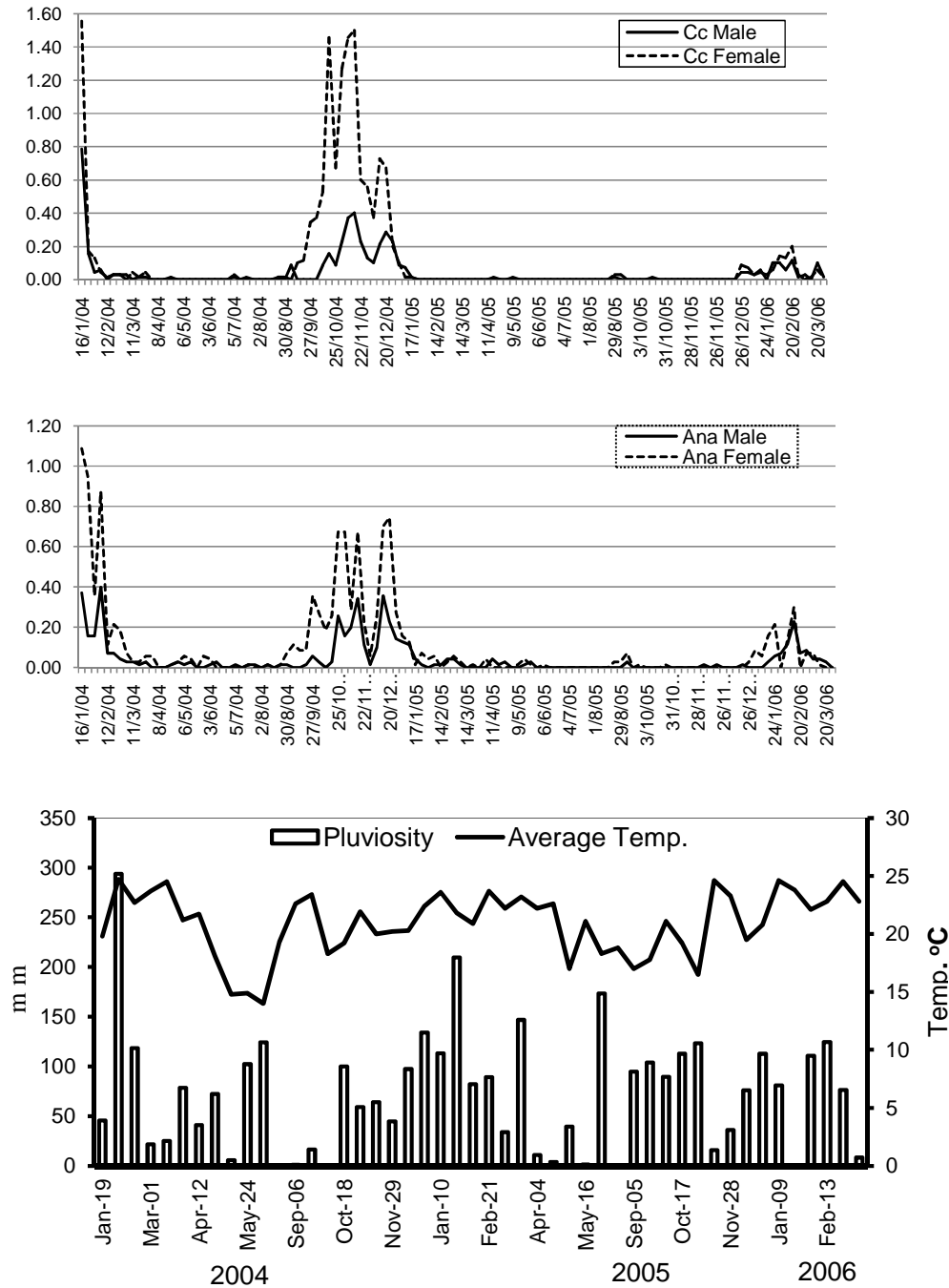


Fig. 2. Flies per trap per day (FTD) of *Ceratitis capitata* (Cc) and *Anastrepha* spp. (Ana) in peach, plum and nectarine orchards, average temperature and volume rainfall during the study period (C). Capão Bonito, SP, Brazil, from January 2004 to March 2006

Table 1. Number of Tephritidae adults emerged from pupae of fruits from different Rosaceae materials. Capão Bonito, SP, Brazil, 2004/2005 and 2005/2006 crops

Variety/species	Nº fruits	Af + Cc	Adults/ fruit	<i>Anastrepha fraterculus</i> (Af)			<i>Ceratitis capitata</i> (Cc)		
				Total	Female	Male	Total	Female	Male
Aurora 1	47	22	0.47	14	8	6	8	4	4
Aurora 2	50	57	1.14	43	18	25	14	5	9
Brasão	25	1	0.04	1	1	0	0	0	0
Diamante	25	51	2.04	12	5	7	39	12	27
Douradão	34	14	0.41	10	3	7	4	3	1
Dourado 1	25	4	0.16	4	1	3	0	0	0
Dourado 2	32	18	0.56	18	5	13	0	0	0
Flor da Prince	22	6	0.27	1	0	1	5	2	3
IAC 680/13	14	4	0.29	1	0	1	3	0	3
Jóia 1	50	26	0.52	11	6	5	15	10	5
Jóia 2	39	32	0.82	7	2	5	25	10	15
Jóia 4	45	7	0.16	7	6	1	0	0	0
Ouromel 2	44	10	0.23	3	2	1	7	4	3
Ouromel 3	25	44	1.76	23	11	12	21	10	11
Regis	25	59	2.36	7	2	5	52	33	19
Tropical	18	13	0.72	4	3	1	9	7	2
Josefina	25	61	2.44	60	34	26	1	1	0
Rosalina	25	40	1.60	40	21	19	0	0	0
Rubro-Sol	25	46	1.84	46	16	30	0	0	0
Carmesim	55	39	0.71	11	2	9	28	18	10
Centenária	113	148	1.31	117	78	39	31	18	13
FLA 87-1	32	13	0.41	0	0	0	13	7	6
FLA 87-7	39	75	1.92	28	5	23	47	24	23
Gema de Ouro	70	52	0.74	28	17	11	24	18	6
Grancuore	88	35	0.40	14	9	5	21	12	9
Harry Pieckstone	54	0	0.00	0	0	0	0	0	0
Januária	77	47	0.61	15	8	7	32	18	14
Kelsey 31	72	6	0.08	0	0	0	6	4	2
Kelsey Paulista	76	4	0.05	0	0	0	4	2	2
Reubennel -1	95	1	0.01	1	0	1	0	0	0
Roxa de Itaquera	49	51	1.04	9	0	9	42	27	15
Mume	100	106	1.06	23	0	23	83	39	44
Okinawa	50	103	2.06	14	8	6	89	52	37
Total	1565	1310	0.84	572	271	301	623	340	283

Due to a change in the volume of fruit production of some Rosaceae species, the 2005-2006 fruit crop was significantly lower than that of the 2004-2005, which in turn negatively affected the population size of fruit flies captured with McPhail traps from November 2005 to March 2006.

From the total of *Anastrepha* females, it was possible to identify 788 intact specimens during collections from traps. Approximately 97.5% of female specimens identified belonged to *A. fraterculus* (Table 2). Other *Anastrepha* species collected have not been reported to feed on Rosaceae species. *Anastrepha grandis* infests Cucurbitaceae fruits, *A. pseudoparallela* infests Anacardiaceae, Passifloraceae, and Myrtaceae fruits; and *A. barbiellinii* was reported in

Cactaceae fruits. *Anastrepha fischeri* does not have any known fruit hosts [25], while *A. daciformis* has only been found on *Schoepfia* (Olacaceae) in a Brazilian Cerrado region [26]. The relative frequency of adults from each species collected in the most important crop season (2004-2005), and species collected in the sum of the two crops, were similar ($t = 0.0516$, $p = 0.959$). Thus, the joint fauna analyses of the infestation were taken into account in the two studied crops. *Anastrepha fraterculus* and *C. capitata* were common, dominant, and constant (Table 2). They were caught in 50.4% and 40.2% of the sampled dates, respectively. The value of the Simpson index (0.50) was medium, influenced by the dominance of the species listed. This value was similar to that obtained for

a mixed fruit orchard in the southern region of the Bahia state (Brazil), in which *A. fraterculus* was predominant [27]. The low adult capture values of other species, as recorded in this study, suggests that the specimens captured migrated from other hosts, seeking shelter, reproduction, or food. The number of flies captured in traps suggests that fruit fly populations were directly affected by host-fruit availability [28].

The reduced value of the Shannon index (0.75) supported the low diversity observed in the orchard, was identical to that obtained in Piracicaba (SP) [29], and was greatly inferior to the value obtained for a mixed orchard in the southern Bahia state (1.35) [27]. Genetically close plants probably do not favor a greater diversity of Tephritidae species. The Hill modified index measures the equitability, or the way abundance is distributed in the community of species [29]. In this study, the value of this index was 0.39 and together with the Margalef index (0.80), it confirmed the dominance of *A. fraterculus* and *C. capitata*, at the expense of other species.

A dominance of *C. capitata* and *A. fraterculus* in guava and sweet orange in commercial orchards was observed in the northwestern region of the state of Rio de Janeiro [30]. In southern Brazil, where average winter temperatures are lower, there is a higher incidence of *A. fraterculus* during the fruiting period of peaches (December and January), with accidental collections of *C. capitata* [31,32].

In eastern and western areas of the state of São Paulo, there are dominances of *A. fraterculus* [12] and *C. capitata* [10], respectively, in peach trees. It is likely that during the fruiting period in the Capão Bonito region, the two species can

satisfactorily explore the local environment and meet their foraging requirements. Furthermore, the São Paulo southwestern region may be a transition region, in which there is no predominance of a single species, due to competitive ability in the agroecosystem.

3.2 Natural Infestation Rate and Fruit Fly Diversity of Peaches, Plums, and Nectarines

Except for "Harry Pickstone" plums (Table 1), the other varieties and rootstocks collected showed infestation by Tephritidae species. From 1,454 pupae of Tephritidae, 1,310 adults emerged (90.1% viability): 669 *C. capitata* and 641 *A. fraterculus*. These values corresponded to 51.1% and 48.9%, respectively of Tephritidae specimens obtained in the experiment (Table 1). In relation to total adults (Fig. 3), we obtained more *C. capitata* females (28.4%) and *A. fraterculus* males (25.2%).

In 23 collected materials, there were reports of both Tephritidae species. "Kelsey 31" and "Kelsey Paulista" plums were exclusively infested by medfly. The peach varieties of "Brasão", "Dourado 1", "Dourado 2" and "Jóia 4"; The nectarine varieties of "Rosalina" and "Rubro-Sol"; and the plum varieties of "Reubennel-1" were infested only by *A. fraterculus*. The peaches "Brasão", "Dourado 1" and "Jóia 4", in addition to plums "Kelsey 31", "Kelsey Paulista" and "Reubennel-1" produced 0.04, 0.16, 0.16, 0.08, 0.05, and 0.01 Tephritidae adults per fruit, respectively (Table 1), and should be considered for improvement programs aiming to resist tephritids. Fruits from "Diamante" peaches, "Josefina" plums, and the "Okinawa" rootstock, had average values above 2.00 adults per fruit.

Table 2. Fauna analysis of fruit flies (Tephritidae) species captured with McPhail traps in a Rosaceae orchard in the municipality of Capão Bonito, SP, Brazil (January 2004 to March 2006). D = dominant; ND = non-dominant; F = frequent; VF = very frequent; W = constant; Y = accessory; VA = very abundant

Species	NS	Relative frequency (%)	No. of collections	Dom.	Abund.	Freq.	Const.
<i>A. fraterculus</i>	768	43.07	64	D	VA	VF	W
<i>A. fischeri</i>	1	0.06	1	ND	VA	F	Y
<i>A. grandis</i>	12	0.67	7	D	VA	F	Y
<i>A. pseudoparallela</i>	5	0.28	5	ND	VA	F	Y
<i>A. barbiellinii</i>	1	0.06	1	ND	VA	F	Y
<i>A. daciformis</i>	1	0.06	1	ND	VA	F	Y
<i>C. capitata</i>	995	55.80	51	D	VA	VF	W

NS= number of specimens; Species richness (S) 7; Simpson index 0.50; Shannon-Wiener index 0.75; Margalef diversity index 0.80; Equitability (Modified hill) 0.39

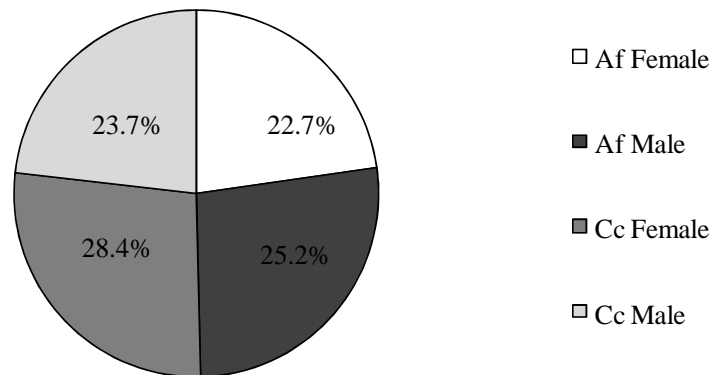


Fig. 3. Comparative percentage by sex of *Anastrepha fraterculus* (Af) and *Ceratitis capitata* (Cc) obtained from fruits of peach, plum and nectarine varieties. Capão Bonito (SP), Brazil, 2004/2005 and 2005/2006

The three varieties of nectarine studied were highly susceptible to *A. fraterculus* attacks, presenting average infestation rates of over 1.60 adult per fruit. This result confirmed the preference for nectarines over peaches and plums in the southwestern region of the state of São Paulo.

There were infestations by both Tephritidae species in fruits from trees grafted onto "Okinawa" and mume rootstocks, but with a dominance of medflies (Table 1). The resistance mechanisms in Tephritidae are dynamic and may be species-specific [33]. The "Fla 86-2" and "Fla 87-7" varieties had the highest average rates of infestation among plum trees (1.74 and 1.92 adult per fruit, respectively), coming close to the rates observed for nectarines.

The results of infestation obtained in this study confirm that *A. fraterculus* adapted to the hosts introduced. The general relative abundance index (RAI) of adults ($Cc/Af + Cc$) was 0.51 and indicated that, in all genetic material sampled there was no dominance of *C. capitata* or *A. fraterculus*. RAI values varying between 0.00 and 1.00 were obtained for peaches, depending on the sample collection area in Argentina, but the Rosaceae family was a standard intermediate for this index [34].

In the municipality of Monte Alegre do Sul, in the eastern region of the São Paulo state, infestations by *A. fraterculus*, *C. capitata*, and *A. obliqua* were observed in peaches, while only

the first two species were recovered in loquats [12]. In the municipality of Presidente Prudente, located in the far-western region of the São Paulo state, there is a predominance of medflies in peaches as related to *Anastrepha* spp. [10].

In eastern São Paulo, the infestation by *C. capitata* in peaches occurs in August and September, with medflies practically absent from peach and loquat orchards in January-July, a period when *A. fraterculus* is dominant [10]. This explains the higher incidence of medflies in the far-western region of the São Paulo state, where fruiting occurs in August and September, when orchards are susceptible to immigrant populations of *C. capitata* from the coffee post-harvest period [9,21].

The fructification of peaches, plums, and nectarines occurs in Capão Bonito in an intermediary period, between those verified for the eastern and the extreme western regions of the São Paulo state, with a much greater balance between infestation rates by both Tephritidae species. Equivalent infestations by *A. fraterculus* and *C. capitata* in peaches were also observed in the Tucumán region (northern Argentina) [13].

Both fruit fly species presented a relative abundance in various parts of Argentina, exploring the same host and coexisting with similar ecological requirements [34]. In Pelotas (RS), a natural infestation by *A. fraterculus* was detected in plums of "Amarelinha", "Pluma", "Santa Rosa", "Reubennel" and "Wade" cultivars,

with damage in fruits measuring approximately 2.0-3.0 cm in diameter [35].

In the municipality of Presidente Prudente (SP), there was no statistical difference in the fruit fly infestation of different cultivars grafted on "Okinawa" [10], when "Aurora-2" reached 23% of fruit infestation by *C. capitata* in 2006. In the present study, fruit flies infested approximately 54% of "Aurora-2" fruits, with a dominance of *A. fraterculus*.

Ample evidence revealed that not all cultivars within a particular fruit species were equally susceptible to infestation. Such information needs to be considered when determining the status of the host plant, and calculating the risk of introduction or spread of flies by infested fruits [33].

4. CONCLUSIONS

Ceratitis capitata and *Anastrepha fraterculus* infest peaches, plums, and nectarines in the southwestern region of the state of São Paulo. The "Brasão", "Dourado 1", "Flor da Prince", "IAC 680-13" and "Jóia 4" peach varieties, in addition to "Kelsey 31", "Kelsey Paulista" and "Reubennel-1" plums, should be considered for improvement programs aiming to facilitate resistance to fruit flies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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