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FRUIT FLIES AND LANCE FLIES (DIPTERA: TEPHRITOIDEA) AND THEIR HOST PLANTS IN A CONSERVATION UNIT OF THE CERRADO BIOME IN TOCANTINS, BRAZIL

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ABSTRACT

Fruit flies (Tephritidae) and lance flies (Lonchaeidae) are specialized in exploring fruits as resource, and the native Angiospermae represent potential hosts for their frugivorous larvae. The aim of this study was to investigate the relationship of fruit fly and lance fly species with native host fruits in the Parque Estadual do Lajeado, Palmas, Tocantins, Brazil. The survey was carried out from May 2011 to Apr 2013, with sampling of fruits to obtain third instar larvae, pupae and adults. We sampled fruits of 18 plant species of which 7 were infested by fruit flies or lance flies, and from which emerged 888 adults of Neosilba spp. (Lonchaeidae), and 81 adults of Anastrepha (Tephritidae). Neosilba zadolicha emerged from fruit of Siparuna camporum, Dyospiros hispida, Annona coreacea, Mouriri pusa, Cheiloclinium cognatum and Salacia crassifolia. Neosilba bifida, N. glaberrima, and Neosilba n. sp.1, emerged from fruits of D. hispida. The species of Anastrepha obtained were: A. serpentina and A. nr. mucronota reared from S. crassifolia and C. cognatum fruits; A. obliqua from S. crassifolia, A. sororcula from Myrcia guianensis, A. turpiniae, and A. zenildae from M. pusa. Of the seven host fruit species, four were infested by Tephritidae, and six by Lonchaeidae. These are the first records for: A. sororcula in fruits of Myrcia guianensis, A. nr. mucronota in Salacia crassifolia and Cheiloclinium cognatum, A. serpentina in Cheiloclinium cognatum, and A. turpiniae and A. zenildae in Mouriri pusa. The associations of fruit flies and lance flies with host fruits is locally and temporally dependent on the flora, since the species of flies require available fruit species. In this survey we observed the highly polyphagous A. obligua, that in this survey to colonize only one species of fruit.

Key Words: biodiversity, cerrado of northern Brazil, frugivory, new records, phytophagy

Resumo

As moscas das frutas (Tephritidae e Lonchaeidae) são um grupo especializado de insetos em explorar frutas como recurso, e as Angiospermas nativas representam potenciais hospedeiros para suas larvas frugívoras. O objetivo deste estudo foi investigar a associação de espécies de moscas de frutas com plantas hospedeiras nativas no Parque Estadual do Lajeado, em Palmas, Tocantins, Brasil. A pesquisa foi realizada a partir de maio de 2011 a abril de 2013, com amostragem de frutas para obter larvas de terceiro instar, pupas e adultos. Foram amostrados frutos em 18 espécies de plantas, sendo sete infestadas por moscas das frutas, das quais emergiram 888 espécimes de Neosilba spp. (Lonchaeidae) e 81 adultos de Anastrepha (Tephritidae). Neosilba zadolicha emergiu de frutos de Siparuna camporum, Dyospiros hispida, Annona coreacea, Mouriri pusa, Cheiloclinium cognatum e Salacia crassifolia. Neosilba bifida, N. glaberrima e Neosilba **sp.n.1** emergiram de frutos de D. hispida. As espécies de Anastrepha obtidas foram: A. serpentina e A. pr. mucronota criadas de frutos de S. crassifolia e C. cognatum; A. obliqua de S. crassifolia, A. sororcula de Myrcia guianensis, A. turpiniae e A. zenildae de M. pusa. Das sete espécies frutíferas hospedeiras, quatro foram infestadas por Tephritidae e seis por Lonchaeidae. Estes são os primeiros registros para: A. sororcula em frutos de Myrcia guianensis, A. pr. mucronota em Salacia crassifolia e Cheiloclinium cognatum, A. serpentina em Cheiloclinium cognatum, A. turpiniae e A. zenildae em Mouriri pusa. As associações de moscas das frutas com as frutíferas hospedeiras são localmente e temporalmente dependentes da flora, uma vez que as espécies de moscas das

frutas precisam de frutos disponíveis, como observado para a espécie altamente polífaga A. obliqua, que neste estudo infestou apenas uma espécie de frutífera.

Palavras Chave: Biodiversidade, Cerrado do Norte do Brasil, frugivoria, fitofagia, novos registros.

Many studies have been conducted to document biodiversity, e.g., Basset et al. (2000), Hawkins & Pausas (2004), and Lewinsohn et al. (2005). Brazil is a country with high biological diversity, and in the Amazon region most species of insects are still unknown (Strikis et al. 2012).

Tephritoid flies have great adaptability to different regions worldwide, and represent the main group of fleshy fruit consumers (White & Elson-Harris 1992). The use of host plants by individual phytophagous species is generally restricted to phylogenetically close groups, often a single family or genus (Strong et al. 1984; Bernays & Chapman 1994), but some species of fruit flies and lance flies are highly polyphagous. Both native and introduced fruits are potential host plants for larvae of frugivorous Tephritidae and Lonchaeidae (Uchôa 2012). Availability of fruits, the high biodiversity and endemism in the Cerrado biome, make these fruit trees potential sites for infestation by fruit flies, since the various fruiting patterns of these host plants assures that fruit is produced throughout the year.

Studies of the associations of species of Tephritidae and their host plants are important to understand their patterns of diversity, ecology and evolution (Aluja et al. 2003), to comprehend their natural history, and to plan strategies to manage fruit fly pests in orchards (Uramoto et al. 2004).

Larvae of *Neosilba* spp. (Lonchaeidae) develop in a wide variety of structures and plant species, including: sprouts, fruits, flowers and buds. Large populations can be found associated with mature fruits (Uchôa 2012). Current studies on *Neosilba* species include taxonomy, biology and ecology (Galeano-Olaya & Canal 2012). These native species of Lonchaeidae under human disturbance may come to use exotic cultivated plant as their hosts. Such disturbances can favor the host range expansion of phytophagous insect species beyond their primary native hosts to include cultivated exotic species (Selivon 2000).

Until the late twentieth century there were no host plant records for species of fruit flies in the State of Tocantins (Zucchi 2008). Bomfim et al. (2007a, b) published the first records. However, this information is still very rudimentary and requires additional studies to more fully elucidate the relationships between fruit flies and lance flies and their hosts, especially in natural environments where there are opportunities to observe additional native host plants.

Protected areas present one of the best strategies to conserve the natural heritage. In these areas natural flora and fauna are preserved, as well as the ecological processes that govern ecosystems, ensuring the maintenance of the stock of biodiversity (Seplan 2005).

The Cerrado is a tropical biome with high plant diversity and endemism, where many unknown interactions between insects and their host plants should occur. Thus, considering the lack of knowledge about frugivorous larvae of Tephritoidea and their association with native host fruit species from Cerrado in the Amazon region (especially in the state of Tocantins), this study aimed to investigate species of fruit flies and lance flies and their association with host fruits in the Conservation Unit of the Parque Estadual do Lajeado, at Palmas, Tocantins, Brazil.

MATERIALS AND METHODS

The study was carried out in the Parque Estadual do Lajeado (PEL), an Integral Protection Conservation Unit in the municipality of Palmas Tocantins, Brazil, bounded by the coordinates S $10^\circ~00'~24''$ -W $48^\circ~17'~03'',~S~10^\circ~00'~13''$ and –W $48^\circ\,15'\,45'',\,S\,10^\circ\,11'\,50''$ and –W $48^\circ\,12'\,56'',\,S\,10^\circ$ 11' 25" and -W 48° 10' 37", S 10° 09' 14" and -W 48° 09' 54". According to Seplan (2005) the area has average temperatures ranging between 25° C and 28° C; the rainfall is concentrated in a 5-6 month period, starting in Oct or Nov and lasting until Mar, with relative humidity ranging from 0 to 40%, and accumulated rainfall of about 1,700 mm per yr. Sampling was carried out from May 2011 to Apr 2013. The search for host fruits occurred biweekly throughout the sampling period, and the collection of fruits occurred according to their availability in the field. All the fruits were collected directly from the trees.

The fruits were kept in a rearing room at the Universidade Federal do Tocantins (UFT), where they were counted and weighed. Then, these fruits were arranged on fiber networks fixed in a wood platform and arranged in plastic trays containing a water slide, where they remained for 30 days to collect the frugivorous third instar larvae of Tephritoidea as they emerged from the fruit. Every 12 h the larvae were removed from the trays, placed in transparent cups (300 mL) containing sterilized sand as a pupation substrate, where the pupae remained until adult emergence occurred. The adults were reared for 3 days on a solution of honey and water (1:5 v:v), to allow full development of the color of their bodies and wings after emergence.

The species of *Neosilba* (Lonchaeidae) were identified by Dr. Laura Gisloti, Universidade Estadual de Campinas (UNICAMP), Campinas-SP, Brazil, according to McAlpine & Steyskal (1982). Voucher specimens were deposited in the Entomological Collection of UNICAMP.

The tephritids were identified in the Universidade Federal da Grande Dourados (UFGD), Dourados-MS, Brazil, using specific identification keys (Stone 1942; Steyskal 1977; Zucchi 2000; Norrbom 1997; Norrbom & Korytkowski 2009). After identification, the Tephritidae were deposited in the Coleção Entomológica do Museu da Biodiversidade (MuBio-UFGD). Dried herbarium specimens (exsiccatae) of the identified host plants were deposited in the Herbarium of Tocantins (HTO) of the Universidade Federal do Tocantins (UFT), at Porto Nacional, Tocantins, Brazil.

To analyze the species richness of fruit fly and lance fly species accumulation curves were plotted using the software EstimateS 8.2 (Colwell 2009) with 100 randomizations. Each point of the curve corresponds to the average of the accumulated species richness in the 100 curves. Each species of fruit collected was considered a sample, totaling 18 samples. The species richness was calculated by the estimators Jacknife 2, and Chao 2. The similarity between species of fruit flies and lance flies regarding exploitation of their host fruits was calculated through cluster analysis using the Bray-Curtis distance by the software Biodiversity Pro 2 (McAleece et al. 1997).

RESULTS

In total 2,682 fruits of 18 species of native plants were sampled. Seven plant species were infested by the fruit fly and lance fly species. These included 888 *Neosilba* (Lonchaeidae) adults recovered, which consisted of the species *N. zadolicha*, *N. pendula*, *N. bifida*, *N. glaberrima*, and *Neosilba* n.sp.1, and 81 adults of *Anastrepha* spp. (Tephritidae): *A. serpentina*, *A. obliqua*, *A.* nr. *mucronota*, *A. sororcula*, *A. turpiniae* and *A. zenildae* (Table 1).

Four of the 7 host species were infested by Tephritidae and 6 by Lonchaeidae. Anastrepha serpentina was recovered from Salacia crassifolia and Cheiloclinium cognatum (Hippocrateaceae), A. obliqua from S. crassifolia; A. nr. mucronota from S. crassifolia and C. cognatum; A. sororcula from Myrcia guianensis (Myrtaceae); and A. tur-

TABLE 1. SPECIES OF FRUIT FLIES AND LANCE FLIES (DIPTERA: TEPHRITOIDEA) AND THEIR NATIVE HOST FRUITS IN THE CONSERVATION UNIT PARQUE ESTADUAL DO LAJEADO, TO, BRAZIL (MAY 2011 TO APR 2013).

Fruit Fly Species	Fruit Tree Species	No. adults	Herbarium of Tocantins (HTO) record No.
A. serpentina	Salacia crassifolia (Mart. ex Schult.) G. Don*	15	6,312 HTO
	Cheiloclinium cognatum (Miers) A. C. Sm.****	10	8,427 HTO
A. obliqua	Salacia crassifolia (Mart. ex Schult.) G. Don**	10	6,312 HTO
A. nr. <i>mucronota</i>	$\begin{array}{llllllllllllllllllllllllllllllllllll$	1	6,312 HTO
	Cheiloclinium cognatum (Miers) A. C. Sm.****	1	8,427 HTO
A. sororcula	Myrcia guianensis (Aubl.) DC.****	12	10,513 HTO
A. turpiniae	Mouriri pusa Gardner****	31	4,575 HTO
A. zenildae	Mouriri pusa Gardner****	1	4,575 HTO
Neosilba zadolicha	Siparuna camporum (Tul.) A.DC.**	6	3,429 HTO
	Diospyros hispida A.DC.**	826	10,565 HTO
	Annona coriacea (Mart.)**	36	5,096 HTO
	Mouriri pusa Gardner**	1	4,575 HTO
	Salacia crassifolia (Mart. ex Schult.) G. Don**	14	6,312 HTO
	Cheiloclinium cognatum (Miers) A. C. Sm.**	1	8,427 HTO
N. pendula	Salacia crassifolia (Mart. ex Schult.) G. Don	1	6,312 HTO
Neosilba bifida***	Diospyros hispida A.DC.	1	10,565 HTO
Neosilba glaberrima	Diospyros hispida A.DC.**	1	10,565 HTO
Neosilba n. sp.1	Diospyros hispida A.DC.	2	10,565 HTO

*First record in the host for Tocantins;

**First record in the host in northern Brazil;

***First record of this lance fly species in northern Brazil;

****First record of these fruit fly species in this host.

piniae and A. zenildae from Mouriri pusa (Memecylaceae).

Five species of Lonchaeidae were recovered as follows: Neosilba zadolicha from fruits of Siparuna camporum (Siparunaceae), Diospyros hispida (Ebenaceae), Annona coriacea (Anonaceae), M. pusa, S. crassifolia and C. cognatum; N. pendula from fruit of S. crassifolia; and N. bifida, N. glaberrima, and Neosilba n. sp.1 from D. hispida fruits. The greatest number of species of fruits (6) were infested by N. zadolicha, the most polyphagous and abundant species (Table 1).

The season of greatest fruit production was from Oct to Mar. *Salacia crassifolia* and *C. cognatum* were the only host fruits simultaneously infested by larvae of *Neosilba* spp. and *Anastrepha* spp.

The host fruits with the highest rates of infestation, taking in account all Tephritoidea, were: S. crassifolia, C. cognatum (Hippocrateaceae), and D. hispida (Ebenaceae). The infestation rate by larvae of Anastrepha species (Tephritidae) was highest in the following host fruits: C. cognatum (32.32 larvae/kg), S. crassifolia (30.01 larvae/kg), and Myrcia guianensis (8.59 larvae/kg). Fruits of *M. guianensis* were 100% infested by *Anastrepha* sororcula. For the species of Neosilba (Lonchaeidae) the greatest rate of infestation occurred in M. pusa (149.35 larvae/kg), D. hispida (123.10 larvae/kg), and A. coriacea (36.73 larvae/kg). One hundred percent of fruits of S. camporum, D. his*pida*, and *A. coriacea* were infested by species of Neosilba (Table 2).

The species accumulation curves were upward, and the curves did not indicate stabilization (Fig. 1). Cluster analysis generated 4 groups based on the use of host fruits: 2 of them formed by *Anastrepha* species, 1 by *Neosilba* species, and a fourth group by *A. obliqua* and *N. pendula* (Fig. 2).

DISCUSSION

The species accumulation curves (Fig. 1) suggest the possibility of finding more species of fruit fly and lance fly species in the fruit species surveyed, if the sampling had been continued. The species richness of frugivorous flies could have been enhanced if we had increased the number of fruit samples from the fruit species evaluated. The estimators Jackknife 2 predicted a species richness of 24, and the Chao 2 estimated a maximum of 27 species for the samples actually made; these estimates are higher than the number of species recovered from fruits sampled in this inventory (n = 10) (Fig. 1).

The similarity in the use of host plant taxa between the different frugivorous fly species (cluster analysis) in this study showed that species of *Neosilba* were more similar to each other than with the species of *Anastrepha*, with the exception of the similarity between *N. pendula* and *A*. *obliqua* (Fig. 2). Interestingly the latter 2 species are widely polyphagous, as pointed out by Uchôa (2012), but herein they attacked a single species of fruit (*S. crassifolia*, Table 1). This may have occurred due the effect of a fire that burned the vegetation (Bomfim & Uchôa unpublished data), probably reducing the populations of the 2 fly species.

The choice of the host plant by insects is not the result of a simple behavior, but represents a dynamic hierarchy of various components, including co-evolutionary aspects (Connell 1980; Aluja & Mangan 2008). The use of host plants may vary at the individual level, in response to changes in the internal physiological conditions of the fly (e.g., the load of eggs) caused by a shortage of preferred hosts or prior experience with a host. In the absence of their preferred host plant, a polyphagous insect may exhibit hierarchies of preference among those currently available. The usage pattern of host plants can vary according to the relative abundance of potential alternate host(s). It is expected that the diversity of fruit trees in the natural forest increases the likelihood of finding monophagous species of fruit flies (Selivon 2000; Aluja & Mangan 2008). Therefore, the association with the host fruit is a local phenomenon and depends on host availability in the ecosystem, as in the cases of Anastrepha obliqua and N. pendula (Araujo & Zucchi 2002); a single species of fruit was infested by the latter 2 species in this survey, despite both species being polyphagous species.

Sixteen interactions between species of fruit flies and lance flies and their host plants sampled in this study were discovered and reported here. This is the consequence of the floristic richness in the Cerrado Biome, and the lack of studies on trophic interactions between Tephritoidea and host plants in the Neotropics. According to Zucchi (2008), the hosts of about 48.70% of *Anastrepha* species reported in Brazil are still unknown.

Prior to this study, host plants were known in the State of Tocantins for the following nine species of fruit flies: A. coronilli, A. fraterculus, A.nr. mucronota, A. obliqua, A. sororcula, A. striata, A. turpiniae, A. zenildae, and C. capitata (Bomfim et al. 2007b). This research has added new associations with hosts for: A. nr. mucronota, A. serpentina, A. sororcula, A. turpiniae, and A. zenildae. Also this research has associated 5 species of *Neosilba* with their host fruits in northern Brazil (Table 1). These results highlight the importance of inventories of frugivorous tephritoids in native host plants, because fruit production by the native plants is interspersed throughout the yr, which provides conditions for the survival of fruit flies. The existence of a wide range of hosts for these flies in Tropical America, with fruit production in different seasons, provide conditions for maintenance of high populations of frugivorous Tephritoidea.

Species of host fruits	No. of Larvae	No. of Fruits	Biomass of fruit (g)	Larvae/fruit	Larvae/kg of fruit	Anastrepha spp. $(\%)$	Neosilba spp.
Calania manufalia (Hinnomatanana)	111	101	2700	1 12	30.01	71	90
Cheiloclinium coanctum (Hynocratescee)	58 85	115	9630	0.74	32.32	11	80
Myrcia guianensis (Myrtaceae)	7 8 78	144	326	0.19	8.59	100	00
Siparuna camporum (Siparunaceae)	18	1952	5957	0.01	3.02	00	100
Diospyros hispida (Ebenaceae)	1101	256	8944	4.30	123.10	00	100
Annona coriacea (Anonaceae)	90	Q	2450	18.00	36.73	00	100
<i>Mouriri pusa</i> (Memecylaceae)	115	109	770	1.06	149.35	66	01
Alibertia edulis Rich (Rubiaceae)	0	41	1482	0	0	0	0
Anacardium occidentale L. (Anacardiaceae)	0	82	2420	0	0	0	0
Brosimum gaudichaudii Tréc. (Moraceae)	0	116	940	0	0	0	0
Caryocar brasiliense Camb. (Caryocaraceae)	0	84	7405	0	0	0	0
Couepia grandiflora Benth. (Chrysobalanaceae)	0	31	362	0	0	0	0
Hancornia speciosa Gomes (Apocynaceae)	0	œ	210	0	0	0	0
Mauritia flexuosa L.f. (Arecaceae)	0	72	3744	0	0	0	0
Byrsonima sp. (Malpighiaceae)	0	140	2289	0	0	0	0
Leandra sp. (Melastomataceae)	0	132	330	0	0	0	0
Passiflora sp. (Passifloraceae)	0	9	241	0	0	0	0
Psidium sp. (Myrtaceae)	0	17	280	0	0	0	0
Total	1,551	3,411	44,579				

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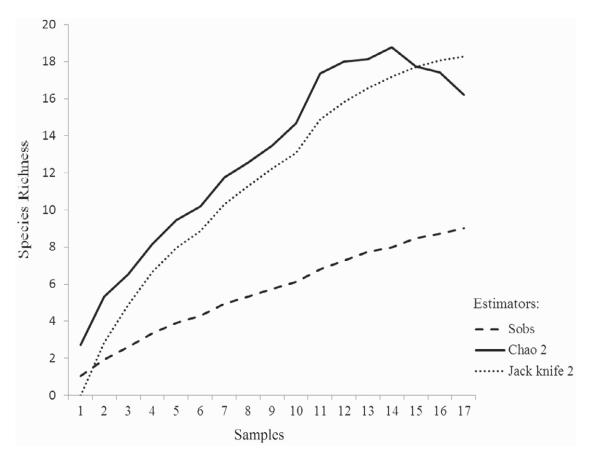


Fig. 1. Accumulation curves for fruit fly and lance fly species (Tephritidae and Lonchaeidae) in the host fruits calculated using Sobs, Chao 2 and Jack 2 estimators, sampled in the Conservation Unit of Parque Estadual do Lajeado, Palmas, to, Brazil (May 2011 to Apr 2013).

Research on fruit flies in other natural reserves in Brazil has revealed important aspects of diversity and trophic interactions with host plants and parasitoids. In a study in the Adolpho Ducke Reserve in Amazonas State, 12 species of Anastrepha were found, of which eight were new species (Costa 2005). In Conservation Unit Vale do Rio Doce (Atlantic Forest), State of Espírito Santo, 21 species of Anastrepha were sampled, and 5 of these were new records for Espírito Santo, and one was a new record for Brazil. In that study 5 new species were found (Uramoto et al. 2008). So, each survey carried out in natural environments in the Neotropical Region has increased new information on Tephritoidea diversity, their host plants and natural enemies.

New Host Fruits of Tephritidae

Anastrepha serpentina was reared for the first time from fruits of *Cheiloclinium cognatum*; A. nr. *mucronota* from *Salacia crassifolia* and *C. cogna*- tum; A. turpiniae and A. zenildae from Mouriri pusa fruits. Here we also reported the first record for A. sororcula in fruits of M. guianensis, a small wild fruit with a diam of 5 mm.

New host fruit of Lonchaeidae

All records of host plants for the species of *Neosilba* in this survey are new associations. The species of *Neosilba* were more abundant and colonized more species of fruit plants than did the species of *Anastrepha*. Other studies have also highlighted the importance of some Lonchaeidae species as primary fruit infesting species, and that several species of *Dasiops*, and *Neosilba* are voracious omnivores (Uchôa et al. 2002; Nicácio & Uchôa 2011; Uchôa 2012).

In all states of the northern region of Brazil there are reports of the occurrence of *Neosilba* species, i.e., Amazonas, Amapá, Acre, Pará, Rondônia, Roraima (Strikis et al. 2011), and Tocantins (Bomfim et al. 2007a, b.). Recent studies indicate that some species of *Neosilba* colonize

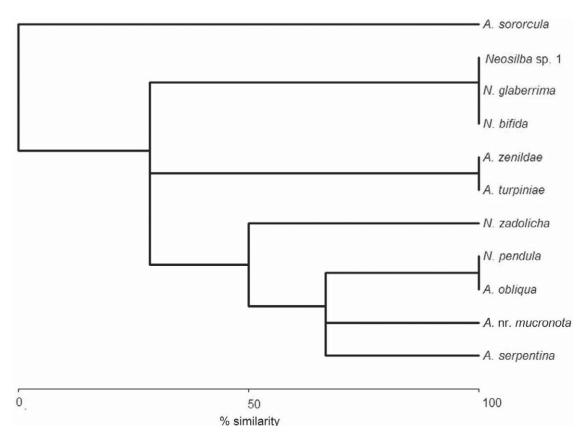


Fig. 2. Analysis of similarity based in the use of host fruits by species of fruit and lance flies (Tephritidae and Lonchaeidae) in the Conservation Unit of Parque Estadual do Lajeado, Palmas-to, Brazil (May 2011 to Apr 2013).

a greater number of host fruit species than the species of *Anastrepha* (Uchôa et al. 2002; Uchôa 2012), and this demonstrates the importance and the need for investigations on the Lonchaeidae in the Neotropical Region.

In this research Neosilba zadolicha infested a greater diversity of host fruits compared to all other obtained species of frugivorous flies. This polyphagous species has already been reported from 17 host species (Strikis et al. 2011; Uchôa 2012). The occurrences reported herein of N. zadolicha in the host plants: S. camporum, D. hispida, A. coriacea, M. pusa, S. crassifolia and C. cognatum, are new records. Neosilba pendula and N. glaber*rima* were individually recorded each in a single host (Salacia crassifolia and Diospyros hispida, respectively - Table 1). However, both species are considered polyphagous (Uchôa 2012). Araújo & Zucchi (2002) reported the polyphagy of N. pendu*la*, and that this species indiscriminately infested native and exotic fruits.

Due to high levels of infestation in native and exotic fruits, lonchaeids are currently considered of economic importance in Brazil (Uchôa et al. 2002; Uchôa 2012; Strikis et al. 2011; Strikis et al. 2012; Gisloti & Prado 2012). In this work, the larvae of some species of *Neosilba* colonized higher number of fruit species than *Anastrepha*. This pattern was also observed before in areas of the Cerrado and the Pantanal (Uchôa et al. 2002; Uchôa & Nicácio 2010). The lonchaeids have been ignored in most studies of frugivorous flies in Brazil, possibly due to lack of taxonomic knowledge.

The fruits of *Diospyros hispida* were colonized only by the species of *Neosilba* (Table 2), suggesting a probable specificity of lonchaeids for this host plant. According to Aluja & Mangan (2008), an alternative explanation for unexpected patterns in the usage of host plants by insects in the field could involve associative learning, and in that case, there is no need for changes in larval characteristics.

Resource Partitioning by Fruit Flies

Sharing of resources between species of *Neosil*ba and those of *Anastrepha* was here observed, both groups (*Anastrepha* species and *Neosilba* species) infested *S. crassifolia* and *C. cognatum*. A similar pattern was observed by Uchôa et al. (2002) who found infestations by species of these 2 genera in *Citrus* spp. (Rutaceae). Braga-Filho et al. (2001) also found that fruits of *S. crassifolia* were natural hosts for species of *Anastrepha* and *Neosilba*, with concomitant infestation. According to Connell (1980), niches occupied by competitors in ecological communities are shaped by mutual co-evolution, which allows many species to coexist.

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