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Scientific Note

Artifices of *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) for survival in umbu, endemic fruit from Brazil

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Abstract. We studied the interaction involving umbu, fruit flies and parasitoids in the Brazilian cerrado biome in Piauí state. The fruits were collected in Baixa Grande do Ribeiro and Uruçuí in the period from February to April 2013 and were analyzed in Laboratory. In this study is being published the first occurrence of *Anastrepha obliqua* (Macquart, 1835) in umbu fruits and two parasitoids, *Aganaspis pelleranoi* (Brèthes, 1924) and *Opius bellus* (Gahan, 1930), in Piauí. Where 77.8 % of the fruits were infested by *A. obliqua*, with 149.4 pupae / kg (2.4 pupae / fruit) and the parasitism rate was 2.7 %. Natural parasitism is not very significant, however, less pupae were found in fruits with parasitized larvae, suggesting that the fly avoids oviposition. Additionally, it was found that the *A. obliqua* performs multiple oviposition in umbu during host selection and multiple infestations is a factor that increases the likelihood of emergence of *A. obliqua* during the colonization of umbu. The tritrophic interaction among *S. tuberosa*, *A. obliqua* and *O. bellus* was discussed.

Keywords: Tritrophic interaction, fruit fly, fruit host, parasitoid.

Umbu (*Spondias tuberosa* Arr. Cam.) is a known host of five species of fruit flies (Diptera: Tephritidae), such as *Ceratitis capitata* (Wiedemann, 1824) (Araujo et al. 2005), *Anastrepha fraterculus* (Wiedemann, 1830) (Alvarenga et al. 2009) *Anastrepha obliqua* (Macquart, 1835) (Araujo et al. 2005; Alvarenga et al. 2009; 2010), *Anastrepha sorocurla* Zucchi, 1979 (Alvarenga et al. 2010) and *Anastrepha zenildae* Zucchi, 1979 (Alvarenga et al. 2009). The species West Indian Fruit Fly, *A. obliqua*, historically represents the most serious pest of *Spondias* spp. (Alvarenga et al. 2010). The *Spondias* infestation by *A. obliqua* has been reported in Argentina, Brazil, Colombia, Mexico, Peru and Caribbean islands (Sivinski et al. 2000). *A. obliqua* was found in *Spondias mombin* L., *Spondias purpurea* L., *Spondias lutea* L., *Spondias venulosa* (Engl.), *Spondias dulcis* Forst., *Spondias cytherea* Sonnerat and *Spondias radkolferi* Donn Sm. (Sivinski et al. 2000). On the other hand, the umbu fruit infestation was only registered in Brazil (Araujo et al. 2005; Lima Junior et al. 2007; Alvarenga et al. 2009; 2010; Bomfim et al. 2010).

The West Indian Fruit Fly has Neotropical origin, being found from Argentina to the southern United States in American continent (Ruiz-Arce et al. 2012; Santos et al. 2020). Its pest status may vary throughout its geographical distribution and depending on the host (Bittencourt et al. 2011; Ruiz-Arce et al. 2012; Santos et al. 2020). The West Indian Fruit Fly has a high diversity of host fruits, infests more than 60 host species in 24 botanical families in American continent (Ruiz-Arce et al. 2012) and 45 hosts species in nine botanical families in Brazil (Zucchi & Moraes 2008). In addition, about 12 hymenopteran parasitoid species have been associated with *A. obliqua* in Neotropical region (Garcia et al. 2020). Information on the tritrophic interaction involving fruit flies, fruits hosts and parasitoids contribute to the knowledge of the ecological, biological and behavioral characteristics of the pest and its natural enemies. Here, we studied the interaction involving umbu, fruit flies and parasitoids in the Brazilian cerrado biome in the Immediate Geographic Region of Uruçuí in the Piauí state, Brazil.

Umbu fruits were collected in the municipalities of Baixa Grande do Ribeiro (7º50'27.33'' S, 45º12'46.08'' W) and Uruçuí (7º23'14.77'' S, 44º31'56.47'' W) in the period from February to April 2013. The sampling was supported by the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN), authorization number A84EACA. Ripe fruits were collected periodically in tree and the ground under tree and transported to the laboratory. The fruits were counted, weighed, labeled by location and date of collection, individually packed in plastic containers under vermiculite and covered with voile and maintained under controlled temperature (25º C). After 10 to 15 days, the vermiculite was screened and the fruits opened to check for the presence of 3rd instar larvae or pupae, the larvae were kept in the fruit pulp until pupation. The pupae obtained were quantified, weighed and individually packed in transparent glass flasks with a diameter of 3.3 cm.

The glass flasks were labeled by location, date of sorting, pupal weight and covered with voile. The insects adults were counted, sexed and fixed in 70% alcohol. The *A. obliqua* was identified at the species level based on morphological characters of females, especially the aculeus, using the taxonomic key of Zucchi (2000). The parasitoids was identified based on morphological characters located in the head and thorax using the illustrated key of Marinho et al. (2018).

The fruit infestation index was determined based on the fruits weight (IWF = number of total pupae/total fruit weight) and the number of fruits collected (ICF = number of total pupae/number of total fruits). In addition, bitrophic interaction was studied by analyzing the influence of umbu fruit weight on *A. obliqua* selection and host exploitation. Subsequently, the involvement of a third trophic level was studied by analyzing the amount of fruits with *A. obliqua* and *Opius bellus* (Gahan, 1930) (Hymenoptera: Braconidae), the infestation of *A. obliqua* in the presence and absence of *O. bellus* and the parasitism index (PI = number of parasitoids)/total pupae *100) of *A. obliqua* by *O. bellus*. Finally, pupae weight was recorded with fruit flies or parasitoids.

From the samples, 1,031 pupae of tefritids from 429 umbu fruits were obtained, from these pupae were obtained 340 specimens of fruit flies (Diptera: Tephritidae) and 36 specimens of parasitoids (Hymenoptera: Braconidae and Figitidae) (Tab. 1). In addition to natural parasitism, the occurrence of microorganisms and dehydration are common factors of biotic and abiotic mortality of tephritids (for review see Carvalho 2005), these causes of mortality were not evaluated in the present study.

Table 1. Fruit fly and parasitoids from umbu fruits.

Insects collected		Males	Females	Total
Fruit fly	*Anastrepha obliqua*	180	160	340
Parasitoids	*Opius bellus*	16	19	35
	Aganaspis pelleranoi	0	1	1
Total		**196**	**180**	**376**

The only species of fruit fly recorded in umbu fruits was *A. obliqua*, suggesting that the unidentified males also belong to the species *A. obliqua*. The species *A. obliqua* is the first fruit flies recorded in the Alto Parnaíba region in the Piauí state. Umbu is the first host fruit registered for *A. obliqua* in the Piauí state. The occurrence of *A. obliqua* in Piauí had already been verified only through Mcphail traps. Parasiticizing *A. obliqua* on umbu fruits, a specimen of *Aganaspis pelleranoi* (Brèthes, 1924) (Hymenoptera: Figitidae) and 35 specimens of *O. bellus* were found.

The average fruit infestation index was 149.4 pupae per kg (2.4 pupae per fruit), with 77.8% of the sampled fruits showing infestation by *A. obliqua* in both locations (Tab. 2). Of the 429 samples, 334 were infested and 95 were exempt from the presence of *A. obliqua*. The infestation index was lower than the previous results recorded in the Caatinga biome, where practically all the umbu fruits sampled were infested by *A. obliqua* (Lima Junior et al. 2007; Bomfim et al. 2010). Most of the sampled fruits weighed more than 15 g (67.4%) and these fruits were preferred by *A. obliqua* (Tab. 3). Therefore, the size of the umbu fruit influences the host selection by *A. obliqua*. Larger fruits provide a more adequate larval support for the survival of the immature phase.

Table 2. Umbu fruit infestation by *Anastrepha obliqua*.

Municipality	Fruits (n)	Weight (Kg)	Pupae (n)	Infested fruits (%)	Infestation pupae/Kg	Infestation pupae/fruit
Baixa Grande do Ribeiro	80	1.1	291	87.5 (70)	264.5	3.6
Uruçuí	349	5.8	740	75.6 (264)	127.5	2.1
Total	**429**	**6.9**	**1,031**	**77.8 (334)**	**149.4**	**2.4**

Table 3. Influence of umbu fruit weight on the natural selection by *Anastrepha obliqua*.

Fruit weight (g)	Infestation	Fruits (n)	Pupae (n)	Pupae/fruit
< 15	Yes	106	300	2,8
	No	34	-	-
= > 15	Yes	228	731	3,2
	No	61	-	-

In the samples, a variable number from zero to 12 pupae per fruit was found, where the presence of one pupa per fruit was recorded in 101 samples (30.2%) and in 233 samples (69.8%) two or more pupae were found by fruit (Fig. 1A). It is known that the females of *A. obliqua* lay only one egg per oviposition (see Aluja 1994), which allows us to conclude that the West Indian Fruit Fly performs multiple ovipositions on umbu fruits during host selection. Our results suggest that a female may perform more than one oviposition per fruit or co-infestation with conspecifics may occur. The fruit can support multiple ovipositions and nutritionally supply several larvae. Pupae in the samples did not show drastic weight fluctuations due to the different population densities (Fig. 1B). However, a low pupal viability was found in the study (39.3%) (Fig. 1C).

Multiple infestations are a factor that increases the probability of emergence of *A. obliqua* adults during the colonization of umbu fruits. It was found that in fruits with higher pupae densities the probability of guaranteeing the perpetuation of the species is higher (Fig. 1D). The lesser the fruit infestation, the greater the risk of no *A. obliqua* emergence (Fig. 1D). In only 23% of the fruits infested with one pupa per fruit, adults emerged while in densities from eight pupae per fruit 100% of the infested fruits allowed the emergence of at least one organism (Fig. 1D). Therefore, high population densities can contribute effectively to the emergence of adults to guarantee the perpetuation of the species. This explains why the female performs multiple ovipositions per fruit or oviposite on fruits infested by conspecifics during host selection.

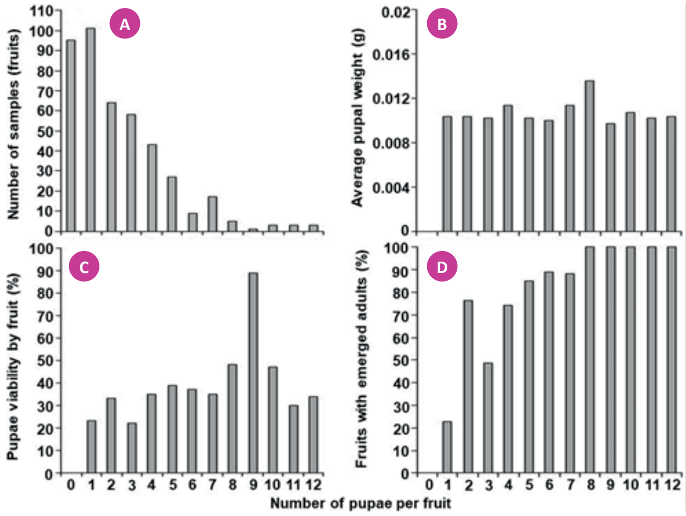

Figure 1. Exploration of umbu fruits by *Anastrepha obliqua* (Macquart, 1835). (A) Levels of infestation in the samples; (B) Pupae weight from different levels of infestation; (C) Pupae viability from different levels of infestation; (D) Contribution to the perpetuation of the species at different levels of infestation.

The West Indian Fruit Fly has a high rate of infestation in umbu fruits, however, natural parasitism in the study region is not very significant (average parasitism: 2.7%), 4.1% in the municipality of Baixa Grande do Ribeiro and 1.4% in Uruçuí, however, parasitism data are higher than those recorded in the Caatinga biome (0.7%) (Bomfim et al. 2010). In addition, less pupae were found in fruits with the presence of parasitoids (Fig. 2), suggesting that the fly avoids oviposition in fruits with parasitized larvae, that is, the presence of the parasitoid may result in greater energy expenditure by *A. obliqua* females in the search for a suitable host fruit. However, fly's behavior is consistent because may limited encounter rate in the natural environment and avoid or escape parasitoid attack. In addition, it was verified that the *A. obliqua* pupae parasitized by *O. bellus* have less weight (i.e., *A. obliqua* pupae: 0.0159 ± 0.0003 (n=242) and pupae parasitized: 0.0083 ± 0.0004 (n=22)), suggesting that the parasitized larvae have less nutritional demand. The parasitoid induce changes in host phenotype, but we don't know what they are the fitness benefits.

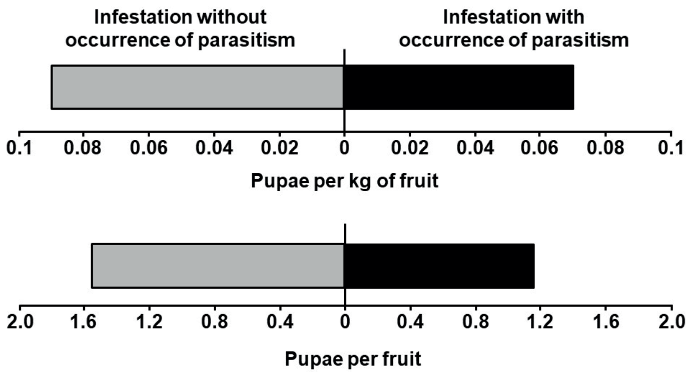

Figure 2. *Anastrepha obliqua* (Macquart, 1835) infestation in umbu fruits with and without parasitism.

The parasitoid *O. bellus*, predominant in this study, is an important parasitoid of *Anastrepha* species in the Neotropical region (Garcia et al. 2020). The parasitoid *O. bellus* has already been found parasitizing *A. obliqua* in guava fruits, *Psidium guajava* L. (Bittencourt et al. 2012), pitanga, *Eugenia uniflora* L. (Aguiar-Menezes & Menezes 2001), star fruit, *Averrhoa carambola* L. (Aguiar-Menezes & Menezes 2001; Pereira et al. 2010), acerola, *Malpighia glabra* L. (Dutra et al. 2013) seriguela, *S. purpurea* (Aguiar-Menezes & Menezes 2001) and cajá, *S. mombin* (Thomazini & Albuquerque 2009; Jesus-Barros et al. 2012; Dutra et al. 2013).

Umbu presents itself as the appropriate host for colonization of fruit flies in the period from February to April (fruit sampling period), since the native vegetation of the cerrado has a lower supply of fruits at the end of the rainy season. As a hypothesis, it suggests that multiple ovipositions are the result of a high population in an environment of low host availability. The West Indian Fruit Fly performs multiple oviposition in umbu during host selection and multiple infestations is a factor that increases the likelihood of emergence of *A. obliqua* during the colonization of umbu. However, not all fruits were infested by *A. obliqua*, so the female had a choice, but did not hesitate to explore an infested host. In this context, several questions are posed: a) Does the fly prefer a host free from competitors or is it encouraged to oviposit in the presence of conspecifics? b) Is the host's pheromone marking (reviewed by Silva et al. 2012) not effective to prevent multiple infestations in this interaction? c) Is multiple infestations a strategy to prevent parasitism and guarantee the perpetuation of the species? These intriguing questions must be investigated for a better understanding of this tritrophic interaction.

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Authors' Contributions

MAS, GCDBS and JGO provided funding support. MAS and GCDBS provided the conceptualization and performed the project administration and supervision. JGO, PLSJ and DSL performed the investigation; MAS, JGO and GNL contributed to the taxonomic identification and formal analysis; MAS, GCDBS, JGO, GNL, PLSJ and DSL wrote the manuscript - original draft, review and editing.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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