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On a poorly known tritrophic interaction: fruit-infesting *Rhagoletotrypeta pastranai* Aczél (Diptera: Tephritidae) and its parasitoid *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) in *Celtis* *ehrenbergiana* (Klotzsch) Liebm. (Rosales: Cannabaceae) fruits

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Sobre una interacción tritrófica pobremente conocida:
Rhagoletotrypeta pastranai Aczél (Diptera: Tephritidae) y
su parasitoide *Utetes anastrephae* (Viereck) (Hymenoptera:
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Abstract: We present information on a poorly known native tritrophic interaction involving *Celtis ehrenbergiana* (Klotzsch) Liebm. (Cannabaceae), the fruit fly *Rhagoletotrypeta pastranai* Aczél (Diptera: Tephritidae), and its parasitoid *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) from Buenos Aires, Argentina. Insects were collected from 70 trees and 950 fruits and immatures were reared in the laboratory. The fruit fly accounted for 41.8% of all pupae (59 out of 141 pupae), whereas the parasitoid accounted for 32.6% of fruit fly pupae (46 braconids out of 141 pupae). Fruit infestation levels by the fruit fly ranged between 0 and 191 pupae/100 g of fruit (mean = 53.3 ± 55.3 pupae/100g of fruit), and percentage infestation levels ranged between 0 and 100% (mean = $18.2 \pm 32.1\%$). Most parasitoid adults emerged after 1-2 months, whereas most fruit fly adults emerged after a 12-mo diapause period. Our results show that both insects are common and abundant at our study area and, despite being non-economically important, highlight the need of studying poorly known species to gain a better insight into the ecology of biotic interactions with multiple interactors.

Keywords: Fruit infestation, Plant-insect interactions, Talares.

Resumen: Presentamos información sobre una interacción tritrófica nativa poco conocida constituida por *Celtis ehrenbergiana* (Klotzsch) Liebm. (Cannabaceae), la mosca de la fruta *Rhagoletotrypeta pastranai* Aczél (Diptera: Tephritidae), y su parasitoide *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) en Buenos Aires,

Argentina. Los insectos fueron colectados a partir de 70 árboles y 950 frutos y la cría de las formas inmaduras se llevó a cabo en laboratorio. La mosca de la fruta representó 41,8% del total de pupas (59 de 141), mientras que el parasitoide representó 32,6% del total de pupas de *R. pastranai* (46 de 141). Los niveles de infestación por mosca de la fruta variaron entre 0 y 191 pupas/100g de frutos (media = $53,3 \pm 55,3$ pupas/100 g de frutos), y los porcentajes de infestación variaron entre 0 y 100% (media = $18,2\% \pm 32,1\%$). La mayoría de los parasitoides adultos emergieron luego de 1-2 meses, mientras que la mayoría de moscas adultas emergieron luego de un período de diapausa de 12 meses. Nuestros resultados muestran que ambos insectos son comunes y abundantes en el área de estudio, y resaltan la necesidad de estudiar especies poco conocidas, a pesar de no presentar importancia económica, para lograr un mejor entendimiento de la ecología de interacciones bióticas con múltiples interactores.

Palabras clave: Infestación de frutos, Interacciones planta-insecto, Talares.

Tritrophic interactions involving insects and plants represent complex systems in which both insect and plant traits modulate direct and indirect interactions within and between higher trophic levels (Tscharnkte & Hawkins, 2002). In particular, interactions involving fruit flies and parasitoids have been largely studied worldwide, due to its economic importance and the possibility of using their parasitoids as biological control agents (Ovruski et al., 2000, 2004). However, non-economically important fruit flies represent *ca.* 95% of more than 4,400 species described, and studies on the basic biology of these species are still necessary (Aluja, 1999; Schliserman et al., 2016).

Celtis ehrenbergiana is a thorny andromonoecious tree, found in dry forests and scrubs from the southern United States to central Argentina (Berg & Dahlberg, 2001). It produces one-seeded, fleshy drupes nearly orange when ripe, $8,5 \pm 0,6$ mm wide, whose seeds are mainly dispersed by birds (Palacio et al., 2014). *Rhagoletotrypeta pastranai* (Diptera: Tephritidae) is the only frugivorous insect recorded to interact with *C. ehrenbergiana* fruits (Aczél, 1954). Indeed, the few known host plants of the genus *Rhagoletotrypeta* Aczél belong to the genus *Celtis* L. (Norrbom, 1994; Ovruski et al., 2005). The only parasitoid reared from *R. pastranai* is *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae), but from *C. iguanaea* (Jacquin) Sargent (Ovruski et al., 2005). To date, parasitoids related to *C. ehrenbergiana* are unknown, and the scarce available records of parasitoids in *Celtis* spp. highlight the need of a deeper work to understand this biotic interaction.

The study was carried out at the San Isidro ranch ($35^{\circ} 09' S$; $57^{\circ} 23' W$), located in the Biosphere Reserve “Parque Costero del Sur”, near the shore of Río de La Plata, Buenos Aires province, Argentina. The area is composed of grasslands and native forest patches, with nearly 10% forest cover. These patches are xeromorphic forests locally named “Los Talares”, which grow on calcareous soil deposits, parallel to the shore of Río de La Plata (Goya et al., 1992). Dominant tree species are *C. ehrenbergiana*, *Scutia buxifolia* Reissek, *Schinus longifolia* (Lindl.) Speg. and *Jodina rhombifolia* (Hook. & Arn.) Reissek (Goya et al., 1992). In phytogeographic terms, this area corresponds to the “Provincia del Espinal”, a region characterized by xeromorphic thorny forests at central

Argentina (Parodi, 1940; Cabrera, 1971). The climate is wet temperate. Annual mean minimum and maximum temperatures of 5.9° C and 27.5° C, respectively. Mean rainfall is about 885 mm, mostly wet in January and February, but without a noticeable dry season.

Fieldwork was carried out between March 28th and April 1st, 2014, during *C. ehrenbergiana* peak fruiting in the study area (Palacio et al., 2014). Seventy fruiting trees were randomly selected across ten forest patches (ca. 80 ha), and 4-20 fruits *per* tree were collected (according to availability). For each tree, average fruit weight was measured by weighting each fruit sample with an Ohaus scale (to the nearest 0.01 g) and then dividing the summed weight by the number of fruits in the sample. In the laboratory, fruit samples were placed in closed styrofoam vessels (750 cc) with a mesh at the top and damp sand (50 g, 5-10 water drops) in the bottom as a pupation substrate under natural conditions. One vessel *per* sampled tree was used (i.e. 70 vessels). Between April 5th and April 25th, 2014, the sand was sieved with a mesh tea strainer to find pupae once a week, and then placed into empty vials. Emerged insects were transferred into vials containing 70% ethanol. Dry fruits were removed after a month. From April 25th, 2014 to April 25th, 2015, insects were counted once a month. Both the fruit fly host and the parasitoid were identified to the species level. All parasitism values and fruit infestation levels reported are based on the number of emerged adults and on the number of pupae *per* 100 g of fruit, respectively. Statistical values are reported as average \pm standard deviation.

A total of 950 fruits were collected (233.8 g; mean fruit weight = 0.23 ± 0.07 g). Only one larva *per* infested fruit was recorded, and one host-parasitoid interaction was recorded between the fruit fly *R. pastranai* and the parasitoid *U. anastrephae*. The latter represents a new record of parasitoid in *C. ehrenbergiana*. The identification of *R. pastranai* adults was based on external characters: scutum with complete lateral white stripe from transverse suture to intra-alar seta, anteriorly with a pair of submedia stripes of dense microtrichia (Aczél, 1954), and a postpronotal lobe entirely white (Norrbom, 1994) (Fig. 1). The identification of *U. anastrephae* adults was also based on external characters: absence of an occipital carina, hind tibia with a sharp ridge located basal-medially, fore wing 2RS shorter than 3RSa, hind wing m-cu absent (Fig. 2). The female has a relatively short ovipositor ~1.6 mm in length (Sivinski et al., 2001). *Utetes anastrephae* is a koinobiont endoparasitoid (the parasitoid allows the host to continue its development and does not kill the host until the parasitoid larva pupates) with a wide distribution ranging from Florida (USA) to Argentina (Wharton & Marsh, 1978; Sivinski et al., 1997). Fruit fly and parasitoid specimens are deposited in the Instituto Fundación Miguel Lillo (IFML) and Museo de La Plata (MLP). The fruit fly accounted for 41.8% of fruit fly pupae (59 out of 141 pupae), whereas the parasitoid accounted for 32.6% of these pupae (46 braconids out of 141 pupae). Fruit infestation levels by fruit fly varied between 0 and 191 pupae/100g of fruit (mean = 53.3 ± 55.3 pupae/100g of fruit), and percentage infestation levels varied between 0.0 and 100.0% (mean = 18.2

$\pm 32.1\%$). Most parasitoids emerged after 1-2 months, whereas most fruit flies emerged after a 12-mo diapause period.

Overall, we report data on species and infestation levels of an almost unknown native tritrophic interaction. As aforementioned, the few known host plants of the genus *Rhagoletotrypeta* belong to the genus *Celtis* (Norrbon, 1994; Ovruski et al., 2005). Our results are in agreement with this and suggest that the *Celtis-Rhagoletotrypeta* interaction may be species-specific. By contrast, the *Rhagoletotrypeta-U. anastrephae* interaction is asymmetric, because the latter parasitizes a wide array of fruit flies associated to several host plants across its geographic range (Leonel et al., 1995; Sivinski et al., 1997; Ovruski et al., 2000). To our knowledge, only one study has quantified infestation and parasitism levels in *Celtis* spp. (Ovruski et al., 2005). Those authors found highly variable *Rhagoletotrypeta* infestation levels among *Celtis* populations. Considering that there is typically one *Rhagoletotrypeta* larva per *Celtis* fruit (Ovruski et al., 2005; this study) and large interspecific variation in fruit size (e.g.: *C. chichape* = 50-60 cm long, *C. ehrenbergiana* = 40-100 mm long, *C. iguanaea* = 120-170 mm long; Berg & Dahlberg, 2001), it is more appropriate to compare percentage infestation levels rather than the number of larvae per mass unit. Ovruski et al. (2005) found infestation levels of *R. pastranai* and *R. parallela* similar to ours in two *C. pubescens* populations from northern Argentina (18.4 and 20.7%). However, one *C. pubescens* population showed almost twofold differences in infestation levels relative to these populations (34.9%; Ovruski et al., 2005). In the same study, one *C. iguanaea* population showed even higher infestation levels (43.7%; Ovruski et al., 2005). Similar to our results, those authors only found *U. anastrephae* as the only parasitoid with parasitism levels of 37.2% in four *Celtis* populations (Ovruski et al., 2005). Given the broad geographic range of the three interacting species and the presumably large variation in infestation levels among populations, further studies are needed to understand the ecology of insects interacting with plants of the genus *Celtis*. Furthermore, our results show that both insects are common and abundant at our study area and, despite being non-economically important, underscore the need of also studying poorly known species to achieve a better understanding of complex biotic interactions.



Fig. 1. *Rhagoletotrypeta pastranai*. A. habitus, lateral view. B. head and thorax, dorsal view.

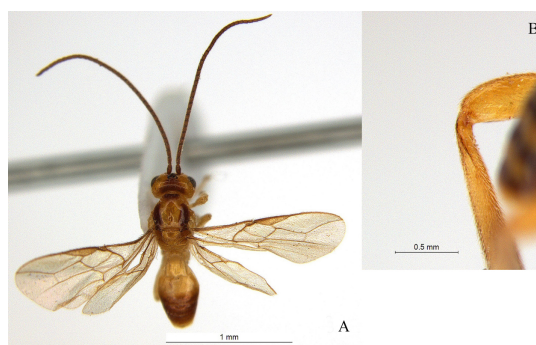


Fig. 2. *Utetes anastrephae*. A. habitus, dorsal view. B. hind tibia with a sharp ridge.

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