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Trichopria anastrephae: A Promising Neotropical-Native Parasitoid for *Drosophila suzukii* Control

Maria Josefina Buonocore-Biancheri ¹, Lorena del Carmen Suarez ^{2,3}, Segundo Ricardo Núñez-Campero ^{4,5} , Marcos Darío Ponssa ¹, Flávio Roberto Mello Garcia ⁶ , Daniel Santiago Kirschbaum ^{7,8} and Sergio Marcelo Ovruski ^{1,*}

- ¹ Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI-CONICET), Biological Control Department, Avenida Belgrano y Pasaje Caseros, San Miguel de Tucumán T4001MVB, Argentina; mjbuonocore@conicet.gov.ar (M.J.B.-B.); ponssa.marcos@conicet.gov.ar (M.D.P.)
- ² Dirección de Sanidad Vegetal, Animal y Alimentos de San Juan (DSVAA)-Gobierno de la Provincia de San Juan, Nazario Benavides 8000 Oeste, Rivadavia, San Juan J5413ZAD, Argentina; lorenasuarez@conicet.gov.ar
- ³ CCT CONICET San Juan, Avenida Libertador General San Martín 1109, San Juan J5400AR, Argentina
- ⁴ Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR-CONICET), Entre Ríos y Mendoza s/n, Anillaco, La Rioja 5301, Argentina; segundo.nc@conicet.gov.ar
- ⁵ Departamento de Ciencias Exactas, Físicas y Naturales, Instituto de Biología de la Conservación y Paleobiología, Universidad Nacional de La Rioja (UNLaR), Avenida Luis de la Fuente s/n, Ciudad de La Rioja, La Rioja 5300, Argentina
- ⁶ Departamento de Ecología, Zoología e Genética, Instituto de Biología, Universidade Federal de Pelotas, Pelotas 96000, RN, Brazil; flavio.garcia@ufpel.edu.br
- ⁷ INTA Estación Experimental Agropecuaria Famaillá, Tucumán Ruta Provincial 301, km 32, Famaillá 4132, Argentina; kirschbaum.daniel@inta.gob.ar
- ⁸ Cátedra Horticultura, Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán, San Miguel de Tucumán 4000, Argentina
- * Correspondence: sovruski@conicet.gov.ar



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Abstract: *Drosophila suzukii* (Matsumura) is an invasive pest mainly affecting berry and stone fruit crops worldwide. In Argentina, it inhabits fruit-growing regions. An eco-friendly management strategy involves biological control by using resident natural enemies, such as the Neotropical-native pupal parasitoid *Trichopria anastrephae* Lima (*Ta*). The study compared the host-killing capacity and the offspring reproductive success of two *Ta* lineages on the puparia of both *D. suzukii* (*Ds*) and *D. melanogaster* (*Dm*) in no-choice and choice tests under laboratory conditions. The host preference and host-switching behaviors were also assessed. One parasitoid lineage was reared on *Ds* (*Ta_{Ds}*), and the second on *Dm* (*Ta_{Dm}*). In no-choice tests, both *Ta* lineages performed similarly on both hosts regarding the percentage of killed hosts and parasitoid offspring survival. The host-killing ability of *Ta_{Dm}* was only significantly lower when *Ds* was offered as a host, relative to *Dm*. In choice tests, *Ta* attacked mainly *Ds* at a 4–9 times *Ds* to *Dm* ratio, but at a 1.5–2 times *Ds* to *Dm* ratio, the host-killing ability was similar between both drosophilids. At an equal host ratio or higher *Dm* ratios, *Ta* preferred the native host. However, it was determined that *Ta* has the potential to parasitize the recently-introduced pest.

Keywords: spotted-wing drosophila; pupal parasitoid performance; parasitoid reproductive success; host preference; host switching behavior; biological control

1. Introduction

Invasions of alien species into new regions usually produce adverse economic, ecological, and social effects [1,2]. The globalized trade of agricultural commodities is among the leading causes of the significant expansion of crop pests over the last decades, threatening worldwide food security [3], which leads to rigorous governmental strategies to either avoid the introduction of invasive species or implement large-scale eradication programs whenever prevention fails [4,5]. In that regard, the distribution range of the spotted-wing

drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), a polyphagous fruit pest native to Southeast Asia, has rapidly expanded in the last decade and became a severe pest in Europe [6], the Americas [7], and throughout Asia [8]. *Drosophila suzukii* is currently a significant pest of berries and stone fruit production worldwide [9]. This invasive pest damages mainly soft-skinned fruit such as *Vaccinium* spp. (blueberry) (Ericaceae), *Fragaria* spp. (strawberry), *Rubus* spp. (raspberry and blackberry), and *Prunus* spp. (cherry, peach, apricot, and plum) (Rosaceae) [10]. In addition, numerous wild and ornamental non-crop fruits have been infested by *D. suzukii* worldwide [11–14]. Unlike other drosophilid flies, the *D. suzukii* female oviposits through the skin of fresh, healthy, and ripening fruits still on the plant due to its serrated and sclerotized ovipositor [15]. Consequently, the larvae cause unmarketable fruit, resulting in high losses to the fruit industry in many countries [16].

Drosophila suzukii invaded Argentina in 2014, spreading throughout fruit-growing regions with highly contrasting climatic conditions, from the humid subtropical rainforests of the north to the semi-desert highlands and lowlands of western and southern Argentina [17]. Such swift expansion could result from two invasion events originating from countries that recorded the pest previously, such as the USA and Brazil [18]. The high environmental adaptability and behavioral and physiological plasticity make *D. suzukii* particularly suited to changing habitats and climatic conditions [19].

Soft fruits, such as berries and cherries, are among the most critical groups of high commercial value fruits that have increased their regional expansion, production, marketing, and export in Argentina. Blueberry (*Vaccinium corymbosum* L.) exports in 2020 reached 11,000 t, accounting for USD 110 million in revenues [20]. Strawberries (*Fragaria × ananassa* Duch.), raspberries (*Rubus idaeus* L.), blackberries (*R. fruticosus* L. and *R. ulmifolius* Schott), and cherries (*Prunus avium* L.) are also highly produced in Argentina [17,21]. The high population growth, thermal plasticity, and broad host range make *D. suzukii* a significant risk to Argentina's soft fruit industry. Although there are few published records of damage caused by this pest, the known data are highly worrisome [17].

Successful Integrated Pest Management (IPM) programs involve an understanding the basic ecology of the target species in both the agroecosystem and the surrounding non-crop areas [22]. Key aspects, such as natural mortality factors influencing pest population dynamics, are essential for implementing control tactics by using biocontrol agents [23,24], and taking into account the heterogeneity of agricultural landscapes [25]. In this regard, several resident parasitoid species have been associated with *D. suzukii* worldwide [10,26–29], but few can overcome its robust immune system [30,31]. Therefore, resident parasitoids with the highest chance of successfully developing on *D. suzukii* are pupal parasitoids [32–34]. Among these parasitoids, *Trichopria anastrephae* Lima (Diapriidae) and *Pachycrepoideus vindemiae* Rondani (Pteromalidae) were the most abundant species parasitizing *D. suzukii* in non-crop areas of the Argentinian northwestern fruit-growing region [35]. The diapriid *T. anastrephae* is a native species to the South American Neotropical region, and it was recorded for Brazil and Argentina from saprophytic drosophilids and *D. suzukii* [36]. It is an endoparasitoid because the egg is placed into the hemocoel of the host pupa [37]. In contrast, the ectoparasitoid *P. vindemiae* is a cosmopolitan species, highly polyphagous, attacking a wide variety of cyclorrhaphous dipterans [38].

Native parasitoids can display genetic variations for host choice and use [39], which is mainly associated with variation in both the physiological and behavioral components of host foraging [40] and through superparasitism [41]. Such novel settings may encourage native parasitoids to include the novel host in their host range through physiological compatibility [42], inducing host switching, i.e., parasitism on the most abundant host available in a choice setting [41,43,44]. Some generalist parasitoid species may display a host-switching behavior to increase their reproductive success [45]; therefore, host preferences may change according to the relative abundance of involved host species [43].

The aims of this study were to compare both the host-killing capacity and the offspring reproductive success of two population lineages of *T. anastrephae* on puparia of *D. suzukii*,

a novel host, and *D. melanogaster*, a resident host, in no-choice and choice tests under laboratory conditions. Likewise, the host preference and host switching were compared in choice tests. We hypothesized that regardless of host origin, the two lineages of *T. anastrephae*, one of them laboratory-reared on *D. suzukii* and the other one on *D. melanogaster*, could both kill *D. suzukii* puparia and successfully develop in the new host. Secondly, the mortality of *D. suzukii* puparia caused by *T. anastrephae* would not be affected by the presence of *D. melanogaster* host puparia in the same habitat. The results are discussed in relation to the feasibility of using *T. anastrephae* in biological control programs against *D. suzukii* within an IPM strategy.

2. Material and Methods

2.1. Insects Rearing

The colonies of *D. suzukii*, *D. melanogaster*, and *T. anastrephae* used in the assays were originated from puparia collected from fallen non-crop peaches (*Prunus persica* (L.) Batsch) in wilderness areas during summer (December) 2019 in Horco Molle (26°55' S, 65°05' W, 600–800 elevation), Tucumán province, northwestern Argentina. Drosophilid and parasitoid colonies were kept at 25 ± 1 °C, 75 ± 5% RH, and 12:12 (L:D) h photoperiod at the Pest Biological Control Department (DCBP, Spanish acronym) from the Biotechnology and Microbiological Industrial Processes Pilot Plant (PROIMI, Spanish acronym), in San Miguel de Tucumán, Tucumán, Argentina. Two *T. anastrephae* population lines were used in the trials. One of them was reared on *D. suzukii* puparia (Ta_{Ds}), whereas the second population line was reared on *D. melanogaster* puparia (Ta_{Dm}). Flies and parasitoids were held in cubical Plexiglas rearing cages (30 cm) with voile screen-covered sidewalls. Adult parasitoids were fed with honey every other day. Adult flies were fed daily with an artificial diet consisting of enzymatically hydrolyzed yeast (MP Biomedicals, LLC, Solon, OH, USA), corn gluten meal (Grupo Arcor S.A., La Reducción, Lules, Tucumán, Argentina), and standard white cane sugar (Ledesma SAAI, Libertador General San Martín, Jujuy, Argentina) in plastic Petri dishes (10 × 1.5 cm, diameter/depth) with a double layer of towel paper. Water was provided ad libitum by 200 mL plastic bottles with yellow absorbent cloth wicks. Four 140 mL disposable plastic cups, with 50 mL larval diet, were used as oviposition devices in each rearing cage. The larval diet was made of corn flour (32.5 g), brewer's yeast (18.5 g), cane sugar (52.5 g), agar-agar (8 g), absolute ethyl alcohol 99.5% (15 mL), vitamin C (1 g), vitamin E (1 g), and Nipagin (methyl 4-hydroxybenzoate) (1.6 g). Agar-agar and corn flour were dissolved in 200 mL and 800 mL of water, respectively. Such ingredient proportions are enough for 500 g of diet. After 24 h, each oviposition device was removed, covered with a cotton voile cloth, and placed in an empty Plexiglass cage. Six days later, puparia were extracted from the larval diet, washed with a 10% sodium benzoate (Pura Química Laboratory, Córdoba, Argentina), and purified water solution. Then, puparia were placed in 500 mL hinged plastic cups with 5.5 cm² of vermiculite (Intersum®, Aislater S.R.L., Córdoba, Argentina) previously sterilized on the bottom as a pupation substrate. These cups were placed in new adult-rearing cages. The procedure was carried out for each drosophilid species. One-day-old host puparia were exposed to parasitoid females for 48 h on 90 mm laboratory filter paper (Cytiva, Shanghai, China) moistened with distilled water inside plastic Petri dishes. The *T. anastrephae* population lines used in the trials were the 40th generation under artificial rearing.

2.2. Experimental Setup

No-choice and choice tests were conducted in an 8 m² room at the DCBP, under the aforementioned controlled laboratory conditions. The no-choice tests were performed to assess both the parasitoid's host-killing capacity (parasitoid performance) and the ability to produce parasitoid surviving offspring (reproductive success) when *D. suzukii* and *D. melanogaster* puparia were singly exposed to parasitoid females from each population lineage. Experiments consisted of five naïve, i.e., never exposed to host puparia, 5-day-old, mated Ta_{Ds} or Ta_{Dm} females exposed to 50 *D. suzukii* or *D. melanogaster* puparia for two days.

As previously described, one-day-old puparia of *D. suzukii* or *D. melanogaster* were provided to parasitoids. Cubical Plexiglas experimental cages (15 cm) with voile screen-covered sidewalls were used for each treatment. Control tests (no parasitoids) were performed simultaneously with treatments to check natural fly mortality and emergence rates. Once the 48 h exposition period ended, Petri dishes were removed from each experimental cage, and host puparia were placed in hinged lid plastic cups with sterilized vermiculite on the bottom. Puparia were kept in cups until adult flies or parasitoids emerged. Ten replicates per treatment and a control were performed. One week after parasitoid emergence, non-emerged host puparia were dissected to corroborate parasitism. A Leica® EZ4D 40× stereomicroscope (Wetzlar, Germany) was used for dissections. The number and sex of the parasitoids, the number of flies, and the number of non-emerged puparia were recorded. Parasitoid performance was calculated using Abbott's percent-corrected host mortality [46], which allows for calculating the parasitoid's host-killing capacity through the relationship between host emergence rates from experimental treatment and control tests. Parasitoid reproductive success was based on the parasitoid offspring emergence, which was calculated as the total number of emerged parasitoids divided by the total number of exposed host puparia. The parasitoid offspring sex ratio was calculated as the percentage of live females recovered from the number of emerged parasitoids.

The dual-choice tests were performed to assess the parasitoid's host-killing capacity and reproductive success, and also the host species preference and host switching behavior when different proportions of *D. suzukii* and *D. melanogaster* puparia were simultaneously offered to parasitoid females from both population lineages. Experiments consisted of five naïve 5-day-old, mated Ta_{D_s} or Ta_{D_m} females exposed to 50 host puparia for 48 h, but at nine different ratios of *D. suzukii* and *D. melanogaster*, as follows: 45:5, 40:10, 35:15, 30:20, 25:25, 20:30, 15:35, 10:40, and 5:45. Puparia of both *D. suzukii* and *D. melanogaster* were placed together in the same standard-size plastic petri dish. Trials were carried out using the same procedure as described above. *Drosophila suzukii* puparia can be easily distinguished from the *D. melanogaster* puparia by the external shape of the anterior spiracles, which have two tubes with plumose-shaped tips on the top [33]. The number and sex of the parasitoids and the number of flies and non-emerged puparia were recorded. Treatments and controls were replicated 10 times. Parasitoid performance and reproductive success (parasitoid emergence rate) were determined. The host species preference was calculated using the formula $E_1/E_2 = C \times N_1/N_2$, as described by [47], where C , a constant, is the preference index for a host species in equal abundance based on the proportion of attacked hosts, which in the current study was 25:25. N_1 and N_2 are the numbers of two host species, i.e., $N_1 = D. suzukii$ and $N_2 = D. melanogaster$, offered to the parasitoid. E_1 and E_2 are the observed numbers of the two host species parasitized, i.e., host parasitism. When the C value is $=0$ or <1 , the preferred host species is #2; when the C value is >1 , the preferred host species is #1, but when the C value is $=1$, there is no preference [48]. Parasitism was calculated as the emerged parasitoids plus non-emerged parasitoids derived from host puparia dissection, divided by the number of offered host puparia. The host-switching behavior was tested to establish whether parasitism was low when a host was uncommon [44]. The switching trials were assessed using the formula $P_1 = C \times F_1 / (1 - F_1 + C \times F_1)$, as described by [49], where F_1 is the proportion of the host species #1, i.e., *D. suzukii*, in a particular trial. The P_1 parameter is the expected ratio of the host species #1 among all parasitized hosts, and C is the constant described above. When host switching occurs, the observed parasitized ratio (E_1/E_2) is higher than expected at a given N_1/N_2 value [50].

2.3. Statistical Analysis

In order to compare the parasitoid's host-killing capacity, the reproductive success, the parasitoid offspring sex ratio, and the host switch index for both Ta_{D_s} and Ta_{D_m} population lineages, Kruskal–Wallis' rank sum tests were performed, with Dunn's post hoc pairwise comparison tests to look for differences between factor levels using the Bonferroni–Holm method for adjustment. Mann–Whitney–Wilcoxon tests were performed with a Bonferroni–

Holm adjustment method to compare the host preference index between Ta_{Ds} and Ta_{Dm} population lineages. The R-4.3.2 statistical software was used for analysis [51]. The box plots were plotted to show the resulting data, which involve median (horizontal line inside the box), mean (X inside the box), interquartile range Q1–Q3 (bottom and top ends of the box), range (minimum: Q0, maximum: Q4; both ends of the whisker on the vertical line outside the box), and raw data dispersal (colored circles). Letters that display the significant difference in figures were included with the R-library ‘rcompanion’ function.

3. Results

3.1. Parasitoid Performance and Reproductive Success

There were significant but small differences when the host-killing effectiveness of *T. anastrephae* was evaluated under no-choice conditions ($H_{(3, n=40)} = 13.384, p = 0.004$). The performance of Ta_{Dm} when *D. suzukii* was offered as a host was significantly lower than that of Ta_{Ds} when *D. melanogaster* was the host (Figure 1A). There were no significant differences between *T. anastrephae* population lineages regarding their performance parasitizing either *D. suzukii* or *D. melanogaster* (Figure 1A). The host-killing capacity of *T. anastrephae* was >80% in all four treatments. There were no significant differences between the reproductive success of the two *T. anastrephae* population lineages or when both fly species were exposed to parasitoid females ($H_{(3, n=40)} = 3.811, p = 0.283$). The mean offspring survival in both parasitoid lineages varied between 50% and 70% (Figure 1B).

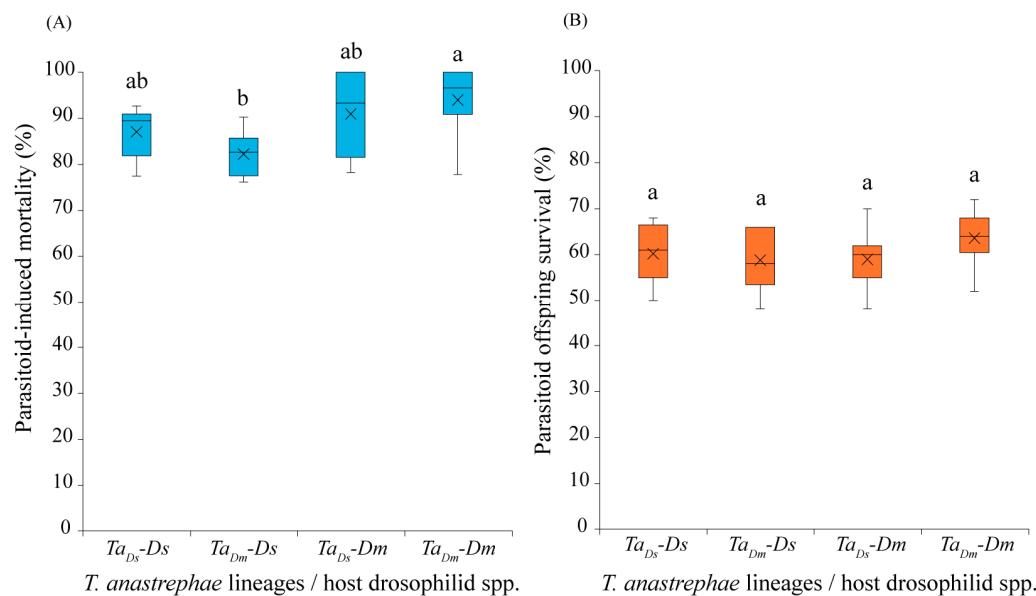


Figure 1. Percentage of host-killing capacity (*Drosophila suzukii* and *D. melanogaster*) (A) and offspring survival (B) of two *Trichopria anastrephae* population lineages under no-choice tests. Meaning of abbreviations: $Ta_{Ds} - Ds$ = *T. anastrephae* reared on *D. suzukii* and recovered from *D. suzukii* puparia, $Ta_{Dm} - Ds$ = *T. anastrephae* reared on *D. melanogaster* and recovered from *D. suzukii* puparia, $Ta_{Ds} - Dm$ = *T. anastrephae* reared on *D. suzukii* and recovered from *D. melanogaster* puparia, $Ta_{Dm} - Dm$ = *T. anastrephae* reared on *D. melanogaster* and recovered from *D. melanogaster* puparia. The same lowercase letters indicate no significant differences at $p > 0.05$ (Dunn's test).

There were significant differences between choice treatments when comparing the host-killing capacity of the Ta_{Dm} lineage ($H_{(17, n=180)} = 118.248, p < 0.001$). The killing capacity of the parasitoid on *D. suzukii* was significantly higher than the *D. melanogaster*-killing parasitoid ability when only the ratio of *D. suzukii* puparia was from 4- to 9-fold higher than that from *D. melanogaster* (Figure 2A). However, when the *D. suzukii* puparia ratio was 1.5- and 2-fold higher than that of *D. melanogaster*, the parasitoid's host-killing capacity was similar for both drosophilid hosts; although when the *D. suzukii:D. melanogaster*

ratio was =1 or <1, females of the Ta_{Dm} lineage killed significantly more puparia of *D. melanogaster* than *D. suzukii* puparia (Figure 2A). The reproductive success of the Ta_{Dm} lineage differed significantly between treatments ($H_{(17, n=180)} = 126.723, p < 0.001$). The parasitoid's surviving offspring sourced from *D. suzukii* puparia was higher than that coming from *D. melanogaster* at 2–9 *D. suzukii* puparia per *D. melanogaster* puparium ratio (Figure 2B). When the *D. suzukii:D. melanogaster* ratio was 1.5-times higher than *D. melanogaster* there were no significant differences between the Ta_{Dm} surviving offspring from both host species. In contrast, when the *D. suzukii:D. melanogaster* ratio was =1 or <1, the parasitoid offspring that emerged from *D. suzukii* puparia was significantly lower than that from *D. melanogaster* puparia (Figure 2B).

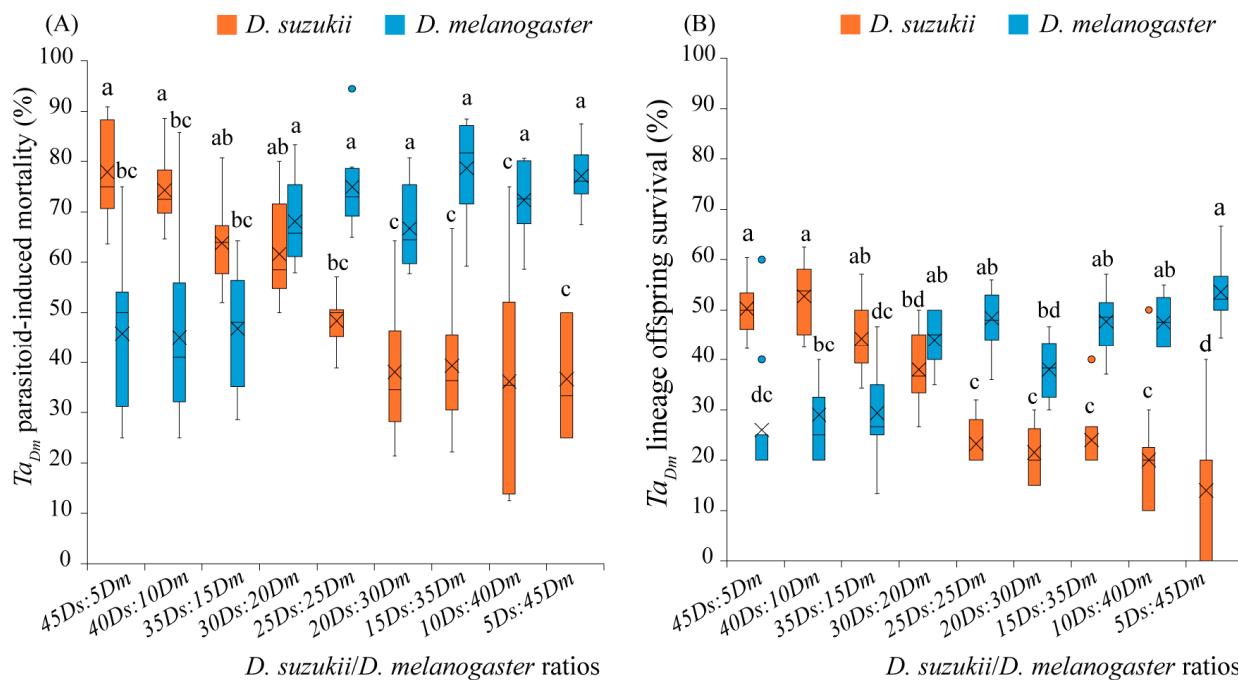


Figure 2. Percentage of host-killing capacity (A) and offspring survival (B) of the *Trichopria anastrephae* population lineage reared on *D. melanogaster* (Ta_{Dm}) at different *D. suzukii* (*Ds*):*D. melanogaster* (*Dm*) ratios under choice tests. The same lowercase letters indicate no significant differences at $p > 0.05$ (Dunn's test).

Similarly to the results with the Ta_{Dm} lineage, there were significant differences between choice treatments when comparing the performance of the Ta_{Ds} lineage ($H_{(17, n=180)} = 141.553, p < 0.001$). The Ta_{Ds} female had a significantly higher capacity to kill *D. suzukii* puparia when its proportion was 4- and 9-fold higher than for *D. melanogaster* (Figure 3A). The Ta_{Ds} lineage performance was similar for the two hosts at ratios of 1.5- and 2-fold more *D. suzukii* puparia than *D. melanogaster*, but at *D. suzukii:D. melanogaster* puparia ratios =1 or <1 there was significantly higher mortality of *D. melanogaster* puparia (Figure 3A). The reproductive success of the Ta_{Ds} lineage differed significantly between treatments ($H_{(17, n=180)} = 133.622, p < 0.001$), and also exhibited a similar trend to that of the Ta_{Dm} lineage concerning tested *D. suzukii:D. melanogaster* puparia ratios. When *D. suzukii* puparia ratios were 2-, 4- and 9-fold higher than that from *D. melanogaster* puparia, there was a significantly higher emergence of parasitoid offspring from *D. suzukii* puparia (Figure 3B). When the *D. suzukii* puparia ratio was 1.5-fold higher than *D. melanogaster* there was similar Ta_{Ds} offspring emergence from both drosophilid hosts. In contrast, at *D. suzukii:D. melanogaster* puparia ratios =1 or <1, the Ta_{Ds} offspring from *D. suzukii* puparia was significantly lower than that from *D. melanogaster* puparia (Figure 3B).

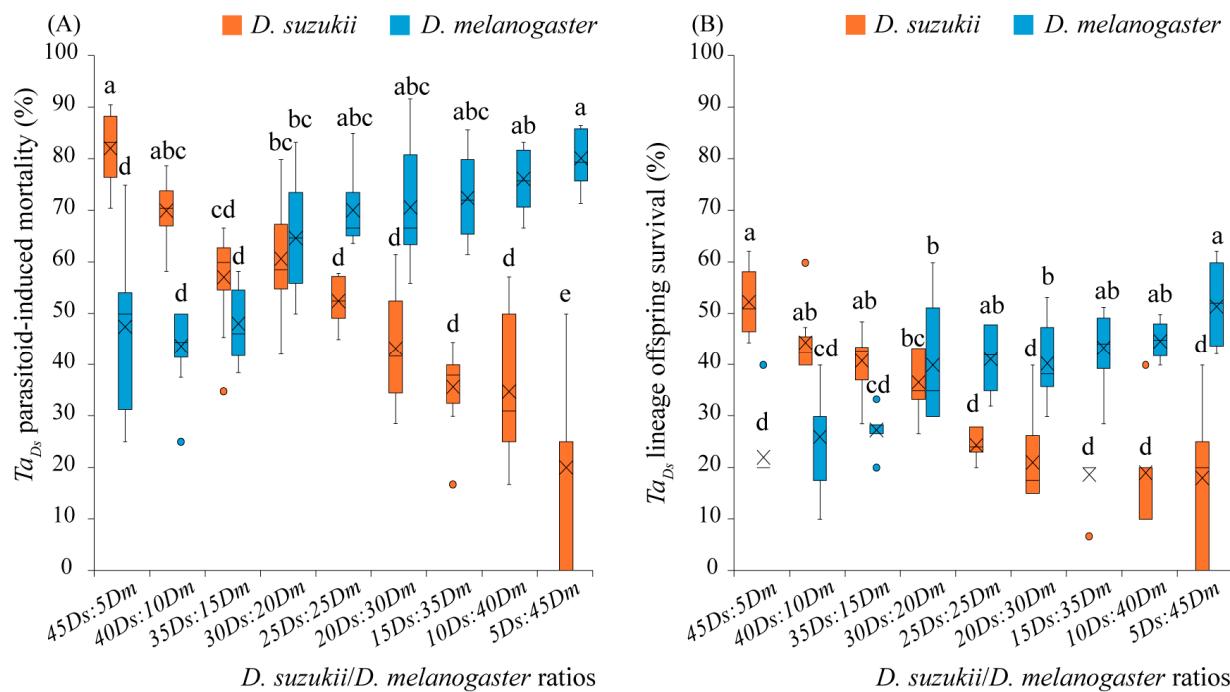


Figure 3. Percentage of host-killing capacity (A) and offspring survival (B) of the *Trichopria anastrephae* population lineage reared on *D. suzukii* (Ta_{Ds}) at different *D. suzukii* (Ds):*D. melanogaster* (Dm) ratios under choice tests. The same lowercase letters indicate no significant differences at $p > 0.05$ (Dunn's test).

The offspring sex ratio of the two *T. anastrephae* lineages was similar in both choice and non-choice tests (Table 1). *Trichopria anastrephae* mainly exhibited a female-biased sex ratio (1.1–1.8:1 females per male), and there was no significant difference in offspring sex ratio when choice tests were compared with each other ($H_{(8, n = 360)} = 12.760, p = 0.120$).

Table 1. Female offspring percentage (sex ratio) recorded from both *Trichopria anastrephae* lineages, one reared on *Drosophila suzukii* (Ta_{Ds}) and the other one on *Drosophila melanogaster* (Ta_{Dm}), at different ratios of *D. suzukii* (Ds) over *D. melanogaster* (Dm).

Parasitoid Lineages/Host Species	Parasitoid Females Offspring Percentage (Median and Range); (Treatments: <i>Drosophila suzukii</i> : <i>Drosophila melanogaster</i> Puparia Ratios)									
	No-Choice Tests		Choice Tests							
	50:0/0:50	45:5	40:10	35:15	30:20	25:25	20:30	15:35	10:40	5:45
$Ta_{Ds} - Ds$	47 (44–62) a	57 (43–67) a	57 (42–67) a	51 (38–77) a	60 (46–80) ab	60 (50–100) ac	65 (25–67) a	67 (33–100) a	50 (0–100) a	100 (0–100) a
$Ta_{Dm} - Ds$	56 (46–61) a	58 (46–70) a	58 (46–70) a	62 (46–73) a	65 (46–80) a	67 (40–80) a	58 (40–80) a	67 (50–67) a	50 (33–100) a	25 (0–100) a
$Ta_{Ds} - Dm$	52 (45–60) a	0 (0–100) a	58 (0–100) a	58 (0–80) a	55 (0–72) ab	60 (0–67) ac	59 (0–67) a	59 (54–72) a	56 (40–75) a	59 (47–64) a
$Ta_{Dm} - Dm$	53 (43–61) a	58 (0–100) a	50 (0–100) a	50 (0–80) a	53 (0–67) b	50 (0–75) bc	51 (0–66) a	64 (59–75) a	50 (37–65) a	58 (48–70) a
Statistical results:										
$H=$	3.91	2.86	1.10	2.13	7.76	9.46	2.26	5.41	1.52	1.51
$df=$	3	3	3	3	3	3	3	3	3	3
$n=$	40	40	40	40	40	40	40	40	40	40
$p=$	0.270	0.410	0.780	0.550	0.050 *	0.020 *	0.520	0.140	0.680	0.680

Different letters in the same column represent significant differences at $p > 0.05$ (Dunn's test). * Significant variation.

3.2. Host Preference and Switching Indexes

In the treatment with an equal proportion of *D. suzukii* and *D. melanogaster*, the parasitism ratio on both drosophilid hosts ranged from 0.55 (0.50–0.61, 0.40–0.67) (Median, IQR (25th–75th percentiles), and minimum and maximum data (range)) for Ta_{Dm} lineage to 0.56 (0.54–0.63, 0.50–0.69) for Ta_{Ds} lineage. The "C" index was <1 , indicating a preference of *T. anastrephae* for *D. melanogaster*. Statistical comparison between both parasitoid lineages indicated no significant differences ($U = 116.0, n = 10, p = 0.427$).

In treatments with unequal proportions of *D. suzukii* and *D. melanogaster*, the observed parasitism was significantly higher than expected at 45:5 and 40:10 *D. suzukii:D. melanogaster* ratios for both parasitoid lineage (Table 2), showing that host switching occurred in both host proportions, in which *T. anastrephae* preferentially attacked *D. melanogaster*. The observed and expected parasitism were not significantly different at 35:15 and 20:30 *D. suzukii:D. melanogaster* ratios for either parasitoid lineage (Table 2), which means that *T. anastrephae* attacked indifferently one or another host. The expected parasitism was significantly higher than observed at the 30:20 *D. suzukii:D. melanogaster* ratio for both parasitoid lineages (Table 2). This suggests that the host switch did not occur because *T. anastrephae* mainly attacked *D. melanogaster*, despite a higher proportion of *D. suzukii* puparia. The observed parasitism was significantly higher than expected at 15:35, 10:40, and 5:45 *D. suzukii:D. melanogaster* ratios for both parasitoid lineages (Table 2). These findings indicate that the host switch occurred because *T. anastrephae* primarily attacked *D. melanogaster* over *D. suzukii*.

Table 2. Host switching behavior by two *Trichopria anastrephae* lineages, one reared on *Drosophila suzukii* (*Ta_{Ds}*) and the other one on *Drosophila melanogaster* (*Ta_{Dm}*), to different ratios of *D. suzukii* over *D. melanogaster*.

Observed (Obs)/ Expected (Exp) Parasitism	Switching Tests: <i>Drosophila suzukii/Drosophila melanogaster</i> Puparia Ratios (Median and Range)							
	45:5	40:10	35:15	30:20	20:30	15:35	10:40	5:45
<i>Ta_{Ds}</i> – Obs	2.5 (1.3–4.3) a	1.6 (0.9–1.8) a	1.3 (1.0–2.0) a	0.8 (0.6–1.2) b	0.6 (0.4–0.8) a	0.5 (0.2–0.7) a	0.3 (0.2–0.8) a	0.3 (0.2–0.7) a
<i>Ta_{Dm}</i> – Obs	1.8 (1.2–3.8) a	2.1 (1.0–3.3) a	1.3 (0.9–2.7) a	0.9 (0.8–1.3) b	0.6 (0.4–0.8) a	0.5 (0.3–0.7) a	0.4 (0.2–0.8) a	0.3 (0.3–0.6) a
<i>Ta_{Ds}</i> – Exp	-1.7 (-1.5–-3.5) b	-3.0 (-2.0–-6.3) b	-7.0 (-6.0–-35.0) a	2.5 (1.9–3.0) a	0.5 (0.5–0.6) a	0.3 (0.2–0.3) b	0.2 (0.1–0.2) b	0.1 (0.06–0.1) b
<i>Ta_{Dm}</i> – Exp	-1.6 (-0.8–-3.1) b	-2.7 (-1.1–-5.0) b	-2.5 (-2.3–-35.0) a	2.6 (2.1–6.0) a	0.5 (0.4–0.6) a	0.3 (0.2–0.3) b	0.2 (0.1–0.2) b	0.1 (0.05–0.1) b
Statistical results:								
<i>H</i> =	29.676	29.657	1.443	29.519	2.043	20.544	18.177	29.796
<i>df</i> =	3	3	3	3	3	3	3	3
<i>n</i> =	40	40	40	40	40	40	40	40
<i>p</i> =	<0.001 *	<0.001 *	=0.695	<0.001 *	=0.564	<0.001 *	<0.001 *	<0.001 *
Obs/Exp results:	Obs > Exp	Obs > Exp	Obs = Exp	Obs < Exp	Obs = Exp	Obs > Exp	Obs > Exp	Obs > Exp

Different letters in the same column represent significant differences at *p* > 0.05 (Dunn's test). * Significant variation.

4. Discussion

The current study provides information on the *D. suzukii*-killing capacity of *T. anastrephae* for two parasitoid lineages faced with *D. suzukii* and the native host *D. melanogaster*, and the subsequent parasitoid reproductive success in terms of the offspring's survival. Based on these parameters, the study reports host preference and host-switching behavior at various ratios of *D. suzukii* and *D. melanogaster*.

The results show that regardless of the population lineage, *T. anastrephae* performed similarly well on *D. suzukii* and *D. melanogaster* with regard to the percentage of killed hosts and offspring survival when there was no choice between hosts. The high mortality of *D. suzukii* recorded in these tests supports previous studies on parasitism levels of a *T. anastrephae* Brazilian population line on *D. suzukii* under laboratory conditions [37,52–55] and in greenhouse trials [56]. Similarly, *Trichopria drosophilae* Perkins, another resident pupal parasitoid found parasitizing *D. suzukii* in North America [57,58], Central America [59], Europe [60–62], and Asia [63] is also able to induce high mortality of *D. suzukii* puparia.

The results also revealed that both lineages of *T. anastrephae* mainly focused on attacking *D. suzukii* puparia at very high proportions of this host relative to *D. melanogaster* in choice situations. However, at higher proportions but no more than twice as many *D. suzukii* over *D. melanogaster*, *T. anastrephae* females attacked both hosts equally. At equal proportions of both hosts, *T. anastrephae* focused its attack on *D. melanogaster*, but the parasitoid intensified its parasitism on *D. melanogaster* at higher proportions of native host over the exotic host in choice tests. In contrast to the present study, the cosmopolitan *T. drosophilae* showed a slight preference to parasitize *D. suzukii* puparia than *Drosophila immigrans* Sturtevant or *D. melanogaster* puparia in laboratory studies when the same host-to-host ratio was assessed [63]. However, the percentages of *T. drosophilae* male and female

offspring recorded from *D. suzukii* puparia individually compared with those emerged from *D. immigrans* were similar [63]. These results suggest that *T. drosophilae*, like *T. anastrephae*, is naturally associated with saprophytic drosophilids. However, the larger size of the *D. suzukii* puparium compared to that of *D. melanogaster* appears to be a factor driving the host preference of *T. drosophilae* for *D. suzukii* [57,58,63–65]. That would not be the case with *T. anastrephae*; despite the small size of *D. melanogaster* puparium, the parasitoid prefers it in an equal host-choice condition. Other biological factors may be influencing host preference by *T. anastrephae*. Probably, olfactory cues associated with the native host puparium play a relevant role in host searching behavior of *T. anastrephae*, in addition to the host-habitat (fruit) olfactory stimuli, which have a significant influence in the long-range host location [56].

The preference for *D. melanogaster* displayed by the *T. anastrephae* in the current studies matches with field survey data recorded from non-crop fruits in wild environments from northwestern Argentina [26,27]. Those wilderness areas involved patches of secondary structure Yungas subtropical rainforest with a mix of feral exotic fruits plus native fruit species. Field studies found that *T. anastrephae* parasitized more resident saprophytic drosophilid puparia than *D. suzukii*. However, it is worth noting that an extremely high abundance of saprophytic drosophilids over *D. suzukii* was always present in the fallen fruits surveyed [27]. Therefore, that a disproportional natural relationship between *D. melanogaster* and *D. suzukii* puparia likely influenced the host preference displayed by *T. anastrephae*, as evidenced by the results of the current tests. There was only host switching to *D. suzukii* when the host ratio was 80–90% *D. suzukii* over *D. melanogaster*. These findings are supported by differences in the evolutionary history between *T. anastrephae* and both hosts because it is a Neotropical-native parasitoid species [66] coevolved in sympatry with saprophytic drosophilid species, such as those of the *D. melanogaster* group [26]. This may account for the close trophic association between *T. anastrephae* and non-pest saprophytic drosophilids, whereas a new trophic association was recently established with *D. suzukii*. Although *T. anastrephae* quickly adapted to *D. suzukii*, the prevalence of puparia from native drosophilid hosts inside fallen fruit in natural environments may reduce the effectiveness of this resident parasitoid on the exotic pest [26]. However, the results of the current study show that although *T. anastrephae* preferred *D. melanogaster* in a choice condition at equal host ratios, *T. anastrephae* was an effective natural enemy as it achieved mortality rates on *D. suzukii* between 40% and 42%. Even at host ratios where *D. suzukii* was disadvantaged, close to 50% of the *D. suzukii* puparia were parasitized by *T. anastrephae*, and the parasitoid offspring was always female-biased. Such findings are significant because they highlight *T. anastrephae* as an important *D. suzukii* mortality agent. In both *Drosophila* species studied, the host mortality caused by *T. anastrephae* was high. An additional mortality factor might be due to superparasitism, particularly when considering the relationship between the low density of exposed hosts and the long host exposure time in the tests. For the current study, verifying whether the host mortality was also caused by superparasitism was not achievable. However, a second step of the study has been planned to ascertain whether superparasitism influenced high host mortality levels reported herein.

Recent studies [35] showed that *T. anastrephae* can also forage *D. suzukii* puparia outside the fruit pulp, where saprophytic dipteran puparia do not prevail. Thus, *T. anastrephae* can parasitize *D. suzukii* puparia in less competitive microhabitats, such as the ground beneath fallen feral peaches and guavas in non-crop areas [35]. Previous studies have shown that most *D. suzukii* larvae pupate on the ground beneath fallen fruit [57–68], and puparia can be attacked by pupal parasitoids [56,69]. These earlier published data, plus information from the current study, reveal that *T. anastrephae* may exert mortality over *D. suzukii* puparia isolated on the ground. Augmentative releases [60] or conservation biological control [70] are strategies that can be focused from a *D. suzukii* area-wide integrated management approach [36,71]. Sustainable pest management must focus on landscape biodiversity conservation and enhanced natural control [72].

5. Conclusions

The results suggest that *T. anastrephae* was effective in killing *D. suzukii* based on its performance relative to the pest and producing reproductively successful offspring. Given the preference of *T. anastrephae* for resident saprophytic drosophilids, the concurrent presence of those hosts in equal or higher ratios to that of *D. suzukii* in the same microhabitat influenced on the performance of the parasitoid. However, the mortality of *D. suzukii* by *T. anastrephae* under such conditions was significant. Optimizing important biological traits can be explored through selective rearing in studies based on the experimental adaptation of resident parasitoids on *D. suzukii*. This would provide a more specific biological control agent targeted at the pest.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14030520/s1>. File S1: Raw Data-Host ratio Treatments; File S2: Raw Data-Host preferences; File S3: Raw Data-Parasitoid sex ratios.

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