Trichopria anastrephae as a Biological Control Agent of *Drosophila suzukii* in Strawberries

Alexandra P. Krüger[1] 🅾 · **Amanda M. Garcez[1]** · **Tiago Scheunemann[1]** · **Dori E. Nava[2]** · **Flávio R. M. Garcia[1,3]**

Abstract

In South America, the resident pupal parasitoid *Trichopria anastrephae* Costa Lima (Hymenoptera: Diapriidae) is a potential biological control agent of the pest *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). In the present study, we (1) examined the behavior of *T. anastrephae* towards different host (*D. suzukii*) and host-substrate (strawberry) cues in choice and non-choice bioassays in laboratory, and (2) examined the density-dependent parasitism of *T. anastrephae* in *D. suzukii*-infested strawberries in a greenhouse. When given a choice, female parasitoids walked longer over chambers with fruits infested with eggs, larvae, or pupae of *D. suzukii*, when compared to healthy uninfested strawberries, and over overripe fruits when compared to unripe or ripe fruits. In the greenhouse assay, we observed an increase in parasitism and a decrease in the number of *D. suzukii* emerging per fruit with an increase in the number of parasitoids released. Our results allow a better understanding of the behavior and parasitism of *T. anastrephae* in *D. suzukii*-infested strawberries and provide useful data for potential biological control programs using this parasitoid.

Keywords Spotted wing drosophila · Parasitoid · Small fruit · Biocontrol

Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive pest species, native to Southeast Asia, that in the last years expanded its range and, now, is spread in Europe, North and South America, and Africa (Bolda et al. 2010; Calabria et al. 2012; Cini et al. 2012; Deprá et al. 2014; dos Santos et al. 2017; Boughdad et al. 2021). After its range expansion, *D. suzukii* rapidly became a major pest in small fruit production, causing an annual economic impact estimated in hundreds of millions of USD, due to yield losses and increased production

costs (Goodhue et al. 2011; Knapp et al. 2021). The potential to cause high damage is mainly due to the female's sclerotized and serrated ovipositor that enables them to lay eggs in ripe and ripening healthy fruits (Lee et al. 2011), short life cycle and high fecundity (Emiljanowicz et al. 2014; Tochen et al. 2014), and its large host range (Lee et al. 2015; Poyet et al. 2015; Kenis et al. 2016). Currently, *D. suzukii* has been controlled by spraying insecticides successively, which can cause insecticide resistance and impacts in non-target species (Desneux et al. 2007; Gress and Zalom 2018). Thus, research effort has been focused on finding alternative approaches, including biological control (Schetelig et al. 2018; Lee et al. 2019; Wang et al. 2020).

Biological controls, such as predators, parasitoids, nematodes, bacteria, fungi, and viruses, were studied and demonstrated potential to be used in Integrated Pest Management programs against *D. suzukii* (Garcia et al. 2017; Schetelig et al. 2018; Lee et al. 2019; Garcia 2020). Biological control agents could reduce *D. suzukii* populations in both crop and non-crop areas promoting a sustained suppression throughout the seasons (Lee et al. 2019). Although resident larval parasitoids associated with *D. suzukii* were found in almost all continents where the pest is present (Rossi Stacconi et al. 2013; Miller et al.

✉ Alexandra P. Krüger
  alexandra_kruger@hotmail.com

1   Crop Protection Department, Federal University of Pelotas (UFPel), Capão do Leão, RS, Brazil

2   Laboratoy of Entomology, Embrapa Temperate Agriculture, Pelotas, RS, Brazil

3   Ecology, Zoology and Genetics Department, Institute of Biology, Federal University of Pelotas (UFPel), Capão do Leão, RS, Brazil

2015; Wollmann et al. 2016; Knoll et al. 2017; Matsuura et al. 2018), few species can overcome *D. suzukii* immune responses. Due to its high hemocyte load, *D. suzukii* can encapsulate eggs from different species of larval parasitoids (Kacsoh and Schlenke 2012). Thus, the most promising parasitoids that can successfully reproduce on *D. suzukii* are pupal parasitoids.

To date, only a few species of parasitoids have been found associated with *D. suzukii* in South America (Wollmann et al. 2016; De la Vega et al. 2021; Garcia et al. 2022), including *Trichopria anastrephae* Costa Lima (Hymenoptera: Diapriidae), a resident pupal parasitoid (Cruz et al. 2011). Since its association with *D. suzukii*, several aspects of *T. anastrephae* parasitism were studied, including parasitism ability in different laboratory conditions (Krüger et al. 2019; Vieira et al. 2019, 2020), competition with another parasitoid (Oliveira et al. 2021), and toxicological effects of insecticides and essential oils (Schlesener et al. 2019). Results from all these studies demonstrated the potential of *T. anastrephae* as a biological control agent of *D. suzukii* and encourage further studies.

Although most studies were performed using naked pupae only, a recent study showed that *T. anastrephae* can discriminate among *D. suzukii*-infested and non-infested blueberry, indicating that this parasitoid can use chemical cues from the interaction among host (*D. suzukii*) and host substrate (fruit) to locate the pest (De La Vega et al. 2021). Similarly, *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae), a pupal parasitoid found parasitizing *D. suzukii* in North and Central America and Europe, is also able to discriminate among non-crop fruits infested and non-infested by *D. suzukii* (Wolf et al. 2020). Furthermore, semi-field and field experiments showed that released *T. drosophilae* can locate and parasitize *D. suzukii* in blueberry, cherry, and raspberry (Rossi Stacconi et al. 2018, 2019; Gonzaléz-Cabrera et al. 2019).

In Brazil, *D. suzukii* imposes a great risk for strawberry production, and heavy economic losses were already reported (Santos 2014; Andreazza et al. 2016), and *T. anastrephae* could be an alternative to control this pest in strawberry orchards. However, it is necessary to evaluate the performance of this parasitoid in *D. suzukii*-infested strawberries. Thus, aiming to evaluate the ability of *T. anastrephae* to parasitize *D. suzukii* in strawberries, we conducted two experiments. In the first experiment, we examined the behavior of this parasitoid towards several host (*D. suzukii*) and host-substrate (strawberry) cues, to identify if *T. anastrephae* can discriminate between infested and non-infested strawberries. In the second experiment, we examined the density-dependent parasitism of *T. anastrephae* in *D. suzukii*-infested strawberries in a confined environment (greenhouse).

Material and methods

Insects rearing

Trichopria anastrephae and *D. suzukii* rearing were conducted under controlled conditions (23 ± 2°C, 70 ± 10% RH, and 12 h of light) at the Laboratory of Entomology, Embrapa Clima Temperado, Brazil. As described elsewhere (Vieira et al. 2019), colonies were initiated from flies and parasitoids collected in nearby organic farms, with new material periodically introduced to maintain colony vigor. Flies were reared on a cornmeal-based diet (Schlesener et al. 2018), and adult flies were held in plastic cages (262 × 177 × 147 mm) (length × height × width), with two side openings (80 × 100 mm), and an opening in the lid (155 × 50 mm), covered with voile cloth. Parasitoids were reared on *D. suzukii* pupae, and adults were held in plastic cages, as the ones described here for *D. suzukii*, and fed pure honey (*ad libitum*).

Behavioral assays

To test the response of *T. anastrephae* females towards different host and host substrate cues, we conducted behavioral assays in a circular four-chamber olfactometer (adapted from Steidle and Schöller (1997), and previously used for *T. drosophilae* by Wolf et al. (2020)). The olfactometer used had a 15-cm diameter and 6-cm height and was divided into four equal-sized chambers. One strawberry (~ 10 g) was placed in one (no choice) or two opposite (choice) chambers of the olfactometer. A plastic mesh was used to cover the olfactometer and a plate (20-cm diameter and 1-cm height) was placed above the mesh. The plate had one hole in the center, where an open 1.5-mL tube containing one parasitoid female (3 to 5 days old, naïve, and mated) was inserted. The parasitoid was placed in the tubes 30–60 min before the assay. After leaving the tube, the parasitoid was able to walk freely in the plate, above the chambers, without contacting the fruits. During the tests, the olfactometer was kept in a white plastic basin, and placed under a light source, for evenly distributed light.

For no-choice and choice bioassays strawberry fruits containing different potential cues were analyzed (Table 1). All fruits were obtained from plants (cv. San Andreas) cultivated without any pesticide spray and showed no signs of insect infestation or fungal contamination before they were treated. For treatments containing *D. suzukii* cues, fruits were collected and individually placed in plastic containers (200 mL) with two couples of *D. suzukii*. After 24 h, *D. suzukii* adults were removed. This treatment induced an infestation of 6–10 eggs per fruit. Infested strawberries were kept for 1 (fruits with eggs), 5 (fruits with larvae), or 8 days (fruits with pupae) before

Table 1 Treatments and rationale tested in a series of no-choice and choice bioassays reflecting cues of *Drosophila suzukii*-infested and non-infested strawberries potentially used by female *Trichopria anastrephae*

Treatment	Rationale
Superficially damaged fruit (also tested against healthy fruits, in choice bioassays)	Compounds released by the fruit after being damaged are used as cues by female parasitoids
Fruit with eggs (naturally infested) (also tested against healthy fruits, in choice bioassays)	Compounds derived from egg-laying and adult flies activities are used as cues by female parasitoids
Fruit with larvae (naturally infested) (also tested against healthy fruits, in choice bioassays)	Compounds derived from larval feeding are used as cues by female parasitoids
Fruit with pupae (naturally infested) (also tested against healthy fruits or fruits with artificially infested pupae, in choice bioassays)	Compounds derived from larval feeding and the presence of host pupae are used as cues by female parasitoids
Fruit with pupae (artificially infested) (also tested against healthy fruits or fruits with naturally infested pupae, in choice bioassays)	Compounds derived from the presence of host pupae are used as cues by female parasitoids
Unripe fruit (also tested against ripe fruits or overripe, in choice bioassays)	Compounds derived from healthy unripe fruits are used as cues by female parasitoids
Ripe fruit (also tested against unripe fruits or overripe, in choice bioassays)	Compounds derived from healthy ripe fruits are used as cues by female parasitoids
Overripe fruit (also tested against unripe fruits or ripe, in choice bioassays)	Compounds derived from healthy overripe fruits are used as cues by female parasitoids

they were used in the bioassays. Non-infested fruits for choice bioassays were collected on the same day, but they were not exposed to *D. suzukii* adults. Artificially damaged fruits were collected and perforated ten times with a "00" entomological pin, which was pierced through the skin only about 1 mm. Fruits with inoculated pupae were kept in the lab for 8 days, then perforated and inoculated with ten 24-h-old *D. suzukii* pupae. Strawberries in different ripening stages were also tested. Unripe (1/3 of the fruit was pink) and ripe (completely red fruit) strawberries were collected the day before being used for bioassays, while for overripe strawberries, fruits were marked in the plant when they were ripe (completely red), but only collected 7–9 days later, when showing signs of overripening (losing their firmness).

Each parasitoid female was observed for 300 s, and the observation began when the female left the tube. If a female did not leave the tube for 50 s or moved for less than 150 s during the observation period, it was considered non-responsive and removed from the analysis. The period that each female walked over each chamber was logged using the open-source software Boris (Behavioral Observation Research Interactive Software) (Friard and Gamba 2016). Each female was used only once. After each observation, the olfactometer was rotated 90° and the plate over the olfactometer was exchanged. Each fruit and olfactometer was used three times, and then, the fruits were removed and the olfactometers were cleaned (with distilled water and neutral soap, rinsed with 70% ethanol, and dried overnight). For each treatment, in both choice and no choice bioassays, between 20 and 37 responsive females were observed. All bioassays were conducted at 23 ± 2°C, 70 ± 10% RH, and between 09:00 and 14:00 h.

Greenhouse assay

We tested the parasitism of different densities of *T. anastrephae* released in walk-in cages (360 × 220 × 200 cm) (length × height × width), placed inside a greenhouse. Inside each walk-in cage, we placed 18 potted strawberry plants (cv. San Andreas), each plant containing at least 3 ripe fruits. An artificial infestation of *D. suzukii* (60 couples, 3–5 days old) was performed in each cage. Since *T. anastrephae* is a pupal parasitoid, they were released inside each cage 5 days later than *D. suzukii* infestation, to allow host development. Parasitoids were released in each cage according to treatments (T1- no release (control), T2- 60 parasitoids, T3- 120 parasitoids, T4- 240 parasitoids, and T5- 360 parasitoids), in the same sex ratio as the maintenance rearing (0.7).

To assess fruit infestation and parasitism, strawberry fruits were collected 5, 10, and 15 days after parasitoid release. In each fruit sampling, 20 fruits were randomly collected and individually placed in plastic containers (250 mL), containing a fine layer (~ 2 cm) of vermiculite. The number of flies and parasitoids from each fruit was recorded 30 days after fruit sampling. The experiment was replicated 4 times between October 2019 and February 2021. No pesticide was sprayed during experiments.

Statistical analysis

For data collected in behavioral experiments, the time that female wasps spent walking over chambers containing fruit vs no fruit, in no choice bioassays, or over the two different treatments, in choice bioassays, was analyzed with Wilcoxon signed rank tests ($p \leq 0.05$). And for data collected in the greenhouse trials, we used a generalized linear model (GLM), with quasipoisson error distribution, to assess the

effects of parasitoid release density and time after parasitoid release on a number of *D. suzukii* or *T. anastrephae* per fruit ($p \leq 0.05$). In case of significance, Tukey's post hoc test was run to compare treatments ($p \leq 0.05$). All statistical analyses were performed in the software R version 4.0.3 (R Core Team 2020).

Results

Behavioral assays

Parasitoid females walked significantly longer over olfactometer chambers with infested strawberry fruits containing larvae, pupae, or inoculated pupae when compared to empty chambers (Fig. 1). Similarly, *T. anastrephae* females also walked longer over chambers containing overripe strawberries than over chambers without a fruit (Fig. 1). However, damaged fruits, fruits containing *D. suzukii* eggs, and unripe and ripe fruits did not affect the length that female parasitoids walked over each chamber of the olfactometer (Fig. 1).

When offered a combination of two fruits containing two different potential cues, parasitoid females walked longer over chambers containing fruits naturally infested with eggs, larvae, or pupae of *D. suzukii*, when compared to healthy uninfested fruits (Fig. 2). Females also walked longer over fruits with naturally infested pupae than inoculated pupae, and over overripe fruits when compared to unripe or ripe fruits (Fig. 2).

Greenhouse assay

Based on the number of insects emerging from strawberry fruits, we did not observe an interactive effect between the number of parasitoids released and time after parasitoid release for either number of *D. suzukii* ($\chi^2 = 1735.20$, $p = 0.3828$) or *T. anastrephae* ($\chi^2 = 338.97$, $p = 0.1592$). However, the number of *D. suzukii* significantly decreased with the increase in the parasitoid release density ($\chi^2 = 1842.50$, $p < 0.0001$, Fig. 3). Compared to the control (no parasitoid release), the number of *D. suzukii* adults emerging per fruit decreased by 21.12, 48.20, 59.48, and 66.38% when 60, 120, 240, or 360 parasitoids were released, respectively. On the other hand, the number of *T. anastrephae* adults emerging per fruit increased with the increase in the release density ($\chi^2 = 374.71$, $p < 0.0001$, Fig. 3). As expected, no


Fig. 1 Length of time (seconds) that *Trichopria anastrephae* females spent walking over olfactometer chambers containing a fruit with different potential cues vs. no fruit (empty). Values are mean ± SE. Sample size is indicated in the brackets. ns indicates no significant difference, while *, **, and *** indicate a significant difference at 5, 1, and 0.1% levels, respectively (Wilcoxon signed rank tests)
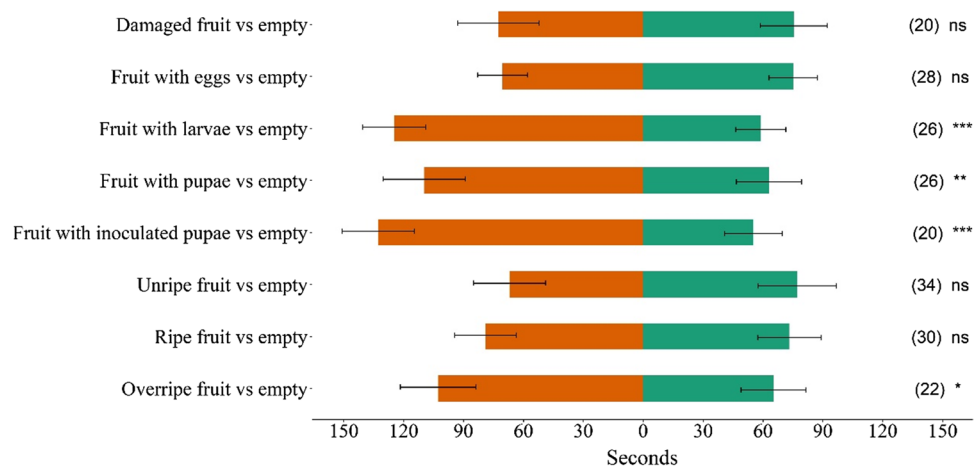
Fig. 2 Length of time (seconds) that *Trichopria anastrephae* females spent walking over olfactometer chambers containing fruits with different potential cues. Values are mean ± SE. Sample size is indicated in the brackets. ns indicates no significant difference, while * and ** indicate a significant difference at 5 and 1% levels, respectively (Wilcoxon signed rank tests)
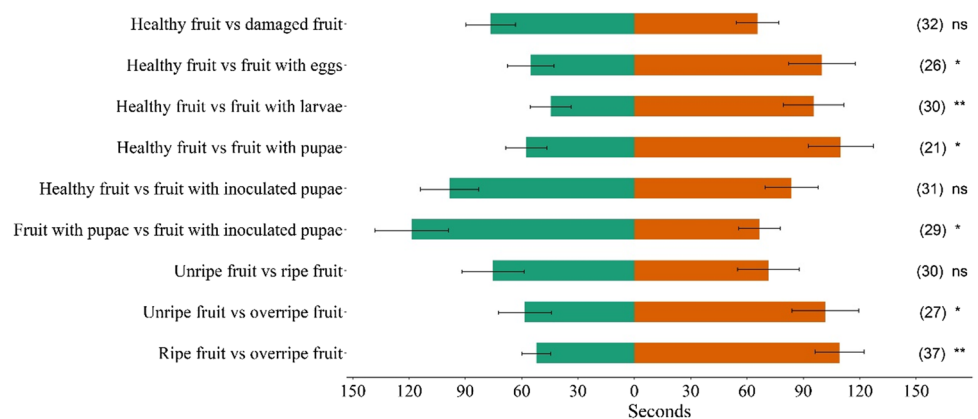
Fig. 3 Number of *Drosophila suzukii* and *Trichopria anastrephae* adults emerging per fruit collected from strawberry plants treated with different release densities of *T. anastrephae*. Values are mean ± SE. Letters are comparing bars of the same color; different letters indicate a significant difference according to the Tukey test ($p \leq 0.05$)
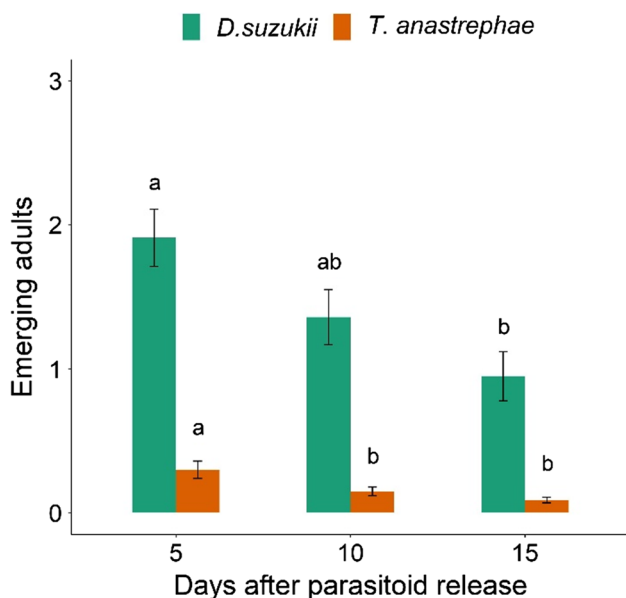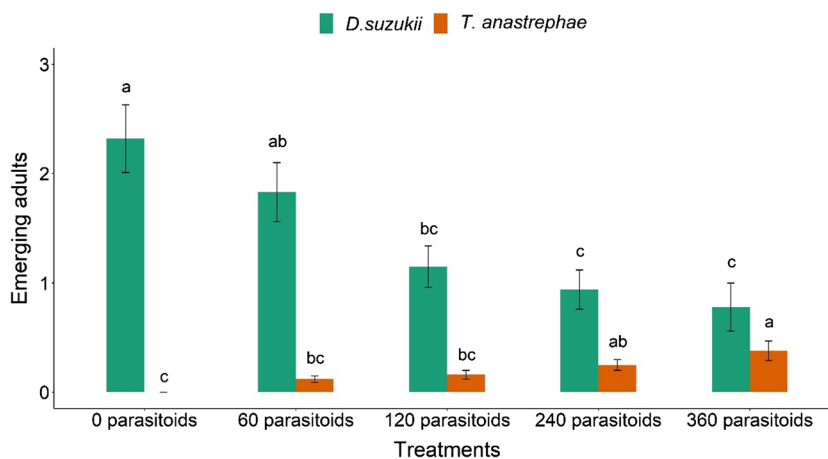
Fig. 4 Number of *Drosophila suzukii* and *Trichopria anastrephae* adults emerging per fruit 5, 10, and 15 days after parasitoid release. Values are mean ± SE. Letters are comparing bars of the same color; different letters indicate a significant difference according to the Tukey test ($p \leq 0.05$)

parasitoid emerged from the control, while 0.38 parasitoids/fruit emerged when 360 parasitoids were released.

The number of *D. suzukii* and *T. anastrephae* were affected by the time after the parasitoid was released ($\chi^2 = 1776.20$, $p < 0.0001$ and $\chi^2 = 350.44$, $p < 0.0001$, respectively, Fig. 4). When fruits were sampled 5 days after parasitoid release, we observed the highest numbers of *D. suzukii* (1.91 ± 0.20) and *T. anastrephae* (0.30 ± 0.06) adults emerging from fruits. However, the number of emerging insects started to decrease for both species 10 days after parasitoid release (1.36 ± 0.19 *D. suzukii*/fruit and 0.15 ± 0.03 *T. anastrephae*/fruit), and number of *D. suzukii* emerging adults reached its lowest 15 days after parasitoid release ($0.95 \pm$

0.17), while the number of *T. anastrephae* were stable after 10 days.

Discussion

In the present study, we evaluated the behavior of *T. anastrephae*, a potential biocontrol agent of *D. suzukii* in Latin America, towards *D. suzukii*-infested and non-infested strawberries, as well as the density-dependent parasitism of *D. suzukii* pupae in strawberries cultivated in a greenhouse. Our results indicate that female parasitoids can recognize fruits infested with larvae and pupae (naturally and artificially infested) or in overripe stage. Moreover, when given a choice, female parasitoids showed preference towards fruits infested with eggs, larvae, and pupae over healthy undamaged fruits, and towards overripe fruits over unripe or ripe fruits. Also, *T. anastrephae* was able to parasitize *D. suzukii*-infested strawberries in the greenhouse and reduce the pest emergence, in a density-dependent manner.

The ability of a parasitoid to search for a host directly affects its efficacy as a biological control agent, and this behavior is often mediated by compounds emitted by the host substrate (plants), the host, and/or interaction among them (Vet and Dicke 1992). Our results show that infested fruits with larvae or pupae of *D. suzukii* and overripe fruits elicit a response in female parasitoids since they preferred to walk over chambers containing these treatments as compared to empty controls. In fact, as a pupal parasitoid, *T. anastrephae* has a better chance of finding a host in fermenting fruits, explaining their preference for fruits in a more advanced decaying stage over empty chambers, even when the fruits were not infested. The use of cues from both uninfested and infested fruits by female parasitoids was shown in the tephritid parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) (Silva et al. 2007; Segura et al. 2012). This larval parasitoid was attracted to fruit cues alone, but the presence of

its host larvae in the fruit enhanced the attraction (Silva et al. 2007). Another experiment with this species showed that even using a larval medium elicits a positive response in female parasitoids, suggesting that byproducts from larval activity are an important source of chemical cues (Segura et al. 2012).

When given a choice, *T. anastrephae* showed a preference for infested strawberries (containing eggs, larvae, or pupae) over healthy fruits. Similarly, this species also shows a preference for infested blueberries when compared to non-infested fruits (De la Vega et al. 2021). The authors showed that the development of *D. suzukii* immature stages inside the fruit results in changes in the odor profile of the blueberry, which may result in a higher attraction of *T. anastrephae* to infested fruits. Another species of the pupal parasitoid, *T. drosophilae*, is also able to distinguish between *D. suzukii*-infested and non-non-infested fruits, in at least five different wild non-crop fruits (Wolf et al. 2020). The larval parasitoid *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) shows a preference for *D. suzukii*-infested cherries, strawberries, and blackberries when compared to non-infested fruits (Biondi et al. 2017). Other larval parasitoids, *Leptopilina japonica* Novkovic & Kimura and *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae), also respond to fruit volatile cues associated with the presence of *D. suzukii* (Biondi et al. 2021).

We also tested fruits containing inoculated pupae. Fruits for this treatment were picked, brought to the lab, and only inoculated and tested 8 days after, to mimic the time taken by *D. suzukii* to become pupae in naturally infested fruits. In no-choice experiments, female parasitoids showed a preference to walk over chambers containing fruits with inoculated pupae compared to empty chambers. But they also spent more time on fruits with naturally infested pupae and overripe fruits than on empty chambers. Thus, it was not clear if females were behaving towards fruits with inoculated pupae due to the presence of pupae or the age of the strawberry. When given a choice between inoculated pupae or healthy fruit (both tested 8 days after picking), female parasitoids did not show any preference, suggesting that the sole presence of host pupae does not seem to be sufficient to elicit a response when compared to old fruits. However, female parasitoids spent more time walking over fruits with naturally infested pupae when compared to fruits with inoculated pupae. For a biological control agent, the foraging process includes host habitat (or substrate) location, host location, host acceptance, and host suitability (Vinson 1976, 1985). Our results suggest that *T. anastrephae* uses cues from overripe strawberries, which may be indirectly associated with the fly infestation since infested fruit become rotten more rapidly; thus, overripe/rotten fruits are more likely to contain hosts than unripe/ripe fruits. In a longer range, volatile compounds released by overripe fruits may direct parasitoids to

habitats with a higher probability of host occurrence (Segura et al. 2012).

Artificially damaged fruits and fruits with eggs did not elicit a response in *T. anastrephae*. These cues are associated with the initial phases of infestation, and, as *T. anastrephae* is a pupal parasitoid, they may not be capable of identifying the egg stage (Hoffmeister and Gienapp 1999; Segura et al. 2012). On the other hand, infested fruits, containing larvae or pupae of *D. suzukii*, elicit a positive response in the parasitoid. The pupal stage, used by *T. anastrephae* as a host, is motionless and does not feed, resulting in a minimum odor emission (Fischer et al. 2001), and our results indicate that the combination of larvae feeding and pupating in the fruit affect the wasp. Probably, the cues responsible for promoting parasitoid response result from the combination of the host substrate (fruit), the host, and the host-associated microorganisms and/or byproducts (Vet and Dicke 1992; Hamby and Becher 2016).

In the greenhouse trial, to guarantee infestation of *D. suzukii*, we released adults of this pest, before releasing parasitoids. The average infestation in our control cage (no parasitoid release) was of 2.32 emerging *D. suzukii* per fruit. This infestation can be considered a low infestation, as found in the early season, since in the same region in Brazil, the average number of emerging *D. suzukii* during the growing season ranges between 5 and 10 adults per strawberry (Santos 2014; Wollmann et al. 2020). The management of *D. suzukii* in the early season is indicated to reduce or delay pest outbreaks (Rossi-Stacconi et al. 2016). Previous studies demonstrated that early augmentative releases of *T. drosophilae* have the potential to suppress the population of *D. suzukii* and reduce pest damage on crops (Rossi-Stacconi et al. 2019). On the other hand, when *T. drosophilae* was tested in a greenhouse with a high infestation of *D. suzukii*, the parasitoid was not effective (Trottin et al. 2014). Here, we showed that *T. anastrephae* can reduce *D. suzukii* emergence depending on the number of parasitoids released, but, ideally, this parasitoid should be also tested in future studies in higher levels of *D. suzukii* infestation.

Despite the initial pest population, the number of released parasitoids is another crucial factor for the success of biological control. In our experiments, the different parasitoid release rates resulted in different impacts on *D. suzukii* and *T. anastrephae* emergence. Although no difference was seen between the control and the lowest number of parasitoids released tested, the emergence of *D. suzukii* decreased when 120 parasitoids or more were released. A previous study showed that releasing up to 3000 *T. drosophilae* adults/ha is not sufficient to impact *D. suzukii* population density; however, releasing 4500 parasitoids/ha resulted in a reduction of 50% of the pest population in a berry field in Mexico (Gonzales-Cabrera et al. 2019). In our study, releasing 240 and 360 parasitoids per cage resulted in a decrease of

approximately 60% in *D. suzukii* emergence. On the other hand, the number of parasitoids emerging per fruit was low (an average of < 1 parasitoid/fruit) even in the highest release ratio. However, it is important to note that this number may be underestimated since we only considered parasitoids emerging from pupae located in fruits collected from plants. Although there were no fruits on the ground to be collected, *D. suzukii* can pupate in the soil (Woltz and Lee 2017), and such pupae could also be parasitized.

Here, the timing of parasitoid release was planned according to the development of *D. suzukii*, to ensure that pupae would be available for a higher parasitoid success. In nature, it is more difficult to predict the most susceptible period of the host since there are several biotic and abiotic factors involved. However, simulated predictions of pest populations were performed for regions in the Northern Hemisphere and allowed researchers to predict periods when populations are composed of different stages, including pupae (Wiman et al. 2014). This information is useful to obtain the highest efficiency from parasitoid releases and to plan early season management of *D. suzukii* (Pfab et al. 2018; Rossi-Stacconi et al. 2019). Such simulations are not yet available for the Southern Hemisphere, but they would help plan biological control programs.

The results presented in this study provide some initial data that can be useful for biological control strategies using *T. anastrephae*. Our results allow a better understanding of the cues used by *T. anastrephae* in host searching, and their ability to parasitize infested strawberries. This indigenous biocontrol agent, despite being generalist, is an alternative to more specialist parasitoid species identified in *D. suzukii*'s native range since the introduction of exotic natural enemies may be challenging due to international regulations and risks imposed to resident biodiversity. Moreover, as a local parasitoid, *T. anastrephae* is expected to be well adapted to local environmental conditions and may not only parasitize *D. suzukii* pupae found in the targeted crop but also in unmanaged adjacent crops and wild fruits, that may serve as a reservoir of *D. suzukii*.

Declarations

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