BIOLOGICAL CONTROL



Effect of the Ages of Parasitoid and Host Eggs on *Telenomus podisi* (Hymenoptera: Platygastridae) Parasitism

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Abstract

This study investigates the influence of parasitoid age and egg age of the hosts Euschistus heros (Fabricius) and Dichelops melacanthus (Dallas) on parasitism of Telenomus podisi Ashmead. Six separate bioassays were conducted: parasitism on eggs of E. heros (bioassay 1) and D. melacanthus (bioassay 2) by T. podisi females of different age (1, 5, and 10 days old); parasitism by T. podisi on eggs of different age (1, 2, 3, 4, and 5 days of embryonic development) of the hosts E. heros (bioassay 3) and D. melacanthus (bioassay 4); preference of T. podisi females for eggs at different embryonic developmental stages (eggs of 1, 2, 3, 4, and 5 days) of the hosts E. heros (bioassay 5) and D. melacanthus (bioassay 6). The age of T. podisi females and their hosts affected parasitism on both E. heros and D. melacanthus eggs. Overall, the parasitism rate was higher in older than younger parasitoids, independent of the tested host species, and host eggs between 1 and 3 days old were similarly parasitized. Thus, in T. podisi mass rearing facilities, it is recommended to use older adults (5 to 10 days old) as mother wasps to increase parasitism on the offered eggs. In addition, when hosts are completely absent in the field, or climatic conditions are unfavorable for release, mass-reared adults can be kept in the laboratory (25°C) for up to 10 days for later release in the field without any impairment of their subsequent parasitism performance.

Introduction

Soybean is one of the world's major crops. However, its yield is frequently reduced because of injuries caused by a stink bug species complex. These species feed directly on pods, affecting the grain's physiological and sanitary quality (Panizzi & Slansky 1985, Corrêa-Ferreira & Azevedo 2002). Among these species, *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) is the most important and abundant stink bug occurring in soybeans in Brazil (Panizzi & Corrêa-Ferreira 1997). In addition, another important stink bug species, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), is increasing in importance. The continuous availability of hosts other than soybean (e.g., maize) all over the year has favored *D. melacanthus* outbreaks in South America. Those pests are usually managed exclusively by chemical (Bueno *et al* 2011, Bueno *et al* 2015a, b, Chiesa *et al* 2016, Panizzi 2013). This overuse of insecticides has trigged rapid selection of resistant pests, reduction of biological control agents, outbreaks of secondary pests among other unfavorable outcomes (Sosa-Gómez *et al* 2001; Sosa-Gómez & Silva 2010).

Alternatively, augmentative biological control (ABC) is a more sustainable pest management tool already available to be used in integrated pest management (IPM). Not only is ABC an appropriate strategy to be used in conventional cropping systems but also in organic farming (Michaud 2018). It has been efficiently applied for more than 100 years in several crops (Cock *et al* 2010) on more than 30 million ha worldwide (van Lenteren *et al* 2018). Among the possible biological control agents that could be used in ABC, egg parasitoids are the most important for stink bug management (Laumann *et al* 2010). These biological control agents attack pest eggs, preventing them from developing and causing injury to the plants (Querino *et al* 2016; Valente *et al* 2016).

Among the different egg parasitoids that can be used in ABC of E. heros and D. melacanthus, Telenomus podisi Ashmead (Hymenoptera: Platygastridae) stands out due to its high efficacy (Peres & Corrêa-Ferreira 2004; Queiroz et al 2018). Although T. podisi preferred eggs of D. melacanthus to those of E. heros (Queiroz et al 2018), host quality, independently of the species, has great influence on parasitism, parasitoid survival and growth. Importantly, parasitoid progeny might not be able to develop if an unsuitable host is chosen (Godfray 1994). Thus, parasitoid fitness and quality is affected by several aspects of the parasitoid-host interaction, which depends on host age and species, among others. Knowing the performance of such parasitoids on hosts with different characteristics could be used to improve the quality of biological control agents in ABC strategies (van Lenteren & Tommasini 2002). Therefore, knowledge of hostparasitoid interactions is essential for an efficient and economic mass rearing of the biological control agent in order to provide the required number of parasitoids to be released in the field and rapidly reduce the number of pests (van Lenteren & Tommasini 2003). Research on the effects of parasitoid age and host quality on parasitoid performance in mass rearing facilities and field conditions is rare (Cingolani et al 2014). Parasitoids usually reduce their offspring with age. Similarly, old eggs are usually less parasitized when compared to younger ones. However, differences in performance of different strains were reported for several platygastrids (Powell & Shepard 1982; Corrêa-Ferreira & Zamataro 1989; Colazza & Rosi 2001) and require further studies. Understanding T. podisi-host interactions particularly the impact of host and parasitoid age on performance and fitness of the parasitoid is essential to provide appropriate recommendations for T. podisi rearing and use in ABC programs. Therefore, this study evaluated the effects of parasitoid and host egg age on T. podisi parasitism as well as female parasitoid preference for host eggs at different embryonic stages of development.

Material and Methods

Laboratory rearing of **Telenomus podisi**, **Euschistus heros**, and **Dichelops melacanthus**

Parasitoid and stink bug species were originally collected in soybean (*T. podisi* and *E. heros*) and maize (*D. melacanthus*) fields in Londrina, PR, Brazil (23°11'11.7"S and 51°10'46.1"W) and kept at Embrapa Soybean Laboratory, where colonies were maintained under controlled environmental conditions ($25 \pm 2^{\circ}$ C, relative humidity 80 ± 10%, and photoperiod of 12/ 12 h L/D).

Telenomus podisi has been reared on *E. heros* eggs for 5 years according to the methodology published by Peres & Corrêa-Ferreira (2004), briefly described in the following. Unparasitized stink bug eggs together with eggs from the laboratory colony previously parasitized by *T. podisi* were glued onto a piece of cardboard (5 cm × 8 cm) and introduced into plastic cages (height 8.5 cm, diameter 7 cm), which were sealed with plastic film. Small drops of *Apis mellifera*-produced honey were placed inside these cages to feed the adults as soon as they emerged. The cages were then closed, and parasitism was allowed for 24 h. Next, eggs were removed and inserted in new pots sealed again with the same plastic film and kept at the same controlled conditions. Adults that emerged from these eggs were used for trials or colony maintenance.

Dichelops melacanthus and E. heros were reared in the laboratory for approximately 5 years. New field insects were introduced each year to maintain colony quality. Rearing was performed according to the methodology published by Panizzi et al (2000) as described briefly in the following. Adults were kept in plastic cages (20 cm × 20 cm × 24 cm high) lined with filter paper. A piece of cloth (raw cotton) placed in each cage served as the substrate for oviposition. The insects were fed with beans (Phaseolus vulgaris L.), soybeans (Glycine max (L) Merril), peanuts (Arachis hypogaea L.), sunflower seeds (Helianthus annuus L.), and privet fruits (Ligustrum lucidum Ait.). Cages were cleaned and food replaced on a daily basis. Collected egg masses were used one half each for colony maintenance and for storage in liquid nitrogen (- 196°C) for up to 1 month to be later use in bioassays 1 and 2 (Silva et al 2008). Fresh eggs were also collected and kept under controlled conditions (temperature of 25 ± 2 °C, relative humidity of 80 \pm 10%, and a photoperiod of 14/10 h L/D) within biochemical oxygen demand (BOD) climatic chambers (ELETROLab®, EL 212 model, São Paulo, SP, Brazil) to be used in bioassays 3, 4, 5, and 6 as described in the following.

Bioassays

Six bioassays were conducted on *E. heros* and *D. melacanthus* eggs as follows: parasitism on eggs of *E. heros* (bioassay 1) and

D. melacanthus (bioassay 2) by T. podisi females of different age (1, 5, and 10 days old); parasitism of E. heros (bioassay 3) and D. melacanthus (bioassay 4) eggs of different age (1, 2, 3, 4, and 5 days of embryonic development) by T. podisi (\leq 48 h old); preference of T. podisi females (\leq 48 h old) for different embryonic developmental stages (1-, 2-, and 3-day-old host eggs) of E. heros (bioassay 5) and D. melacanthus eggs (bioassay 6). All trials were performed under controlled conditions (temperature of 25 ± 2°C, relative humidity of 80 ± 10%, and photoperiod of 14/ 10 h L/D) within BOD climatic chambers (ELETROLab[®], EL 212 model, São Paulo, SP, Brazil).

For parasitism on eggs of *E. heros* (bioassay 1) and *D. melacanthus* (bioassay 2) by *T. podisi* females of different age, 1-, 5-, and 10-day-old females were used. For parasitism of *E. heros* (bioassay 3) and *D. melacanthus* (bioassay 4) eggs of different age (1, 2, 3, 4, and 5 days of embryonic development) by *T. podisi* (\leq 48 h old); preference of *T. podisi* females (\leq 48 h old) for different embryonic developmental stages (1-, 2-, and 3-day-old host eggs) of *E. heros* (bioassay 5) and *D. melacanthus* eggs (bioassay 6), up to 48-h-old females were used. In all bioassays, females were previously mated (kept in the presence of males until they were individualized) with no parasitism experience.

Parasitism of **E. heros** and **D. melacanthus** eggs by **T. podisi** females of different age

A separate bioassay was carried out for each host species in a completely randomized design with three treatments (1-, 5-, and 10-day-old female parasitoids from the same laboratory generation) and seven replicates. Each replicate consisted of the mean result of three *T. podisi* females individualized in plastic microtubes (12-mm diameter × 75-mm height) (seven replicates × three individualized females per replicate = 21 individualized females evaluated per treatment) following the methodology proposed by Queiroz *et al* (2017) for other parasitoid species of the genus *Telenomus* with droplets (100 µl) of pure honey placed on the walls of plastic microtubes for adult feeding.

Eggs of *E. heros* (bioassay 1) and *D. melacanthus* (bioassay 2) ($25 \pm 10 \text{ eggs}$) were glued on white cardboards (1 cm × 6 cm) and used according to the respective treatments. Cardboards with eggs were inserted into the microtubes containing individualized 1-, 5-, or 10-day-old *T. podisi* females (previously mated) and exposed to parasitism for a period of 24 h. Next, parasitoid females were removed from the microtubes, which were sealed with PVC plastic film and kept in a BOD climatic chamber until evaluation under the same conditions in which parasitism occurred.

Parasitism of *E. heros* and *D. melacanthus* eggs of different age by *T. podisi*

Similar to bioassays 1 and 2, a separate bioassay was carried out for each host species in a completely randomized design with five treatments (1-, 2-, 3-, 4-, and 5-day-old host eggs) and four replicates consisting of the mean results of five individualized parasitoid females each. Eggs of both *E. heros* (bioassay 3) and *D. melacanthus* (bioassay 4) were collected from the insect colony and maintained in BOD climatic chambers for 5, 4, 3, and 2 days until assembly of the experiment. One-day-old eggs came directly from insect rearing before they were used in the experiments.

Females of *T. podisi* (\leq 48 h old) were individualized into plastic microtubes containing droplets of pure honey for feeding. Subsequently, a mean of 25 ± 10 eggs of *E. heros* (bioassay 3) and *D. melacanthus* (bioassay 4) of different age (1, 2, 3, 4, and 5 days old) were glued on white cardboards (1 cm × 6 cm) and assigned to the respective treatments. Cardboards with eggs were inserted into the microtubes containing a *T. podisi* female, and parasitism was allowed for 24 h. After this period, the females were removed and the tubes containing the eggs were sealed with PVC plastic film and kept under the same conditions until evaluation.

Preference of **T. podisi** females for different embryonic developmental stages of **E. heros** and **D. melacanthus** eggs

A separate bioassay was carried out for each host species in a completely randomized design with three treatments (1-, 2-, and 3-day-old *E. heros* or *D. melacanthus* eggs) and 15 replicates (one arena per replicate). A free choice test (in which parasitoids had to choose between host eggs of 1, 2, and 3 days) was performed in arenas, adapted from Thuler *et al* (2007) and Queiroz *et al* (2018), composed of polyethylene bottles (2-cm diameter × 4-cm height) containing six plastic microtubes arranged at equal distances in the lower part of the bottle, and one microtube placed at the top of the arena (Fig 1).

Twenty-five eggs of *E. heros* or *D. melacanthus* (1, 2, and 3 days old) were glued on white cardboards (1 cm × 6 cm). Then, two cardboards of each host egg age were introduced into microtubes, with cards of the same age placed on opposite sides (Fig 1). Six newly emerged *T. podisi* females (\leq 48 h old, mated, with no previous parasitism experience) were released into the top of each arena and parasitism was allowed for 24 h, according to the methodology developed by Thuler *et al* (2007) for evaluation of host preference by *Trichogramma* parasitoids and later adapted by Queiroz *et al* (2018) for evaluation of host preference by parasitoids of the genus *Telenomus*. After a 24-h period, cardboards were removed and kept in BOD climatic chambers until emergence of adults.



Fig 1 Arena adapted from Thuler *et al* (2007) used in the parasitoid host preference test (Queiroz *et al* 2018).

Data analysis

The number of parasitized eggs was calculated as the number of emerged parasitoids plus the number of adult parasitoids completely developed but dead inside the host (observed by means of dissections). Parasitism (%) was calculated as the number of parasitized eggs / (number of parasitized eggs + number of non-parasitized eggs) × 100. Parasitoid emergence (%) was the number of eggs with parasitoid emergence / number of parasitized eggs × 100. Sex ratio (female proportion) of the progeny was the number of females/(number of females + number of males). The results were submitted to exploratory analysis to evaluate the normality assumption of residues (Shapiro & Wilk 1965), homogeneity of variance of treatments, and additivity of the model to allow the application of analysis of variance (ANOVA) (Burr & Foster 1972). The means were then compared by Tukey's test using the statistical analysis program SAS (SAS Institute 2009).

In the bioassays studying the preference of *T. podisi* females for different embryonic developmental stages of *E. heros* and *D. melacanthus* eggs, the number of parasitized eggs in each different age eggs was evaluated and compared to total parasitism observed in the arena (parasitism distribution %). The number of parasitized eggs was analyzed using chi-square statistics (Sas Institute 2009).

Results

The age of *T. podisi* females affected parasitism (%) of both *E. heros* (F = 9.42, df = 18, p = 0.0016) and *D. melacanthus* (F = 11.62, df = 18, p = 0.0006) eggs. In general, the number of parasitized eggs increased with parasitoid age from 17.4 to 21.5 *E. heros* eggs (F = 11.02, df = 18, p = 0.0008) and from 18.0 to 25.0 *D. melacanthus* eggs (F = 13.41, df = 18, p = 0.0003). The highest parasitism (%) was recorded for 10-day-old female parasitoids with average parasitism (%) of

75.7% and 85.1% for *E. heros* and *D. melacanthus*, respectively. In contrast, parasitoid emergence (%) of the progeny did not differ between eggs parasitized by 1-, 5-, or 10-day-old *T. podisi* for both *E. heros* (F = 1.14, df = 18, p = 0.3406) and *D. melacanthus* (F = 0.97, df = 18, p = 0.3994). The sex ratio was inferior only for parasitoids emerging from *D. melacanthus* eggs when parasitized by 1-day-old *T. podisi* (0.41) (F = 32.01, df = 18, p < 0.0001). All other sex ratios were higher than 0.75 (Table 1).

Similar to parasitoid age (Table 1), host egg age (Table 2) also affected *T. podisi* parasitism (%) on both *E. heros* (*F* = 19.4, df = 15, p < 0.0001) and *D. melacanthus* (*F* = 74.67, df = 15, p < 0.0001) eggs. In general, *T. podisi* was able to parasitize eggs of all studied ages (up to 5 days old). However, *E. heros* eggs of 1, 2, 3, and 4 days old (*F* = 19.4, df = 15, p < 0.0001) and *D. melacanthus* eggs of 1, 2, and 3 days old (*F* = 74.67, df = 14.67, df = 15, p < 0.0001) were highly parasitized when compared to older eggs (Table 2).

Progeny emergence (%) was affected by host age with both *E. heros* (F = 8.05, df = 14, p = 0.0014,) and *D. melacanthus* eggs (F = 9.07, df = 15, p = 0.0006). Emergence (%) was lower when *T. podisi* parasitized 1-dayold eggs of either host. However, emergence was still higher than 75%, with an average of 81.9 and 75.5% on *E. heros* and *D. melacanthus* eggs, respectively. Progeny sex ratio was not impacted by egg age (F = 1.49, df = 14, p = 0.2571 for *E. heros* eggs and F = 0.65, df = 15, p = 0.6350 for *D. melacanthus* eggs) (Table 2).

Concerning *T. podisi* host preference among *E. heros* eggs of different ages, parasitism was higher on 2-day-old eggs (48.6% of the total parasitized *E. heros* eggs) compared to parasitism on 1-day-old eggs (19.2% of the total parasitized *E. heros* eggs) (χ^2 = 7.12, df = 29, *p* = 0.0114). *Telenomus podisi* parasitism was intermediate on 3-day-old eggs (32.2% of the total parasitized *E. heros* eggs). Thus, no age preference was found between 2-day-old and 3-day-old eggs (χ^2 = 1.68, df =

29, p = 0.2436). Neither between 1-day-old and 3-day-old eggs ($\chi^2 = 1.99$, df = 29, p = 0.1585) (Fig 2A).

No *T. podisi* preference was observed between *D. melacanthus* eggs of 1-day-old (34.6% of the total *D. melacanthus* parasitized eggs), 2-day-old (29.4% of the total *D. melacanthus* parasitized eggs), and 3-day-old (35.6% of the total *D. melacanthus* parasitized eggs) eggs ($\chi^2 = 0.83$, df = 29, p = 0.6598) (Fig 2B).

Discussion

Different biotic and abiotic variables may affect fitness of a mass-reared parasitoid and consequently its quality and efficiency in ABC programs (McDougall & Mills 1997; Thomson & Hoffmann 2002; van Lenteren & Tommasini 2002; van Lenteren & Tommasini 2003). Analyses of parasitism of T. podisi females of different age as well as parasitism of eggs (E. heros and D. melacanthus) at different embryonic developmental stages significantly contribute to the understanding of parasitoid-host interactions. Knowing this information enables to adjust parasitoid release recommendations in order to improve its field performance. For example, time between field releases depends upon the interval the biological control agent can be active in the field as well as the lifespan of adults and parasitism capacity over eggs of different ages. Similarly, T. podisi parasitism on eggs at different embryonic developmental stages will be directly related to its capacity to control the pest as well as its release strategy in the field. Moreover, this information is helpful in the parasitoid rearing procedures. Parasitism of T. podisi of different ages indicates how long adults can be stored (Vinson & Iwantsch 1980; van Lenteren & Tommasini 2002; Zhou et al 2014). Therefore, this understanding is essential for efficient T. podisi mass rearing and its field release in ABC programs against stink bugs.

Table 1	Parasitism of Euschistus heros (bioassa	y 1) and Dichelops melacanthus	(bioassay 2) eggs by	<i>Telenomus podisi</i> females o	of different age (1, 5
and 10 da	ays old).				

Biological characteristic		Female parasitoid age (days)			Statistic			
		1	5	10	CV	F	df _{error}	p
E. heros	Number of parasitized eggs	17.4 ± 1.0 b	16.8 ± 0.8 b	21.5 ± 0.4 a	10.9	11.02	18	0.0008
	Parasitism (%)	67.3 ± 2.9 ab	60.3 ± 2.8 b	75.7 ± 1.7 a	9.8	9.42	18	0.0016
	Emergence (%)	85.0 ± 2.6 a	83.0 ± 2.5 a	88.1 ± 2.0 a	7.4	1.14	18	0.3406
	Sex ratio (female proportion)	0.83 ± 0.05 a	0.89 ± 0.04 a	0.89 ± 0.02 a	12.6	0.66	16	0.5291
D. melacanthus	Number of parasitized eggs	18.0 ± 0.7 b	22.2 ± 0.7 a	25.0 ± 1.3 a	11.7	13.41	18	0.0003
	Parasitism (%)	67.0 ± 3.8 b	80.3 ± 2.3 a	85.1 ± 2.0 a	9.4	11.62	18	0.0006
	Emergence (%)	67.6 ± 3.7 a	65.6 ± 2.5 a	61.1 ± 4.0 a	14.0	0.97	18	0.3994
	Sex ratio (female proportion)	0.41 ± 0.03 b	0.76 ± 0.05 a	0.85 ± 0.03 a	16.09	32.01	18	< 0.0001

Mean \pm SEM, followed by the same letter in each line did not differ statistically (Tukey's test, p > 0.05).

Biological characteristic	Host egg age (days)				Statistic			
		1	2	3	4	5	-	
Euschistus heros	Number of parasitized eggs	21.4 ± 0.6 a	22.2 ± 0.7 a	22.5 ± 1.1 a	19.2 ± 1.7 a	11.9 ± 0.6 b	CV = 10.6% F = 18.06 $df_{error} = 15$ p < 0.0001	
	Parasitism (%)	85.5 ± 2.2 a	88.2 ± 2.6 a	87.4 ± 4.4 a	76.0 ± 6.2 a	47.8 ± 2.6 b	CV = 10.2% F = 19.4 $df_{error} = 15$ p < 0.0001	
	Emergence (%)	81.9 ± 2.1 b	93.8 ± 1.0 a	90.7 ± 1.2 a	95.1 ± 1.7 a	93.0 ± 2.4 a	CV = 3.7% F = 8.05 $df_{error} = 14$ p = 0.0014	
	Sex ratio (female proportion)	0.91 ± 0.02 a	0.90 ± 0.01 a	0.92 ± 0.01 a	0.91 ± 0.01 a	0.86 ± 0.03 a	CV = 3.9% F = 1.49 $df_{error} = 14$ p = 0.2572	
Dichelops melacanthus	Number of parasitized eggs	23.2 ± 1.2 a	20.5 ± 0.3 a	19.9 ± 0.9 ab	15.9 ± 1.1 b	4.2 ± 0.9 c	CV = 11.2% F = 63.83 $df_{error} = 15$ p < 0.0001	
	Parasitism (%)	90.6 ± 3.8 a	79.4 ± 1.2 a	78.4 ± 2.9 a	63.7 ± 4.2 b	16.9 ± 3.7 c	CV = 10.2% F = 74.67 $df_{error} = 15$ p < 0.0001	
	Emergence (%)	75.5 ± 2.4 b	87.6 ± 2.3 a	92.3 ± 2.1 a	93.5 ± 2.6 a	91.7 ± 3.0 a	CV = 5.6% F = 9.07 $df_{error} = 15$ p = 0.0006	
	Sex ratio (female proportion)	0.80 ± 0.08 a	0.81 ± 0.03 a	0.81 ± 0.08 a	0.84 ± 0.06 a	0.84 ± 0.06 a	CV = 16.9% F = 0.65 $df_{error} = 15$ p = 0.6350	

Table 2 Parasitism of *Euschistus heros* (bioassay 3) and *Dichelops melacanthus* (bioassay 4) eggs of different age (1, 2, 3, 4, and 5 days of embryonic development) by *Telenomus podisi* (\leq 48 h old).

Mean \pm SEM, followed by the same letter in each line did not differ statistically (Tukey's test, p > 0.05).

Overall, our results indicate that both parasitoid age and host (egg) age affect parasitism (Vinson & Iwantsch 1980; Papaj 2000; Queiroz et al 2019). Older parasitoids (10 days old) showed higher parasitism than younger ones (1 day old), independent of the offered host. Older parasitoids are usually less selective, lowering the quality threshold below which hosts are rejected (Cingolani et al 2014). Alternatively, 1-day old parasitoids might not have mature oocytes so that the peak of oviposition occurs after a specific time (\geq 5 days) (Abram 2016). Differently from parasitoid, host aging reduced parasitism. A lower nutritional value of older eggs can explain the lower parasitism of those eggs compared with younger ones. Optimal foraging theory predicts that all organisms should specialize on nutritionally more rewarding items (Stephens & Krebs 1986). Since the eggs used in our experiments (to study host age effects) were neither frozen nor made unviable by other means, nutrients of older eggs had been consumed by the developing stink bug nymphs (Vinson 1997) lowering host quality to the parasitoid. Moreover, host eggs at different embryonic developmental stages may differ in surface, size, structure of the chorion, and other egg characteristics such as changes in color during embryonic development, size and volume (Cônsoli *et al* 1999, Queiroz *et al* 2018). All of these differences affect not only parasitoid handling time and exploitation, but also host suitability to parasitism and parasitoid development (Cônsoli *et al* 1999). In addition, changes in the external characteristics of stink bug eggs may be related to a hardening of the chorion as embryonic development proceeds, limiting the penetration capacity of the *T. podisi* ovipositor (Pak *et al* 1986; Borges Filho *et al* 2017).

Although lower parasitism rates were observed for older eggs (5 days old for *E. heros* and 4 and 5 days old for *D. melacanthus*), parasitism (%) higher 63% still



Fig 2 Preference of *Telenomus* podisi female (\leq 48 h old) for different embryonic developmental stages (host eggs of 1, 2, or 3 days) of (A) *Euschistus heros* (bioassay 5) and (B) *Dichelops melacanthus* eggs (bioassay 6). Mean ± SEM, followed by the same letter for each host species did not differ statistically (chi-square test, p >0.05).

occurred with eggs of 4 days and even eggs close to stink bugs emergence (5 days) can be parasitized at levels of 16.9 to 47.8% what is a positive feature to the parasitoid use in ABC. The observed slight preference of T. podisi for 2-day-old E. heros eggs and the complete lack of parasitism preference regarding *D. melacanthus* eggs confirms the ability of T. podisi to efficiently use 1- to 3-day-old eggs as hosts. Studies indicate that insect parasitoids are in many ways adapted physiologically and biochemically for survival and development in a living host, thus allowing larval stages of parasitoids to exhibit high nutritional efficiency even in considerably poorer hosts (Fisher 1981; Wiegart & Peterson 1983; Slansky 1986). This could also be the case with T. podisi, making it a generalist parasitoid on eggs of a variety of species of agricultural stink bugs of agricultural interest (Corrêa-Ferreira & Moscardi 1995), able to parasitize even 5day-old stink bug eggs.

Considering the importance of synchronizing the release of *T. podisi* females with stink bug eggs in the field as one of the most important challenges for a successful ABC program (van Lenteren et al 2018), T. podisi has high potential to succeed in the field. The ability to parasitize up to 5-day-old host eggs and to keep their oocytes viable even after host deprivation, thereby maintaining parasitism capacity and the period of fertility, is clearly an advantage when using T. podisi in ABC for stink bug management. In field conditions, it is possible that parasitoid females have difficulties finding host eggs regardless of age (Biever 1972). The biological characteristics of the parasitoid that allow it to parasitize up to 5-dayold eggs as well as maintaining the ability to parasitize host eggs even after the absence of hosts for up to 10 days is not only an advantage for ABC in the field, but also for mass rearing in the laboratory. To meet the needs for insect rearing or for ABC in the field, our results suggest that T. podisi adults can be stored for up to 10 days at 25°C for later use without any impairment of their subsequent parasitism performance. Moreover, stink bug eggs do not need to be collected every day in insect colonies since the parasitism rate of T. podisi is the same on 1- to 3-day-old eggs.

It was expected that host quality and parasitoid age would affect the sex ratio of progeny (Schwartz & Gerling 1974; Charnov et al 1981; Houseweart et al 1983). Usually, sperm stock in a female's spermatheca decreases over time as a result of sperm depletion, sperm death, sperm digestion by the female, or sperm disintegration while stored in the spermatheca (Cunningham et al 1971). As a consequence, the number of males in the progeny could be expected to increase with parasitoid aging since unfertilized females produce only male individuals due to their reproductive mode of arrhenotokous parthenogenesis (Pratissoli et al 2014). Moreover, mated females can control their progeny's sex ratio by choosing when to release sperm during oviposition (Cingolani et al 2014). Usually, the production of males is associated with low-quality hosts, given that females suffer more when they develop in low-quality hosts (King 1987). Assuming a lower nutritional quality of older eggs, a higher number of males in the progeny originating from older host eggs may be expected. Surprisingly, sex ratio was not greatly impacted neither by parasitoid age nor by host age with the exception of 1-day-old T. podisi parasitizing D. melacanthus eggs. Thus, even in the absence of stink bug eggs, T. podisi sperm remained viable for up to 10 days, and sex ratio always remained higher than 0.60, which is considered adequate for ABC programs (van Lenteren & Bueno 2003). Similarly, parasitoid emergence was not impacted by parasitoid age and only slightly influenced by host age. Parasitoid emergence was always higher than 75%, which can be considered adequate for ABC programs (Bueno et al 2012; van Lenteren et al 2018).

Thus, we conclude that the reported biological characteristics of T. podisi presented in this work can improve parasitoid mass-rearing procedures as well as its release in the field. In summary, in mass-rearing facilities for T. podisi, the use of older adults (5 to 10 days old) as mother wasps increases parasitism of the offered eggs. Additionally, stink bugs eggs could be collected in intervals of 3 days since similar parasitism was recorded in up to 3-day-old eggs. This contributes to reducing parasitoid production costs since host egg collection is currently done on a daily basis in most insect rearing facilities, which is more labor intensive. The observed biological characteristics are also important for the parasitoid's success in field releases. Since host deprivation for up to 10 days did not reduce parasitism by older wasps, parasitoid field release can be performed at an early stage of pest infestation (low level of pest numbers in the field). Also, when hosts are completely absent in the field, or climatic conditions are unfavorable for the release, mass-reared adults can be kept in the laboratory (25°C) for up to 10 days for later release in the field with a little negative effect their subsequent parasitism performance. This knowledge is crucial to maintain the quality of T. podisi in the laboratory as well as in field releases for biological control of stink bugs.

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