

Egg parasitoid wasps as natural enemies of the neotropical stink bug *Dichelops melacanthus*

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Abstract – The objective of this work was to determine the potential of five species of Scelionidae wasps – *Telenomus podisi*, *Trissolcus basalis*, *Trissolcus urichi*, *Trissolcus teretis* and *Trissolcus brochymenae* – as natural enemies of the neotropical stink bug *Dichelops melacanthus*, and to determine if the presence of eggs of other stink bug species influences the parasitism and development of the parasitoids. Two kinds of experiments were done in laboratory: without choice of hosts (eggs of *D. melacanthus*) and with choice (eggs of *D. melacanthus* and of *Euschistus heros*). Biological parameters, including proportion of parasitism, immature survivorship, progeny sex ratio, immature stage development period, and host preference were recorded. All the evaluated parasitoids can parasitize and develop on *D. melacanthus* eggs. The first choice of eggs did not influence the proportion of *D. melacanthus* eggs parasitized by *Tr. basalis*, *Tr. teretis* or *Tr. brochymenae*. However, *D. melacanthus* eggs as the first choice of *Te. podisi* and *Tr. urichi* increased, respectively, 9 and 14 times the chance for parasitism on eggs of this species. Behavioral and ecological aspects of parasitoids should be considered prior to their use in biological control programs.

Index terms: *Telenomus*, *Trissolcus*, biological control, corn pests, host preference, host suitability.

Vespas parasitoides de ovos como inimigos naturais do percevejo neotropical *Dichelops melacanthus*

Resumo – O objetivo deste trabalho foi determinar o potencial de cinco espécies de vespas Scelionidae – *Telenomus podisi*, *Trissolcus basalis*, *Trissolcus urichi*, *Trissolcus teretis* e *Trissolcus brochymenae* –, como inimigos naturais do percevejo neotropical *Dichelops melacanthus*, e determinar se a presença de ovos de outras espécies de percevejo tem influência sobre o parasitismo e o desenvolvimento dos parasitoides. Foram realizados dois tipos de experimentos em laboratório: sem escolha de hospedeiros (ovos de *D. melacanthus*) e com escolha (ovos de *D. melacanthus* e de *Euschistus heros*). Foram registrados parâmetros biológicos que incluíram: proporção de parasitismo, sobrevivência de imaturos, razão sexual da progênie, tempo de desenvolvimento dos estágios imaturos e preferência de hospedeiro. Todas as espécies de parasitoides avaliadas podem parasitar e desenvolver-se em ovos de *D. melacanthus*. A escolha inicial de ovos não influenciou a proporção de ovos de *D. melacanthus* parasitados por *Tr. basalis*, *Tr. teretis* ou *Tr. brochymenae*. Entretanto, para *Te. podisi* e *Tr. urichi*, a escolha inicial por ovos de *D. melacanthus* aumentou em 9 e 14 vezes, respectivamente, a chance de parasitismo nesta espécie. Aspectos comportamentais e ecológicos dos inimigos naturais devem ser considerados antes de sua utilização em programas de controle biológico.

Termos para indexação: *Telenomus*, *Trissolcus*, controle biológico, pragas do milho, preferência de hospedeiro, disponibilidade do hospedeiro.

Introduction

Stink bugs are key pests for many crops due to the damage caused by their feeding habits and to the worldwide distribution of many species (Panizzi & Slansky, 1985; Panizzi, 1997).

In South and Southwestern Brazil, the neotropical stink bug *Dichelops melacanthus* (Dallas) (Hemiptera:

Pentatomidae) is one of the major pests for corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.), and a secondary pest for soybean [*Glycine max* (L.) Merrill] (Ávila & Panizzi, 1995; Chocorosqui & Panizzi, 2004; Manfredi-Coimbra et al., 2005). The impact of *D. melacanthus* in cultivated areas has increased in recent years mainly because the extensive adoption

of crop rotation in no-tillage cultivation systems, favors stink bugs by offering a continuous food supply and by enhancing their ability to survive in adverse periods of the year (winter or dry seasons) under crop debris (Chocorosqui & Panizzi, 2004; Carvalho, 2007).

The technology available for *D. melacanthus* management is based on chemical control with insecticides (Ávila & Panizzi, 1995). Therefore, the development of alternative methods for population management, such as biological control, may help to reduce the pest impact in a more sustainable context.

Scelionidae egg parasitoid wasps are important natural enemies of stink bugs, and some species of this wasp family are being used in many countries for their biological control, especially the cosmopolitan *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Clarke & Walter, 1995; Corrêa-Ferreira & Moscardi, 1996; Corrêa-Ferreira, 2002; Ehler, 2002). These parasitoid wasps have biological attributes and behavioral responses to host density that could lead to density-dependent parasitism and efficient local regulation of stink bug populations (Laumann et al., 2008). In Brazil, Corrêa-Ferreira & Moscardi (1996) showed that inoculative liberation of *Tr. basalis* can control stink bug populations, and this technology is being used in microbasin production systems in Southern Brazil (Corrêa-Ferreira, 2002).

There is little understanding of natural parasitism by egg parasitoids in *D. melacanthus*. In Paraná state, Brazil, eggs of this species can be parasitized by *Te. podisi*, *Tr. basalis*, *Trissolcus brochymenae* (Ashmead), and *Trissolcus urichi* (Crawford) with parasitism indexes from 30 to 60% (Corrêa-Ferreira & Moscardi, 1995). In the Western region of Brazil, *Te. podisi* has been registered attacking eggs of *D. melacanthus* (Carvalho, 2007). Other non-scelionid parasitoid insects registered on egg masses of *D. melacanthus* are: *Ooencyrtus* sp. (Hymenoptera: Encyrtidae), *Neorileya ashmeadi* (Crawford) and *Neorileya* sp. (Hymenoptera: Eurytomidae) (Corrêa-Ferreira & Moscardi, 1995). Despite this information, there are no data on the biology and biological potential of these parasitoids in eggs of *D. melacanthus*.

The objective of this work was to study the biological aspects of five species of Scelionidae wasps parasitization on eggs of *D. melacanthus*, and to determine if the presence of eggs of other species of stink bug influence the parasitism and the development of the parasitoids.

Materials and Methods

Parasitoid wasp colonies were started with insects collected in soybean crops and natural vegetation in the Distrito Federal, Brazil (15°47'S; 47°55'W). The insects were reared in laboratory under continuous rearing for approximately 25 generations. The scelionid species selected for this study (*Telenomus podisi*, *Trissolcus basalis*, *Tr. brochymenae*, *Tr. urichi*) were previously reported attacking *D. melacanthus* and others species of stink bugs eggs such as *Euschistus heros*, *Piezodorus guildinii* and *Nezara viridula* in the field (Corrêa-Ferreira & Moscardi, 1995; Medeiros et al., 1998). Although *Trissolcus teretis* (Johnson) was not reported using *D. melacanthus* as a host it was also selected because it is usually found in stink bug eggs in Central Brazil (Medeiros et al., 1998).

The parasitoids were maintained using eggs of the brown stink bug *Euschistus heros* (Fabricius) as hosts, in a controlled environmental chamber in plastic cages, 25-cm² flask tissue culture, angled neck, (ICN Biomedicals, Irvine, USA). Host eggs were exposed to parasitoids for 24 hours and then removed and placed in glass tubes, 7.5 cm long x 1.3 cm diameter, for incubation. Droplets of honey were offered to adult wasps as food.

Euschistus heros and *D. melacanthus* were reared on sunflower, *Helianthus annuus* (L.), soybean, raw peanut, *Arachis hypogaea* L., and seeds and pods of green bean, *Phaseolus vulgaris* L. *Dichelops melacanthus* were also fed on Chinese privet, *Ligustrum lucidum* Aiton. Separate 8-L plastic containers were used for nymphs and adults. The eggs were collected daily and separated in Petri dishes until nymph emergence. Stink bugs and parasitoids were both reared under 14-hours photophase at 26.0±1°C and 65±10% relative humidity. The *E. heros* colony

was started from insects collected in soybean and in natural vegetation in Distrito Federal, Brazil, and the *D. melacanthus* colony was started from insects from the entomology laboratory of Embrapa Soja, previously collected in Londrina, Paraná state, Brazil.

To test the capacity of each parasitoid species to successfully parasitize and develop using *D. melacanthus* as a host, egg masses from the laboratory colony with 20 eggs were offered to females of each parasitoid species ($n=20$ for each parasitoid species, using one egg mass per parasitoid female). The insects used in the experiment were naive females with 24 to 48 hours at the adult stage, which were maintained for 24 hours with males for copulation before the experiments were initiated. Egg masses and parasitoid females were placed in plastic Petri dishes (4.5 cm diameter) in a rearing chamber, under the same conditions described above; a droplet of honey was added as food for the wasps. After 24 hours, the parasitoids were removed from the arena and the egg masses were maintained in the rearing chamber for evaluations of parasitism and development of parasitoid immature stages. Daily observations under stereoscopic microscopy Zeiss SV6, (Carl Zeiss Microimaging GmbH, Munich, Germany) were performed to evaluate the number of parasitized eggs, number of parasitoids emerged from egg masses, sex ratio of the progeny, and immature stage development period. These data were used to calculate: parasitism (number of parasitized eggs per number of eggs offered), estimated by color change (Medeiros et al., 1998); immature survivorship, which is the proportion of eggs that successfully complete the immature development (number of adults per number of egg parasitized); sex ratio (number of females/total number of progeny); and immature stage development period (days from the parasitism of the eggs to adult emergence). When egg color did not offer a clear indication and the parasitoid did not complete development, the eggs were dissected under stereoscopic microscopy in order to confirm parasitism.

To evaluate how the parasitoids recognize and select *D. melacanthus* eggs in the presence of another host, an *E. heros* egg mass was offered

along with the *D. melacanthus* egg mass. Both egg masses contained the same number of eggs: 20 eggs per mass. This experiment was carried out following the same procedures as described before. To determine if the parasitoid species showed any host first choice preference, the movements of female wasps in the arenas was observed until they chose one of the egg masses offered, made physical contact with it and drummed it with their antennae for at least 60 seconds. The alternative host *E. heros* was selected because it is one of the most common stink bugs species of cultivated areas in Brazil.

Parasitism preference was evaluated by maintaining the parasitoid females for a period of 12 hours in the arenas. After this time, they were removed, the egg masses were separated by host species in Petri dishes, and the egg masses were observed daily to compute the number of eggs of each parasitized host and the number of parasitoids emerging from the egg masses of each host. With these data, calculations were performed for each egg mass and host and parasitoid species, and the parasitism and immature survivorship was determined as described above.

The mean proportion of parasitism, immature survivorship and sex ratio (proportion of females) of the different parasitoid species were analysed using generalized linear models (GLM) (Lee et al., 2006) with binomial distribution and log link function. When overdispersion of the data was observed, a quasi-likelihood method was used to estimate the heterogeneity factor. Multiple comparisons through orthogonal contrasts were made to determine which means were significantly different, and Bonferroni error corrections were applied. Immature stage development period (days) was analysed by Kruskal Wallis ANOVA and Dunn's multiple comparison test to evaluate significance between mean values of the parasitoid species.

Parasitoid first choice in the experiments with two hosts were analyzed using generalized linear models (GLM) with binomial distribution and logit link function, and an estimate of *D. melacanthus* egg mass choosing probability was calculated. The hypothesis of nonpreference (50% of choice for egg masses of each stink bug species) was

tested by the χ^2 Wald test. The mean proportion of parasitism and immature survivorship, in each combination of host/parasitoid in the experiment with possibility of host choice were compared using GLM analyses, as described above. Additionally, to study if the parasitoid first choice influenced the number of parasitized eggs of each host, GLM analyses were done using first choice as covariate, and number of parasitized eggs as response variable. A binomial distribution was adjusted to the response variable, and a quasi-likelihood method to the heterogeneity factor estimation was applied to correct for data overdispersion. From this model, the odds ratios and their confidence interval (95%) were calculated to determine the chance, after the parasitoid first choice, to parasitize *D. melacanthus* eggs instead of *E. heros* eggs, that is: the odds ratio was calculated to estimate the possible influence of the first selection of a host on the number of parasitized eggs of *D. melacanthus*. All tests were performed at 5% significance level.

Results and Discussion

All the studied parasitoid species successfully parasitized and developed on eggs of *D. melacanthus*. The mean proportion of parasitized eggs only differed between *Tr. urichi* and *Te. podisi*, and the first species showed a significantly higher proportion of parasitism (Table 1). Immature survivorship was different between *Tr. brochymenae* and *Tr. basalis*, with the last one showing lower survivorship. The sex ratio of the progeny was mostly female biased, except for *Tr. urichi*. The immature development period varied from 11 to 13 days and was significantly different among the species, except between *Tr. basalis* and *Tr. urichi*.

The parasitoids showed a differential ability to identify and use *D. melacanthus* eggs as a suitable host, and the proportion of parasitism reported here, for some of the species, showed differences with the parasitism previously reported in other hosts (Pacheco & Corrêa-Ferreira, 1998; Sujii et al., 2002; Laumann et al., 2008). Nevertheless, the present work corroborates previous observations for different species of Scelionidae (Kivan & Kilic, 2002, 2004), indicating that, despite being oligophagous or polyphagous (Austin et al., 2005), scelionid wasps show variability in their ability to recognize and select different species of stink bugs as hosts. Interestingly, *D. melacanthus* was shown to be a suitable host for *Tr. teretis*, a species not recorded previously as parasitizing eggs of this stink bug in the field.

When eggs of *E. heros* and *D. melacanthus* were offered at the same time, *Te. podisi*, *Tr. teretis* and *Tr. brochymenae* showed a significant first choice preference for eggs of *E. heros* ($\chi^2 = 5.00$, $p = 0.03$; $\chi^2 = 5.56$, $p = 0.02$; and $\chi^2 = 8.89$, $p = 0.003$, respectively). However, *Te. basalis* and *Tr. urichi* showed no preference for eggs of either stink bug species (Figure 1).

Trissolcus brochymenae parasitized a higher proportion of *E. heros* eggs (Figure 2 A). This species was the only one to show a difference in the proportion of parasitized eggs according to the host. However, immature survivorship was higher in *E. heros* eggs for *Te. podisi*, *Tr. urichi*, *Tr. teretis* and *Tr. brochymenae* (Figure 2 B). The preference observed for *E. heros* eggs by these species could be related to the higher survival of the immature stage of these parasitoids in *E. heros* eggs. *Trissolcus basalis* and *Tr. urichi* showed no host preference, and this could be related to the preference of these species for other stink bugs, such as *Nezara viridula* and *Chinavia* spp., respectively (Sujii et al., 2002). The continuous

Table 1. Biological parameters of five egg parasitoids species on *Dichelops melacanthus* eggs⁽¹⁾.

Species	Proportion of parasitism	Immature survivorship (proportion)	Sex ratio (proportion of females)	Development period (days)
<i>Telenomus podisi</i>	0.41±0.38a	0.64±0.39ab	0.78±0.17a	13.24±0.85a
<i>Trissolcus basalis</i>	0.71±0.38ab	0.64±0.23a	0.53±0.23ab	11.25±0.97b
<i>Trissolcus urichi</i>	0.89±0.17b	0.60±0.20ab	0.49±0.18b	11.74±0.74b
<i>Trissolcus teretis</i>	0.62±0.46ab	0.69±0.24ab	0.77±0.11a	12.38±0.49c
<i>Trissolcus brochymenae</i>	0.74±0.30ab	0.80±0.21b	0.69±0.18a	12.03±0.18d

⁽¹⁾Means±standard deviation followed by equal letters do not differ by Dunn's multiple comparison test, at 5% probability.

rearing of the parasitoids in *E. heros* eggs does not appear to modify the host preference. Scelionidae wasps show an innate host preference (Sujii et al., 2002), and if the rearing in a host had any influence, the preferential first choice for *E. heros* eggs would be observed in all the species. These results agree with those found by Pacheco & Corrêa-Ferreira (1998), Sujii et al. (2002) and Kivan & Kilic (2002, 2004) for other stink bug and scelionid wasp combinations.

The selective response toward a specific host (*E. heros*) for three of the five species evaluated could suggest that these parasitoids use cues from host egg masses to identify those that would

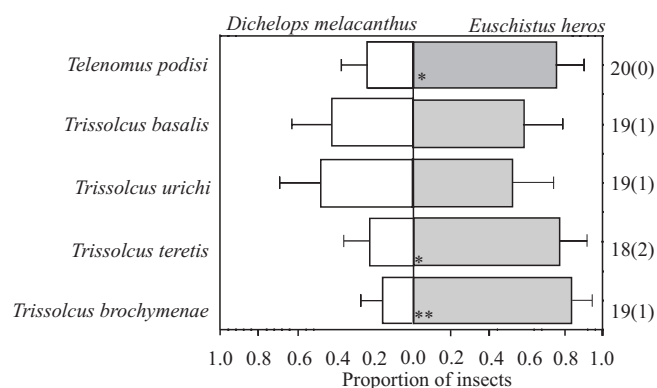


Figure 1. First choice of the parasitoid species evaluated in experiments with two hosts (*Euschistus heros* and *Dichelops melacanthus*). Bars indicate the 95% confidence interval. Numbers on the right side on the figures are the total number of replicate insects tested. Numbers in brackets represent the number of insects that did not respond to the treatment tested. * and ** Significant by the χ^2 Wald test, respectively at 5 and 1% probability.

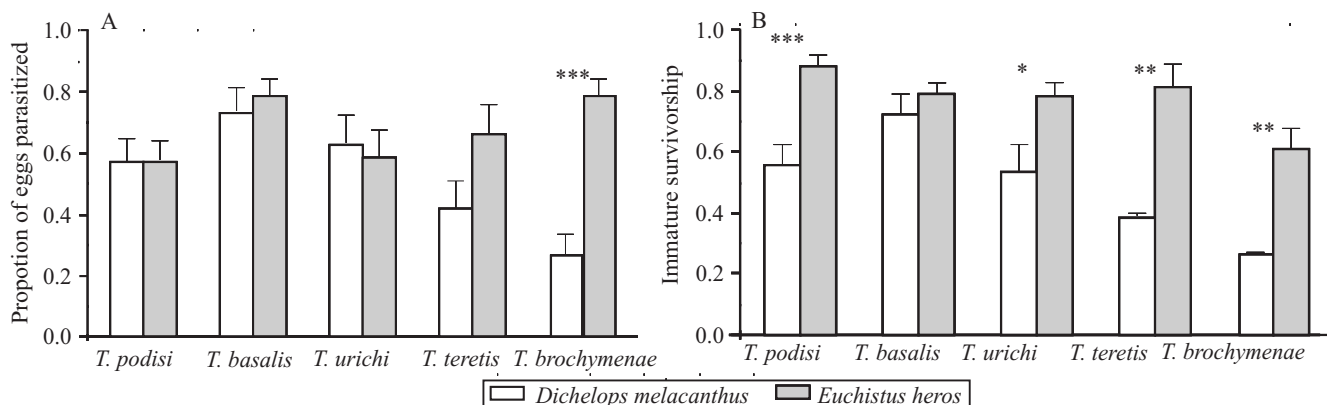


Figure 2. Mean \pm standard-error of: A, proportion of parasitized eggs; B, immature survivorship of different parasitoids on *Euschistus heros* or *Dichelops melacanthus* egg mass. *, ** and *** Significant by GLM analyses respectively by 5, 1 and 0.1%.

maximize their reproductive success. These cues could be related to physical (color, size, number and disposition of eggs, chorion thickness, corion ornamentation) and chemical (semiochemical) characteristics of the egg masses (Bin et al., 1993; Borges et al., 1999).

When the parasitoid female first choice was related to the number of eggs of each host, the first choice of *Te. podisi* had a significant influence in the number of *D. melacanthus* eggs parasitized (Table 2 and Figure 3 A). The chance, estimated by the odds ratio, to parasitize eggs of *D. melacanthus* increased nine times when the first choice was for eggs of this species (Table 2). The number of *D. melacanthus* eggs parasitized by *Tr. urichi* was also significantly influenced by the female first choice (Table 2 and Figure 3 C), and first choice increased the chance of parasitizing eggs of this species by approximately 14 times.

For *Tr. basalis*, *Tr. teretis* and *Tr. brochymenae*, the first choice of the females did not influence the number of parasitized eggs of *D. melacanthus* or *E. heros*. However, for *Tr. teretis* the proportion of *E. heros* parasitized eggs after the female choice was marginally nonsignificant (Table 2 and Figure 3 B, D and E).

The proportion of eggs parasitized by scelionid wasps in different hosts may be modified by the presence of two or more hosts (Pacheco & Corrêa-Ferreira, 1998; Sujii et al., 2002), in some cases increasing parasitism on nonpreferred hosts (Pacheco & Corrêa-Ferreira, 1998). The results of the present work showed that, even in the presence of an alternative host, used to rear parasitoids in

the laboratory during several generations, the first choice of hosts in three out of the five species did not affect the parasitism in *D. melacanthus* eggs.

However, the effect of the first and second trophic level in the foraging behaviour of the parasitoids could modify the host/parasitoid interaction. For the plant-stink bug-scelionid wasp trophic system, it has been shown that volatile substances of different plant species, induced by different stink bugs, can act as synomones attracting females of *Tr. basalis* (Colazza et al., 2004) or *Te. podisi* (Moraes et al., 2005, 2008). In addition, there is also some evidence that these parasitoids may selectively respond to semiochemicals from stink bugs (Borges et al., 2003; Conti et al., 2004; Salerno et al., 2006; Silva et al., 2006; Laumann et al., 2009) or substrate-borne vibratory signals used in the stink bug sexual communication (Laumann et al., 2007).

The Scelionidae species evaluated in this work are potentially effective natural enemies of *D. melacanthus*. At least *Tr. basalis*, *Tr. teretis* and *Tr. brochymenae* have this potential and could be used in areas where populations of this stink bug species co-exist with other ones. However, before using these parasitoids in biological control of *D. melacanthus*, specific information about mass rearing, host search behavior in different host densities and influence of different species

Table 2. Probability of five scelionid wasps to parasitize *Dichelops melacanthus* eggs instead of *Euschistus heros* eggs, estimated by the odds ratio.

Parasitoid first choice	Odds ratio	Confidence Interval (95%)	Significance
<i>Telenomus podisi</i>			
<i>Euschistus heros</i>	0.5617	0.7966– 3.9780	0.4732
<i>Dichelops melacanthus</i>	9.2579	3.1858–26.9030	0.0370
<i>Trissolcus basalis</i>			
<i>Euschistus heros</i>	0.4834	0.2684– 0.8706	0.2170
<i>Dichelops melacanthus</i>	4.5251	1.5158–13.5083	0.1675
<i>Trissolcus urichi</i>			
<i>Euschistus heros</i>	0.5491	0.2023– 1.4899	0.5480
<i>Dichelops melacanthus</i>	14.9041	4.6239–48.0398	0.0210
<i>Trissolcus teretis</i>			
<i>Euschistus heros</i>	0.0882	0.0239– 0.3250	0.0626
<i>Dichelops melacanthus</i>	0.2728	0.0809– 0.9200	0.2853
<i>Trissolcus brochymenae</i>			
<i>Euschistus heros</i>	0.8376	0.3291– 2.1314	0.8495
<i>Dichelops melacanthus</i>	0.7330	0.2343– 2.2933	0.7850

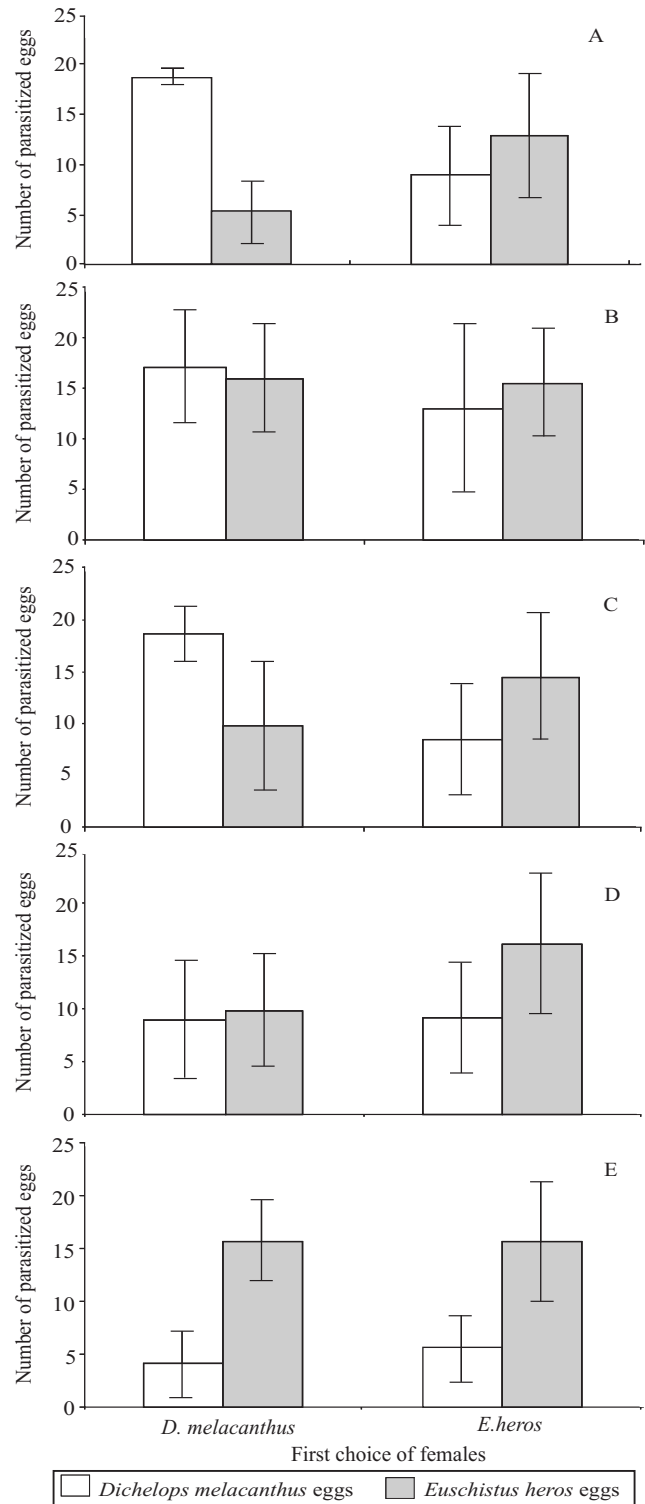


Figure 3. Relation between first choice of the parasitoid females and number of parasitized eggs (mean±standard deviation), in experiments where the two hosts were offered simultaneously to the parasitoids. A, *Telenomus podisi*; B, *Trissolcus basalis*; C, *Trissolcus urichi*; D, *Trissolcus teretis*; and E, *Trissolcus brochymenae*.

complexes in tri-trophic interactions, as well as other relevant biological and ecological aspects need to be established.

Conclusions

1. Scelionidae wasps may successfully use *Dichelops melacanthus* eggs as hosts.
2. The parasitoids show interspecific behavioral differences.
3. For *Trissolcus basalis*, *Trissolcus teretis* and *Trissolcus brochymenae*, the presence of eggs of different species of stink bugs does not influence the performance of the parasitoids, evaluated on *Dichelops melacanthus*.

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