Monthly Parasitism Rate by Tachinid Flies and Egg Allocation on the Body of Dichelops furcatus

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Monthly parasitism rate by tachinid flies and egg allocation on the body of *Dichelops furcatus*

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Abstract

Monthly parasitism rates and oviposition behavior of tachinid flies parasitizing *Dichelops furcatus* (F.) (Hemiptera: Pentatomidae) adults were determined. Weekly surveys, with duration of 2 hr each, were performed during 1 yr at the National Wheat Research Center in Passo Fundo, Rio Grande do Sul, Brazil. Stink bugs were collected from different plants and from crop residues, and taken to the laboratory to examine whether or not they were parasitized (presence of fly eggs). Number and egg allocation (dorsal and ventral surfaces) on stink bug bodies were determined. Stink bugs were kept inside plastic boxes to collect the parasitoid pupae and to identify the flies after emergence. Four tachinid (Diptera: Tachinidae) species were obtained as follows: *Ectophasiopsis gradata* (Wiedemann), *Cylindromyia* sp., and *Gymnoclytia* sp. 1 and sp. 2. The parasitism rate ranged from 2.5 to 7.0% (during Apr–Aug), and from 12 to 59% (Sep–Mar). Eggs were laid preferably on the dorsal surface (82%), and the majority of them (42%) under the wings. The number of fly eggs per parasitized stink bug ranged from 1.0 to 2.0.

Key Words: Hemiptera; Pentatomidae; Tachinidae; egg incidence; oviposition; biocontrol

Resumo

Taxa anual de parasitismo e comportamento de oviposição de moscas tachinídeos parasitando adultos de *Dichelops furcatus* (F.) (Hemiptera: Pentatomidae) foram determinados. Amostragens semanais, com duração de 2 hr cada, foram realizadas durante 1 ano no Centro Nacional de Pesquisa em Trigo da Embrapa em Passo Fundo, Rio Grande do Sul, Brasil. Percevejos foram coletados em diferentes plantas e resíduos de culturas agrícolas e levados para o laboratório onde foi determinado se os indivíduos estavam parasitados (presença de ovos de moscas) ou não. Número e alocação (superfície ventral e dorsal) dos ovos no corpo do percevejo foram determinados. Os percevejos foram mantidos em caixas plásticas para coletar as pupas dos parasitoides e então identificar as moscas após sua emergência. Quatro espécies de tachinídeos (Diptera: Tachinidae) foram obtidos, sendo essas: *Ectophasiopsis gradata* (Wiedemann), *Cylindromyia* sp., and *Gymnoclytia* sp. 1 and sp. 2. A taxa anual de parasitismo variou de 2,5 a 7,0% (de abr-ago) e de 12 a 59% (de set-mar). Ovos foram preferencialmente ovipositados na superfície dorsal do percevejo (82%) e a maioria deles (42%) sob as asas. O número de ovos das moscas por percevejo parasitado variou de 1,0 a 2,0.

Palavras Chave: Hemiptera; Pentatomidae; Tachinidae; incidência de ovos; oviposição; controle biológico

A large number of stink bugs (Pentatomidae) are considered important pests of many crops cultivated in the world. Among them, *Dichelops furcatus* (F.) (Hemiptera: Pentatomidae), a neotropical species referred to as the green-belly stink bug in Brazil, has been reported to cause damage to the wheat crop (*Triticum aestivum* L. [Poaceae]) in southern Brazil (Chocorosqui & Panizzi 2004; Panizzi et al. 2016). For example, wheat plants infested during the vegetative and reproductive stage (booting) for 18 days with 16 stink bugs per m of row plants, incurred significant reduction of grain yield. Similarly, infestations during booting with 8 stink bugs per m caused abnormal seedlings to increase significantly (Panizzi et al. 2016).

Stink bug (nymphs and adults) are attacked and parasitized by several species of parasitoids, mostly by flies (Diptera: Tachinidae) (Guimarães 1997). In the southern region of Brazil the following species of tachinids are reported to parasitize different species of pentatomids: *Gymnoclytia paulista* Townsend (Panizzi & Corrêa-Ferreira 1997), *Hyalomyodes* sp. (Panizzi & Oliveira 1999), *Phasia* spp. (Corrêa-Ferreira et al. 1998; Farias et al. 2012), *Cylindromyia* sp. (Panizzi & Corrêa-Ferreira 1997; Farias et al. 2012), and *Trichopoda giacomellii* (Blanchard) (= *Eutrichopodopsis nitens* Blanchard) (Panizzi & Herzog 1984).

Trichopoda giacomellii is one of the most common natural enemies of adults and late instar nymphs of the southern green stink bug *Nezara viridula* (L.) (Diptera: Pentatomidae) (La Porta 1987; Ferreira et al. 1991; Liljesthrom 1991; Nunes et al. 1998). In addition, it also can parasitize other important pentatomid pests, such as *Euschistus heros* (F.), *Piezodorus guildinii* (Westwood), *Chinavia* spp., *Edessa meditabunda* (F.), and *Thyanta perditor* (F.) (all Hemiptera: Pentatomidae) (Panizzi & Herzog 1984; Nunes et al. 1998). *Trichopoda giacomellii* may reduce the longevity and fecundity of their stink bug host, mainly when they parasitize them during nymphal and earlier adult stages (Corrêa-Ferreira et al. 1991).

Only 1 report on the parasitization of *D. furcatus* by 1 species of tachinid fly (*Phasia* sp.) was found in the literature (Nunes et al. 1998). No further studies have been conducted to evaluate the incidence of tachinid parasitism on *D. furcatus*. Therefore, we studied the monthly incidence of tachinid parasitoids on *D. furcatus*, and mapped the egg allocation on the different areas of the body surface (dorsal and ventral).

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Material and Methods

Weekly surveys of *D. furcatus* were conducted in the experimental area of the Embrapa National Wheat Research Center located in Passo Fundo, RS, Brazil (28.2500°S; 52.4000°W) from Dec 2014 to Nov 2015. A total of 51 surveys were conducted, with a duration of 2 hr each. Adults of *D. furcatus* were manually collected from soybean, *Glycine max* (L.) Merrill (Fabaceae), maize, *Zea mays* L. (Poaceae), and wheat, *Triticum aestivum* L. (Poaceae) plants, and from the weed plants, *Brassica rapa* L. (Brassicaceae), *Brachiaria plantaginea* (Link) Hitch. (Poaceae), and *Digitalia sanguinalis* (L.) Scop. (Poaceae), from a native plant, Brazilian pepper-tree, *Schinus terebinthifolius* Raddi (Anacardiaceae), and from crop residues.

Adults were taken to the laboratory and observed visually to determine if they were parasitized (adults with at least 1 egg on the exoskeleton were considered to be parasitized). In addition, the number and the spatial allocation of the eggs on the stink bug body were determined; for allocation, we considered the dorsal and ventral surfaces. The dorsal surface was separated into different sites: under the wings (hidden), head, pronotum, scutellum, and above the wing. The area under the wings was divided into 2 distinct portions, the forewings (hemelytra) that cover the abdomen, i.e., the coriaceous anterior portion, and the membranous posterior portion (Fig. 1).

After that, the stink bugs were placed inside plastic boxes (11 × 11 × 3.5 cm) lined with filter paper containing fresh green bean pods, *Phaseolus vulgaris* L. (Fabaceae), and raw shelled peanuts, *Arachis hypogaea* L. (Fabaceae) as food. The plastic boxes were kept in a walk-in growth chamber (25 ± 2 °C, 65% ± 5% relative humidity, and 14:10 h L:D photoperiod) and observed daily for 45 d. At this time, the parasitoid pupae were collected and placed inside Petri dishes (5 cm diam) lined with moistened filter paper, and kept in the growth chamber for the emergence of adults. Adult flies were identified and later deposited at the entomological collection of the Embrapa National Wheat Research Center.

Data on the incidence of tachinid parasites on *D. furcatus* were calculated to determine the parasitism rate during each month. For this, we used the presence or absence of fly eggs to estimate the parasitism, as applied in other studies (Harris & Todd 1980; Eger 1981). The number of insects parasitized, number of fly eggs, and mean number (± SE) of fly eggs deposited per stink bug were calculated for each mo. In addition, the number of eggs deposited on different body surfaces were recorded and used to calculate the percentages (± SE) for each surface (number of eggs per surface per total number of eggs × 100). The percentage of eggs laid on the dorsal vs. ventral surfaces, and the percentage of eggs laid under the 2 portions of the forewings (hemelytra), coriaceous anterior portion vs. membranous posterior portion were compared using Pearson's Chi-Square test (χ^2).

Results

Four species of Tachinidae flies were found parasitizing *D. furcatus* in natural conditions; the most abundant was *Ectophasiopsis gradata*

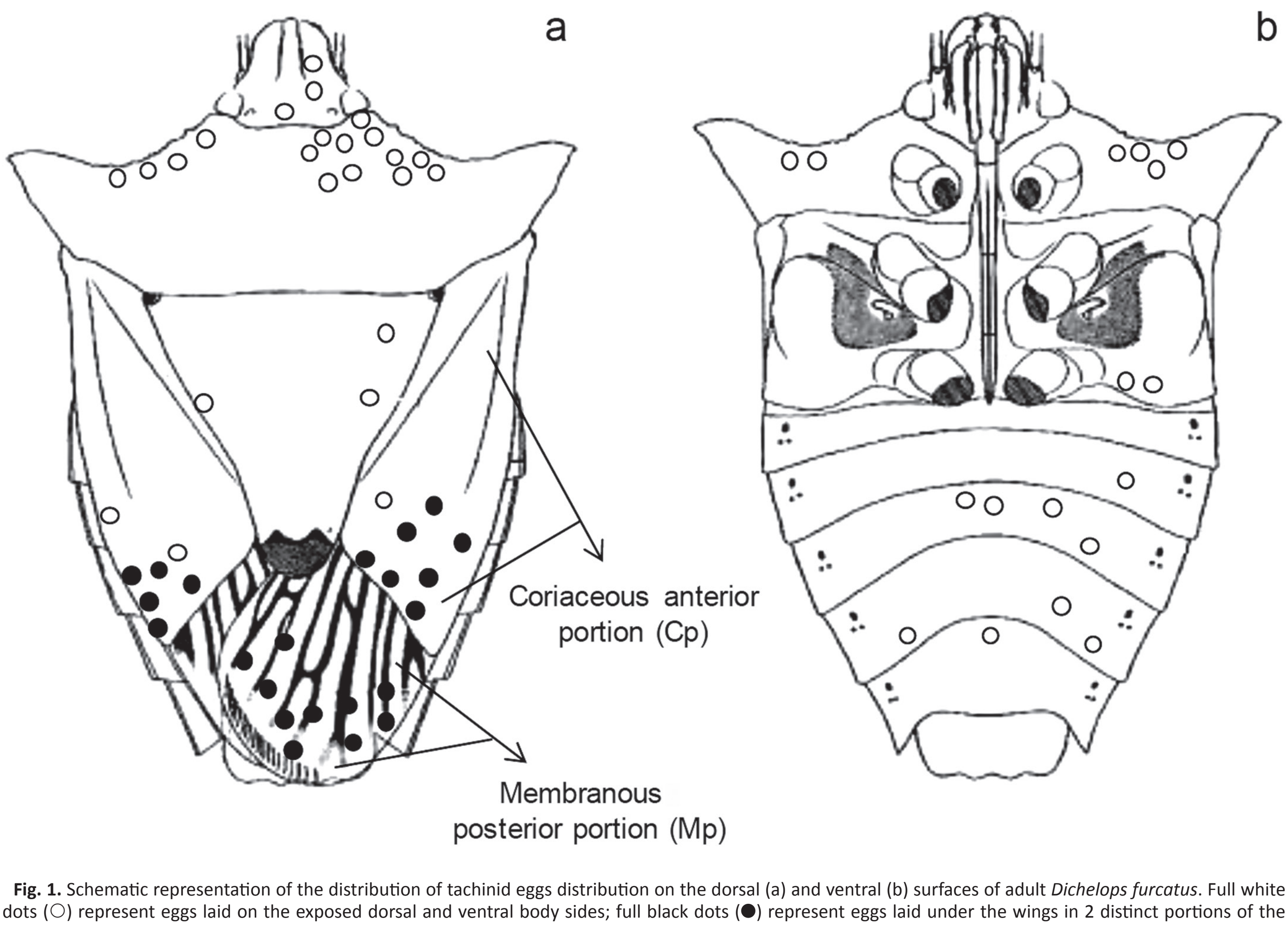

Fig. 1. Schematic representation of the distribution of tachinid eggs distribution on the dorsal (a) and ventral (b) surfaces of adult *Dichelops furcatus*. Full white dots (○) represent eggs laid on the exposed dorsal and ventral body sides; full black dots (●) represent eggs laid under the wings in 2 distinct portions of the forewings (hemelytra) that cover the abdomen, i.e., the coriaceous anterior portion (Cp) and the membranous posterior portion (Mp).

(Wiedemann), (Diptera: Tachinidae) (74%) [n = 52], followed by *Cylindromyia* sp. (16%) [11], and *Gymnoclytia* sp. 1 and sp. 2 (10%) [7] (Fig. 2a-d). In total, 1,143 *D. furcatus* were field collected from Dec 2014 to Nov 2015. Of these, the majority (992 individuals, 87%) were not visibly parasitized, i.e., without presence of tachinid eggs attached on the body surface; only 151 stink bugs were found to be visibly parasitized (13%) (Table 1).

In total, we observed 220 fly eggs allocated on different areas of the stink bug body. The majority of the eggs (90%) were found from Dec to Mar and from Sep to Nov (Table 1). The mean number of eggs was 1.4 per stink bug, with the highest value of 9 eggs on 1 male. On 70% of the parasitized stink bugs, only a single egg was found attached to the body. When compared by mo, the number of eggs per stink bug ranged from 1.0 to 1.4 eggs, except in Dec 2014 and Nov 2015, when this number was higher, with 2.0 and 1.8 eggs per stink bug, respectively (Table 1).

The parasitism rate varied greatly among the months of the survey. In the hottest periods of the year (Dec to Mar and Sep to Nov), associated with presence of crop plants (soybean and wheat), the rates ranged from 12 to 59%; while in the coldest months (Apr to Aug), associated with absence of crops, the parasitism rates decreased (range from 2.5 to 7%) (Fig. 3).

Considering the entire period of the survey, the parasitoids showed a strong and significant preference to deposit eggs on the dorsal surface (82%) compared to the ventral surface (18%) of the body (χ^2 =

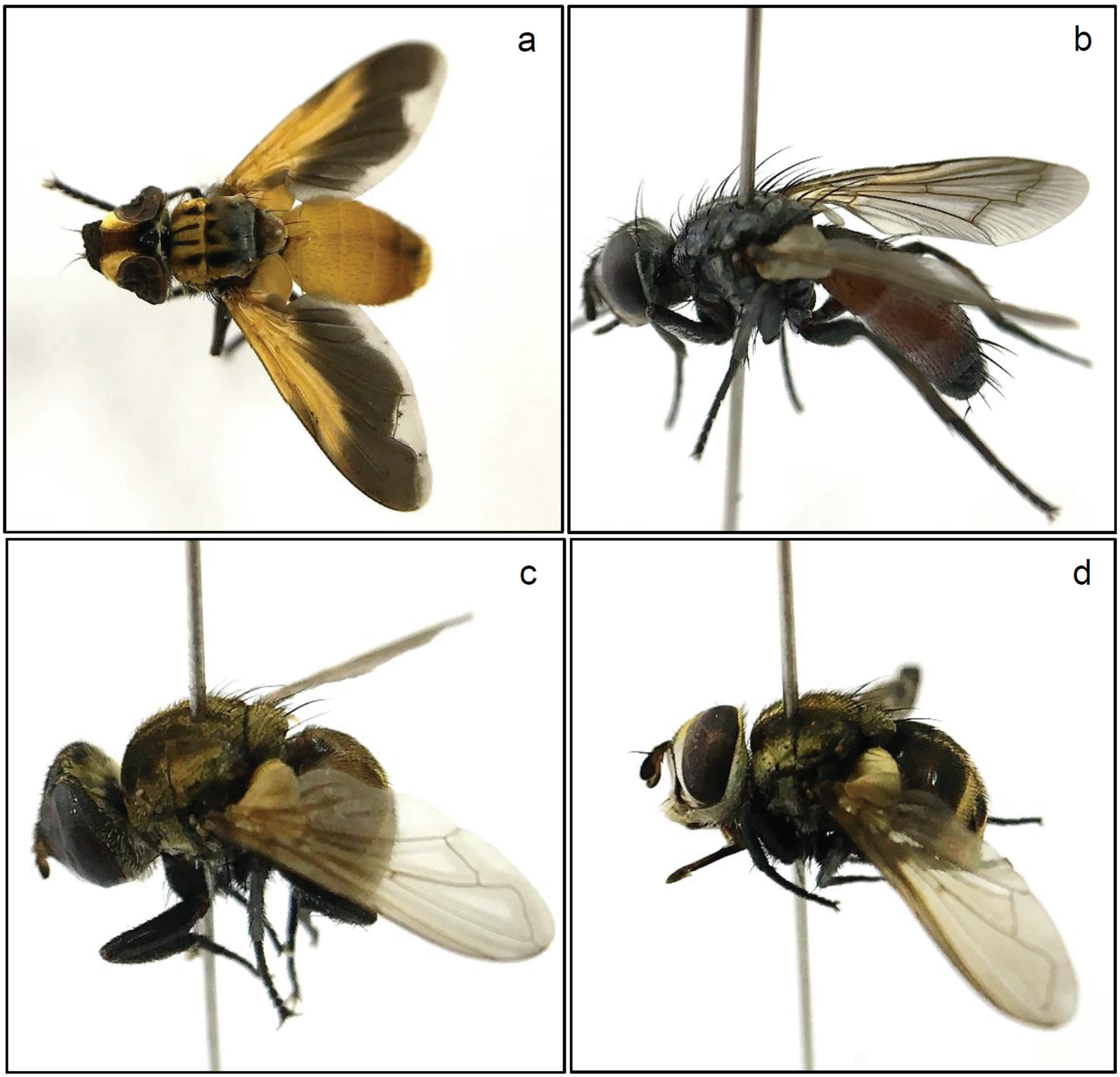

Fig. 2. Species of parasitoids (Diptera: Tachinidae) obtained in the laboratory parasitizing adults of the stink bug *Dichelops furcatus* in Passo Fundo, RS, Brazil. *Ectophasiopsis gradata* (a), *Cylindromyia* sp. (b), *Gymnoclytia* spp. (c,d).

Table 1. Monthly incidence of tachinid parasitism on *Dichelops furcatus* adults collected from Dec 2014 to Nov 2015, in Passo Fundo, RS, Brazil.

Month	Total number of insects collected	No. of insects parasitized (total; range)	No. of fly eggs (total; range)	No. of fly eggs per stink bug (mean ± SE)
Dec 2014	56	12 (0–6)	24 (0–9)	2.0 ± 0.7
Jan 2015	41	7 (0–5)	10 (0–8)	1.4 ± 0.2
Feb 2015	33	5 (0–2)	7 (0–4)	1.4 ± 0.2
Mar 2015	83	12 (0–4)	13 (0–5)	1.1 ± 0.1
Apr 2015	83	6 (0–4)	6 (0–4)	1.0 ± 0.0
May 2015	120	5 (0–3)	6 (0–4)	1.2 ± 0.2
Jun 2015	133	3 (0–2)	3 (0–2)	1.0 ± 0.0
Jul 2015	120	4 (1–1)	4 (1–1)	1.0 ± 0.0
Aug 2015	120	3 (0–2)	3 (0–2)	1.0 ± 0.0
Sep 2015	150	18 (1–9)	21 (1–12)	1.2 ± 0.1
Oct 2015	114	23 (2–9)	30 (2–16)	1.3 ± 0.2
Nov 2015	90	53 (3-19)	93 (6-39)	1.8 ± 0.1
Mean (total)	95.3 (1,143)	12.6 (151)	18.3 (220)	1.4

40.9, $P < 0.001$). On the dorsum, the greatest number of eggs were deposited under the wings (42%) compared to the head (3.2%), pronotum (30%), scutellum (2.7%), and on the dorsum of the wings (3.6%) (Fig. 4). No significant differences were observed between the number of eggs laid under the membranous posterior portion of the hind wings (54%) compared to the coriaceous anterior portion (46%) ($\chi^2 = 0.64$, $P = 0.424$).

Discussion

No previous study has been conducted to evaluate the incidence of tachinid flies parasitizing *D. furcatus* under natural conditions in the neotropics. The majority of the studies on tachinid parasitism of stink bugs in this region involve the incidence of parasitism of *N. viridula* by different species of tachinid flies, mostly *T. giacomellii* and *Trichopoda pennipes* (F.) (Jones et al. 1996; Sands & Coombs 1999; Salerno et al. 2002; Tillman 2010).

We observed 4 species of tachinid flies in 3 different genera attacking *D. furcatus*, as follows: *Ectophasiopsis*, *Cylindromyia*, and *Gymnoclytia*. This is the 1st report of these genera parasitizing *D. furcatus*. There are relatively few studies of tachinid parasitism of pentatomids in the neotropics. Only 1 other study reported tachinid flies parasitizing *D. furcatus* by an unidentified species (Nunes et al. 1998). The species *Cylindromyia brasiliana* Townsend was found parasitizing *Dichelops melacanthus* (Panizzi & Corrêa-Ferreira 1997), and *Tibraca limbativen-*

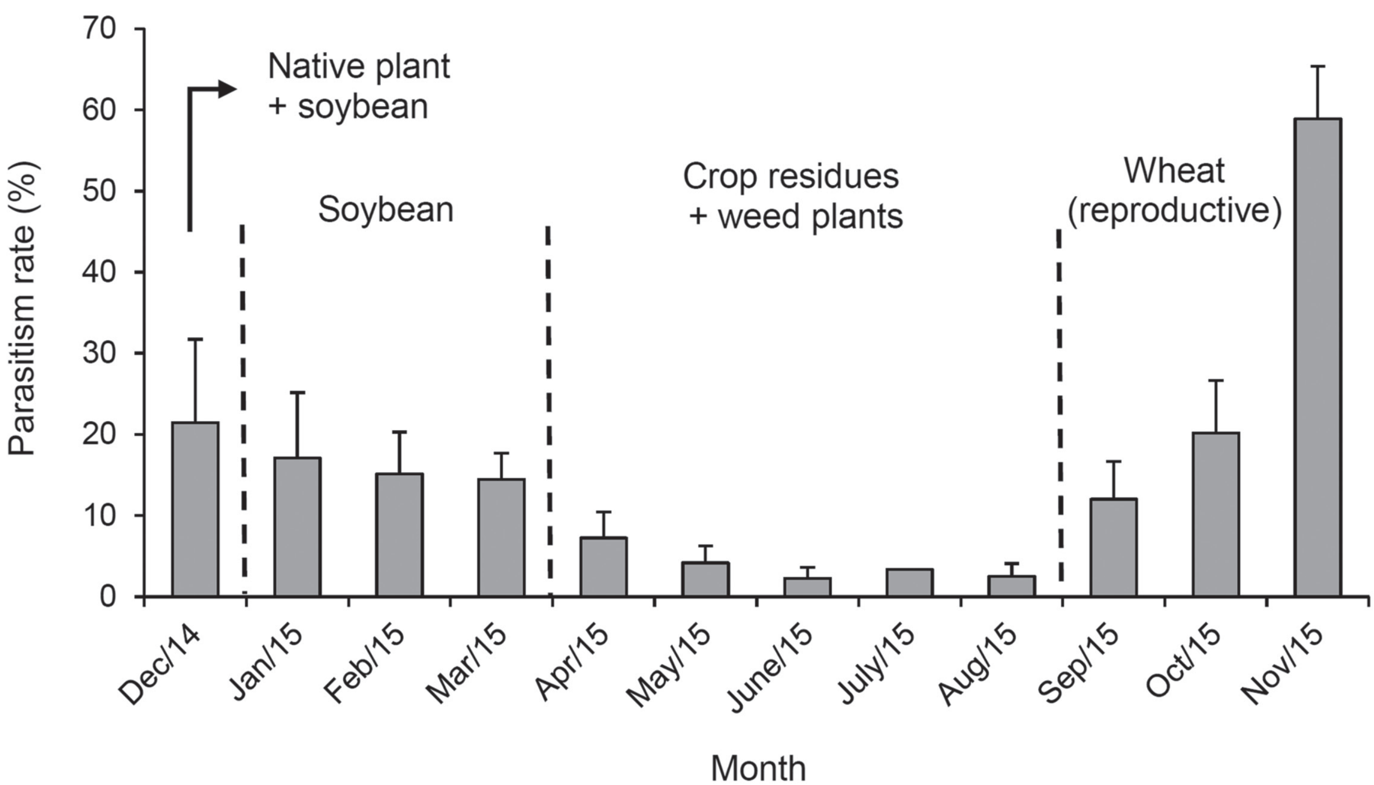

Fig. 3. Monthly parasitism rates (mean % ± SE) of *Dichelops furcatus* adults by all species of tachinid flies collected from different plants and from crop residues from Dec 2014 to Nov 2015, in Passo Fundo, RS, Brazil (dashed lines separate different plants and crop residues from where stink bugs were collected).

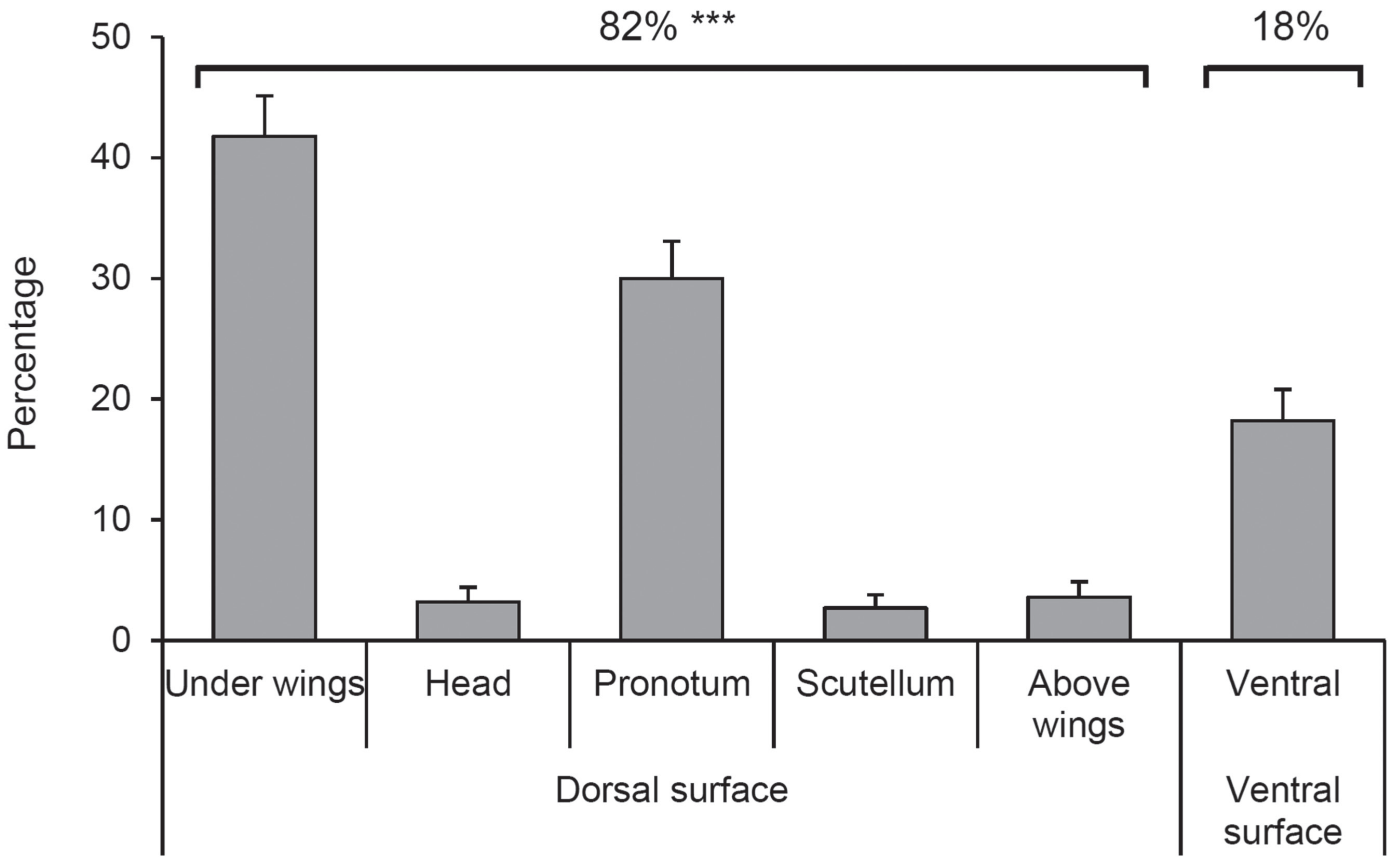

Fig. 4. Allocation of the eggs of tachinid flies (% ± SE) on different areas of the body of adult *Dichelops furcatus* collected from different plants and from crop residues during Dec 2014 to Nov 2015, in Passo Fundo, RS, Brazil (dorsal surface - under the wings, head, pronotum, scutellum, and above the wings; and ventral surface). Based on examination of 151 parasitized individuals. ***Significant difference between dorsal vs. ventral surfaces using Pearson's Chi-Square test ($P < 0.001$).

tris (Stål) (Farias et al. 2012), while *Gymnoclytia paulista* Townsend reportedly parasitizes *E. heros* and *N. viridula* (Panizzi & Corrêa-Ferreira 1997).

The parasitism rate on *D. furcatus* was largely variable according to season. During autumn to winter (end of Mar to late Sep) this species rarely was found on plants (wild hosts or vegetative wheat plants), remaining sheltered underneath crop residues, entering into partial dormancy. In fact, this behavior of concentrating in shelters during the unfavorable condition yielded greater number of stink bugs captured monthly at this time (Table 1). This behavior probably facilitated its escape from parasitism by tachinid flies at this time. The Neotropical brown stink bug, *E. heros,* presents the same behavior during mid-winter to mid-spring (Panizzi & Niva 1994). Similarly, during the hottest months, when the stink bugs remained exposed on the plants, the incidence of parasitism of *D. furcatus* by tachinid flies increased constantly, similar to what was reported to *E. heros* (Panizzi & Oliveira 1999).

A different behavior is observed for *N. viridula* during the coldest months of the mild winter in northern Paraná state, Brazil. In this case, the stink bugs concentrate mostly on wild plants, becoming an easy target for tachinid flies. Even under less than favorable conditions, the parasitism rate increased substantially, reaching 100% (Panizzi & Oliveira 1999).

In our literature review, few studies were found quantifying the oviposition site preference by tachinid parasites on stink bugs (e.g., Beard [1940] in the coreid bug *Anasa tristis* (De Geer) (Hemiptera: Coreidae) and Eger [1981] in the pentatomids *Brochymena* spp. (Hemiptera: Pentatomidae)]. We observed that the majority of eggs were laid on the dorsal surface of the body, mostly under the wings, and on the pronotum. Similarly, Eger (1981) observed that the majority (97%) of the *Trichopoda plumipes* (F.) (Hemiptera: Tachinidae) eggs were deposited on the dorsal surface of *Brochymena* spp. and 91% of those were deposited on hidden sites (eggs not visible when the host is at rest), which included the abdominal tergites and hind wings. These results are different from those obtained by Beard (1940), who reported that nearly 75% of the *T. pennipes* eggs were found on the ventral surface of the abdomen of *A. tristis*. Some tachinid species (*Trichopoda* spp.) are known to preferably oviposit on males' bodies rather than on females' bodies (Todd & Lewis 1976, Liljesthrom 1991). However, in our study on the tachinid species parasitizing *D. furcatus*, we did not compare the parasitism rate between genders. This prevents us from knowing of any possible preference by the flies to parasitize a particular gender.

According to Eger (1981), the preference to deposit eggs on hidden sites (i.e., under the wings) may be a strategy used by the parasitoid to prevent dislodging of the egg by the host, and to protect them against unfavorable environmental conditions, such as direct sunlight, and perhaps egg parasitoids. We also suggest that this preference may be a strategy to oviposit on a less rigid area (i.e., with thinner cuticle), which probably facilitates the penetration of the larvae inside the stink bug's body after hatching. This could be an advantage compared to, for example, oviposition on the pronotum; even though many eggs were observed on the latter surface, the larvae may face a stronger barrier to penetration. In the present study, the parasitized stink bugs were not dissected after death to check if larvae were present internally. However, Eger (1981), by doing this, recovered a low number (8%) of larvae of *T. plumipes* from adult stink bugs carrying hatched eggs, suggesting low success of larvae developing in the host.

In conclusion, our results revealed several species of tachinid flies parasitizing *D. furcatus* in southern Brazil, and that this parasitism is usually underestimated because the tachinid eggs are mostly laid on hidden areas of the stink bug's body. These results also suggest that these parasitoids may play an important role in mitigating numbers of *D. furcatus* in cropping systems. Future studies should concentrate on evaluating the impact of these parasitoids on these stink bug pest populations.

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