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Tachinids in conservation biological control of phytophagous Pentatomidae

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Abstract Several stink bugs species constitute an important pest complex in soybean worldwide. Tachinidae parasitoids are an important tool to be exploited in conservation biological control programs. We evaluated parasitism of pentatomids in different periods of their life-history: diapause (late autumn and winter), start of activity (spring) and full activity (late summer and early autumn). During the growing seasons 2017–2018 and 2019–2020, we collected stink bugs from soybean, alfalfa and wheat crops and natural vegetation, of three species (*Nezara viridula*, *Edessa meditabunda* and *Diceraeus furcatus*) and reared them in laboratory until pupal formation and adult emergence of tachinid. We examined the relationship among pentatomid abundance and multiple measures of parasitism: through observation of tachinid eggs externally attached to stink bugs body upon field collection and through further verification of parasitism in the laboratory. We also examined the preference of tachinids regarding host sex. Seven tachinid species were detected. *Neobrachelia* cf. *edessae* parasitizing *E. meditabunda* was recorded for the first time in Argentina. Only in two periods of *N. viridula* life history (diapause and start of activity period) the presence of tachinid eggs externally attached to the stink bugs cuticle was a reliable estimation of successful parasitism. Parasitism was highest on *N. viridula*, followed by *E. meditabunda* and lower on *D. furcatus*. Parasitism of stink bugs was highest during the start of activity and during diapause. During the full activity period in 2017–2018 male hosts were more parasitized than females. This work provides field results valuable for designing tools for pentatomid conservation biological control programs.

Keywords Tachinidae · Parasitoids · Biological control · Stink bugs · Soybean

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Introduction

Phytophagous hemipterans belonging to the family Pentatomidae, commonly known as stink bugs, have been identified as one of the most significant pest complexes in soybean and other important extensive and horticultural crops within the Neotropical region (Schaefer and Panizzi 2000). Both juveniles and adults cause direct damage by feeding on stems, flowers, pods and seeds, reducing soybean crops yield and seed quality. The Pampas Region of Argentina has about 18,000,000 ha with annual crops: 45% corresponds to soybean crops, 25% to corn crops, 18% to wheat, the reminder other crops such as sunflower, sorghum and other winter cereals. In this region, the most frequently found species are *Nezara viridula* (Linnaeus), *Piezodorus guildinii* (Westwood), *Edessa meditabunda* (Fabricius), and *Diceraeus furcatus* (Fabricius) (Hemiptera: Pentatomidae) (Azcuy & Fernández 2020). The seasonal population abundance of these insects fluctuates from year to year. However, *N. viridula* and *P. guildinii* are generally the most abundant species. *N. viridula* feeds on a wide variety of crops and it has a cosmopolitan distribution (Casmuz et al. 2021). *P. guildinii* is a Neotropical species which damages mainly cultivated Fabaceae such as soybeans and alfalfa. Furthermore, *E. meditabunda* exhibits a similar behavior to *N. viridula*, while *D. furcatus* shows preferences for cultivated grasses such as maize, sorghum and wheat (McPherson 2018). Currently, the control of stink bug pests is accomplished through repeated applications of high doses of pesticides with broad spectrum of action, as pyrethroids and organophosphates (Conti et al. 2021). In addition, stink bugs are attacked by a diverse complex of natural enemies, mainly parasitoids of eggs and adults, that can exert significant biological control of populations of these pests (Bueno et al. 2023).

Among these natural enemies, tachinid flies are one of the most important groups of biological control agents against stink bugs. They have been studied for classical biological control programs, primarily targeting defoliating lepidopteran pests in the Neotropical region (Dindo and Grenier 2022). There are approximately 8,600 species of tachinids described. However, it is estimated that there are still thousands of undiscovered species (O'Hara et al. 2020). They can be found in all types of habitats all around the world and are the most important non-hymenopteran parasitoids (Stireman et al. 2019). Few tachinids are known to be specialists attacking a single host species, the majority of them can parasitize several different species, usually belonging to the same order. Some tachinids, like those in the subfamily Phasiinae, commonly exploit pheromones of their hosts as host-finding kairomones, or even volatiles given off by a damaged plant that is being fed upon (Aldrich et al. 2006). All tachinids are endoparasitoids with three larval stages. Adult flies feed on nectar from both floral and extrafloral nectaries, as well as sugary liquids produced by secretions from the spikelets of certain Poaceae and other plants wounds. They can also be potentially important pollinators (Martel et al. 2021).

Despite potential of tachinid flies in the biocontrol of agricultural pests, research on their biology and ecology is limited, and the difficulty in taxonomic identification of species leads to an underestimation of the diversity of the group (Liljesthröm and Ávalos 2015). Even more, many tachinids are difficult to rear under laboratory conditions, which complicates the use of these natural enemies in inoculative biological control strategies. Due to these, conservation biological control may be the most suitable alternative. Conservation biological control is based on habitat manipulation strategies with the aim of actively benefit natural enemies, and the implementation of mitigation actions against the practices harmful to them (Eilenberg et al. 2001). The development of protocols for a more rational use of pesticides, that includes a reduction on the frequency of applications based on monitoring of not only pest densities but also natural enemies' presence, is of primary importance.

In the current study we evaluated parasitism of pentatomids during two growing seasons (May 2017 to April 2018 and May 2019 to March 2020), separated into different periods of their life history: diapause, start of activity and full activity. This information is interesting for the design of biological control programs, mainly to improve the synchronization of the population of the pest and its parasitoids. Additionally, we compared the accuracy of two different ways to estimate stink bugs parasitism in order to estimate the reliability of field observation of tachinid eggs attached to the stink bugs tegument as a method of estimating actual parasitism. The estimation of parasitism by observing the eggs (method 1) is a simple way that can be easily performed in field but may underestimate parasitism when compare to laboratory verification (methods 2), which is a more accurate estimate of parasitism, but demands more time, expertise and specific equipment to be carried out. The possibility to rely on a simple reliable way to detect parasitism and the presence of these natural enemies in the field will contribute to develop monitoring protocols that may stimulate farmers to adopt strategies to maintain/attract tachinids and promote the benefits of biological control.

Materials and methods

The study was conducted at the Experimental Field, J.F. Villarino, of the Faculty of Agricultural Sciences of the National University of Rosario (UNR), with a surface of 507 hectares, located in Zavalla, Santa Fe province, Argentina (33°01′45″S, 60°53′21″W). The study spanned over two growing seasons: May 2017 to April 2018, and May 2019 to April 2020. The experimental field consisted of plots with soybean, corn, wheat and pasture crops. As in the rest of the Pampas region, the presence of wild vegetation is limited to the edges of crops, under fences and internal roads.

During field samplings, three collections of stink bugs (one per month) were carried out in each of three different periods of their life history: diapause (late autumn and winter; May to July); start of activity period (spring; October to December); and full activity (late summer and early autumn; March to May). Soybean crops are grown from November to April in Argentina. No monitoring was carried out during September as it is considered a transitional month, in which stink bugs can be both in diapause or starting their activity in alternative plant hosts, and so those stink bugs cannot be accurately assigned to a particular life history period. During January and February, no crops were monitored: stink bugs are generally absent in summer crops in the region due to the usual high temperatures and because crops are in a vegetative phenological stage and, therefore, they do not have pods on where they mainly feed. However, patches of natural vegetation were monitored to verify the presence of stink bugs on non-cultivated vegetation. In 2020, monitoring during the full activity period was interrupted due to the implementation of lockdown measures related to the SARS-CoV-2 pandemic (COVID-19).

The crops and types of vegetation patches sampled and the methods used for adult stink bugs collection varied according to the particularities of stink bug species and their life history period. During diapause, *D. furcatus* and *E. meditabunda* were collected on soybean stubble and accompanying dry vegetation and corn lot borders. We used a metal ring of 0.25 m^2, placed every 20 m on a 100 m long transect. Stink bugs were collected from six transects. *Nezara viridula* adults were collected manually under the bark of cultivated trees (*Platanus* × *hispanica* Mill. ex Münchh. (Proteales: Platanaceae) and *Eucalyptus camaldulensis* Dehnh. (Myrtales: Myrtaceae) intersected in two transects of 100 m each, along tree transects located near crop fields, with an average of 17.5 trees per transect. During the start of the activity period, adults of the three mentioned stink bug species were collected in natural vegetation, wheat (*Triticum aestivum*) (three lots of 5 ha each, in average) and alfalfa (*Medicago sativa*) (5 ha) crops using a sweep net in. Five net swings of 0.4 cm every 20 m, along a 100 m transect were performed, over six transects in total. During the full activity period, adults of the three stink bug species were collected in soybean fields (phenological stage R5) (six lots of 5 ha each, on average), using a one-meter vertical beat sheet (Drees and Rice 1985). Beats were performed every 20 m along 100 m transects, over six transects in total.

The collected adults were taken to the laboratory and reared using a methodology adapted from Aquino et al. (2019) at the brood chamber of the Agricultural Science University, UNR, under controlled conditions of temperature (25 ± 2 °C), RH (70 ± 10%), and photoperiod (L:D 14:10). Stink bugs of the same species were placed in groups of five, in 8 cm diameter by 6 cm high plastic containers, with filter paper lining the base and a voile fabric cover. They were fed with green beans (*Phaseolus vulgaris* L.) and raw peanuts (*Arachis hypogaea* L.) (Fabales: Fabaceae), and wet cotton discs were provided for hydration. This methodology was carried out until the formation of tachinid pupae or until the death of the stink bugs. All dead stink bugs from which no parasitoids were observed were dissected to confirm the absence of tachinid larvae inside the stink bug body. Dissections were done in Petri dishes with saline solution under a stereomicroscope. Obtained pupae were separated and placed in containers with voile covers and soil at the base as a substrate until adult fly emergence.

Parasitism percentages were calculated according two verification criteria. The first criteria was: egg observations, calculated as a percent of stink bugs with tachinid eggs externally attached to their bodies; and the second criteria was: parasitism verified in laboratory, calculated as a percent of stink bugs from which tachinid pupae were obtained in the laboratory + dissected stink bugs with tachinid larvae.

In order to evaluate the use of field observation of eggs attached to the pentatomid tegument as a reliable estimation of parasitism, Pearson correlation analysis was performed between both verification criteria, for each period of life history and pentatomid species, in both growing seasons (n = 6). Two-way ANOVAs were performed to compare parasitism percentages verified in laboratory (dependent variable), between pentatomid species and life history periods, and the interactions between these factors. Tukey test was used for post-hoc analysis. Pearson correlation analysis was performed between abundance of stink bugs and percentages of parasitism, verified in the laboratory (dependent variable). The variables were normalized using arcsine square root transformation for parasitism and logarithm transformation for stink bugs abundance. One-way ANOVA was performed to analyze parasitism percentages (verified in laboratory) between stink bug sex for each species and means were compared using Tukey test. Taxonomic determination of parasitoid adults was carried out by Rodrigo de Vilhena Perez Dios, and of stink bugs by Celina Fernández and Eduardo Punschke.

Results

A total of 3,153 adult stink bugs were collected during both sampling growing seasons (851 *D. furcatus*; 870 *E. meditabunda*; 1432 *N. viridula*). It is worth mentioning that, although *P. guildinii* is also part of the complex of pentatomid pests and numerous specimens were collected and reared (n = 947), only one tachinid pupa formation was recorded from this host, which did not complete development, making the tachinid species impossible to be identified. During January and February, no pentatomid adults were found in the monitored patches of natural vegetation.

Table 1 Stink bugs abundance in two growing seasons, across their life history periods. Methods used for adult stink bugs collection varied according to the particularities of stink bug species and their life history period

	Diapause	Start of activity	Full activity
2017–2018			
D. furcatus	9.57 m^{-2}	1.19 m^{-2}	1.59 m^{-1}
E. meditabunda	11.10 m^{-2}	0.42 m^{2}	1.16 m^{-1}
N. viridula	28.67 per 100 m	2.19 m^{-2}	8.77 m^{-1}
P. guildinii	6.00 m^{-2}	3.93 m^{-2}	3.03 m^{-1}
2019–2020			
D. furcatus	6.36 m^{-2}	0.58 m^{-2}	0.90 m^{-1}
E. meditabunda	4.62 m^{-2}	0.73 m^{-2}	0.87 m^{-1}
N. viridula	87.67 per 100 m	1.13 m^{-2}	4.33 m^{-1}
P. guildinii	0	0.39 m^{-2}	0

In every studied period of the life history of the stink bugs and in both growing seasons, *N. viridula* was the most abundant, mainly during diapause in 2019, except for spring of 2017 when *P. guildinii* was the most abundant. *Diceraeus furcatus* abundance remained low in both growing seasons. However during the diapause period the number of adults was slightly higher than in the start activity and full activity periods. Abundance of *E. meditabunda* remained low throughout the 2019–2020 campaign, while it was higher during full activity and diapause in the 2017–2018 campaign (Table 1).

Seven different species of tachinids were recorded, belonging to six different genera (Table 2). *Neobrachelia* cf. *edessae* was recorded for the first time parasitizing *E. meditabunda* in Argentina. Each tachinid species was found parasitizing only one stink bug species except for *Trichopoda gradata* which parasitized *D. furcatus* and *N. viridula*.

We found a significant positive correlation between both parasitism verification methods (eggs *vs*. laboratory observation), only for *N. viridula*, in diapause and start of activity periods (Table 3). No significant correlation was found for *E. meditabunda* nor for *D. furcatus*. In diapause the parasitism verified in laboratory was higher than parasitism verified by eggs observation for the three species. In contrast, during the start and full activity, parasitism verified by eggs observation were 1.9 times higher and 1.3 times higher than laboratory verification,

Table 2 Tachinid parasitoids obtained from field collected pentatomid adults during different periods of their life history: diapause (late autumn and winter: May to August), start of activity (spring: October to December), and full activity (late summer and early autumn: March to May). (*) First record from Argentina

Pentatomid hosts	Life history period	Tachinid parasitoid		
		Genus/species	Subfamily	Tribe
Edessa meditabunda	Diapause Start of activity Full activity	*Phasia* sp. Latreille	Phasiinae	Phasiini
	Full activity	*Neobrachelia* cf. *edessae* (*) (Townsend)	Phasiinae	Parerigononi
Diceraeus furcatus	Start of activity Full activity	*Cylindromyia brasiliana* (Townsend)	Phasiinae	Cylindromyiini
	Diapause	*Trichopoda gradata* (Wiedmann)	Phasiinae	Gymnosomatini
	Diapause	*Gymnoclytia* sp. Brauer and Bergenstamm	Phasiinae	Gymnosomatini
Nezara viridula	Diapause Start of activity Full activity	*Trichopoda pictipennis* Bigot	Phasiinae	Gymnosomatini
	Diapause	*Trichopoda gradata* (Wiedmann)	Phasiinae	Gymnosomatini

respectively, showing changes in the pattern of relationship between the two variables.

A significant negative correlation between abundance of stink bugs and percent parasitism was found for *N. viridula* in diapause. Only one significant positive correlation was found between *N. viridula* abundance and its parasitism percentage in full activity. The correlation between the abundance of *E. meditabunda* and *D. furcatus* and their parasitism percentage were not significant (Table 4).

No significant relationship was found between life history period and pentatomid species ($F_{4,39}=0.93$; $p=0.458$). Parasitism percentages were significantly different among species ($F_{2,39}=3.22$; $p=0.050$) but not among periods of the stink bug life history ($F_{2,39}=0.36$; $p=0.696$). *Diceraeus furcatus* had the lowest parasitism percentages compared to *N. viridula* and *E. meditabunda* (Fig. 1). Parasitism varied significantly between host sex for *N. viridula* during the full activity and start of activity period in 2017–2018: males were more parasitized than females. We found no significant differences in parasitism between sexes of *D. furcatus* and *E. meditabunda* (Table 5).

Discussion

Tachinids are one of the most important natural enemies of *N. viridula, E. meditabunda* and *D. furcatus*, with great potential to be used in integrated pest management programs, mainly through conservation biological control strategies. No tachinids were obtained from *P. guildinii*. Several authors mention that particularly this stink bug species is the only one of the stink bug pest complex that is not attacked by these parasitoids (Liljesthröm and Ávalos 2015; Aquino et al. 2019).

The correct taxonomic identification of the species of natural enemies plays a crucial role in the development of conservation biological control strategies since these are strengthened not only by increasing the abundance of natural enemies, but also their diversity. In this sense, it is worth noting that the actual number of tachinid species reported parasitizing stink bugs is likely underestimated due to the scarcity of studies on this group, and difficulties in accurate identification (Dios 2020). In this study, the most abundant genus was *Trichopoda*, which includes common species in the region. *Neobrachelia* cf. *edessae* (Townsend) is reported for the first time in Argentina parasitizing adults of *E. meditabunda*. More studies on the taxonomy of the Neotropical genus *Neobrachelia* are needed, as the boundaries of some species are not well defined. The species *N. edessae* was reared from *E. meditabunda* in Uruguay (Guimarães 1977) but its

Table 3 Correlation between percentage of pentatomid parasitism estimated through direct tachinid egg observation and through further verification of parasitism in the laboratory, estimated from stink bugs collected from field in different periods of their life history. Values in parentheses indicate SE. Bold letters: significant correlation between variables (Pearson correlation test p ≤ 0.05)

Species	Periods of life-history											
	Diapause				Start of activity				Full activity			
	Egg observation	Parasitism verification	*r*	p-value	Egg observation	Parasitism verification	*r*	p-value	Egg observation	Parasitism verification	*r*	p-value
N. viridula	29.64 (±15.34)	32.19 (±14.62)	**0.99**	**0.001**	67.48 (±11.68)	35.18 (±16.90)	**0.95**	**0.014**	13.55 (±7.04)	11.73 (±4.06)	0.88	0.314
E. mediabunda	0.52 (±0.52)	13.80 (±3.62)	0.55	0.255	3.00 (±3.00)	22.23 (±10.87)	− 0.31	0.616	0	10.66 (±2.45)	0.00	0.999
D. furcatus	1.47 (±0.81)	3.41 (±1.08)	0.74	0.090	21.31 (±11.06)	14.58 (±8.37)	0.56	0.324	1.19 (±1.19)	1.86 (±1.03)	0.70	0.509

biology and efficiency as a biological control agent against this pest are poorly known. In this study, no external evidence of *N. edessae* was obvious in the parasitized stink bugs (i.e., the eggs attached to the host's body were not visible), which may result in an underestimation of the presence of this natural enemy in the field.

Tachinids egg laying strategies are varied. Some species deposits eggs on foliage near the host insect. Then, hatched maggots are ingested during feeding by the host, which then develop inside the host. This is common in tachinids that parasitize caterpillars. The adult flies of other tachinid species glue their eggs onto the body of the host, and conspicuous white eggs can be seen on the host body. After the eggs hatch, the maggots penetrate into the host body. Adult females of the relatively few species which lay eggs in the host body have piercing structures derived from female sternites, which are used to pierce the host integument and to guide ovipositor into the cut, and insert the eggs inside the host body (Dindo and Nakamura 2018). For the particular case of tachinids that parasitize stink bugs, some of them lay their eggs on the cuticle of stink bugs, like *Trichopoda* and *Gymnoclytia* species, while others like *Cylindromyia*, *Neobrachelia* and *Phasia*, insert their eggs inside the bodies of adult hosts and so eggs are not visible externally. However, for the management of pentatomid pest species in extensive crops, estimation of parasitism is often done by directly observing the presence of eggs on the stink bug cuticle (Panizzi and Oliveira 1999; Anderson et al. 2020). While this technique can be easily implemented in the field, it should not be generalized for all species, as it could lead to incorrect estimations of biocontrol levels. The accuracy depends on the oviposition strategy of each species, on the host species, and on other factors. In this study we found that only in two situations was the presence of tachinid eggs externally attached to the cuticle of the stink bugs a reliable indicator of parasitism, with a significant correlation between both estimation methods for *N. viridula* in diapause and start of activity. However, different life history periods exhibited different patterns: in diapause the observation of parasitoid eggs would underestimate the parasitism observed in the laboratory, but in the start of activity and full activity the observation of eggs overestimates actual parasitism. Probably in these periods mortality of eggs and/or larvae of tachinids is caused by

Table 4 Correlation between stink bugs abundance and parasitism through their life history. Bold letters: significant correlation between variables (Pearson correlation test p ≤ 0.05)

Species	Period of life-history					
	Diapause		Start of activity		Full activity	
	r	p-value	*r*	p-value	*r*	p-value
N. viridula	**– 0.88**	**0.021**	– 0.07	0.930	**1.00**	**0.038**
E. meditabunda	0.63	0.182	– 0.60	0.286	– 0.25	0.840
D. furcatus	0.28	0.537	– 0.79	0.109	–	–

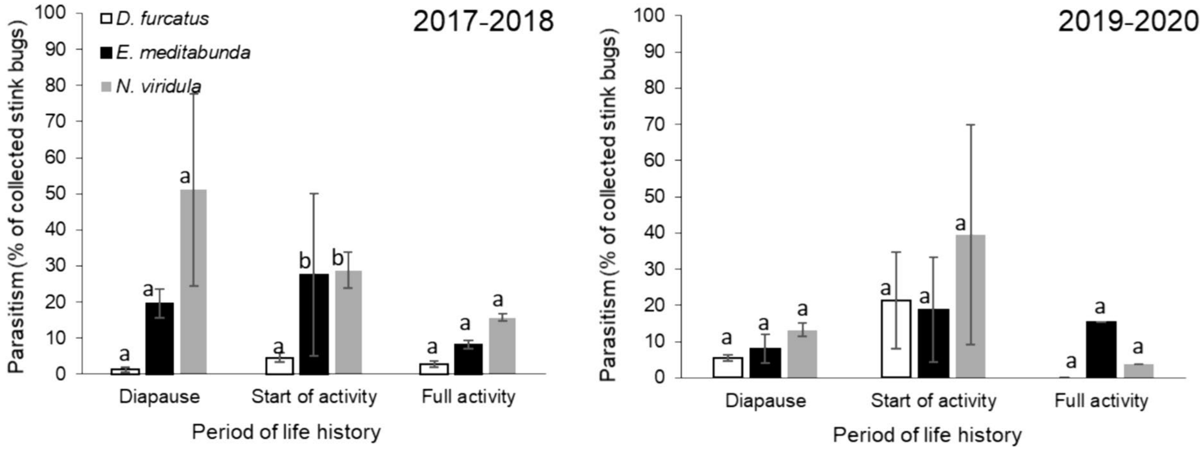

Fig. 1 Parasitism by tachinids (mean percentages of parasitism ± SE) of three pentatomid species, during different periods of their life history diapause (late autumn and winter: May to August), start of activity (spring: October to December), and full activity (late summer and early autumn: March to May), during two agricultural campaigns. Different letters denote significant differences (Tukey test p ≤ 0.05) between stink bugs species for each period of life-history

dehydration or high temperatures in the field. Additionally, although these eggs are sometimes lost as they fall off of the host, the presence of eggs on the body of *N. viridula* may be a good indicator of actual parasitism by tachinids, but not for the rest of the stink bugs species. Subsequent studies should determine whether the differences we found between both parasitism estimation methods remain stable through many agricultural growing seasons and different agroecosystems, and up to what level of accuracy can parasitism be estimated by observing the presence of tachinid eggs attached to the stink bug body. Finally, field observation of stink bugs with tachinid eggs on their tegument could be incorporated into a sampling plan, where the abundance of stink bugs and the level of parasitism are the variables to be considered in a decision chart that suggests different possible actions (Greco et al. 2011).

Fluctuations in parasitism levels throughout the periods of the life history of stink bug hosts may be attributed to several factors, including abundance, overwintering strategies, weather events, and crop management among others (Liljesthröm and Bernstein 1990; Panizzi and Oliveira 1999; Zerbino and Panizzi 2019). Regarding weather events, the Pampas Region of Argentina began to experience a period of drought in the summer of 2018 and it deepened in 2019, which affected crops and natural vegetation, and stunted soybean crops growth. Pentatomid species, despite their similarities, behave differently in relation to changes in agroecosystems (Panizzi et al 2022). During the fall, *N. viridula* concentrates on double-cropping soybean (Stella et al. 2018). The loss of crops due to drought further exacerbated this fact in the fall of 2019, increasing relative abundance of stink bugs making them susceptible to massive parasitism. Tachinids remain

Table 5 Pentatomid parasitism (mean ± SE) discriminated between host sexes, in different periods of their life history. Bold letters: significant differences (Tukey test p ≤ 0.05), df: 1,5. Collections in full activity in 2020 were interrupted due to lockdown measures related to the SARS-CoV-2 pandemic (COVID-19)

Species	Periods of life-history											
	Diapause				Start of activity				Full activity			
	% par. ♀	% par. ♂	*F*	p-value	%par. ♀	%par. ♂	*F*	p-value	% par. ♀	% par. ♂	*F*	p-value
2017–2018												
N. viridula	54 ± 45	48 ± 48	0.02	0.894	18 ± 8	40 ± 7	**17.38**	**0.014**	13 ± 1	19 ± 1	**9.32**	**0.037**
E. meditabunda	17 ± 7	22 ± 9	0.56	0.497	5 ± 5	44 ± 51	1.28	0.320	10 ± 4	8 ± 1	0.76	0.433
D. furcatus	2 ± 2	1 ± 2	0.40	0.562	5 ± 3	4 ± 3	0.35	0.585	2 ± 3	4 ± 5	0.07	0.809
2019–2020												
N. viridula	15 ± 6	12 ± 2	1.10	0.352	13 ± 17	37 ± 55	0.70	0.449	6 ± 0	2 ± 0	–	–
E. meditabunda	9 ± 8	7 ± 7	0.01	0.911	17 ± 23	20 ± 26	0.01	0.923	0	31 ± 0	–	–
D. furcatus	7 ± 4	4 ± 4	1.15	0.343	27 ± 41	15 ± 15	0.09	0.779	0	0	–	–

inside the stink bugs until the diapause ends. Parasitoids may enter in diapause following the signals received from their host. As it was demonstrated for other tachinid species (Jadhav & Armes 2013), parasitoid diapause may be induced by the physiological changes in host. At the end of winter, stink bugs concentrate on the few food sources that still remain available: natural vegetation, wheat and alfalfa crops (which are even scarcer in extreme droughts). There, tachinids could easily locate them, leading to high parasitism rates, as observed in spring, which regulated the stink bug population and reduced it to lower densities in full activity. Therefore, tachinid density may also have decreased, as did parasitism rates at full activity.

In general, after diapause, *D. furcatus* and *E. meditabunda* move to wheat and corn fields and also natural vegetation. However *N. viridula* is not common on wheat and corn fields in the study area. Corrêa-Ferreira et al. (1984) also documented higher parasitism on *N. viridula* during winter months in the northern State of Paraná, Brazil. Despite this is a region where stink bugs remain active throughout the winter instead of entering diapause, unlike in the Pampas Region, Argentina, stink bugs aggregate on the few available host plant (Agostinetto et al 2018).

At the end of diapause, *E. meditabunda* and *D. furcatus* aggregate on the first host plants species they find in the area, where they may be easily found by tachinids. Panizzi and Oliveira (1999) reported similar results for *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae), an emerging pest in the region and the most abundant in Brazilian soybean crops (Aquino et al. 2019; Saldanha et al. 2024), which displays a winter survival strategy similar to *D. furcatus* and *E. meditabunda* in the Pampas region, taking refuge in grassy areas and stubble during the winter. In this way they manage to escape late summer parasitism as they are on different host plants at that time. Information about stink bugs life histories provides useful knowledge for designing habitat management strategies favoring tachinids presence in the field, and their permanence near crops. Research is still much needed (Panizzi and Lucini 2024).

Parasitism of *D. furcatus* was significantly lower than *E. meditabunda* and *N. viridula*. In the Neotropical Region, *D. furcatus* has been the species with the lowest relative abundance within the soybean stink bugs complex, not only in the surveyed years, but also across decades (Massaro et al. 2022). This may have affected the synchronization of host-parasitoid dynamics (Godfray 1994), driving down abundance of tachinids that prefer to parasitize *D. furcatus*. However, increases in damage caused by *D. furcatus* to other crops, such as corn and wheat (Panizzi and Lucini 2016; Jacobi et al. 2022; Panizzi et al. 2022) emphasizes the importance of studying the biology and ecology of its natural enemies, for the design of sustainable management tools.

The impact of stink bug control by parasitoids is often evaluated during the periods of full activity of pests, which are associated with critical phenological stages in soybeans as full pod and beginning-seed development of soybeans (i.e., R4 and R5 on the scale proposed by Fehr and Caviness 1977), during the summer and early autumn months. However, parasitism obtained in this study during the start of the activity period (spring) was high, denoting that stink bugs control must be taken into account not only in the critical period of crops. Upon adult fly emergence from their winter diapause, they immediately require food sources (nectar and pollen) to activate their sexual development. It is well known that consumption of a sugary source like nectar increases survival, fertility, and longevity of parasitoids (Coombs 1997; Dindo and Grenier 2022). Montero (2008) recorded floral visitation events of *T. giacomelli* on *Baccharis punctulata* L. (Asterales, Asteraceae) and *Senecio grisebachii* Baker (Asteraceae). In that study adult flies were recorded feeding on the nectaries of flowers. The implementation of conservation biological control programs seeks to promote the presence of natural enemies throughout the growing season and over years. The presence of refuges and food sources such as flowers for adult flies during unfavorable periods is crucial for synchronization between the biological cycles of stink bugs and tachinids to optimize the synchronization between biological cycles of stink bugs and tachinids.

There is limited knowledge about the mechanisms of survival of the parasitoid within diapausing stink bugs. However, what has been observed in this study is that, from all the parasitized stink bugs collected during the diapause period, the emergence of parasitoids did not occur until the stink bug diapause was broken. This suggests a possible hypothesis that tachinid larvae enter diapause alongside their host and only resume their development when environmental conditions remain suitable over time.

Like in this study, Liljesthröm (1991) found a clear preference of *T. giacomelli* males of *N. viridula* under controlled conditions. It is known that *T. pectipenis* is attracted to the pheromone that the male *N. viridula* releases to attract females (Mitchell and Mau 1971). However, other factors could hold greater relevance in attracting female Tachinidae, such as insect aggregation pheromones due to the presence of a food source, especially during diapause emergence or critical crop periods (Borges and Blassioli-Moraes 2017; Dindo and Nakamura 2018).

The present work provides field results on Tachinidae parasitoids of economically significant pentatomids in soybean among other important crops, which are valuable for implementing a conservation biological control program of stink bugs. Such management strategies require taking advantage of the abilities of all tachinid species present in an agroecosystem at different times of the year, and their relationship with suitable plant diversity.

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Declarations

Conflict of interest We also declare no potential conflicts of interest.

Research involving human and animal participants This research was conducted in accordance with ethical guidelines for the use of animals in research.

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