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Release of the egg parasitoid *Telenomus podisi* to manage the Neotropical Brown Stink Bug, *Euschistus heros*, in soybean production

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ABSTRACT

Eushistus heros is one of the major pests in soybean in South America. Not only has insecticides constantly failed on its control but also triggered different negative side effects related to the overuse of chemicals. Among the biocontrol agents that can be used in Augmentative Biological Control, *Telenomus podisi* stands out due to its high parasitism capacity on eggs of its hosts. Therefore, this study aimed to assess *T. podisi* release strategies and moments (release done together with the detection of first *E. heros* adults in the area as well as together with fungicide sprays), in order to reach optimal parasitoid field performance. For this purpose, a replicated experiment was conducted with a soybean crop located in Londrina, Paraná, Brazil, during 2017/18 and 2018/19 crop seasons. Treatments consisted of 18750 released *T. podisi* pupae (released either inside cardboard capsules or as unprotected pupae sprinkled in bulk, at different moments) and were evaluated against insecticide spraying strategies. At harvest, yield and quality of the seeds were measured. Overall, our results clearly indicate that *T. podisi* pupae can be released either encapsulated or unprotected, with similar efficacy. Their release in the field increased *E. heros* egg parasitism to 70% and 50%, in 2017/2018 and 2018/2019, respectively. Thus, *T. podisi* can be efficiently used to control stink bug eggs. However, since the number of stink bugs \geq 0.5 cm in the field highly depends on its migration from neighboring fields, it is important to use *T. podisi* inside integrated pest management.

1. Introduction

Stink bugs are the most important pest group causing soybean (*Glycine max*) yield loss in South America (Bueno et al., 2015). They are piercing-sucking insects, feeding directly from soybean pods, seriously affecting crop yields by impairing the physiological and sanitary quality of the seeds (increased percentage of dead embryos, fungi contamination besides weight and size reduction of seeds and grains) (Corrêa-Ferreira and Azevedo, 2002). Of this group, the Neotropical Brown Stink Bug, *Euschistus heros* (Hemiptera: Pentatomidae) is the most important species due to its high frequency, abundance and management difficulties (Panizzi and Corrêa-Ferreira, 1997; Panizzi, 2013). Currently, soybean growers can only rely on chemical insecticides to control this pest. However, the overuse of insecticides has trigged rapid selection of resistant populations, reduction of biological control agents,

and outbreaks of secondary pests, among other undesirable consequences (Sosa-Gómez et al., 2001; Sosa-Gómez and Silva, 2010). Therefore, a more sustainable stink bug management is of major theoretical and practical interest.

Among the most environment-friendly and sustainable pest management tools available, augmentative biological control stands out, a method that has been applied for over 100 years (Cock et al., 2010) on more than 30 million ha worldwide (van Lenteren et al., 2018). Egg parasitoids have wide use in augmentative biological control and can be considered the most important stink bug biocontrol agents (Koppel et al., 2009; Laumann et al., 2010). Among the different species of egg parasitoids that can be used in augmentative biological control of *E. heros, Telenomus podisi* (Hymenoptera: Platygastridae) is noteworthy due to its high parasitism and control efficacy against its hosts (Peres and Corrêa-Ferreira, 2004; Queiroz et al., 2018; Silva et al., 2018). Despite

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its huge potential to control stink bugs, large-scale field release of *T. podisi* is still limited due to the lack of information regarding release technologies such as how and when the parasitoids are best released (Pinto and Parra, 2002), especially when extensive crops such as soybean are concerned.

Ground release of parasitoid pupae was the first standard method in countries where parasitoid production and labor costs were low (Huffaker, 1977). Later, this method was replaced by aerial release of parasitoid pupae in larger areas (Bouse and Morrison, 1985). Egg parasitoid pupae can be either released inside capsules, which are usually made of cardboard or other organic material, or spread directly, without protection, over the field in bulk (Smith, 1994; Pinto and Parra, 2002; Pinto et al., 2003). Parasitoid field success highly depends on climatic conditions, the number of released parasitoids, time and frequency of releases, as well as the parasitoid distribution method (Hassan, 1994), which is virtually undeveloped for *T. podisi* release to control E. heros in soybean fields. Better understanding of these interacting parameters may help growers to choose the optimal T. podisi release strategy. Therefore, this study was carried out to evaluate different T. podisi release strategies at different moments, in order to reach the best T. podisi field performance.

2. Materials and methods

2.1. Laboratory rearing of T. podisi

Telenomus podisi pupae used in the trials was originated from insect colonies kept at Embrapa Soybean (one of the units of the Brazilian Agricultural Research Corporation), Londrina, State of Paraná, Brazil and reared on *E. heros* eggs. *Telenomus podisi* and *E. heros* colonies were kept under controlled environmental conditions inside Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at $80 \pm 10\%$ humidity, temperature of 25 ± 2 °C, and a 14:10 h (L:D) photoperiod. Those procedures were according to methodologies described by Peres and Corrêa-Ferreira (2004) for *T. podisi* rearing and Panizzi et al. (2000) for *E. heros* rearing; and are briefly summarized in the followings.

Euschistus heros were originally collected in soybean field in Embrapa Soybean Experimental Farm, Londrina, State of Paraná, Brazil (23° 11′ 11.7″ S and 51° 10′ 46.1″ W). The populations was kept in the laboratory for approximately 4 year during which new field insects were introduced each year to maintain colony quality. Those insects were kept in plastic screen cages ($20 \text{ cm} \times 20 \text{ cm}$ sides $\times 24 \text{ cm}$ tall) (Plasvale Ltda., Gaspar, State of Santa Catarina. Brazil) lined with filter paper and fed *ad libitum* with a mixture of beans (*Phaseolus vulgaris* L.; Fabaceae), soybeans (*Glycine max* L. Merr.; Fabaceae), peanuts (*Arachis hypogaea* L.; Fabaceae), sunflower seeds (*Helianthus annuus* L.; Asteraceae) and privet fruits (*Ligustrum lucidum* Aiton; Oleaceae). A Petri dish (diameter 9 cm) with a cotton wad soaked in distilled water was added to each cage. Cages were cleaned, food replaced, and egg masses collected on a daily basis. The eggs were then used for *T. podisi* rearing or colony maintenance.

Telenomus podisi was collected originally also from soybean fields in Embrapa Soybean Experimental Farm, Londrina, Stated of Paraná, Brazil (23° 11′ 11.7″ S and 51° 10′ 46.1″ W). The colony has been kept in the laboratory for approximately 5 years. It has been reared on *E. heros* eggs (aged \leq 24 h) glued to pieces of card (5 cm × 8 cm). When parasitoid was close to emergence (1 day before), new eggs (aged \leq 24 h) were introduced into plastic cages (8.5 cm high and 7 cm in diameter) together with the eggs already parasitized by *T. podisi* close to parasitoid emergence. Small drops of *Apis mellifera*-produced honey are placed inside these tubes to provide food for the adults when they emerge. The tubes are then closed, and after adult emergence, the eggs allowed to be parasitized for 24 h. After 24 h, the eggs recently parasitized were removed to other cages starting a new parasitoid cycle. Adults that emerge from these eggs are used for trials as well as for colony maintenance.

2.2. Field trial description

The experiment was carried out under field conditions during two consecutive soybean seasons (2017/2018 and 2018/2019) in the municipality of Londrina ($23^{\circ}28'49.19''S$ 50°59'04.79''W in 2017/2018 and $23^{\circ}30'11.47''S$ 51°00'47.54''W in 2018/2019) in the northern of the state of Paraná (PR), Brazil. In the crop season of 2017/2018, the trial was carried out in a randomized block design with three treatments (3 ha per treatment) (Fig. 1A). Treatments were: 1) Treatment with *T. podisi* pupae released inside cardboard capsules released when the first *E. heros* adults were sampled in the field. Such treatment was named as 'Capsule/bugs' which received 32 capsules/ha (each containing approximately 195 pupae of *T. podisi* one day from emergence – totaling 6250 pupa/ha). As previously mentioned, those capsules were randomly spread after the capture of the first *E. heros* adults in the ground cloth (week 1), followed by identical releases in week 2, and week 3 (a total of



Fig. 1. Distribution of treatment field during 2017/18 trial (A) and 2018/19 trial (B).

18750 pupae per hectare); 2) Treatment named as 'Integrated Pest Management (IPM)'. In this treatment insecticide was sprayed only when the stink bug population reached Economic Threshold (ET) of 2 stink bugs (body length ≥ 0.5 cm)/meter (sampled with the use of ground cloth) (Bueno et al., 2015). Ground cloth measurements were taken by quietly unrolling a 1-m long cloth between 2 adjacent rows of soybean in a way that cover the ground and one the rows and briskly shaking the plants of the uncover row over the cloth (Rudd and Jensen, 1977). 3) Treatment named as 'Prophylactic Use of Insecticide (PUI)'. This treatment was composed by the spray of insecticide mixed with fungicides on a calendar basis following growers common practice.

In the crop season of 2018/2019, a trial was carried out in a randomized block design with five treatments (10 ha per treatment) (Fig. 1B). Treatments were: 1) 'Capsule/bugs' as previously described for the 2017/2018 trial; 2) Treatment was named as 'Pupa/bugs'. This treatment received 6250 unprotected pupae (without the use of the capsules) of T. podisi one day from emergence. Those pupae were randomly bulk-released in tracks 32 m apart, after the first E. heros adult capture in the ground cloth (week 1). Then, it was followed by identical releases in week 2 and week 3 (18750 pupae per hectare). 3) Treatment named as 'Capsule/fungicide'. This treatment was similar to 'capsule/ bugs', but with a different moment for the release. Parasitoid release in this treatment was triggered by the time when fungicide was sprayed (instead of the time when the first bug was captured in the ground cloth). 4) Treatment named as 'Pupa/fungicide'. This treatment was similar to 'Pupa/bugs', but with a different timing of release, triggered by the time when fungicide was sprayed (instead of when the first bug was captured in the ground cloth) and. 5) 'IPM' treatment as previously described for the 2017/2018 trial.

2.3. Soybean sowing, cultivars and plant protection management

Soybean was sown on October 25, 2017 (cultivar 'P95R51 RR', maturity group 5.1, indeterminate growth) for the 2017/2018 trial, and on November 12, 2018 (cultivar 'BS 2606 IPRO', maturity group 6.0, indeterminate growth) for the 2018/2019 trial at 15 seeds per meter. In the first season (2017/2018) an insecticide against caterpillars (*Bacillus thuringiensis* 13.44 g.a.i. ha⁻¹; Dipel® 400 mL ha⁻¹) was applied twice in all treatments together with herbicides to isolate the effect of stink bug infestation. In the second trial (2018/2019 trial) insecticides for caterpillars were not used because cultivar 'BS 2606 IPRO' is a Bt soybean which efficiently controlled the most important caterpillars of the crop. Herbicides (glyphosate 1440 g.a.i. ha⁻¹; Roundup® 3L ha⁻¹), and

fungicides (azoxystrobin + cyproconazol 93.33 g.a.i. ha^{-1} ; Priori Xtra® 300 mL ha^{-1}) were applied equally in all treatments to isolate the effect of stink bug infestation in the experiments. During the soybean season, herbicides were applied twice (2 and 5 weeks after soybean emergence) and fungicides were applied three times. The first application was made at the early soybean development stage of R₃ (Fehr et al., 1971) followed by two applications at intervals of 21 days.

The insecticide used for controlling stink bugs (IPM and PUI treatments) was thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}), applied according to each treatment description. Spraying of all pesticides (herbicides, fungicides, and insecticides) was adjusted to a volume of 150 L ha^{-1} using a hollow cone nozzle, model TXVK-8 tip. Spraying was carried out under appropriate environmental conditions (winds below 6 km h^{-1} , relative humidity above 50%, and a maximum temperature of 25 °C).

2.4. Assessment of the stink bug population, Telenomus podisi parasitism and soybean yield

Samples were taken weekly, starting from soybean development stage V₅ until soybean maturation. For this, a ground cloth (1.0 m \times 1.4 m) positioned horizontally on the ground, parallel to the soybean rows, was used for sampling (Fig. 2). The ground cloth needs to be large enough to cover all ground as well as the adjacent soybean row. Each treatment was divided into four areas of pseudoreplicates. In each replicate, four random samples were taken from 1-m row sections, counting stink bugs >0.5 cm (corresponding to adults and nymphs from 3rd to 5th instars), stink bugs smaller than 0.5 cm (corresponding to nymphs from 1^{rst} to 2nd instars), and identifying all individuals to species. In addition, in each replicate, 10 egg masses of stink bugs were collected and taken to the laboratory for later evaluation of parasitism. 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) x 100.

At full maturity of soybean grains (R_8 development stage) (Fehr et al., 1971), plants were collected from 5-m sections of the two central rows of each replicate. These samples were then threshed individually and evaluated. Weight and moisture content of each sample were recorded, and values were then corrected for yield adjusted to 13% seed moisture.



Fig. 2. Ground cloth use during sampling.

2.5. Tetrazolium test

The tetrazolium test was conducted according to the methodology described by França-Neto et al. (1998). Briefly, this procedure included two sub-samples of 50 seeds per plot, wrapped in a paper substrate filter moistened with sterile H₂O equivalent to 2.5 times the weight of the seeds. The samples were placed in controlled environmental conditions (environmental chambers) for 16 h at 25 ± 2 °C. The seeds were then immersed in a solution of 0.075% tetrazolium salts (2,3,5-triphenyl tetrazolium chloride) and placed in an oven at 40 °C for 2 h and 30 min

in the dark. This procedure differentiates living tissues from dead tissues in embryos of seeds on the basis of dehydrogenase activity, a respiratory enzyme. After seed hydration, the activity of dehydrogenase increases, resulting in the release of hydrogen ions, which reduces the solution of colorless tetrazolium salt into a red formazan compound called Formazan. Bright red spots indicate living cells, whereas dead cells remain colorless. After a defined period, the seeds were washed in running water and individually inspected for stink bug damage. A scale of 6–8 (%) indicates the percentage of seeds with sufficiently severe damage to make them unviable.



Fig. 3. Number of stink bugs \geq 0.5 cm per meter (A and B), its egg parasitism (%) (C and D) and the number of stink bugs < 0.5 cm per meter (E and F) during the 2017/18 (A, C and E) and the 2018/19 (B, D and F) crop seasons. Arrows indicate the moment of application of *T. podisi* pupae or insecticides in each treatment. Means followed by the same letter, in each soybean phenological stage, did not differ statistically (Tukey test at 5% probability).

2.6. Data analysis

Applying soybean values of US\$ 0.61/soybean kilogram and an average value of insecticide application of US\$ 15.81/hectare/application (FAOstat, 2020), an economic analysis was carried out following the methodology described by Corrêa-Ferreira et al. (2010). Since the commercial use of *T. podisi* was only recently approved there is no reference to its market prices. Therefore, the total of three releases was considered to be US\$ 15.81/hectare in order to be commercially competitive with insecticides, what is now the closest price estimate available. Net income was considered as total yield minus the cost of stink bug control used for each treatment, converted to kilograms of soybean (25.92 kg of soybean per insecticide application).

Net income as well as all collected data were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance for each treatment (Burr and Foster, 1972), and, if necessary, transformed to perform ANOVA. For 2018/2019 crop season, the percentage of dead embryos was transformed by \sqrt{X} . The treatment means were then compared by Tukey test at the 5% probability level (SAS Institute, 2001).

3. Results

During all evaluated soybean seasons, *E. heros* was the most abundant stink bug, representing >90% of all individuals collected. Other stink bug species were *Dichelops melacanthus* and *Piezodorus guildinii* (Hemiptera: Pentatomidae). In 2017/2018, stink bugs were first detected late in the season [R₂ soybean development stage (Fehr et al., 1971)] (Fig. 3A). In 2018/2019, stink bugs were first detected early in the season [V₉ soybean development stage (Fehr et al., 1971)] but population just started to grow at R_{5.1} soybean development stage (Fehr et al., 1971)] (Fig. 3B). Thus, IPM treatment required two insecticide sprayings in 2017/2018 while only one was necessary in 2018/2019. It is important to point out that, at R₇ soybean development stage (Fehr et al., 1971), the number of stink bugs (\geq 0.5 cm) increased in both crop seasons in all tested treatments, regardless of biological control or insecticide treatment (Fig. 3A and B).

In 2017/2018, the first *E. heros* adults were captured in the ground cloth in R_2 (Nov/23/2017), which triggered *T. podisi* releases to be started in the following week, R_3 soybean development stage (Fehr et al., 1971) (Dec/1/2017) (Fig. 3A). After the third *T. podisi* release, at $R_{5.2}$ soybean development stage (Fehr et al., 1971) (Dec/28/2017), parasitism (%) in the *T. podisi* treatment (capsule/bugs) reached 70%, while both in the IPM and PUI treatments, parasitism was close to zero (Fig. 3C). Parasitism (%) in the capsule/bugs remained higher than values recorded for IPM and PUI until $R_{5.4}$ soybean development stage (Fehr et al., 1971) (Jan/10/2018). Later, at $R_{5.5}$ soybean development stage (Fehr et al., 1971) (Jan/18/2018) parasitism increased naturally in all treatments and no differences could be observed between

treatments until the end of the crop season (Fig. 3C).

Similarly, in the second season (2018/2019), the first *E. heros* (\geq 0.5 cm) was captured in the ground cloth in V₉ (Dec/11/2018). However, stink bugs kept being recorded in low numbers (close to zero) until R₃ (Jan/3/2019). Therefore, the release of *T. podisi* started only at R₄ soybean development stage (Fehr et al., 1971) (Jan/10/2019) for both treatments in which the parasitoids were released when the first adults were captured in the ground cloth (capsule/bugs and pupa/bugs). In contrast, parasitoid release started earlier, at the soybean development stage of R₃ (Jan/3/2019), for both treatments in which parasitoids were released and pupa/bugs). In contrast, parasitoid release started earlier, at the soybean development stage of R₃ (Jan/3/2019), for both treatments in which parasitoids were released together with the first fungicide spraying (capsule/fungicide and pupa/fungicide) (Fig. 3B).

Parasitism (%) increased faster for treatments with earlier parasitoid releases (together with fungicide sprays) reaching 40% at evaluation performed at $R_{5.1}$ (Jan/17/2019). However, at evaluation performed at $R_{5.5}$ (Jan/31/2019), similar parasitism (%) among parasitoid releases done with fungicide sprays and with first *E. heros* captured in the ground cloth were recorded, all higher than parasitism observed in the IPM treatment (Fig. 3D). It is worth to emphasize that the number of stink bugs (<0.5 cm) per meter was very low in both crop seasons, being less than two and one insect per meter during the 2017/2018 and 2018/2019 seasons, respectively (Fig. 3E and F).

Yields (kg ha⁻¹) as well as their net income obtained over the two seasons of study allowed us to assess the impact of different T. podisi release strategies on stink bug populations and, consequently, the soybean yield. In 2017/2018 season, the lower population of stink bugs, which was generally observed in the insecticide treatment areas (IPM and PUI) from R_{5.2} to R₆, especially immediately after spraying (Fig. 3A) resulted in a higher yield from PUI compared with treatment capsule/ bugs. However, PUI did no differ from IPM and IPM did not differ from capsule/bugs (Table 1). In the 2018/2019 season, the lower population of stink bugs observed for IPM from $R_{5.4}$ to $R_{5.5}$ (Fig. 3B) did not result in a higher yield (Table 1). Neither were any differences concerning net income value observed between T. podisi and insecticide treatments (Table 1). Moreover, in examining the seed quality obtained in both crop seasons (2017/2018 and 2018/2019), we note that IPM had lower damage in 2017/2018 and the treatments were statistically equal in 2018/2019. It is important to point out that in both seasons, less than 6% of seeds were non-viable (embryos killed by stink bug sucking) (Table 1).

Together, our results did not reveal a great difference in yield or net income between areas of insecticide and *T. podisi* treatment (Table 1). Only in the first season (2017/2018), a lower yield in the *T. podisi* treatment was observed. In the second season (2017/2018), seed quality was good in the *T. podisi* treatment when pupae were released with first fungicide spraying or with first adults captured in the ground cloth. Here, less than 6% of seeds were recorded as non-viable in all treatments, similar to the IPM treatment (Table 1). Thus, regardless the

Table 1

Yield, net income (kg/ha) and percentage of dead embryos (scale 6–8 in tetrazolium test) obtained in two different soybean seasons (2017/2018 and 2018/2019). Londrina, Paraná, Brazil.

Treatment		Crop season 2017/2018			Crop season 2018/2019		
		Yield (Kg.ha ⁻¹)	Net $income^1$ (Kg.ha ⁻¹)	Dead embryo (%)	Yield (Kg.ha ⁻¹)	Net income ¹ (Kg.ha ⁻¹)	Dead embryo (%) ²
Capsule/bugs		$3253.0 \pm 177.4 \text{ b}$	$3227.1 \pm 177.4 \text{ b}$	3.3 ± 0.8 a	3083.1 ± 74.3^{ns}	3057.2 ± 74.3^{ns}	$2.5\pm0.3^{\text{ns}}$
Pupa/bugs		-	_	-	3414.1 ± 279.5	3388.2 ± 279.5	3.3 ± 1.0
Capsule/fungicide		-	_	-	3508.1 ± 159.9	3482.2 ± 159.9	3.0 ± 0.7
Pupa/fungicide		-	_	-	3453.0 ± 118.0	3427.1 ± 118.0	2.8 ± 0.6
IPM		$3855.7 \pm 143.4 \text{ ab}$	$3803.9 \pm 143.4 \text{ ab}$	0.7 ± 0.2 b	3320.7 ± 121.3	3294.8 ± 121.3	4.3 ± 0.5
PUI		4661.5 ± 153.1 a	4583.75 ± 153.1 a	$1.8\pm0.6~ab$	-	_	-
Statistic	F	11.21	10.41	6.11	0.70	0.90	0.78
	р	0.0229	0.0260	0.0357	0.6693	0.4941	0.5565
	df _{error}	7	7	7	12	12	12

Means (\pm SE) followed by the same letter in the column were not statistically different from each other applying the Tukey test, at *p* > 0.05. ¹Net income calculated by considering US\$ 0.61/soybean kilogram and insecticide application average value of US\$ 15.81/hectare/application (FAOstat, 2020). ²Original means followed by statistics performed on the data were transformed by \sqrt{X} . –Treatment not evaluated in the crop season.

number of insecticide applications used in IPM or PUI areas, yield or net income did not differ between *T. podisi* and insecticide treatments (Table 1).

4. Discussion

Telenomus podisi has been extensively studied over the last years in laboratory and other controlled conditions to allow their proper use for stink bug management in soybean, mainly due to its high parasitism capacity (Silva et al., 2018). Accumulated knowledge indicates that several biotic and abiotic variables might influence the fitness of a mass-reared parasitoid and consequently its efficiency in augmentative biological control programs (van Lenteren and Bueno, 2003; Castellanos et al., 2019). In extensive crop areas such as soybean, abiotic variables such as high temperatures, or biotic factors such as predation rates of released pupae by ants and other arthropods are the most important impacts on biocontrol agents in field conditions (Pratissoli et al., 2002; Pinto and Parra, 2002). In contrast to many European countries where biological control is used, Brazil has an enormously diverse fauna, including many ant species. These ants can prey on natural enemies when they are exposed and susceptible, reaching 100% predation within a few hours after release (Parra, 2014). Therefore, it is important to determine whether the release of protected or unprotected parasitoid pupae is the better strategy (Pinto and Parra, 2002). Consequently, the results of this study provide important information for the improvement of field recommendations for augmentative biological control of stink bugs using T. podisi. Overall, our results clearly indicate that T. podisi can be released using both encapsulated and unprotected pupae with similar efficacy. Their release in the field efficiently increased stink bug egg parasitism to 70% and 50% in the crop seasons of 2017/2018 and 2018/2019, respectively. The number of parasitized eggs in this study was calculated as the number of emerged parasitoids plus the number of adult parasitoids that were fully developed but dead inside the host (observed by dissections). Considering this, the efficacy of T. podisi to control stink bugs eggs may have been even higher. During the parasitization process a parasitoid can kill stink bug embryos simply by introducing its ovipositor into the host egg, even without depositing eggs, resulting in dead host eggs with undefined contents (Ganesalingam, 1966). This type of damage to the host was not evaluated or accounted for as a parasitoid effect in our work.

Successful results of egg parasitoid release have also been reported for other Platygastridae species. For example, Mineo and Lucido (1976) recorded that releases of Gryon muscaeformis (Hymenoptera, Platygastridae) in hazel resulted in higher parasitism rates on Gonocerus acuteangulatus (Heteroptera: Coreidae) eggs. Also, mass-releases of Trissolcus basalis(Hymenoptera: Platygastridae) in soybean fields resulted in significant increases in parasitism on eggs of Nezara viridula (Hemiptera: Pentatomidae) (Thomas et al., 1972; Lee, 1979). Years later, T. basalis was also reported as a successful augmentative biological control program to control N. viridula in Brazil, adopted in the 1980s and 1990s in soybean fields (Corrêa-Ferreira, 2002). Similarly, early-season releases of Telenomus gifuensis (Hymenoptera: Platygastridae) were found to increase parasitism on eggs of Scotinophara lurida (Heteroptera: Pentatomidae), leading to higher rates than recorded in untreated areas (Hidaka, 1958). However, as far as we know, our study is the first to evaluate T. podisi control of stink bugs, comparing release strategies in soybean fields. Euschistus heros, which was dominant in the present study (over 90% of the insects), is the most abundant species in most of the important soybean areas in Brazil such as the northern state of Paraná and the Midwest region (Cividanes and Parra, 1994) and T. podisi is regarded as its most important biological control agent (Pacheco and Corrêa-Ferreira, 2000) emphasizing the importance of our findings.

The overuse use of pesticides, particularly against stink bugs, has been a great challenge in the soybean production system in Brazil and other soybean production areas of South America (Bueno et al., 2013; Panizzi, 2013). Therefore, even though the yield resulting from the

T. podisi treatment in the 2017/2018 trial was lower, the more intense insecticide use in the PUI treatment must be taken into consideration because the overuse use of pesticides may lead to human or environmental contamination as well as faster pest resurgence and selection for insect resistance (Meissle et al., 2010; Tang et al., 2010). These negative effects are not accounted for in net income analyses due to difficulties of evaluating their consequences. The overuse use of synthetic insecticides often promotes selection for pest resistance because they impose significant selection pressure on the pest population (Metcalf, 1986; Kogan, 1998; Pimentel, 2005). Thus, the long-term benefits of a more environment-friendly pest control strategy, such as the use of T. podisi, may make this strategy worthwhile, even at the risk of a small reduction in productivity. For instance, the organic market usually brings higher prices, which may compensate for possible small yield losses. To be eligible for the organic market, biological control is essential since synthetic pesticides are not allowed in organic production. Furthermore, organic farming has demonstrated environmental benefits at farm level, which increases the value of biological control treatments. Moreover, the similarity in yield, net income, and bean quality observed in 2018/2019 reinforces the assumption that more intensive insecticide use does not necessarily increase yield or quality of the seeds. In fact, the intensity of damage observed in both crop seasons was less than 6%, which is still acceptable for the production of seeds, where standards are more demanding than in grain production. Thus, it is very important to emphasize that "prophylactic" stink bug control adopted by many growers will neither increase yields nor net income, but generally raise production costs, increase the problem of pest resistance, and have greater impact on natural enemies and human health.

In the second season, the moment of both T. podisi releases (at the occurrence of the first stink bug adults or with first fungicide spraying) had a similar overall effects. There are two possible explanations for this outcome. First, the time difference between these two strategies was only seven days and therefore, considering a T. podisi parasitism phase of 15-20 days (Silva et al., 2018), the first parasitoids released at the time of the first fungicide spraying were probably still alive when the first adults were captured in the ground cloth a week later. Second, a ground cloth, a sampling tool developed and efficient for insecticide spray purposes, might not be the best tool to be adapted to determine the best moment for parasitoid release. Synchronization of stink bug eggs with parasitoid adults in the field is crucial for determining parasitism effectiveness (Orr, 1988). Therefore, a well-defined pest monitoring method still needs to be better studied in future research to be used for the determination of the best moment for parasitoid releases. It is important mainly considering the extensive crop areas of Central Brazil, as in the states of Goiás, Mato Grosso, Mato Grosso do Sul, Bahia, Maranhão and others (Parra, 2014). The use of pheromones and remote sensing are possible alternatives but their application still needs to be further studied in order to precisely determine the best time for T. podisi release in the field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Adeney de Freitas Bueno: Writing - original draft. Érica Caroline Braz: Writing - original draft. Bruna Magda Favetti: Writing - original draft. José de Barros França-Neto: Writing - original draft. Gabriela Vieira Silva: Writing - original draft.

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