Communication

Release of adults and pupae of *Telenomus podisi* (Hymenoptera: Scelionidae) for the management of *Euschistus heros* (Hemiptera: Pentatomidae) in soybean

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Abstract: *Euschistus heros* (Hemiptera: Pentatomidae) is one of the main pests of soybean. Egg parasitoids such as *Telenomus podisi* (Hymenoptera: Scelionidae) can be highly effective in attacking *E. heros* eggs, preventing the establishment at levels of economic damage. This study evaluated two release methods of *T. podisi* in soybean. The treatments compared results from release of *T. podisi* adults fed with honey in cardboard capsules and the release of pupae in bulk. Four aerial releases per area were carried out via drone (7,500 females/ha) distributed at 10 points. The population density of *E. heros*, productivity and reproductive aspects of soybean were evaluated. Total population of *E. heros* was lower in adult release treatment and male population was lower with pupae release. Thousand grain weight had higher values in fed adults treatments compared to release of pupae and control. Number of damage grains was lower in both release treatments compared with the control area. Therefore, the use of biological control with inundative release of *T. podisi*, regardless of the release method, is recommended within IPM programs, supporting production parameters and benefiting the sustainability of the agroecosystem.

Keywords: biological control; integrated pest management; egg parasitoids; sustainability

1. Introduction

The brown stink bug, *Euschistus heros* (Hemiptera: Pentatomidae) is one of the main pests of soybean (*Glycine max* L.) and lowers yield through feeding on both pods and grains [1]. It is the most abundant species in soybean fields in the Neotropical region, which includes Brazil, Argentina and Paraguay [2]. The demands for more sustainability in crops and the low availability of active ingredients for the management of soybean pest pentatomids make biological control grow annually between 10 and 15% in the world [3] and even more in Brazil [4]. Egg parasitoids, such as *Telenomus podisi* (Hymenoptera: Scelionidae), even though being generalists, are highly effective in management of *E. heros* eggs, presenting 80% of parasitism in the field and limiting population increases of the pest at the beginning of its development (egg stage) [5]. These wasps are 1 mm long, black in color and are reared in specialized laboratories for large-scale production, using eggs from the natural host (*E. heros*) [6, 7]. In 2019, *T. podisi* was registrated for commercial use in Brazil [8], increasing the management tactics for pentatomids within Integrated Pest Management (IPM) programs.

The mass release of *T. podisi* is usually carried out at the end of the soybean vegetative stage [9], in order to prevent the population of pentatomids from reaching the crop at levels of economic damage in the critical stage (reproductive stage). Because of advances in technology and the size of cultivated areas, the use of drones for the release of parasitoids has been increasing in agriculture. Parasitoids can be released from drones as pupae (eggs parasitized by *T. podisi*) or as adults. The pupal stage can be more easily manipulated in the release process, reducing labor and contributing to the homogeneous distribution of parasitized eggs in the crop [10]. However, because pupae are immobile, they may suffer greater predation or be more affected by adverse environmental conditions.

The release close to the emergence of adults is a key factor for the efficiency of this management method [10]. Alternatively, the release of adults may be more advantageous because of their ability to disperse more easily and avoid

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possible predators and unfavorable environmental conditions, being able to locate shelter in the field [11]. It is known that parasitoids first prioritize the location of food sources and then carry out reproductive activities (parasitism) [12]. Therefore, supplying a food source inside the release capsules may benefit survival and host-finding, as it reduces the insect's exposure time in the agricultural environment, but may also increase labor costs. The lack of studies on release of different stages of *T. podisi* may limit the use of parasitoids among producers or limit its effectiveness. Thus, it is necessary to evaluate the influence of these different methods on the *E. heros* population and on soybean yield in the absence of other control methods. Therefore, we assessed in this work two release methods of *T. podisi*, comparing pupae and fed adults release, allowing us to identify effective tools to manage *E. heros* in soybean crops.

2. Results

2.1. E. heros field density

The average of total population of E. heros in the areas of fed adults and pupae release did not differ significantly from each other. The control area and pupae release also did not differ statistically (F: 4.58; P: 0.025) (Table 1). The male population of E. heros in the treatment where adults were released was statistically similar with the other two area. However, the male population in the area of pupae release was significantly less than the number found in the control area (F: 3.94; P: 0.038) (Table 1). The population of females (F: 0.49; P: 0.62) and nymphs (F: 1.48; P: 0.253) of E. heros did not differ among the three treatments.

Table 1. Average of total population, population of males, females and nymphs of Euschistus heros per sample collected in the field in the areas corresponding to the treatments. Means followed by the same letter in the column do not differ at a 5% significance level.

Treatment	***Euschistus heros* average population**			
	Total	**Males**	Females	Nymphs
Area 1[a]	1.66±0.09 b	1.41±0.13 ab	1.59±0.07 a	1.62±0.32 a
Area 2[b]	1.72±0.14 ab	1.35±0.11 b	1.52±0.14 a	2.44±0.14 a
Area 3[c]	2.06±0.05 a	1.73±0.06 a	1.66±0.05 a	2.06±0.47 a

[a]Area 1: release of fed T. podisi adults. [b]Area 2: release of T. podisi pupae. [c]Area 3: control (no release of T. podisi and no pesticide application).

2.2. Soybean reproductive parameters and productivity

The number of pods per plant, number of grains per plant, number of grains per pod and productivity did not differ among treatments (F: 0.89; P: 0.414; F: 0.91; P: 0.407; F: 1. 12; P: 0.331; F: 1.9; P: 0.157 respectively) (Table 2). However, the thousand grain weight differed among all treatments, with the area of fed adults release having the highest value (F: 26.68; P < 0.01). The number of damaged grains also differed among treatments, (F: 11.91; P < 0.01), where both release methods showed better results in comparison with the control area (Table 2).

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Table 2. Productivity and reproductive parameters of soybean samples collected in the areas corresponding to the treatments. Means followed by the same letter in the column do not differ at a 5% significance level.

	Parameters					
Treatment	**Nº pods/plant**	**Nº grains/plant**	**Nº grains/pod**	Productivity (bags/ha)	Thousand grain weight (g)	Nº damage grains
Area 1[a]	84.08±11.09	200.88±25.54	2.43±0.04	12.70±1.58	155.03±1.50 a	6.5±1.52 a
Area 2[b]	68.36±5.78	162.88±15.36	2.35±0.05	9.50±0.91	148.26±1.67 b	5.37±0.65 a
Area 3[c]	70.64±5.50	166.80±12.96	2.35±0.04	9.20±0.72	138.50±1.64 c	12.87±1.17 b

[a]Area 1: release of honey fed Telenomus podisi adults. [b]Area 2: release of T. podisi pupae. [c]Area 3: control (no release of T. podisi and no pesticide application).

3. Discussion

Given the efficiency of *T. podisi* parasitism on eggs of pest pentatomids, especially *E. heros* [13], the difference in the total and male population of brown stink bugs between areas can best be explained by the density of parasitoids released in the field. In general, it is recommended to release at least 15,000 *T. podisi* females per hectare throughout the season, which can be divided into three or four applications [14]. To reach the recommended dose, four releases of 7500 females per hectare were carried out in this study. Therefore, the high dose of released parasitoids was sufficient to decrease the population of *E. heros* in the treated areas. In our work, releases were performed late (R4 stage). Stink bugs usually arrive at soybean crops at the end of the vegetative stage and remain until the end of the reproductive stage [9]. When released in early dates, *T. podisi* shows more efficacy to control nymphs and adults as well [15]. However, despite being released in the middle of reproductive stage, the parasitoids lowered the populations of *E. heros* in comparison with the control area. Thus, our research demonstrates that biological control programs with egg parasitoids can be an useful management tactic even with high density of pentatomids in soybean.

Historically, parasitoids of the genus *Trichogramma* are released in the adult phase [11, 16]. However, the release of pupae in bulk has showed equal or greater efficiency mainly in unfavorable environmental conditions [11, 17-19]. When comparing the population of *E. heros* males and some soybean parameters, our results demonstrated that pupae and adult release are similar. Altought previous feeding of adults parasitoids has not been deeply studied before, mainly with the genus *Telenomus*, the presence of sugars (honey) can contribute to increasing the effectiveness of parasitoids in the field by increasing fecundity and longevity [20-22]. However, the labor of preparing the capsules manually by offering honey to the parasitoids one by one must be evaluated. Considering that and the parameters that we studied, the release of pupae would be more efficient and practical, especially in extended areas of soybean crops. Additioanlly, the capsules can improve the chance of contamination by fungi or lead to drowning of the parasitoids [23]. To give more solid conclusions, further studies with cost and time of both methods should be evaluated. Studies comparing release of adults with pupae have not previously been reported and are important to develop new techniques for biological control programs.

Bioassays carried out in the field can be influenced by external factors that are difficult to control and that affect the quality and productivity of the crop [15], such as high or low temperatures, rainfall, type of variety, migration of new pests, etc. In addition, as the soybean cultivar used and the agronomic management carried out in the field were the same in the three experimental areas, similarities in productive reproductive parameters between treatments were found.

The excessive use of pesticides can act as a selection pressure, leading to pest resistance [24-26]. Therefore, the use of biological control in order to reduce the use of synthetic products in IPM programs can be beneficial to the environment and human health, even without influencing the final productivity [24, 27, 28] and, as observed in our work, increase the quality of soybean grains. Reducing the application of pesticides would also be possible because the population of *E. heros* was smaller than in the control area. In addition, continuous releases throughout the seasons may increase the natural population of the parasitoid, further contributing to the management of target pests [29, 30].

4. Materials and Methods

4.1. Rearing of E. heros and T. podisi in the laboratory

The parasitoids used in these experiments were reared in the laboratory of the Research Group on Integrated Pest Management in Agriculture (AGRIMIP) at the School of Agricultural Sciences (FCA/UNESP) in the municipality of Botucatu (22°50'42"S 48°26'03.9 "W). The rearing of *T. Podisi* was maintained under controlled conditions of light (14:10 L:D), relative air humidity (70±5%) and temperature (25±2°C). The parasitoids were reared in *E. heros* eggs, the preferred host [13]. The rearing of *E. heros* was carried out under the same conditions at the same site of the parasitoids. The brown stink bugs were maintained on a diet consisting of green bean pods (*Phaseulus vulgaris* L.) and raw peanuts (*Arachis hypogaea* L.) in 25 × 25x12 cm plastic boxes, closed with voile fabric [31]. Eggs collected from *E. heros* were glued onto paper cards based on the demand for releases in the field. Adults of *T. podisi* were placed along with *E. heros* eggs into three-liter plastic pots and closed with plastic film and an elastic band. Pure honey was provided as food for the adult parasitoids with the aid of a brush. Twice a week, eggs of *E. heros* were offered to *T. podisi* in the rearing units, allowing parasitism for 24 hours, before adults were removed and added to new pots to continue the rearing and obtain the necessary quantity for release in the field.

4.2. Release of *T. podisi* in the field

The releases of *T. podisi* were carried out in the 2021/2022 soybean crop at the experimental unit of the Fazendas de Ensino, Pesquisa e Extensão (FEPE) in São Paulo State University located in the municipality of Botucatu (22°49'24.5"S 48°25'51.8 "W). The experiment consisted of three treatments of one hectare each: area 1) release of fed *T. podisi* adults in biodegradable cardboard capsules; area 2) *T. podisi* pupae released in bulk and area 3) control treatment (without release of biological agents or application of pesticides). For the adult treatment, pure honey was used as an energy source. When the first adults began to emerge, a fillet of pure honey was deposited inside of the capsules before releasing them. To synchronize the release of adults and pupae treatments, parasitized eggs containing *T. podisi* pupae (destined for treatment 2) were placed at 22 °C in Biochemical Oxygen Demand (BOD) climatic chambers to control emergence time. When the first adults began to emerge, the pupae stored at 22 °C were moved to 25 °C, and released into the field afterwards. The soybean cultivar sown was Neo 610 IPRO (Neogen®) and the plants were at the same phenological stage (R4) and under the same planting conditions in all areas of the experiment. Pesticides were not applied in any area of study. In areas 1 and 2, four releases of 7500 females each were carried out, distributed at 10 points. Aerial releases were performed with the DJI Phantom 4 Pro model drone, equipped with a dispenser for bulk release with a capacity of 200 ml of *T. podisi* pupae or a dispenser adapted for individual release of cardboard capsules containing parasitoid adults. In both areas, release occurred at a height of 30 meters and a distribution range of 25 meters, with flights carried out in the longitudinal direction of the plot to improve the distribution of pupae and adults of *T. podisi* [32]. The releases were carried out in the morning, with the absence of strong winds, under mild temperatures and high relative humidity, allowing thermal adaptation of the parasitoids in the plots throughout the day.

4.3. *E. heros* field density

The average density of the total population, males, females and nymphs of *E. heros* in the treated areas was evaluated using the beat cloth method, with a sampling of 10 points per area. This method was performed at the same time of day one day before each release and three days after each release, totaling eight evaluations throughout the bioassay. The beating cloth (1.0x1.4 m) was positioned horizontally below the soybean planting line, hitting the plants on the cloth so that the insects present would wall onto the cloth to be counted. In each beating, one meter of soybean plants was evaluated.

4.4. Soybean reproductive parameters and productivity

To evaluate the reproductive parameters and productivity, 25 soybean plants from each area were collected across the hectare to evaluate the number of pods per plant, number of grains per pod and thousand grain weight. In addition, the number of grains damaged was determined using the tetrazolium test. For this test, two subsamples of 50 seeds from each treatment were obtained. The seeds were soaked in water for about 16 hours at 25 °C until they reached about 2.5 times their initial weight. After this period, they were immersed in a tetrazolium salt solution at a concentration of 0.075%, kept in a BOD chamber at 30 °C in the dark for 3 hours and then washed to interpret the results [33, 34]. From the color of the seeds, it was possible to identify living and dead tissues, based on the activity of dehydrogenase enzymes, allowing the identification of damage caused by pentatomids. The grains that contained whitish spots were counted as damaged. To calculate the productivity of each treatment, the number of plants per hectare and the average grain weight were also collected, using the formula (equation 01) to generate bags per hectare.

$$Productivity = (\frac{plants}{hectare} * \frac{pods}{plant} * \frac{grains}{plant} * average\ grain\ weight)/60000 \quad \text{(eq. 01)}$$

4.5. Statistical analysis

Data on *E. heros* population density, productivity and soybean reproductive parameters of treatments were submitted to exploratory analyzes to evaluate the assumptions of normality [36] the homogeneity of variance of treatments [37] and the additivity of the model to allow the application of ANOVA. Subsequently, the means were compared by Tukey's test (P<0.05) using the statistical software Minitab 19.

5. Conclusions

Based on our results, we recommend the release of both methods of *T. podisi* to manage *E. heros* in soybean, despite no significant effect on the final productivity of the evaluated area.

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