EGG STOCKING OF *Euschistus heros* (FABRICIUS) (HEMIPTERA: PENTATOMIDAE) TO MAXIMIZE PRODUCTION OF THE EGG PARASITOID *Telenomus podisi* ASHMEAD (HYMENOPTERA: SCELIONAE)

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SUMMARY

Among the phytophagous insects that attack the soybean crop, the brown bug, Euschistus heros *(Fabricius, 1794) (Hemiptera: Pentatomidae) stands out because of its competitive ability in relation to other pentatomids and because it is the most abundant species in the Neotropics, including Brazil. However, the use of* Telenomus podisi *Ashmead (Hymenoptera: Scelionae) as biological control tool have been studied. This work aimed to study how 3 different materials and 6 storage periods, at 4 temperatures of parasitized and non-parasitized eggs of* E. heros *could interfere with the biological characteristics of* T. podisi*. The best biological parameters were observed when the eggs were stored in nitrogen tank in aluminum at 60 days (0.90 ± 0.02 and 0.98 ± 0.02), while the lowest were observed at 60 days when stored in tubes in biochemical oxygen demand (BOD) (0.00 and 0.00). The highest values of egg-adult period are observed when eggs were stored in tubes for 30 days in freezer (16.86 ± 0.37) and BOD (16.90 ± 0.15). When the pupae of the parasitoid were stored in aluminum tubes at the same temperatures mentioned above for 1, 2, 3, 4 and 5 days, only adults emerged when stored in refrigerator and BOD, presenting highest levels of emergence and sex ratio in BOD. These experiments may contribute to an increased production and the availability of the biological agent throughout the year, regardless of climatic conditions.*

Introduction

The cultivation of grains in Brazil occupies a large area of the country, with special emphasis laid on soybean cultivation, *Glycine max* L., one of its most important export products. The estimated production during 2019/2020 was 122 million tons, the largest in the world and seeing a 6.1% increase from the 2018/19 crop (Conab, 2020). However, production can be further increased with the reduction of losses from insect pests, mainly bedbugs and caterpillars.

The imbalance caused by excessive use of plant protection products, mainly in the absence of technical positioning, has changed the dynamics of insect occurrence, with population outbreaks of pests, which were previously considered secondary due to suppression factors such as natural enemies (Bueno *et al*., 2013; Czepak *et al*., 2013).

The change in the agricultural landscape is concerning to producers with regard to pest control. In this context, a group of insects that cause great damage to the soybean crop are the phytophagous insects, mainly of the Pentatomidae family, considered key pests that cause serious damage, both in the yield of oil extracted from the grains and in the quality of the grains themselves (traits such as "shriveling") (Panizzi and Lucini, 2016; Tuelher *et al*., 2016). Among the phytophagous insects that attack the soybean crop, the brown bug, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) stands out because of its competitive ability in relation to other pentatomids and because it is the most abundant species in the Neotropics, including Brazil (Krinski *et al*., 2013).

In this context, in order to manage pests, an interdisciplinary and multidisciplinary approach integrating various control methods more sustainable for man and the environment may be feasible. Complementary tactics for the successful control of insect-pests can be incorporated within the premises of

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ALMACENAMIENTO DE HUEVOS DE *Euschistus heros* (FABRICIUS) (HEMIPTERA: PENTATOMIDAE) PARA MAXIMIZAR LA PRODUCCIÓN DEL PARASITOIDE DEL HUEVO *Telenomus podisi* ASHMEAD (HYMENOPTERA: SCELIONAE)

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RESUMEN

Entre los insectos fitófagos que atacan el cultivo de soja, la chinche marrón, Euschistus heros *(Fabricius, 1794) (Hemiptera: Pentatomidae) se destaca por su capacidad competitiva en relación a otros pentatómidos y por ser la especie más abundante en el Neotrópico, incluyendo Brasil. Sin embargo, se ha estudiado el uso de* Telenomus podisi *Ashmead (Hymenoptera: Scelionae) como herramienta de control biológico. Este trabajo tuvo como objetivo estudiar cómo 3 materiales diferentes y 6 periodos de almacenamiento, a 4 temperaturas de huevos parasitados y no parasitados de* E. heros *podían interferir en las características biológicas de* T. podisi*. Los mejores parámetros biológicos se observaron cuando los huevos se almacenaron en tanque de nitrógeno en aluminio a los 60 días (0,90 ± 0,02 y 0,98 ± 0,02), mientras que los más bajos se observaron a los 60 días cuando se almacenaron en tubos en demanda bioquímica de oxígeno (DBO) (0,00 y 0,00). Los valores más altos del periodo huevo-adulto se observan cuando los huevos se almacenaron en tubos durante 30 días en congelador (16,86 ± 0,37) y DBO (16,90 ± 0,15). Cuando las pupas del parasitoide se almacenaron en tubos de aluminio a las mismas temperaturas mencionadas durante 1, 2, 3, 4 y 5 días, sólo emergieron adultos cuando se almacenaron en frigorífico y DBO, presentando los niveles más altos de emergencia y proporción de sexos en DBO. Estos experimentos pueden contribuir a aumentar la producción y la disponibilidad del agente biológico durante todo el año, independientemente de las condiciones climáticas.*

ESTOCAGEM DE OVOS DE *Euschistus heros* (FABRICIUS) (HEMIPTERA: PENTATOMIDAE) PARA MAXIMIZAR A PRODUÇÃO DO PARASITOIDE DE OVOS *Telenomus podisi* ASHMEAD (HYMENOPTERA: SCELIONAE)

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RESUMO

Dentre os insetos fitófagos que atacam a cultura da soja, destaca-se o percevejo marrom, Euschistus heros *(Fabricius, 1794) (Hemiptera: Pentatomidae), por sua capacidade competitiva em relação aos demais pentatomídeos e por ser a espécie mais abundante na região Neotropical, incluindo o Brasil. Entretanto, o uso de* Telenomus podisi *Ashmead (Hymenoptera: Scelionae) como ferramenta de controle biológico tem sido estudado. Este trabalho teve como objetivo avaliar como 3 materiais diferentes e 6 períodos de armazenamento, em 4 temperaturas de ovos parasitados e não parasitados de* E. heros *poderiam interferir nas características biológicas de* T. podisi*. Os melhores parâmetros biológicos foram observados quando os ovos foram armazenados em tanque de nitrogênio em alumínio aos 60 dias (0,90 ± 0,02 e 0,98 ± 0,02), enquanto os menores foram observados aos 60 dias quando armazenados em tubos em demanda bioquímica de oxigênio (BOD) (0,00 e 0,00). Os maiores valores de período ovo-adulto são observados quando os ovos foram armazenados em tubos por 30 dias em freezer (16,86 ± 0,37) e BOD (16,90 ± 0,15). Quando as pupas do parasitoide foram armazenadas em tubos de alumínio nas mesmas temperaturas citadas acima por 1, 2, 3, 4 e 5 dias, apenas adultos emergiram quando armazenados em geladeira e BOD, apresentando maiores níveis de emergência e razão sexual em BOD. Esses experimentos poderão contribuir para o aumento da produção e da disponibilidade do agente biológico durante todo o ano, independente das condições climáticas.*

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integrated pest management (IPM). In this sense, the use of biological control as a more sustainable form of pest management is becoming more important. The method has a high potential for success, since it deals with the release of natural enemies that can reduce the pest population to levels below that of economic damage (Van Lenteren *et al*., 2017).

Among the insects used in biological control programs, the egg parasitoid *Telenomus podisi* (Ashmead, 1893) (Hymenoptera: Scelionae) has shown promising results. Since *E. heros* is one of its main hosts and a frequent pest in soybean crops, studies have been conducted with this parasitoid (Silva *et al*., 2018).

The conditions that induce and regulate the hibernation and diapause processes in egg parasitoids are also of extreme interest because the control of these processes can increase the efficiency of their large-scale multiplication to be used as biological controls, as advocated for other egg parasitoid species (Pastori *et al*., 2013; Ghosh and Ballal, 2017).

The mass production of *T. podisi* is limited by the absence of hosts during autumn and, mainly, winter. Therefore, it is necessary to develop alternative methods of mass production for areas where hosts are not available during these periods, ensuring the survival of the natural enemy. One method successfully used for egg parasitoid production during summer off-season in southern Brazil is the storage of host eggs in liquid nitrogen, as demonstrated for *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) (Doetzer and Foerster, 2013). However, for other species of bedbugs, there are still very few data available on the potential use of cold storage of

eggs as a method of mass production of parasitoids.

Therefore, the objective of this work was evaluate the biological aspects of the parasitoid on *E. heros* eggs stored at low temperature, as well as to determine the ideal temperature and best means of eggs storage, that keep the effective action of *T. podisi*, to increase their duration of availability on the market, as well as to plan inundative releases by monitoring phytophagous pentatomids in soybean cultivation. These are fundamental steps for the commercial development for the recommendation of the use of *T. podisi* in biological control.

Materials and Methods

The experiments were conducted in the Research group on integrated pest management in agriculture - AGRIMIP laboratories and experimental field, located in the School of Agronomy – FCA, Campus of Botucatu of São Paulo State University "Júlio de Mesquita Filho" – UNESP, SP (latitude 22º 53' 09" S longitude 48º 26' 42" W).

Rearing of Euschistus heros

The adult bedbugs were held in square, plastic cages (4.5L), with the lid covered with a tulle-type fabric to allow ventilation. The bottom of the cage was covered with filter paper, on which moistened cotton and the diet composed of fresh bean pods (*Phaseolus vulgaris* L.) and peanut grains (*Arachis hypogaea* L.) were placed. On the sides, small rectangles of tulle-type fabric (10 × 10cm) were attached as oviposition substrate.

Rearing maintenance was performed twice a week to replace food, remove dead insects and exuvia, and three times to remove eggs. The eggs removed from the adult cages were placed in a plastic capsule (6cm diameter) containing a moistened piece of cotton wool, until they hatched. From the second instar, the nymphs were placed in square plastic cages (4.5L) containing the same diet as the adults. When they reached adulthood, they were sexed and transferred to new cages, each of which held up to 300 adults per cage. The rearing was maintained under controlled conditions of 25 ± 2°C, 70 ± 10% relative humidity and 14/10h photoperiod (light/dark).

Breeding and multiplication of the parasitoids

The adults of *T. podisi* were reared in eggs of *E. heros*, which were glued on rectangular cards made of cardboard and placed in plastic jars sealed with PVC plastic film, along with recently emerged *T. podisi* females – where parasitism was allowed for 24h. After this period, the eggs were removed and transferred to a new pot until emergence. Honey was offered as a food source for the parasitoids. The rearing was maintained under controlled conditions of 25 ± 2°C, 70 ± 10% relative humidity and 14/10h photoperiod (light/dark).

Storage of fresh eggs of E. heros

The eggs were stored in a liquid nitrogen tank (NL), freezer (F), refrigerator (G) and biochemical oxygen demand (BOD) (B) at the temperatures, -196ºC, -18ºC, 5ºC and 15ºC, respectively.

Due to the low oviposition of eggs, the experiment was readjusted. The number of replicates was increased to 10 and the number of eggs per replicate to 30, with one more storage condition and two more storage periods of 5 and 10 days. The eggs were stored in portions, so eggs for the 60-day storage period were stored first, starting with the treatments T1.1 (cryogenic tube + NL), T1.2 (cryogenic tube + freezer), T1.3 (cryogenic tube + refrigerator) and T1.4 (cryogenic tube + BOD). This was followed with T2.1 (cryogenic tube + aluminum foil + NL), T2.2 (cryogenic tube + aluminum foil + freezer), T2.3 (cryogenic tube + aluminum foil + refrigerator) and T2.4 (cryogenic tube + aluminum foil + BOD). Finally, T3.1 (aluminum foil + NL), T3.2 (aluminum foil + freezer), T3.3 (aluminum foil + refrigerator), and T3.4 (aluminum foil + BOD) were used. The first number represents the stored period (1–3 corresponds to 60 days, 4–6 to 45 days, 7–9 to 30 days, 10–12 to 15 days, 13–15 to 10 days and 16–18 to 5 days) and the second number, the storage temperature (1= NL [-196°C], 2= freezer [-18°C], 3= refrigerator [5°C] and 4= BOD [15°C]). After the storage period, the eggs were removed and incubated at a temperature of 30°C for 5 seconds to maintain their physiological quality. After this process, 30 eggs were glued per rectangular cardboard card (2 × 8cm). These cards were placed in flat bottom tubes (2 × 8cm) sealed with PVC plastic film. In each tube was placed a female of *T. podisi* of 48h and a drop of honey, which served as food for the female. After 24h, the cells with eggs were removed and placed in new flat bottom tubes until the emergence of *T. podisi* and subsequent evaluation of parasitism, development time from egg to adult, emergence, sex ratio, and longevity of adults.

The experimental design was entirely randomized and the treatments were arranged in a 4 × 3 factorial arrangement (four storage temperatures and three storage materials), with 10 replicates per treatment.

Evaluation of E. heros *eggs parasitized by* T. podisi *stored at low temperature*

According to the results of item 3.3, fresh eggs were stored in aluminum foil in liquid nitrogen for 5 days. After this period, these eggs were removed and incubated at a temperature of 30°C for 5 seconds to maintain their physiological quality. Following this process, 30 eggs were placed in flat bottom tubes (2 × 8cm) sealed with PVC type plastic film. In each tube was placed a 48h female of *T. podisi* and a drop of honey, which served as food for the female. After 24h, the eggs were removed and divided into 20 treatments with 10 repetitions each. The treatments were NL5, F5, G5 and B5; NL4, F4, G4 and B4; NL3, F3, G3 and B3; NL2, F2, G2 and B2; NL1, F1, G1 and B1. The letter represents the place and temperature of storage (idem item 3.3) and the numbers corresponds to the period in days. Thus, the eggs parasitized by *T. podisi* when they reached the 10th day of development, (which corresponds to the pupal stage) were stored for 1, 2, 3, 4 and 5 days at each temperature, then removed and submerged at 30°C for 5 seconds to maintain their physiological quality. After this process, 30 eggs were glued per rectangular cardboard card (2 × 8cm). These cards were placed in flat bottom tubes (2 × 8cm), sealed with PVC plastic film until the emergence of *T. podisi*, and subsequent evaluation of parasitism, development time from egg to adult, emergence, sex ratio and longevity of adults was carried out.

To evaluate the fitness of females of the F1 generation, the 48h females that emerged from this last bioassay were individually placed in flat bottom tubes containing a rectangular cardboard (2 × 8cm) with 30 fresh eggs and a honey drop for feeding and observation of parasitism. Although most eggs were parasitized in all treatments, only adults from the refrigerator (5°C) and BOD (15°C) emerged.

Results and Discussions

For the bioassays performed, the following parameters were evaluated: parasitism, development time from egg to adult, emergence, sex ratio, and longevity of adults, as observed in the Tables I to VI.

TABLE I
BIOLOGICAL CHARACTERISTICS OF *Telenomus podisi* REARED IN *Euschistus heros* EGGS STORED IN BOD (15 ±2ºC) IN DIFFERENT FORMS AND PERIODS

		15°C		
	Days	Tube	Tube+Aluminum	Aluminum
Parasitism	5	0.43 ± 0.05 B ns	0.62 ± 0.05 A ns	0.65 ± 0.05 AB ns
	10	0.77 ± 0.01 A ns	0.68 ± 0.07 A ns	0.59 ± 0.03 B ns
	15	0.61 ± 0.04 A ns	0.77 ± 0.04 A ns	0.77 ± 0.04 A ns
	30	0.77 ± 0.05 A a	0.30 ± 0.02 B b	0.71 ± 0.04 AB a
	45	0.09 ± 0.03 C b	0.38 ± 0.06 B a	0.02 ± 0.01 C b
Viability	5	0.43 ± 0.07 AB b	0.63 ± 0.07 AB a	0.69 ± 0.04 A a
	10	0.68 ± 0.09 A ns	0.77 ± 0.05 A ns	0.63 ± 0.04 AB ns
	15	0.64 ± 0.04 A ns	0.49 ± 0.06 B ns	0.58 ± 0.05 AB ns
	30	0.69 ± 0.05 A a	0.00 ± 0.00 C c	0.53 ± 0.04 B b
	45	0.24 ± 0.11 BC b	0.66 ± 0.09 AB a	0.00 ± 0.00 C c
	60	0.00 ± 0.00 C ns	0.20 ± 0.07 C ns	0.00 ± 0.00 C ns
Sex ratio	5	0.33 ± 0.14 BC ns	0.92 ± 0.02 A ns	0.88 ± 0.02 A ns
	10	0.68 ± 0.15 AB ns	0.96 ± 0.01 A ns	0.83 ± 0.07 A ns
	15	0.80 ± 0.10 A ns	0.67 ± 0.10 AB ns	0.68 ± 0.15 A ns
	30	0.87 ± 0.04 A a	0.00 ± 0.00 C b	0.94 ± 0.02 A a
	45	0.20 ± 0.13 C b	0.67 ± 0.13 AB a	0.00 ± 0.00 B b
	60	0.00 ± 0.00 C a	0.40 ± 0.16 B a	0.00 ± 0.00 B a
Egg-Adult (Days)	5	12.63 ± 1.42 AB ns	13.00 ± 0.15 A ns	13.52 ± 0.09 B ns
	10	11.40 ± 1.29 AB ns	12.41 ± 0.15 A ns	13.41 ± 0.25 B ns
	15	13.25 ± 0.15 A ns	12.55 ± 0.24 A ns	12.24 ± 0.15 C ns
	30	16.90 ± 0.15 A a	0.00 ± 0.00 B b	15.30 ± 0.14 A a
	45	7.05 ± 2.89 B b	12.89 ± 1.44 A a	0.00 ± 0.00 D c
	60	0.00 ± 0.00 C b	8.00 ± 2.72 A a	0.00 ± 0.00 D b

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability. ns: not significant.

TABLE II
BIOLOGICAL CHARACTERISTICS OF *Telenomus podisi* REARED IN *Euschistus heros* EGGS STORED IN REFRIGERATOR (5 ±2ºC) IN DIFFERENT FORMS AND PERIODS

		5°C		
	Days	Tube	Tube+Aluminum	Aluminum
Parasitism	5	0.30 ± 0.03 B b	0.55 ± 0.06 B a	0.36 ± 0.05 B b
	10	0.71 ± 0.04 A ns	0.57 ± 0.06 AB ns	0.41 ± 0.04 B ns
	15	0.59 ± 0.05 A ns	0.57 ± 0.05 AB ns	0.61 ± 0.04 A ns
	30	0.74 ± 0.06 A ns	0.77 ± 0.03 A ns	0.67 ± 0.03 A ns
	45	0.37 ± 0.07 B b	0.59 ± 0.03 AB a	0.45 ± 0.02 B ab
	60	0.08 ± 0.03 C b	0.30 ± 0.06 C a	0.21 ± 0.03 C a
Viability	5	0.54 ± 0.08 A b	0.63 ± 0.05 NS b	0.84 ± 0.03 A a
	10	0.62 ± 0.04 A ns	0.65 ± 0.03 NS ns	0.65 ± 0.06 AB ns
	15	0.66 ± 0.04 A ns	0.42 ± 0.03 NS ns	0.40 ± 0.04 C ns
	30	0.63 ± 0.08 A a	0.66 ± 0.05 NS a	0.43 ± 0.06 C b
	45	0.74 ± 0.09 A ns	0.63 ± 0.06 NS ns	0.55 ± 0.03 BC ns
	60	0.15 ± 0.08 B b	0.58 ± 0.11 NS a	0.45 ± 0.06 BC a
Sex ratio	5	0.56 ± 0.13 A ns	0.94 ± 0.03 A ns	0.82 ± 0.09 NS ns
	10	0.93 ± 0.02 A ns	0.95 ± 0.02 A ns	0.68 ± 0.13 NS ns
	15	0.86 ± 0.09 A ns	0.84 ± 0.07 A ns	0.78 ± 0.10 NS ns
	30	0.79 ± 0.09 A ns	0.94 ± 0.02 A ns	0.88 ± 0.06 NS ns
	45	0.68 ± 0.12 A ns	0.67 ± 0.14 AB ns	0.91 ± 0.03 NS ns
	60	0.10 ± 0.10 B c	0.46 ± 0.13 B b	0.95 ± 0.05 NS a
Egg-Adult (Days)	5	13.56 ± 0.25 A ns	13.63 ± 0.18 AB ns	13.26 ± 0.23 B ns
	10	12.64 ± 0.17 A ns	12.21 ± 0.18 AB ns	12.92 ± 0.22 B ns
	15	12.90 ± 0.14 A ns	12.32 ± 0.21 AB ns	13.92 ± 0.31 B ns
	30	14.30 ± 1.60 A ns	14.87 ± 0.16 A ns	15.32 ± 0.22 A ns
	45	12.77 ± 1.44 A ns	14.35 ± 0.23 AB ns	15.52 ± 0.31 A ns
	60	4.03 ± 2.05 B b	11.36 ± 1.91 B a	15.53 ± 0.43 A a

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability. ns: not significant.

Biological characteristics of T. podisi *reared on* E. heros *eggs stored in different types of materials and storage periods at low temperature*

Despite a general association that low temperature storage affects the biological characteristics of insects (Chown and Terblanche, 2006; Van Baaren *et al*., 2006; Hance *et al*., 2007), the results reported here indicate that storage of *E. heros* eggs for 60 days at -196°C and -18°C in aluminum does not compromise their use as a rearing substrate for *T. podisi*. Storage of parasitoids and their rearing host at low temperatures has proven to be a valuable method of great interest to biological control companies for several reasons (Van Lenteren and Tommasini, 2003).

First, it contributes to the reduction of production costs and to the increase of the life span of the biological agents in order to provide a constant and sufficient quantity of insects for applied biological control programs. Secondly, the types of storage can provide substrate for multiplication of the biological agent when it is not in abundant quantity in nature in adverse seasons. Finally, storage allows greater planning for the release of natural enemies in the field as and when a need for control is perceived (Leopold, 1998; Van Lenteren and Tommasini, 2003).

The successful storage of eggs in liquid nitrogen (-196°C) has been tested many times and is well documented in the literature, but storage at -18°C or 5°C (obtained in freezer and refrigerator respectively), is still poorly addressed (Cosi *et al*., 2010).

For eggs stored in BOD (15 ± 2°C) there was no statistical difference in parasitism until 15 days of storage. Only the eggs stored in tubes or aluminum tubes showed satisfactory results when observed during the 30-day period. For emergence, the best results were observed at 30 days in tube, 10 and 45 days in tube + aluminum and until day 15 in aluminum.

The percentages of the sexual ratio observed in tubes were not different in the periods 10, 15 and 30 days, being observed only in 5, 45, and 60 days – with there being no emergence of adults in the last one. For those stored in tube + aluminum condition, only in the emerged insects of 60 days was there noticed a low sex ratio, excluding the period of 30 days that no adult emerged. For the adults emerged from the eggs stored in aluminum foil until 30 days, there was no significant difference. No adults emerged from the eggs stored for 45 and 60 days.

The egg to adult parameter in the period 45 and 60 days did not have enough data to perform statistical analysis. There was no emergence in the tube + aluminum at 30 days, so it was incorrect to compare it to the other days. For eggs stored in the refrigerator at 5 ± 2°C, no statistical difference was observed in the periods 10, 15 and 30 days of storage. Eggs stored for 30 days in all forms were found to be the best treatments.

In addition, a decrease in parasitism was observed after longer periods of storage of host eggs in tubes at 5°C, indicating that in addition to temperature, the duration of exposure and the material used are essential components of tolerance to low temperature storage in insects (Tables I and II) (Corrêa-Ferreira and Oliveira, 1998; Colinet and Boivin, 2011)

For emergence, the best results were observed in eggs stored up to 45 days in tube and tube + aluminum, while for eggs stored in aluminum, only those stored for up to 10 days showed significant emergence. For eggs stored for 60 days in the tube and tube + aluminum, there was a low sex ratio, which indicates that there is a possibility that the tube interfered with this parameter for this period, since the eggs stored in aluminum for 60 days and other periods had no significant difference between them or when compared to the other stored forms.

For the egg adult parameter, it was noted that there was no significant difference in any stored period when compared to the storage forms, except at 60 days, which cannot be stated due to the small sample number of eggs stored in tubes. When observed within each stored form, it was noted that neither the eggs stored up to 15 days in aluminum nor those stored in aluminum tube had any significant difference. The period of 30 days was the best storage period for the emergence of insects.

For eggs stored in the freezer at -18 ± 2°C, there was no statistical difference in eggs stored in tube + aluminum, regardless of the period of storage. For eggs stored in tube and aluminum, the best results were observed in eggs stored for 30 and 60 days, respectively. The eggs stored in tube + aluminum and only in aluminum had the best results when observed the period above 30 days (Table III).

For the parameter emergence, it was noted that eggs stored in different forms between the period of 15 and 60 days did not display significant difference, only exceptions being that at 5 days aluminum had a better result, while for 10 days of storage, the eggs stored in tube and tube + aluminum resulted in greater emergence (Table III).

The adults that emerged from the eggs stored in aluminum foil for 60 days had a higher number of females compared to the other forms stored during the same period. For the other storage periods there was no difference. It was observed that in the eggs stored in tubes, there was little difference, with only the eggs stored at 5 and 60 days being below the other periods. For the eggs stored in aluminum, only the period of 10 days performed below the other periods. When the period of 30 to 60 days in aluminum foil was observed, a greater number of females were noticed. A greater number of females are considered important for biological control programs, because the males do not contribute directly to the decline populations of the pest (Navarro, 1998). For the egg-adult period, only the adults emerging from eggs stored in a tube for 30 days had a longer developmental period, and the rest, regardless of the form and period, were not different from each other.

Interestingly, for the same storage period (60 days), the storage temperature of -18°C (freezer) displays similar efficacy as that found in eggs at -196°C, when parasitism was observed (Tables III and IV).

TABLE III
BIOLOGICAL CHARACTERISTICS OF *Telenomus podisi* REARED IN *Euschistus heros* EGGS STORED IN FREEZER A -18 ±2ºC IN DIFFERENT FORMS AND PERIODS

		-18°C		
	Days	Tube	Tube+Aluminum	Aluminum
Parasitism	5	0.33 ± 0.07 C b	0.74 ± 0.03 NS a	0.75 ± 0.03 AB a
	10	0.72 ± 0.03 AB ns	0.81 ± 0.05 NS ns	0.53 ± 0.07 C ns
	15	0.55 ± 0.08 BC b	0.82 ± 0.05 NS a	0.80 ± 0.04 AB a
	30	0.90 ± 0.03 A a	0.75 ± 0.05 NS b	0.82 ± 0.02 AB ab
	45	0.41 ± 0.07 C b	0.78 ± 0.05 NS a	0.69 ± 0.04 BC a
	60	0.48 ± 0.10 BC b	0.76 ± 0.09 NS a	0.86 ± 0.03 A a
Viability	5	0.38 ± 0.06 B b	0.55 ± 0.04 BC ab	0.63 ± 0.03 B a
	10	0.71 ± 0.06 A a	0.59 ± 0.06 ABC	0.39 ± 0.06 C B
	15	0.62 ± 0.08 AB ns	0.40 ± 0.07 C ns	0.62 ± 0.05 B ns
	30	0.69 ± 0.03 A ns	0.58 ± 0.06 ABC	0.62 ± 0.03 B ns
	45	0.65 ± 0.09 AB ns	0.82 ± 0.04 A ns	0.84 ± 0.03 A ns
	60	0.56 ± 0.08 AB ns	0.65 ± 0.07 AB ns	0.61 ± 0.06 B ns
Sex ratio	5	0.40 ± 0.13 B ns	0.86 ± 0.03 NS ns	0.83 ± 0.06 AB ns
	10	0.88 ± 0.07 A ns	0.77 ± 0.09 NS ns	0.57 ± 0.14 B ns
	15	0.61 ± 0.13 AB ns	0.47 ± 0.15 NS ns	0.75 ± 0.13 AB ns
	30	0.92 ± 0.03 A ns	0.85 ± 0.07 NS ns	0.94 ± 0.02 A ns
	45	0.70 ± 0.09 AB ns	0.73 ± 0.09 NS ns	0.91 ± 0.01 A ns
	60	0.36 ± 0.13 B b	0.75 ± 0.13 NS a	0.96 ± 0.01 A a
Egg-Adult (Days)	5	12.35 ± 1.42 NS ns	14.37 ± 0.23 NS ns	14.14 ± 0.10 NS ns
	10	12.08 ± 1.36 NS ns	13.81 ± 0.27 NS ns	13.69 ± 1.53 NS ns
	15	13.01 ± 1.47 NS ns	12.04 ± 1.35 NS ns	14.55 ± 0.27 NS ns
	30	16.86 ± 0.37 NS a	15.32 ± 0.27 NS ab	14.71 ± 0.17 NS b
	45	13.35 ± 1.51 NS ns	14.29 ± 0.14 NS ns	14.67 ± 0.18 NS ns
	60	12.21 ± 1.37 NS ns	14.05 ± 1.57 NS ns	14.05 ± 0.24 NS ns

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability. ns: not significant.

TABLE IV
BIOLOGICAL CHARACTERISTICS OF *Telenomus podisi* REARED IN *Euschistus heros* EGGS STORED IN LIQUID NITROGEN AT -196ºC IN DIFFERENT FORMS AND PERIODS

		-196°C		
	Days	Tube	Tube+Aluminum	Aluminum
Parasitism	5	0.32 ± 0.04 C b	0.66 ± 0.03 AB a	0.63 ± 0.04 B a
	10	0.76 ± 0.07 A ns	0.64 ± 0.04 AB ns	0.39 ± 0.05 C ns
	15	0.63 ± 0.06 AB ns	0.49 ± 0.05 B ns	0.62 ± 0.04 B ns
	30	0.82 ± 0.03 A a	0.68 ± 0.04 AB b	0.66 ± 0.04 B b
	45	0.44 ± 0.05 BC b	0.73 ± 0.04 A a	0.62 ± 0.04 B a
	60	0.67 ± 0.08 AB b	0.61 ± 0.08 AB b	0.90 ± 0.02 A a
Viability	5	0.73 ± 0.09 AB a	0.75 ± 0.03 AB a	0.63 ± 0.03 B a
	10	0.51 ± 0.07 B b	0.75 ± 0.05 AB a	0.64 ± 0.08 B ab
	15	0.84 ± 0.04 A ns	0.71 ± 0.06 AB ns	0.75 ± 0.05 B ns
	30	0.74 ± 0.03 AB a	0.56 ± 0.03 B b	0.74 ± 0.04 B a
	45	0.75 ± 0.04 AB ns	0.79 ± 0.03 A ns	0.74 ± 0.05 B ns
	60	0.73 ± 0.05 AB b	0.78 ± 0.09 AB ab	0.98 ± 0.02 A a
Sex ratio	5	0.50 ± 0.14 NS ns	0.90 ± 0.02 AB ns	0.63 ± 0.09 NS ns
	10	0.78 ± 0.11 NS ns	0.88 ± 0.06 AB ns	0.65 ± 0.11 NS ns
	15	0.67 ± 0.12 NS ns	0.43 ± 0.14 C ns	0.72 ± 0.12 NS ns
	30	0.94 ± 0.02 NS ab	0.99 ± 0.01 A a	0.83 ± 0.07 NS b
	45	0.94 ± 0.02 NS a	0.55 ± 0.14 BC b	0.62 ± 0.14 NS ab
	60	0.75 ± 0.13 NS ns	0.71 ± 0.12 ABC ns	0.88 ± 0.06 Ns ns
Egg-Adult (Days)	5	13.28 ± 0.36 C ns	13.38 ± 0.11 NS ns	12.72 ± 0.09 C ns
	10	12.57 ± 0.11 C ns	13.26 ± 0.21 NS ns	13.49 ± 0.19 BC ns
	15	12.70 ± 0.12 C ns	12.63 ± 0.12 NS ns	13.54 ± 0.23 B ns
	30	15.57 ± 0.17 A ns	14.04 ± 0.08 NS ns	14.43 ± 0.11 A ns
	45	14.46 ± 0.15 B ns	13.10 ± 0.16 NS ns	13.68 ± 0.23 AB ns
	60	12.82 ± 0.20 C ns	12.78 ± 1.44 NS ns	13.55 ± 0.24 B ns

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability. ns: not significant.

Usually, the detrimental effects caused by chilling are reduced by rapid freezing and a lower storage temperature. At -18°C, slow and partial freezing may lead to the formation of small crystals, which, when the material is thawed, will cause damage to the eggs in the form of cellular rupture (Canet, 1989). However, the detrimental effects of parasitoids reared in stored eggs have also been observed as a function of storage time, and not only as a function of temperature (Peverieri *et al*., 2014). Thus, 60 days may not be enough to reduce the nutritional quality of eggs stored in aluminum for the development of *T. podisi*.

In addition, the possibility of being able to store host eggs in a domestic appliance, such as the freezer (-18°C), as opposed to liquid nitrogen, may be an economically attractive alternative (Peverieri *et al*., 2014).

The eggs stored in aluminum foil for 60 days showed higher parasitism when compared to the other forms of storage and within the same form, regardless of the period observed. For the eggs stored in tube + aluminum, only the eggs stored for 15 days showed less parasitism, while for other periods there was no difference.

The preservation of *E. heros* eggs in liquid nitrogen (-196°C) or in a -18°C freezer requires protection against damage caused by low temperature and to prevent this damage, aluminum foil is used (Corrêa-Ferreira and Oliveira, 1998). However, as the results indicate, between 10 and 30 days, there was no difference when compared to eggs stored in tubes (Table IV).

Again, it was found that the eggs stored for 60 days in aluminum foil had higher emergence of adults compared to the other forms and with the periods within the same form of storage. For eggs stored in tubes and tubes + aluminum, there was a lower emergence at 10 and 30 days, respectively. When observed within the storage forms tube and aluminum, there was no difference regarding the stored period. However, when the tube + aluminum was evaluated at 30 days, a greater number of emerged females were observed.

For the egg-adult parameter, there is no difference when comparing eggs stored in the same period but different forms. For tube + aluminum there is also no difference when observed throughout the storage period. Adults emerging from *E. heros* eggs stored in aluminum for 5 and 10 days needed less time to complete their development. However, those emerging from eggs stored in tubes for 30 and 45 days required longer time when compared to other storage periods and with the tubes.

A hypothesis raised was that the tube + the aluminum would offer greater protection for the eggs and this would reflect in the biological characteristics. However, this hypothesis was not validated because at various times this form of storage was equal to only using the tube or the aluminum, making the utilization of two materials useless if there is no clear advantage.

In short, the most efficient treatment to stock eggs of *E. heros* for longer is the combination of nitrogen tank with aluminum foil (T3.1), showing high parasitism (0.90 ±0.02), viability (0.98 ±0.02) and no significant difference of sex ratio between the stocking methods (tube, tube + aluminum foil and aluminum foil). The freezer still interesting for shorter stock periods, until 45 days, because of lower viability at 60 days.

Biological characteristics of T. podisi *stored in the pupal stage at different storage periods at low temperature*

Storage of adults generally leads to a greater and faster reduction in biological characteristics than storage of immatures (Van Lenteren and Tommasini, 2003). Therefore, parasitoids are usually stored as pupae since this stage is immobile and well protected from being impacted by handling and subsequent abrasions inside their cocoon. Despite the immobility, the pupal stage is metabolically very active. Larval tissues undergo histolysis and are reassembled into the adult form. Therefore, it is not surprising to observe variable tolerance to cold storage within this specific stage (Colinet and Boivin, 2011).

The parasitism data do not fall short of those of the

TABLE V
Telenomus podisi REARED IN *Euschistus heros* EGGS STORED IN LIQUID NITROGEN AT -196ºC IN ALUMINUM FOIL FOR 5 DAYS

Parasitism					
T(ºC)	Day 1	Day 2	Day 3	Day 4	Day 5
-196	0.61 ±0.05 A a	0.31 ±0.05 A b	0.57 ±0.04 A a	0.54 ±0.03 A a	0.67 ±0.08 AB a
-18	0.71 ±0.06 A ab	0.34 ±0.04 A d	0.55 ±0.06 A bc	0.49 ±0.06 A cd	0.81 ±0.07 A a
5	0.69 ±0.06 A a	0.30 ±0.04 A c	0.54 ±0.04 A ab	0.48 ±0.05 A bc	0.71 ±0.03 AB a
15	0.57 ±0.05 A a	0.29 ±0.05 A b	0.63 ±0.05 A a	0.46 ±0.03 A ab	0.60 ±0.06 B a

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability.

TABLE VI
BIOLOGICAL CHARACTERISTICS OF *Telenomus podisi* REARED IN *Euschistus heros* EGGS STORED IN THE PUPA STAGE AT 5ºC AND 15ºC OVER TIME

	Emergence				
T(ºC)	Day 1	Day 2	Day 3	Day 4	Day 5
5	0.43 ±0.10 B ab	0.54 ±0.07 A a	0.53 ±0.07 A ab	0.30 ±0.04 B ab	0.06 ±0.02 B b
15	0.86 ±0.03 A a	0.74 ±0.10 A a	0.75 ±0.03 A a	0.81 ±0.04 A a	0.86 ±0.04 A a
	Sex ratio				
	Day 1	Day 2	Day 3	Day 4	Day 5
5	0.74 ± 0.12 A ab	0.91 ± 0.05 A a	0.64 ± 0.10 B b	0.70 ± 0.09 A ab	0.65 ± 0.15 A ab
15	0.93 ± 0.01 A a	0.88 ± 0.05 A a	0.89 ± 0.02 A a	0.82 ± 0.05 A a	0.89 ± 0.03 A a
	Egg-adult				
	Day 1	Day 2	Day 3	Day 4	Day 5
5	11.67 ± 1.95 A a	14.11 ± 0.48 A a	15.34 ± 0.26 A a	14.74 ± 0.17 A a	11.82 ± 2.61 A a
15	14.32 ± 0.12 A a	13.07 ± 0.22 A a	13.81 ± 0.14 B a	13.77 ± 0.28 A a	13.92 ± 0.16 A a

Means ± Standard error of the mean followed by the same uppercase letter in the column and lowercase in the row, do not differ statistically from each other by the Tukey test at 5% probability.

previous experiment, because the conditions are the tables if we consider that the eggs used were stored in NL for 5 days and in aluminum foil (Table V). The temperatures described in the table are those that were used subsequently.

There was a higher emergence of adults from eggs stored at 15°C, except for a storage time of 2 and 3, where there was no significant difference. There was also a low emergence of adults after 4 days of storage (Table VI). It should be noted that for eggs stored at -196°C and -18°C there was no emergence of adults because of which there were no data for evaluation of the other biological parameters. For sex ratio, there was only a difference between temperatures when stored at 3 days, where it could be observed that a greater number of females emerged when stored at 15°C (Table VI). Pupae stored for 3 days at the temperature of 5°C, took longer to emerge when compared to the storage temperature of 15°C.

The results obtained indicate that aluminum is better than the other materials for storing eggs for a longer period and at lower temperatures, while the tube delivers results when used for storage at temperatures of 5°C and 15°C and for a storage period of up to 30 days.

It is also important to report that the most effective method for storing host eggs at low temperature is currently liquid nitrogen; however, it can be observed that the freezer, a more accessible appliance, also delivered great results, often being similar to those found in liquid nitrogen. Thus, the freezer could be a great alternative to the former.

Pupae of *T. podisi* when stored in liquid nitrogen (-196°C) or freezer (-18°C) did not emerge. It can be inferred then that these temperatures are unfeasible for storage of insects in this phase of development. However, when stored in refrigerator and BOD (5 and 15°C respectively), the emergence of the biological agent can be observed. It can also be pointed out that regarding emergence in the BOD (in relation to the refrigerator), the former was superior in three periods of storage (1, 4, and 5 days). The emergence of *T. podisi* was not harmed by the storage of pupae at 15°C in any of the 5 days and at 5°C between 2 and 3 days. The highest levels demonstrated by BOD treatments shows the efficiency of this method. Although, the delay of egg-adult period, presents at 5ºC (refrigerator) for 3 days of storage, could be used in cases that this delay is necessary to wait for a day with better release conditions. Our results have a theoretical and practical application, as they can be applied to year-round egg production and storage, and serve as a theoretical basis to advance studies on low-temperature storage of host eggs and biological agents.

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