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BIOLOGICAL CONTROL

Use of Low Temperature Storage to Preserve Host and Parasitoid to Improve the Rearing of *Telenomus podisi* (Hymenoptera: Platygastridae) on *Euschistus heros* (Hemiptera: Pentatomidae) Eggs

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Abstract
Successful parasitoid rearing is crucial for augmentative biological control. A low temperature preservation protocol allowing the availability of host and parasitoid year-round was evaluated in this study in four bioassays: (1) host eggs [*Euschistus heros* (Fabricius)] stored at − 196, − 80, and − 20°C for up to 70 days prior to exposure to *Telenomus podisi* Ashmead parasitism; (2) *Euschistus heros* eggs removed from storage at − 196°C after 70 days and kept at 5°C for up to 9 days prior to exposure to *T. podisi* parasitism; (3) *Telenomus podisi* adult emergence of insects stored as pupae at 5°C; and (4) fitness of adults of *T. podisi* stored at 5°C. Higher parasitism was observed in parasitoids reared on *E. heros* eggs stored at − 196 and − 80°C. Host eggs removed from − 196°C and stored at 5°C for up to 6 days did not impact *T. podisi* parasitism and development. Storage of *T. podisi* pupae for more than 7 days negatively affected parasitoid biology. Storing *T. podisi* adults at 5°C for up to 6 days does not alter the biological parameters of the parasitoid. Thus, parasitoids can be stored as pupae or adults as well as its host *E. heros* eggs. Our findings can be applied to improve the feasibility of year-round insect production.

Introduction

Research on alternative methods to avoid the excessive use of pesticides has increased worldwide in recent years (Mélo-Filho & Guenther 2015, Souza *et al* 2015). Among these alternatives, augmentative biological control (ABC) by seasonal release of invertebrate and/or microbial organisms is a sustainable option for pest management (van Lenteren *et al* 2017). Today, ABC is applied to more than 30 million ha worldwide (van Lenteren *et al* 2017). However, efficient and economic mass rearing of biological control agents is essential for the success of ABC programs (Corrêa-Ferreira & Moscardi 1993, van Lenteren & Tommasini, 2002). A major constraint is the relatively short shelf life of most insects used in ABC, compared with pesticides. This means that due to a lack in efficient storage methodology (Colinet & Boivin 2011), biological control agents must be produced shortly before their intended use (Macedo *et al* 2006).

Among the different natural enemies that are used in ABC, *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) stands out due to its ability to control several stink bug species of economical importance at the egg stage, thus avoiding any injuries to plants in the field (Koppel *et al* 2009, Querino *et al* 2016, Valente *et al* 2016). *Telenomus podisi* has been successfully reared in the laboratory using eggs of *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) as hosts (Peres & Corrêa-Ferreira 2004). However, interruptions in parasitoid rearing might cause

problems for maintaining the required insect production level to support ABC releases (Pratissoli *et al* 2003).

For the optimization of mass rearing necessary to carry out ABC in the field (López & Botto 2005, Doetzer & Foerster 2007), appropriate storage techniques for parasitoids and hosts are necessary to increase parasitoid availability throughout the year. Additionally they can reduce insect production costs, making the use of biological control agents more attractive than other crop protection alternatives (Colinet & Boivin 2011).

Low production costs are essential for making biological control agents including *T. podisi* economically viable tools in ABC (Bernardo *et al* 2008, Macedo *et al* 2006, Carvalho *et al* 2008, Colinet & Boivin 2011; Queiroz *et al* 2017). Therefore, appropriate storage techniques are needed to increase the parasitoids' shelf life (Macedo *et al* 2006; Queiroz *et al* 2017). Shelf life increase is often inversely proportional to production costs and can therefore be decisive for the success or failure of ABC (Macedo *et al* 2006; Queiroz *et al* 2017).

Initial studies on storage procedures of *T. podisi* (Nakama & Foerster 2001) and *E. heros* eggs (Favetti *et al* 2014) showed promising results for the optimization of mass rearing. However, additional trials, such as those carried out in the present study, are important to complement existing knowledge on the storage of those organisms under different conditions without changing their biological characteristics. In order to evaluate the effects of different storage techniques on insect quality, possible negative effects resulting in host or parasitoid damage must be considered (Colinet & Boivin 2011). Negative effects depend on storage temperature and duration of exposure (Lysyk 2004), which need to be evaluated in detail. For the evaluation of insect quality after storage at low temperature, a number of biological and reproductive parameters must be taken into account such as parasitism, emergence, longevity, fecundity, and number of viable offspring. The objective of this work was to evaluate the storage of *E. heros* eggs and *T. podisi* pupae and adults under various conditions in order to optimize mass rearing of this parasitoid in the laboratory.

Material and Methods

This study was carried out in the laboratory of Embrapa Soybean, Londrina, State of Paraná. The study consisted of four independent bioassays: (1) evaluation of *E. heros* eggs stored at −196, −80, and −20°C for 20, 40, 50, and 70 days for later use in *T. podisi* rearing; (2) evaluation of *E. heros* eggs removed from storage at −196°C after 70 days and kept at 5°C for 0 (control), 1, 2, 3, 4, 5, 6, 7, 8, and 9 days; (3) evaluation of pupae of *T. podisi* stored at 5°C for 0 (control), 7, 14, 21, and 28 days; and (4) evaluation of adults of *T. podisi* stored at 5°C for 0 (control), 2, 4, and 6 days. After each storage period, all treatments were moved to controlled conditions [25 ± 2°C; 80 ± 10% relative humidity (RH); 14/10 h L/D photoperiod] inside Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil).

Parasitoid and host colonies

Insects used in this study were obtained from colonies kept at Embrapa Soybean, Londrina, State of Paraná, Brazil, under controlled conditions inside BOD climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 25 ± 2°C, 80± 10% RH, and a 14/10 h photoperiod (L/D). *Euschistus heros* and *T. podisi* were reared according to Silva *et al* (2008) and Peres and Corrêa-Ferreira (2004), respectively, as briefly described in the following.

Euschistus heros was originally collected from soybean plants in Londrina, State of Paraná. The population was kept in the laboratory for approximately two years during which new field insects were introduced each year to maintain quality. The insects were kept in cages (20 cm × 20 cm sides × 24 cm tall) made of plastic screen and lined with filter paper. A Petri dish with a cotton wad soaked in distilled water (9 cm of diameter) was added to each cage. Insects were fed with dry soybean seeds (*Glycine max* L.); fresh green bean pods (*Phaseolus vulgaris* L.); sunflower seeds (*Helianthus annuus* L.); raw shelled peanuts (*Arachis hypogaea* L.); and water. Cages were cleaned, food was replaced, and egg masses were collected three times a week. After each collection, the egg masses were transferred to acrylic boxes (11 cm × 11 cm × 3.5cm) lined with filter paper moistened with sterile-distilled water (Gerbox®). After eclosion, second instar nymphs were transferred to new cages identical to those previously described. The laboratory-reared insects were then used for experiments as well as for colony maintenance.

Telenomus podisi was also originally collected in soybean fields in Londrina, State of Paraná. The population was kept in the laboratory for approximately five years where it was reared on eggs of *E. heros*. Host eggs were glued to pieces of cardboard (2 cm × 8 cm) and introduced into glass tubes (8 cm long and 2 cm Ø) together with eggs already parasitized by *T. podisi* close to adult emergence. Small drops of honey were placed inside the tubes to provide food for the adults as soon as they emerged. The tubes were then closed, and the eggs allowed to be parasitized for 24 h. Adults that emerged from these eggs were used for trials as well as colony maintenance.

Host storage (bioassays 1 and 2)

Bioassay 1: storage of Euschistus heros eggs

The experiment was carried out in a 3 × 4 factorial, completely randomized design, with three temperatures (− 196, − 80, and − 20°C) and four storage periods (20, 40, 50, and 70 days) and replicated four times. Each replicate was composed of ten individualized *T. podisi* females (≤ 48 h old) (n = 40 females/treatment).

Approximately 24,000 *E. heros* eggs (≤ 24 h) were collected during laboratory rearing as previously described. Eggs were divided, placed in aluminum foil, and stored in liquid nitrogen (− 196°C), in an ultrafreezer (− 80°C), or in a common freezer (− 20°C) for 20, 40, 50, and 70 days. After removing *E. heros* eggs from storage, they were defrosted at 25°C for 1 h. Then, the eggs were glued onto cardboard cards (1.5cm × 5cm, approximately 25 eggs per card). Each card was exposed to one female of *T. podisi* (≤ 48 h old, previously mated) in a Duran tube (1.5 ml) for parasitism under controlled conditions (25 ± 2°C; 80 ± 10% RH; 14/10 h L/D photoperiod). The females were fed with honey. Parasitism was allowed to continue for 24 h after which the cards were kept in climate chambers under controlled conditions (25 ± 2°C; 80 ± 10% RH; 14/10 h L/D photoperiod) until parasitoids emerged. The evaluated parameters were the following: percentage of parasitism of stored *E. heros* eggs, emergence (%) and sex ratio [number of females/(number of males + number of females)] of *T. podisi* emerged from stored eggs of *E. heros*, and duration of egg-to-adult development (days) of *T. podisi* in stored eggs.

Bioassay 2: storage of Euschistus heros eggs at 5°C after a 70-day storage at − 196°C

The experiment was carried out in a completely randomized experimental design with 11 treatments including five replicates, each composed of five females with approximately 200 eggs (n = 25 females/treatment). The tested treatments were the following: (1) fresh *E. heros* eggs (control); (2) eggs recently removed from liquid nitrogen; (3) storage at 5°C for 1 day; (4) storage at 5°C for 2 days; (5) storage at 5°C for 3 days; (6) storage at 5°C for 4 days; (7) storage at 5°C for 5 days; (8) storage at 5°C for 6 days; (9) storage at 5°C for 7 days; (10) storage at 5°C for 8 days; (11) storage at 5°C for 9 days.

Initially, 10 g of *E. heros* eggs (≈ 20,000 eggs) was stored in liquid nitrogen (− 196°C) for 70 days. Then, the eggs were transferred to BOD, adjusted to refrigerator temperature (5 ± 2°C, 80 ± 10% RH, and 14/10 h L/D photoperiod), where they remained for one of the tested periods of each treatment. Approximately 1 g (2000 eggs) of *E. heros* eggs was used for each treatment. The evaluation methodology and the evaluated biological parameters were the same as described for bioassay 1.

Parasitoid storage (bioassays 3 and 4)

Bioassay 3: storage of late-formed Telenomus podisi pupae (1–2 days before emergence)

The third experiment was carried out in a completely randomized design with five treatments (*T. podisi* pupae stored at 5°C for 0, 7, 14, 21, and 28 days) and five replicates. For each replicate, approximately 80 parasitized *E. heros* eggs containing parasitoid pupae were glued onto white cardboard cards, then placed in flat-bottomed glass tubes (8 cm × 2 cm diameter), which were then sealed with plastic wrap. The tubes were stored in BOD climate chambers at 5°C, 80 ± 10% RH, and 14/10 h L/D photoperiod for periods of 0 (control), 7, 14, 21, and 28 days to evaluate the impact of storage on parasitoid emergence and adult survival. Every 7 days, five replicates were withdrawn from storage and transferred to a second climatic chamber (BOD at 25 ± 2°C, 80 ± 10% RH, 14/10 h L/D photoperiod) to evaluate the emergence (%) of adults, the duration from egg-adult (days, excluding storage time), and longevity of emerged females (days).

Along with the treatments, we stored 200 additional eggs at the same temperatures and time periods. This was done to evaluate parasitism of *T. podisi* adults raised from stored pupae on eggs of *E. heros* and to assess possible sublethal storage effects on stored parasitoids. Using the natural host for this evaluation was essential to simulate field conditions after parasitoid release.

Parasitism of *T. podisi* adults from stored pupae was evaluated for newly emerged females (≤ 48 h, previously mated), which were individually placed in Duran tubes (1.5 ml) containing a droplet of honey as food. Egg masses with approximately 50 eggs of *E. heros* were fixed with non-toxic white glue (Tenaz®) onto labeled white cardboard cards (2.5 cm × 5 cm). The cards were individually introduced into the tubes, and parasitism was allowed for 24 h. The experiment was performed in a BOD climate chamber set to a temperature of 25 ± 2°C, a relative humidity of 80 ± 10%, and a photoperiod of 14/10 h L/D.

After parasitism, cards were removed and kept at the same conditions until adults emerged. We evaluated the following *T. podisi* biological parameters in the F1 generation: parasitism (%), duration of parasitoid egg-to-adult period (days), parasitoid emergence (%), and offspring sex ratio. To determine the duration of the egg-to-adult period and

the longevity of parental females, emergence and death rate of adults were observed daily.

Bioassay 4: storage of Telenomus podisi adults

The fourth experiment was carried out in a completely randomized design with four treatments and nine replicates. Each replicate was composed of 18 newly emerged (≤ 48 h, previously mated), adults (nine females and nine males), which were placed in individual glass tubes (8 cm × 2 cm) and stored at 5°C in BOD climate chambers for 0 (control) 2, 4, and 6 days. Mortality was recorded for each storage period by counting dead and live individuals. After each period, the insects were transferred to another BOD chamber (25 ± 2°C, 80 ± 10% RH, and 14/10 h L/D photoperiod) to evaluate parasitism of adults after the storage period. Host eggs were exposed to parasitism according to the methodology described in bioassay 3. The evaluated parameters were the following: number of live males, number of live females, longevity and percentage of parasitism of parental females, and emergence, egg-adult period and sex ratio of the F1 generation.

Statistical analysis

The results of the four bioassays were analyzed by exploratory analyses to assess the assumptions of normality (Shapiro & Wilk 1965), variance homogeneity of the treatments (Burr & Foster 1972), and the additivity model for implementation of ANOVA. The results of bioassays 1, 3, and 4 were compared by the Tukey test and results of bioassay 2 were compared by Scott-knott (5% error probability), using the statistical analysis program SAS (SAS Institute 2009).

Results

Host storage (bioassays 1 and 2)

Bioassay 1: storage of Euschistus heros eggs

Factorial analysis revealed an interaction between temperature and storage period for parasitism (Table 1). Unexpectedly, parasitism was lower at − 196°C (46.17%) than at − 80°C (64.12%), however with no difference with parasitism at − 20°C (52.67%) (Table 1) after the shortest storage period of 20 days. In contrast, parasitism (%) was higher at − 196 and − 80°C at longer storage periods (40 to 70 days) than at − 20°C. The colder temperatures are therefore more appropriate for the conservation of *E. heros* eggs for subsequent parasitism by *T. podisi* (Table 1). The lowest parasitism rate was observed for eggs stored for the longest storage period (70 days) at the highest temperature (− 20°C) (Table 1).

Table 1 *Telenomus podisi* parasitism (%) at 25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h L/D on stored eggs of *Euschistus heros* after different storage periods and temperatures (bioassay 1).

Storage (days)	Temperature		
	− 196°C	− 80°C	− 20°C
20	46.17 ± 3.11 Bb	64.12 ± 1.92 Aa	52.67 ± 3.06 ABa
40	51.91 ± 3.21 Aab	42.75 ± 4.78 ABb	37.12 ± 2.99 Bb
50	43.97 ± 4.93 Ab	44.94 ± 4.12 Ab	19.99 ± 2.11 Bc
70	60.04 ± 2.98 Aa	59.38 ± 5.61 Aa	4.88 ± 1.47 Bd
CV (%)	16.25		
$F_{temp*stor}$	16.43		
$p_{temp*stor}$	< 0.0001		

Means ± SEM followed by the same lowercase letter in columns or uppercase letter in rows did not statistically differ (Tukey test, $p > 0.05$).

Regarding the other evaluated parameters (emergence, sex ratio, and egg-adult period), there was no interaction between temperature and storage time (Table 2). Storage temperature impacted parasitoid emergence and duration of *T. podisi* egg-adult period but did not change its sex ratio (Table 2). The emergence of *T. podisi* from *E. heros* eggs stored at − 20°C (46.59%) was lower compared to − 80 and − 196°C (60.03 and 65.70%, respectively) (Table 2). The duration of *T. podisi* egg-adult development was directly proportional to the increase of temperature, with longer duration (14.27 days) for *T. podisi* reared on *E. heros* eggs stored at − 20°C and shorter duration for parasitoids reared on eggs stored at − 80°C (13.94 days) and − 196°C (13.47 days) (Table 2). Sex ratio of the parasitoid offspring did not differ between temperatures (Table 2). Storage time did not impact *T. podisi* biological parameters evaluated in this bioassay (Table 2).

Bioassay 2: storage of Euschistus heros eggs at 5°C after 70-day storage at − 196°C

Storage time of *E. heros* eggs at 5°C, after removal from liquid nitrogen (− 196°C), impacted the biological parameters of *T. podisi* (Table 3). The percentage of parasitism varied between treatments, with the highest values observed at the shortest storage times (1 and 2 days, 54.29 and 49.36%, respectively). Emergence also varied according to storage time. However, more than 60% of the parasitoids emerged in all treatments with the exception of the 3-day treatment (57.91%) (Table 3).

Egg-to-adult development time was shortest in the control (12.8 days) and ranged from 13.36 to 14.79 days in all other treatments. The sex ratio of parasitoid offspring

Table 2 Influence of temperature and storage time of *Euschistus heros* eggs on emergence, sex ratio, and egg-adult period of *Telenomus podisi* [bioassay 1, performed at 25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h (L/D)].

	Parameter	Emergence (%)	Sex ratio	Egg-adult period
Temperature (°C)	− 196	65.70 ± 2.91a	0.86 ± 0.03^{ns}	13.47 ± 0.04c
	− 80	60.03 ± 3.31a	0.86 ± 0.01	13.94 ± 0.06b
	− 20	46.59 ± 3.68b	0.90 ± 0.02	14.27 ± 0.08a
Storage (days)	20	59.27 ± 4.05^{ns}	0.87 ± 0.03^{ns}	13.93 ± 0.14^{ns}
	40	63.45 ± 4.88	0.91 ± 0.02	13.98 ± 0.12
	50	54.91 ± 4.26	0.87 ± 0.03	13.85 ± 0.12
	70	52.12 ± 4.28	0.85 ± 0.04	13.82 ± 0.11
	CV (%)	21.67	12.06	2.05
Statistical analysis	F_{temp}	9.95	0.67	30.85
	p_{temp}	0.0004	0.5155	< 0.0001
	F_{stor}	1.92	0.75	0.83
	p_{stor}	0.1443	0.5283	0.4865
	$F_{temp*stor}$	1.56	0.43	0.52
	$p_{temp*stor}$	0.1871	0.8536	0.7866

Means ± SEM followed by the same letter in columns for each parameter did not statistically differ (Tukey test, $p > 0.05$).

^{ns} ANOVA not significant.

(Table 3) varied from 0.72 to 0.90 accordingly to storage time at 5°C.

Parasitoid storage (bioassays 3 and 4)

Bioassay 3: storage of late-formed Telenomus podisi pupae (1–2 days before emergence)

The storage of *T. podisi* pupae for 7 days at 5°C did not impact adult emergence (%). However, when the storage period was 14 days or longer, *T. podisi* emergence from stored pupae was reduced (Table 4). Similarly, the duration of egg-adult development (excluding storage period) was longer when storing *T. podisi* pupae at 5°C for 21 and 28 days (Table 4). Adult longevity did not differ between treatments (Table 4).

In the second parasitoid generation (F1), parasitism (%) of adults emerged from pupae stored for 7 days (23.2%) and 14 days (24.1%) was lower compared with the control (42.9%) (Table 4). Parasitoid emergence (%) of the following generation was not impacted by pupae storage. In contrast, the duration of parasitoid egg-adult development was longer after 7 and 14 days of pupae storage (Table 4) and sex ratio was lower after 14 days of storage (Table 4).

Bioassay 4: storage of Telenomus podisi adults

The survival of *T. podisi* males and females was not influenced by storage of adults at 5°C for different periods of time (up to 6 days) and was more than 93% for both genders in all treatments (Table 5). Parasitism rate of survivors was the same (Table 6). Storage did not affect female longevity, which was similar or superior to that of the control (Table 6). In the following generation (F1), parasitoid emergence (%) and sex ratio also did not differ between storage times (Table 6). In contrast, the duration of egg-adult development was longer after storage for 6 days, compared with the control (zero storage days) (Table 6).

Discussion

Despite a general association of low temperature storage with major fitness costs of insects (van Baaren *et al* 2005, 2006, Chown & Terblanche 2006, Hance *et al* 2007), our results indicate that storage of *E. heros* eggs for 70 days at − 80 and − 196°C does not compromise their suitability as rearing substrate for *T. podisi*. Parasitism capacity of *T. podisi* was not impaired by pupae storage at 5°C for 7 days. Adults of this species can be stored for 6 days at 5°C with no detectable injury to its biology or parasitism capacity. Storage of parasitoids and their rearing host at low temperatures has proved to be a valuable method and is desirable for biocontrol production companies for various reasons (van Lenteren & Tommasini 2003). Firstly, it helps to decrease production costs and increase the shelf life of natural enemies in order to provide a steady and sufficient supply of insects for ABC programs. Secondly, storage facilities can build up reserve supplies of entomophages to compensate for periods of low production or unexpected high demand. Lastly, storage allows synchronized field releases of natural enemies during critical stages of pest outbreaks (McDonald & Kok 1990, Leopold 1998, Venkatesan *et al* 2000, van Lenteren & Tommasini 2003).

Table 3 Biological parameters of *Telenomus podisi* reared on eggs of *Euschistus heros* stored in liquid nitrogen for 70 days and subsequently kept at 5°C for different periods [bioassay 2, performed at 25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h (L/D)].

Storage at 5°C	Parasitism% (number of parasitized eggs)	Emergence (%)
Fresh	42.96 ± 3.53 (15.5) a	76.75 ± 4.31 b
Freshly drawn by N_2	45.61 ± 2.12 (16.7) a	82.33 ± 0.93 a
1 day	54.29 ± 4.44 (18.5) a	84.81 ± 0.67 a
2 days	49.36 ± 5.07 (18.7) a	70.71 ± 1.85 c
3 days	40.59 ± 3.40 (14.0) b	57.91 ± 2.60 d
4 days	36.98 ± 2.56 (12.4) b	63.49 ± 2.41 d
5 days	38.32 ± 2.60 (12.9) b	72.49 ± 3.51 c
6 days	44.45 ± 2.32 (13.7) a	75.12 ± 2.16 b
7 days	31.52 ± 1.59 (10.3) b	72.72 ± 2.59 c
8 days	32.73 ± 4.35(10.2) b	68.68 ± 2.00 c
9 days	35.35 ± 3.37 (12.2) b	69.39 ± 3.51 c
CV (%)	18.4	8.14
F	4.34	8.58
p	0.0003	< 0.0001
Storage at 5°C	Egg-adult period (days)	Sex ratio
Fresh	12.80 ± 0.08 c	0.73 ± 0.04 b
Freshly drawn by N_2	13.60 ± 0.12 b	0.88 ± 0.01 a
1 day	13.36 ± 0.07 b	0.90 ± 0.01 a
2 days	13.52 ± 0.13 b	0.89 ± 0.01 a
3 days	14.32 ± 0.14 a	0.80 ± 0.04 b
4 days	14.24 ± 0.09 a	0.72 ± 0.07 b
5 days	14.16 ± 0.19 a	0.75 ± 0.02 b
6 days	14.28 ± 0.08 a	0.88 ± 0.02 a
7 days	14.52 ± 0.08 a	0.84 ± 0.07 a
8 days	14.79 ± 0.28 a	0.83 ± 0.04 a
9 days	14.48 ± 0.12 a	0.86 ± 0.04 a
CV (%)	2.28	11.80
F	17.73	2.31
p	< 0.0001	0.0277

Means ± SEM followed by the same letter in columns for each variable did not statistically differ (Scott-Knott, $p > 0.05$).

In the literature, there are several reports of successful development of egg parasitoids in host eggs that have undergone freezing or heating (Wajnberg & Hassan 1994). Eggs of various Heteroptera species can be stored at low temperatures and still be parasitized by Platygastridae species (Orr 1988). However, to our knowledge, Favetti *et al* (2014) provided the only study of *E. heros* egg storage for later *T. podisi* parasitism. The authors reported the successful storage of *E. heros* at liquid nitrogen (− 196°C) for six months without impairing *T. podisi* parasitism. Thus, complementing the results of Favetti *et al* (2014) and taking into consideration that production and conservation of host eggs are limiting factors in soybean stink bug ABC programs, our results are of great theoretical and practical interest.

Our study indicates that storage at − 80°C in an ultra-freezer is as efficient as at − 196°C, requiring liquid nitrogen. It is important to note that, although parasitism did not exceed 65% in all treatments, the number of parasitized eggs was higher than reported in the literature (Pacheco & Corrêa-Ferreira 2000). Moreover, the possibility to store host eggs at temperatures obtained by mechanical devices (− 80°C), as opposed to liquid nitrogen, can be an economically attractive alternative (Peverieri *et al* 2015).

Unexpectedly, for short storage periods (up to 20 days), even storage temperature of − 20°C (freezer) provides the same efficacy. Usually, damaging effects caused by the cooling are mitigated by fast freezing and lower storage temperatures. At − 20°C, partial freezing can lead to freeze-thaw recrystallization causing damage on the host stored tissue (Canet 1989). However, detrimental effects of parasitoids reared on stored host eggs have been observed not only as a function of temperature but also as a function of time of storage (Peverieri *et al* 2015). Thus, short period of time as 20 days might not be enough to reduce nutritive quality of the stored host for the development of *T. podisi*. Similar results were reported by Peverieri *et al* (2015) that noted reduction of

Table 4 Biological parameters of *Telenomus podisi* from stored pupae at 5°C for different periods and of its second generation (F1) (bioassay 3, performed at 25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h photoperiod (L/D)).

Storage (days)	Egg-adult period (days)[1]		Emergence (%)	Female longevity (days)[2]
0	15.0 ± 0.0 c		45.3 ± 2.6 a	19.5 ± 0.7[ns]
7	15.0 ± 0.0 c		40.5 ± 3.1 a	24.3 ± 2.4
14	16.0 ± 0.3 c		16.4 ± 1.8 b	20.9 ± 1.9
21	18.0 ± 0.0 b		1.6 ± 0.8 c	–
28	22.0 ± 0.8 a		1.2 ± 0.5 c	–
CV (%)	3.66		21.53	17.04
F	698.08		107.71	1.84
p	< 0.0001		< 0.0001	0.2141
Storage (days)	Egg-adult period (F1)	Parasitism (%) (F1)	Sex ratio (F1)	Emergence (%) (F1)
0	14.8 ± 0.1 b	42.9 ± 1.5 a	0.86 ± 0.03 a	69.3 ± 4.6[ns]
7	15.3 ± 0.1 a	23.2 ± 1.7 b	0.82 ± 0.03 a	71.5 ± 3.8
14	15.5 ± 0.1 a	24.1 ± 2.3 b	0.17 ± 0.05 b	55.7 ± 9.3
CV (%)	1.5	12.5	25.97	19.45
F	8.87	34.98	1.98	1.81
p	0.0074	< 0.0001	< 0.0001	0.2186

Means ± SEM followed by the same letter in columns did not statistically differ (Tukey test, $p > 0.05$).

– data not available.

[1] Egg-adult period excluding the storage period.

[2] Longevity of the *T. podisi* females emerged from stored pupae at 5°C.

[ns] ANOVA not significant.

the progeny production of *Gryon pennsylvanicum* (Asmead) (Hymenoptera: Platygastridae) only after one-month storage of *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) eggs.

The success of storing eggs in liquid nitrogen (– 196°C) has been tested many times and is well documented, but storage at – 80 or – 20°C produced in mechanical freezers is still poorly understood (Cosi *et al* 2010). Successful storage results were reported for eggs of *Graphsoma lineatum* (Linnaeus) (Hemiptera: Pentatomidae) parasitized by *Trissolcus simoni* (Mayr) (Hymenoptera: Platygastridae) after five years of storage in liquid nitrogen (Gennadiev & Khlistovskii 1980). Orr (1988) reported the storage of *Dendrolinus pini* (Linnaeus) (Hemiptera: Pentatomidae) eggs for *Telenomus tetratonus* Thomson (Hymenoptera: Platygastridae) parasitism. Testing the response to stored eggs of several Pentatomidae species, the author also did not find a preference of *Trissolcus grandis* (Thompson) (Hymenoptera: Platygastridae) for fresh eggs over eggs stored for more than 270 days at – 20°C, although parasitism decreased when eggs were stored for over 180 days. Here, we found a similar decrease in parasitism after longer storage periods of host eggs (*E. heros*) at – 20°C, indicating that, in addition to temperature, duration of exposure is an essential component of cold storage tolerance in insects (Colinet & Boivin 2011).

Several biological characteristics recorded for *T. podisi* support the best storage conditions for *E. heros* eggs at lower temperatures (– 80 and – 196°C) including a shorter egg-adult period of the parasitoid. An extended duration of the egg-adult period of *T. podisi* reared on *E. heros* eggs stored at higher temperatures may be due to compensatory action of parasitoid larvae when feeding on lower-quality hosts, hence allowing pupation and sufficient weight gain for development

Table 5 Survival of males and females of *Telenomus podisi* stored as adults at 5°C for different periods (bioassay 4).

Storage (days)	Number of live males (live males %)	Number of live females (live females %)
0	9.0 ± 0.0[ns] (100)	9.0 ± 0.0[ns] (100)
2	8.7 ± 0.2 (96.2)	9.0 ± 0.0 (100)
4	8.6 ± 0.2 (95)	9.0 ± 0.0 (100)
6	8.4 ± 0.2 (93.7)	8.9 ± 0.1 (98.7)
CV (%)	6.59	1.85
F	1.59	1.00
p	0.2114	0.4055

[ns] ANOVA not significant.

Table 6 Biological parameters of *Telenomus podisi* stored as adults at 5°C and of its second generation (F1) (bioassay 4, performed at 25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h (light/dark)).

Storage (days)	Female longevity (days)	Parasitism (%)	Egg-adult period (days) (F1)
0	22.5 ± 2.1 b	55.9 ± 4.7^{ns}	12.9 ± 0.2 b
2	27.4 ± 0.5 ab	50.4 ± 4.0	13.5 ± 0.1 ab
4	30.4 ± 1.9 a	50.5 ± 4.0	13.2 ± 0.2 ab
6	26.3 ± 1.4 ab	42.9 ± 3.5	13.7 ± 0.1a
CV (%)	12.16	16.30	2.39
F	4.06	1.08	3.75
p	0.0331	0.2141	0.0447
Storage (days)	Emergency (%) (F1)		Sex ratio (F1)
0	81.7 ± 3.6^{ns}		0.83 ± 0.04^{ns}
2	73.1 ± 2.1		0.87 ± 0.03
4	78.3 ± 4.2		0.88 ± 0.01
6	77.7 ± 3.5		0.80 ± 0.05
CV (%)	8.81		8.86
F	1.93		0.95
p	0.3949		0.4484

Means ± SEM followed by the same letter in columns did not statistically differ (Tukey test, $p > 0.05$).
^{ns} ANOVA not significant.

to adults (Zhenwei & Qiyao 1988, Behmer 2009). The quality of the food consumed by insects directly influences their biological, physiological, and behavioral characteristics (Nation 2001).

Preservation of *E. heros* eggs in liquid nitrogen (− 196°C) or in a − 80°C ultrafreezer requires their protection from desiccation in aluminum foil (Corrêa-Ferreira & Oliveira 1998), often with a variable number of eggs stored per portion. When removing a recipient with stored eggs from liquid nitrogen (− 196°C) or an ultrafreezer (− 80°C), unused eggs are usually kept at 5°C for several days until later usage in parasitoid rearing. However, it was previously unknown how the duration of 5°C storage following liquid nitrogen storage impacts *T. podisi* parasitism. Our results indicate that *E. heros* eggs can be kept at 5°C for up to 6 days after liquid nitrogen storage without impairing *T. podisi* parasitism, providing a viable solution to avoid economic loss and to optimize host production in parasitoid mass rearing.

An important finding of our study is that the sex ratio of *T. podisi* offspring was not affected by storage of *E. heros* eggs at any of the three tested temperatures and generally remained at 0.86 or higher, with a minimum of 0.72. Higher numbers of females are considered important in ABC because males do not contribute directly for parasitism-induced declines in pest populations (Navarro 1998; Queiroz *et al* 2017). A similar sex ratio was considered to be of acceptable quality in mass production of other egg parasitoids such as *Trichogramma pratissolii* (Querino & Zucchi) (Hymenoptera: Trichogrammatidae) (van Lenteren 2003).

Apart from host availability, one of the major difficulties in mass rearing of egg parasitoids in the laboratory is the necessity of releasing them immediately after adult emergence, due to the adults' short life span (Queiroz *et al* 2017). Although *T. podisi* has a longer life span than *T. remus*, this still applies to both species. Therefore, in addition to host egg storage, strategies for parasitoid storage (pupae or adult) must be developed in order to optimize parasitoid production costs and improve its availability to consumers (Leopold 1998, Gardner *et al* 2012; Queiroz *et al* 2017).

Storage of adults usually leads to higher and faster reduction in fitness than storage of immatures (van Lenteren & Tommasini 2003). Therefore, parasitoids are usually stored as pupae because this stage is immobile and well protected from being impacted by handling and desiccation inside its cocoon. Despite its immobility, the pupa stage is metabolically very active. The larval tissues undergo histolysis and are reassembled in the adult form. Therefore, it is not surprising to observe a highly variable cold storage tolerance within this specific stage (Colinet & Boivin 2011). For this reason, it is important to assess the quality of insects produced after storage as well as the damage that such storage may cause to different insect species (van Lenteren & Tommasini 2003, Colinet & Boivin 2011). For example, cold storage (8°C) of *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) for 2 and 16 days produces similar percentages of emergence, but their ability to fly was much lower after storage for 16 days (van Lenteren & Tommasini 2003).

For *T. podisi*, it was observed a positive linear relationship between pupal the age of pupae stored at low temperature and emergence rate (Foerster *et al* 2004). It was suggested that parasitoids stored at 12 and 15°C as young pupae spent more energy to complete their development, affecting their emergence and longevity (Foerster *et al* 2004). Adding to Foerster *et al* (2004), our research indicates that the late-

formed *T. podisi* pupae can be stored at 5°C for 7 days without impairing their biological characteristics, a finding that can be of great practical use for mass rearing. Longer storage periods of pupae drastically reduced parasitoid emergence from stored pupae and is not advisable for insect rearing. According to Foerster & Doetzer (2006), prolonged storage of pupae reduces parasitoid emergence due to the negative effects on pupae nutrition, insufficient respiration, or changes in hardness and thickness of host structures, which can directly or indirectly impair parasitoid survival and emergence.

Pupa viability is essential when evaluating insect storage (Pereira *et al* 2009). The present study showed that the storage of *T. podisi* pupae for 7 days is feasible despite the observed reduction of parasitism in adults emerged from stored pupae. Parasitism capacity of adults emerged from stored pupae was null when the storage was longer than 14 days at 5°C, which can be explained by their higher energy expenditure for development, thus decreasing later activities such as parasitism. Similar results were previously reported by Foerster *et al* (2004) for *T. podisi* females, which did not parasitize *E. heros* eggs when stored for long periods at 15°C. Concerning parameters related to the F1 generation, an increase in the egg-adult period corresponding to egg storage was observed, which was already verified for other species (Tezze & Botto 2004).

According to Foerster *et al* (2004), *T. podisi* adults can be successfully stored because of a dormancy period that occurs in colder seasons/periods (Mansingh 1971). The possibility of storing those stages of natural enemies (at dormancy) has been previously studied. However, most studies did not lead to practical applications, because of unacceptably high mortality during artificially induced dormancy (van Lenteren & Tommasini 2003). Our results indicate the possibility of *T. podisi* adult storage at 5°C for 6 days without impairing parasitism, providing greater flexibility in *T. podisi* mass production (Gardner *et al* 2012)

The appropriate conditions for parasitoid storage vary according to several factors (López & Botto, 2005). According to the results obtained in the present study, it can be concluded that adults of *T. podisi* are in fact as resistant as or even more resistant to storage than parasitoid pupae. Parasitism capacity was not impaired regardless of storage time, neither was the emergence of parasitoids, which was always higher than 70%.

Therefore, our results allow the overall conclusion that (1) temperatures of − 80 and − 196°C provide better storage conditions for *E. heros* eggs for subsequent *T. podisi* rearing; (2) *E. heros* eggs removed from liquid nitrogen can be kept at 5°C for up to 6 days without impairing the biological parameters of *T. podisi* reared on those eggs; (3) the storage of *T. podisi* pupae at 5°C is only feasible for 7 days with reduction of parasitism of the emerged adults; and (4) storing *T. podisi* adults at 5°C for up to 6 days does not alter the biological parameters of the parasitoid.

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