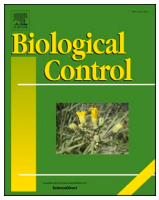
Biological Control 219 (2026) 106094

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



# Double trouble for the stink *bug Bagrada hilaris*: Harnessing sterile eggs to enhance parasitoid efficacy in its biocontrol

Chiara Peccerillo [a,*], Chiara Elvira Mainardi [b], Nathalie Ramualde [c], Alessandra Paolini [d], Massimo Cristofaro [d], Gianfranco Anfora [a], René F.H. Sforza [c]

[a] Center of Agriculture, Food and Environment (C3A), University of Trento, 38010 San Michele all'Adige, Italy
[b] Department of Environmental Biology, University of Rome "La Sapienza", 00185 Rome, Italy
[c] European Biological Control Laboratory, United States Department of Agriculture (EBCL-USDA-ARS), 810 Avenue du Campus Agropolis, 34980 Montferrier, France
[d] Biotechnology and Biological Control Agency (BBCA-Onlus Foundation), Via Angelo Signorelli 105, 00123 Rome, Italy

## HIGHLIGHTS

- *B. hilaris* non-fertilized eggs are a viable substrate for the development of parasitoid *Gryon aetherium*.
- Parasitoids emerging from non-fertilized eggs exhibit normal vitality.
- Non-fertilized eggs are suitable for parasitization up to 15 days, about 10 days more than the fertilized ones.
- Sentinel non-fertilized eggs could improve parasitoid monitoring and pest management.
- Sterile insect technique and classical biological control showed synergistic interaction in laboratory conditions.

## ARTICLE INFO

*Keywords:*
*Gryon aetherium*
Painted bug
Sterile insect technique
Gamma radiation
Sentinel eggs
AW-IPM

## ABSTRACT

Invasive agricultural pests such as *Bagrada hilaris,* a major pest of Brassicaceae crops, pose significant threats to global food security and require innovative management strategies. This study explored the potential application of the synergism between the Sterile Insect Technique (SIT) and Classical Biological Control (CBC) for the implementation of Area-Wide Integrated Pest Management to control this pest. SIT involves the release of sterilized males to reduce pest populations by producing nonviable offspring. Eggs produced by females mated with sterilized males (SIT eggs) serve as a novel substrate for parasitization by the oophagous parasitoid *Gryon aetherium*, thereby enhancing the effectiveness of CBC. Our findings demonstrated that these sterile eggs exhibit extended viability for parasitization maintaining their suitability for up to 15 days under laboratory conditions, compared with untreated eggs, which are available for parasitization for up to a maximum of 5 days. Parasitoid emergence rates from SIT eggs were comparable to those from control eggs, with no significant impact on the sex ratio and the longevity of offspring. These findings may suggest favorable ecological implications for the application of the two techniques by presenting a scenario in which the population of the harmful species is subject to demographic control through the combined release of sterile males and the biological control agent. SIT eggs also have the potential as "long-life sentinel eggs" for monitoring the parasitoid population and identifying natural enemies already present in the invaded areas.

## 1. Introduction

Among the agricultural pests that have recently stood out for the extensive damage caused to economically important crops, there are several species of bugs belonging to the pentatomid family (Conti et al., 2021). In addition to the best-known *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) and *Nezara viridula* L. (Hemiptera: Pentatomidae), in recent years also *Bagrada hilaris* Burmeister (Hemiptera: Pentatomidae) has rapidly expanded its distribution beyond its area of origin, raising the interest in its control and monitoring. *Bagrada hilaris*

* Corresponding author.
*E-mail addresses:* chiara.peccerillo@gmail.com (C. Peccerillo), chiaraelviramainardi@gmail.com (C.E. Mainardi), nramualde@ars-ebcl.org (N. Ramualde), ale.paolini1@gmail.com (A. Paolini), m.cristofaro55@gmail.com (M. Cristofaro), gianfranco.anfora@unitn.it (G. Anfora), rsforza@ars-ebcl.org (R.F.H. Sforza).

https://doi.org/10.1016/j.biocontrol.2026.106094
Received 7 December 2025; Received in revised form 20 April 2026; Accepted 20 June 2026
Available online 21 June 2026

represents not only a damaging agricultural pest to brassicaceous crops in its area of origin, such as South Africa, India and Pakistan (Kavita et al., 2014; Carvajal et al., 2019), but also as a newly introduced invasive alien species in the Americas. It invaded first southwestern U.S., including California, New Mexico (Bundy et al., 2012), and Arizona (Reed et al., 2013) then became a serious threat to Central and South America (Sánchez-Peña, 2014; Faúndez, 2018; Carpintero et al., 2021). In Europe, although it has not been recorded on the continent, it has been detected as a pest that damages the economy of caper plants in the Mediterranean islands of Malta and Pantelleria (Italy) (Carapezza, 1981). Its adaptability to new environments, polyphagy, and climate-related suitability may facilitate further spread (Carvajal et al., 2019).

The feeding method mediated by the piercing-sucking mouthparts causes circular chlorotic lesions to the leaves that eventually result in the destruction of apical meristems, thus blocking growth of the terminal part of the shoot (Colazza et al., 2004). Moreover, the mechanical feeding damage is amplified by a characteristic gregarious behavior of nymphal instars (Huang et al., 2014). To date, the only methods to control this species rely on multiple applications of broad-spectrum synthetic insecticides such as pyrethroids and neonicotinoids (Guarino et al., 2017).

Economic and environmental unsustainability, combined with the emergence of pest resistance to such substances (Palumbo et al., 2016), raises the urgent need for the development and application of targeted, sustainable and more effective control techniques (Hendrichs et al., 2009; Lance and McInnis, 2021). Among these, it is important to mention classical biological control (CBC), which, in the cases of *H. halys* and *N. viridula,* has already been applied in the field with the release of host-specific oophagous parasitoids (Falagiarda et al., 2023; Sabbatini-Peverieri et al., 2020; Simaz et al., 2023; Oliveira et al., 2024; Gard et al., 2022). In addition, the potential use of the sterile insect technique (SIT) is being evaluated for both species, either independently (Horrocks et al., 2020) or in combination with biological control (Roselli et al., 2023).

Sterile insect technique requires the collection and the mass rearing of pests in areas of infestation. After sexing the individuals, the males are sterilized through methods that may include the use of gamma or x-rays. Subsequently, a previously computed quantity of irradiated males is released at regular intervals in localized areas where the pest population is present. In this way, wild females mating with field-released irradiated males will lead to sterile egg production resulting in a gradual decrease in the number of individuals in the target population through birth control (Knipling, 1955; Lance and McInnis, 2021). However, this technique can be applied in combination with biological control (Roselli et al., 2023). In the case of *H. halys*, Roselli et al. (2023) proved that a SIT approach, in addition to reducing the population density of the target species, could provide a valuable resource for parasitoid oviposition through the eggs laid by wild females mated with irradiated males, which is useful for improving the establishment and multiplication of selected egg parasitoids. By deliberately relying on the control model developed for the two pentatomids mentioned above, similar screening was carried out on *B. hilaris.*

To prevent the risk of releasing harmful adult individuals into the environment, the SIT is widely applied to control holometabolous insects which cause damage during the larval stages (Lance and McInnis, 2021; Klassen, Curtis and Hendrichs, 2021). In the case of *B. hilaris*, despite it presents an incomplete metamorphosis, this threat is overcome since the major damage on host plants is caused by insects during the nymphal stages (Huang et al., 2014). Therefore, the application of SIT to this species may still be considered a viable option and has been the subject of recent investigations. A preliminary study evaluating the physiological and behavioral effects of gamma radiation for potential SIT applications indicated that irradiation at 80 Gy reduces fertility while preserving male mating ability and longevity (Cristofaro et al., 2022; Mainardi et al., 2025). In parallel, the oophagous parasitoid *Gryon aetherium* Talamas (Hymenoptera: Scelionidae), originally collected in Pakistan from *B. hilaris* eggs (Mahmood et al., 2015), has been investigated as a potential biological control agent (Martel et al., 2019). Unlike other pentatomids, the bagrada bug lays eggs individually by burying them in the soil (Taylor et al., 2014; Martel and Sforza, 2021), and *G. aetherium* is able to locate and successfully parasitize eggs buried at depths of approximately 2 mm (Martel and Sforza, 2021).

Based on these elements, the present study aimed to investigate the feasibility of combining SIT and classical biological control for the management of *B. hilaris*. Specifically, we evaluated the ability of *Gryon aetherium* to parasitize and successfully develop on eggs produced by females mated with males irradiated at 80 Gy. In addition to their reduced hatching rate (Cristofaro et al., 2022), these eggs may remain available for parasitization for a longer period than eggs from untreated individuals, which typically hatch within approximately 5 days (Atwal, 1959). For clarity, eggs produced by females mated with irradiated males are hereafter referred to as “SIT eggs”, whereas eggs produced after mating with untreated males are referred to as “control eggs”.

The parasitoid was subsequently exposed to SIT eggs under various conditions, and key biological parameters, such as the longevity and fertility of the offspring emerging from these eggs, were evaluated to identify any differences compared to those parasitoids that developed on normally fertile eggs. Therefore, the aim of this study was to assess whether SIT eggs can support the successful parasitization, development and performance of *Gryon aetherium* in comparison with eggs from untreated individuals.

## 2. Material and Method

### 2.1. Insects rearing and eggs collection

The laboratory colonies of *B. hilaris* and *G. aetherium* used in this study were maintained in the quarantine greenhouse of the European Biological Control Laboratory (USDA-ARS-EBCL) at Montferrier-sur-Lez (France) under official agreement No. R76-2019-162, issued by the French Ministry of Agriculture (Martel and Sforza, 2021).

### 2.2. The host colony

*Bagrada hilaris* colony was obtained in 2016 from a colony maintained at the University of California, Riverside, USA, and reared in mesh insect cages (60 × 60 × 60 cm, 680 μm opening mesh; BugDORM®, Taiwan) in a quarantine greenhouse (T: 25 ± 5 °C). Natural daylight was supplemented with sodium lamps (L/D 12:12; RH: 30 ± 20%). Depending on plant availability, insects were fed with greenhouse-grown (22 °C, RH: 50 ± 20) white mustard (*Sinapis alba*), broccoli (*Brassica oleracea* var. *italica*), and field-collected false arugula (*Diplotaxis erucoides*). To enable oviposition, three Petri dishes (9 cm Ø) filled with fine sand (DIALL®, yellow sand, granulometry = 200 μm, Spain) were placed in each cage, and eggs were collected by sifting the sand every two days through a sieve (Hogentogler®, mesh size = 425 μm, USA).

To obtain a colony with irradiated individuals, 50 males were sent every 2 months to Rome (Italy) through a DHL with a “5 level” packing procedure to be subjected to irradiation. Five groups of 10 three day-old males were confined in Petri dishes (9 cm Ø) and were irradiated at 80 Gy (dose rate 2.92 Gy/min) at the 60Co gamma facility Calliope at ENEA (Italian National Agency for New Technologies, Energy, and Sustainable Economic Development, Rome) (Baccaro et al., 2019). At the end of the process, all irradiated material was brought back to the EBCL quarantine using the same procedure. Upon arrival, adults were released into mesh insect cages (60 × 60 × 60 cm, 680 μm opening mesh; BugDORM®, Taiwan) under the same rearing conditions as the control colony containing 50 virgin females ≤ 3 days of age. Egg collection took place every two days in the same way as for non-SIT egg collection.

### 2.3. The parasitoid colony

*G. aetherium* colony was obtained from Pakistan (Mahmood et al., 2015) and has been maintained since 2016 in transparent plastic insect-proof boxes (18 × 12 × 5.5 cm). Aeration was ensured through holes in the lid (Ø = 50 mm), covered with nylon netting. The rearing boxes were kept in a rearing chamber (22 ± 0.5 °C, RH 50 ± 5 %, L/D 12:12), and drops of diluted acacia honey (Lune de Miel ®, France) were dropped on the mesh as an *ad libitum* food source. The parasitoid colony was propagated by exposing freshly collected *B. hilaris* eggs to female parasitoids twice a week.

### 2.4. Eggs exposure and data collection

Female parasitoids up to a 3-day age previously mated and naive were exposed to SIT eggs under three different conditions. The first experiment, aimed at verifying the suitability of SIT eggs as a suitable parasitizing substrate, involved the exposure of fresh eggs (less than 48h old) in a no-choice setting.

The second experiment focused on identifying the possible preferences between SIT and fertile eggs, both fresh, in a choice context. A third experiment, named "Suitability for parasitization of aged SIT eggs", involved the exposure of different-aged eggs eggs oviposited at different time periods (5, 8, 10, 12, 15, and 20 days previously) to test the ability of the parasitoid to parasitize and reproduce through a mature substrate. It is important to note that in this experiment, a control with untreated eggs was not carried out. This choice is justified by the insufficient numerical availability of untreated aged eggs, as under the chosen experimental conditions they generally hatch within 5 days.

In the no-choice and the age of SIT eggs suitability experiments, *B. hilaris* eggs, either SIT or control, were glued (UHU® stick, Germany) in groups of 10 on a cardboard patch (1.5 x 0.75 cm) (Martel et al., 2019). Eggs were stored in a Petri dish (3 cm Ø) sealed with Parafilm© to prevent dessication (T: 25 ± 5 °C; RH: 30 ± 20%). Four mated and naive *G. aetherium* females were added into the Petri dishes when the preset days for egg age were reached. After 24h, all parasitoids were removed and the eggs were placed in an incubator (Percival Scientific, model E-30B, USA) (T: 30 °C, RH 50%, L:D 12:12) to accelerate the development time and emergence of potential parasitoids (Martel and Sforza, 2021). The four removed females were then exposed to five control eggs for 24 h to perform a fertility check and to consider only replicates with parasitoids that had parasitized at least one of the eggs exposed in this part of the trial (all replications were considered valid except one in the control, where the tested female was found dead). In choice experiments, 10 SIT eggs and 10 control eggs were glued onto two cardboard patches in contact with each other (1.5 x 0.75 cm), placed in a Petri dish and exposed to four females of *G. aetherium*. After two hours, the parasitoids were removed, and the two pieces of cardboard were isolated individually in two Petri dishes and placed in the incubator at 30 °C. An exposure time of two hours was chosen to detect a preference, allowing the individuals to have sufficient time to oviposit in the first chosen eggs, but not enough time to complete parasitization of all exposed eggs.

All eggs in the Petri dishes placed in the incubator were removed after 20 days, a sufficient time to allow the emergence of offspring (Martel and Sforza, 2021), and moved to the freezer at − 18 °C. Following a 7-day freezing period, the emerged parasitoids were counted and sexed through a stereomicroscope (Stemi 508, Zeiss®, Germany) located inside the quarantine facility. Parasitization was considered effective only when the larval development of *G. aetherium* led to the emergence of the adult (apparent parasitism). Eventually emerged *B. hilaris* nymphs were also considered within the choice experiment.

### 2.5. Offspring fertility

Alongside the no-choice experiment, the ability of emerged females to mate and parasitize was also tested. Trials that provided a minimum of one individual female that emerged from one of the 10 previously exposed eggs were selected. Fifteen replications formed by groups with an average of 2 females were obtained. Each group of females obtained from the selected replicates was isolated in a Petri dish with a drop of honey and a number of males in a 2:1 sex ratio. After 24hr, cardboard with 10 glued control eggs was added. Twenty-four hours later, the individuals were removed, and the eggs were placed back in the incubator (T: 30 °C, RH 50%, L:D 12:12) to await potential hatching.

### 2.6. Offspring Longevity

A cardboard patch with approximately 200 eggs of *B. hilaris* (control and SIT eggs were kept separately) was exposed individually to 50 non-virgin and naive *G. aetherium* females at 26 °C RH: 30 ± 20% L:D 12:12 for four days. The parasitoids were then removed and eggs transferred to the incubator. In order to identify the exact date of emergence, a check of the new adults was carried out daily. Then, the emerged parasitoids were sexed and isolated one by one in a cylindrical insect-proof box of 9 cm in diameter (from control eggs: *n* of females = 28, *n* of males = 30; from SIT eggs: *n* of females = 30, *n* of males = 30). As nourishment, drops of honey and water were provided *ad libitum* on the cap through a mesh. The emerged parasitoids were kept in the boxes under the same climatic conditions as the initially exposed eggs. A check on the vitality of the individuals was carried out each day to identify the precise day of death.

### 2.7. Data analysis

All statistical analyses were performed on R 4.1.1 (R Core, 2023). The data were always checked for normality using a Shapiro–Wilk test before conducting any statistical test and presented with their means and 95% of confidence intervals. In the no-choice the analysis of differences between the two sexes of the parasitoid were tested with a two-way ANOVA test followed by Tukey's HSD post hoc test. A survival analysis was performed using the Cox proportional hazards model to check the differences in the longevity of female and male parasitoids emerged from control and SIT eggs. In the choice test, an analysis was performed on the emergence of *G. aetherium* and *B. hilaris* in the two treatments through a Wilcoxon signed rank test with continuity correction. An initial analysis of the suitability for parasitization of aged SIT eggs experiment took into account the parasitization rate across all trials, defining positive replicates as those in which at least 1 in 10 eggs was parasitised. Replicates in which none of the females were able to parasitise the eggs were considered negative. A subset of the positive replicates was then selected to study the average number of parasitised aged eggs in each sample and the statistical differences have been identified through the Kruskal-Wallis test followed by a Dunn test with Bonferroni correction as post hoc using the "dunn.test" package (Dinno, 2015). To assess whether egg age influences offspring sex ratio, we fitted a binomial generalized linear model using the number of females and males as a two-column response. All plots were generated using the "tidyverse" package (Wickham et al., 2019).

## 3. Results

### 3.1. No-choice and fertility tests

The outcomes of the no-choice test analysis showed significant differences in the mean number of female and male parasitoids emerging from control eggs (n=31) (diff = 3.58, 95% CI = 2.10–5.06, $p < 0.001$), presenting a significantly higher value of emerging males. The same pattern also occurred for the exposure to SIT eggs (n=32) (diff = 3.66,

95% CI = 2.20–5.12, p < 0.001). Comparing the number of parasitoids for both sexes in the different treatments, no significant difference was obtained between either males (diff = -0.57, 95% CI = -2.04–0.90, p = 0.745) or females (diff = -0.64, 95% CI = -2.12–0.83, p = 0.665) emerging from control eggs and SIT eggs. The mean of females emerged from the control eggs is 23.83±4.63% and from the SIT eggs is 21.05 ±5.55%, meaning that in control eggs, the sex ratio corresponded to approximately 3 males for every female, while in SIT eggs the ratio was about 4 males per female. The results of this test indicate that, in addition to having a similar sex ratio, the total number of adults that hatched from the SIT eggs does not differ significantly from that recorded for the control eggs. Concerning the test of offspring fertility, 12 of 15 of the groups of females obtained from SIT eggs were able to parasitize the exposed eggs, indicating that 80% of them are able to mate and reproduce successfully.

### 3.2. Longevity test

The analysis on the lifespan of individuals of both sexes emerged from SIT and control eggs showed a tendency for females to live longer than males in both treatments although these differences do not reach significance (HR = 1.30, p = 0.321). Moreover, the model revealed no significant effects of treatment (p = 0.979), or its interaction with the sex (p = 0.544) on longevity. In particular, the longevity of females emerged from control eggs was 51.21±10.66 days and from SIT eggs was 54.33±6.50 with an average difference of 3.12 days, while in males from control eggs the lifespan was of 46.43±12.58 days and from SIT eggs was of 49.33±10.99 days with an average difference of 2.9 days. Overall, the model was not significant (Likelihood ratio test, p = 0.7), indicating that neither sex nor treatment influenced survival.

### 3.3. Double choice test

The results of the choice test analyses indicated a significant difference in the number of host nymphs hatched between the two treatments (Fig. 1a), with a consistently lower number emerging from SIT eggs (4.12±1.15%) than that from the control eggs (30.28±4.26%) (V = 369, p < 0.001). The total number of parasitoids emerged (females and males) from SIT eggs (16.39±3.72%) was significantly lower than that from control eggs (28.06±5.56%) (V = 177, p = 0.033) (Fig. 1b). Although the no-choice experiment revealed no difference in parasitism between the two treatments, this second outcome suggests a preference for the control eggs over the SIT ones when they are offered together.

### 3.4. Suitability for parasitization of aged SIT eggs

An initial analysis of this assay aimed to investigate how many replicates out of the total number of replicates showed that at least one adult parasitoid emerged from the aged SIT eggs (Fig. 2). It is noticeable that as the exposed eggs become older day after day, the number of positive replications decrease, but not in a linear way: for 2-day-old eggs (n=32), the number of positive replicates is 96.9%, after 5 days (n = 36) it is 88.9%, and then drops steeply to 35.5% for 8-day-old eggs (n=31) and rises again to 53% at 10 days (n=28). The lowest values occur after 12 (n=34) and 15 (n=18) days with 14.7% and 11.1% of positive replicates. Parasitization of 20-day-old eggs (n=19) has not been observed.

By focusing on the replicas that had at least 1 parasitised egg in 10 (Fig. 3) and analyzing the parasitization rate of the 10 exposed eggs, we noticed a decrease in the parasitization rate as the age of the exposed eggs increased. In this case, the average number of emerging parasitoid offspring decreases as the age of the eggs increases. The analysis revealed significant differences between ages 2d and 5d with 10 (2d: Z = -4.922, p = 1.282e-05, 5d: Z = -3.239 p = 1.195e-03), 12d (2d: Z = -4.060 p = 7.348e-04, 5d: Z = -2.958, p = 4.644e-02) and 15d (2d: Z = -2.682, p = 1.097e-01, 5d: Z = -1.951 , p = 7.647e-01).

A binomial generalized linear model was used to assess the effect of egg age on the offspring sex ratio. The analysis indicated a slight negative relationship between egg age and the proportion of female offspring (Estimate = -0.025), suggesting a tendency toward a more male-biased sex ratio with increasing egg age. However, this effect was extremely weak, as indicated by the minimal reduction in deviance (null deviance = 130.3; residual deviance = 130.2), suggesting that egg age does not meaningfully affect sex ratio.

## 4. Discussion

The objective of this study was to test the potential combination of SIT and CBC under laboratory conditions to develop a control strategy for *B. hilaris* by evaluating the ability of the oophagous parasitoid *G. aetherium* to parasitize eggs fertilized by sperm of irradiated males. The results indicate that eggs produced by females mated with sterile males can serve as a substrate for parasitization by *G. aetherium* and can support parasitoid development with good fitness levels. As shown in previous studies, egg non-viability does not necessarily prevent parasitoid females from recognizing host eggs and accepting them as oviposition substrates. Similar observations have been reported for masses of *H. halys* and other pentatomid eggs killed by freezing or irradiation, including cases involving *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae) (McIntosh et al., 2019; Roselli et al., 2023),

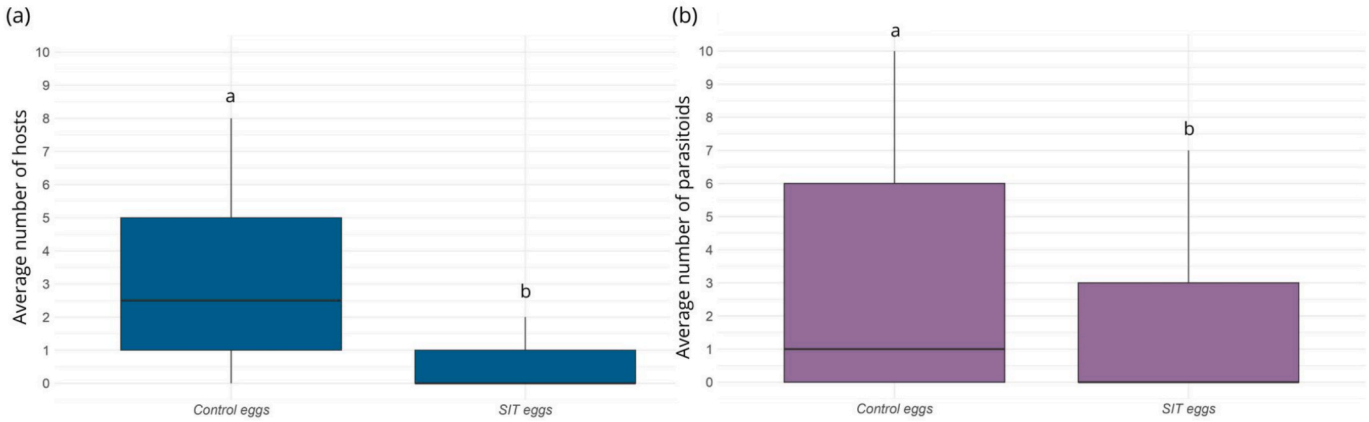



**Fig. 1.** Boxplot describing values for the number of emerged hosts (*Bagrada hilaris*) (a) and parasitoids (*Gryon aetherium*) (b) in the double-choice experiment from the control eggs and SIT eggs (n = 36). The box plots represent the interquartile range, with a horizontal bar being the median. The bottom whisker and the upper whisker represent the minimum and maximum values, respectively. Different letters indicate significant differences for each behavioral parameter between treatments.

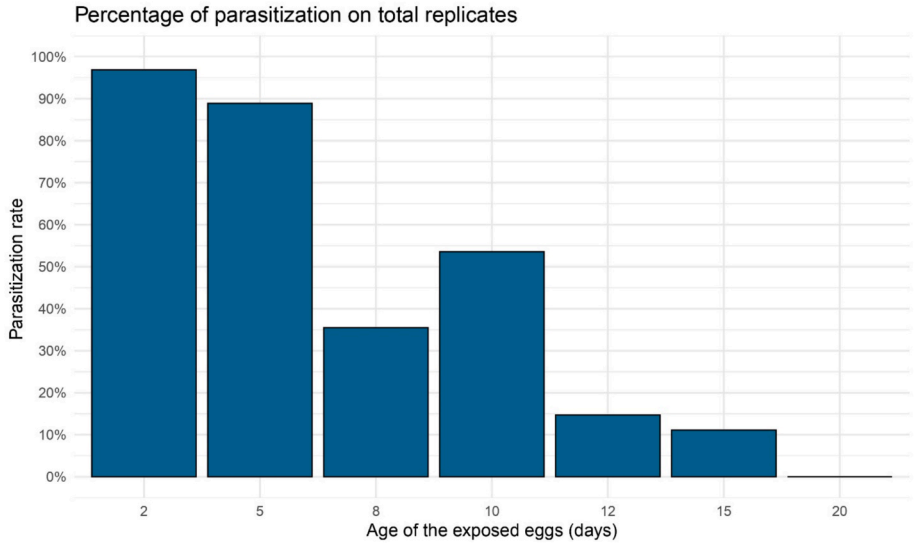



**Fig. 2.** Percentage of insect emergence of *Gryon aetherium* across SIT eggs of different days. Bars represent the proportion of samples in which emergence occurred (emerg = "y") relative to the total number of observations for each day. Values are expressed as percentages, with 100% indicating emergence in all replicates and 0% indicating no emergence.

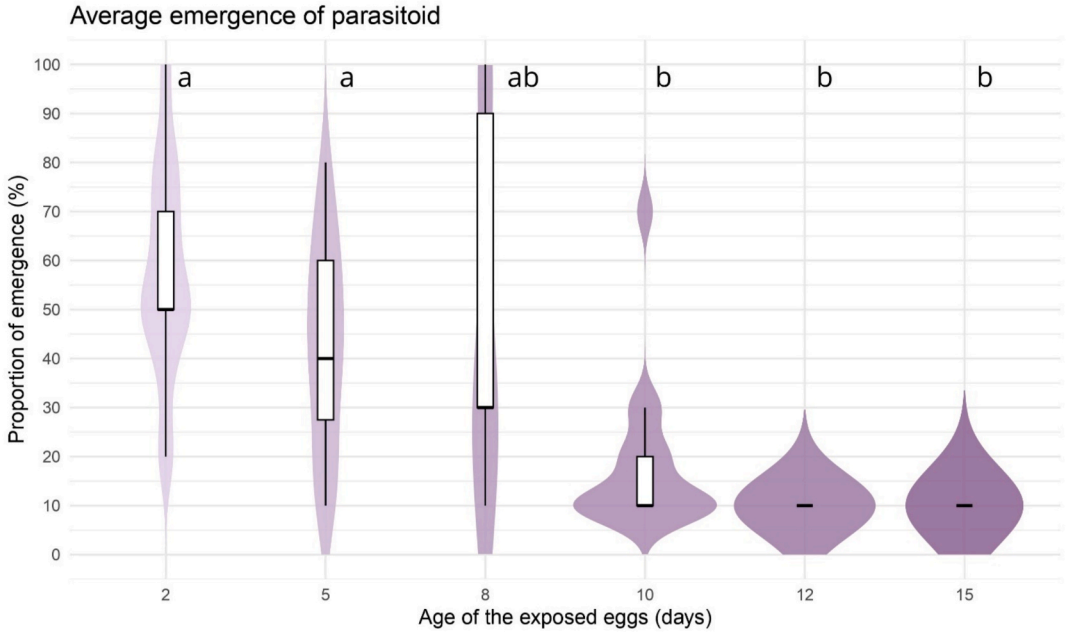



**Fig. 3.** Violin plot with boxplot of the average percentage of parasitoid (*Gryon aetherium*) from eggs of different days of age. The analysis was performed on the subset of replicates that presented at least one parasitoid emergence from ten exposed eggs (2 d, n = 31; 5 d, n = 32; 8 d, n = 11; 10 d, n = 15; 12 d n = 5; 15 d n = 2). Different letters indicate statistically different values.

*Trissolcus euschisti* Ashmead (Hymenoptera: Scelionidae) (Cornelius et al., 2021), and *Anastatus bifasciatus* Geoffroy (Hymenoptera: Eupelmidae) (Stahl et al., 2019). From a biological perspective, parasitoids hatched from SIT eggs showed longevity comparable to those emerging from control eggs. These results may suggest favorable ecological implications for the combined use of the two methods in a scenario where the target population is subjected to demographic control through the release of sterile males and species-specific parasitoids. One advantage identified in this study is the longer suitability of SIT eggs as a parasitizable substrate.

Although young eggs are preferred by other oophagous parasitoids of *Gryon spp*, (Rocha et al., 2006; Morrill and Almazan, 1990; Olson and Nechols, 1995; Noda, 1993; Romeis, Shanower and Madhuri, 2000), *G. aetherium* was previously shown to maintain an unchanged parasitization rate on 4-day-old eggs compared to eggs that were 24 h old (Martel et al., 2019). After this period, the embryos of *B. hilaris* reach maturity in normal eggs under laboratory conditions (Deep et al., 2014). Therefore, the use of SIT eggs may increase the likelihood of parasitization, as they appear to remain suitable for longer than non-SIT eggs. In our experiments, the average parasitization rate per sample did not differ significantly from fresh eggs (48 h) to 8-day-old eggs, with only an 8% decrease in the number of emerged individuals (Fig. 3). However, the proportion of positive replicates in the 8-day-old test subset was lower than that in fresh eggs, decreasing from 96.9% to 35.5% (Fig. 2). 10-day-old SIT eggs remained suitable for parasitoid development in more than half of the exposed individuals, with a 17.5% increase in

positive results compared to 8-day-old eggs. Mature eggs were stored at the same temperature range used for *B. hilaris* rearing (25–30°C) to approximate conditions favorable for host embryo development. From approximately 10 days onward, the eggs became visibly dehydrated and shriveled, which was associated with a marked decline in the parasitization rate. Nevertheless, a minimal hatching rate was observed after 15 days. This could facilitate the parasitization process by giving the parasitoid more time to locate the eggs buried individually in the soil. In addition to the physical location of the eggs, the ability of egg parasitoids to locate their hosts may also be influenced by chemical signals, including kairomones associated with the host eggs or chemical traces left by females during oviposition, as reported for other systems involving pentatomids and egg parasitoids (Peri et al., 2006; Colazza et al., 2010; Aartsma et al., 2021; Chiappini et al., 2021). The hypothesis that SIT eggs have a different chemical composition could be a possible reason for the egg parasitoid's preference for the control eggs in the double-choice experiment. To obtain accurate results on this issue, it is necessary to carry out analyses of the organoleptic properties of the SIT eggs.

The temporal availability of the SIT eggs, combined with the similar longevity of the emerged parasitoids, may be important in a context where the host undergoes extended periods of inactivity. Each year, for several months, *B. hilaris* slows its metabolism and does not oviposit, remaining in sheltered areas. In a potential biological control program, identifying the optimal timing for BCA release is necessary (Shea and Possingham, 2000; Stack et al., 2020). In our study, parasitoid longevity may have improved the likelihood of host encounters at the end of quiescence. The maintenance of longevity under host paternal sterility supports the rationale for this approach, although natural conditions are likely to be more challenging than those in controlled chambers with *ad libitum* food. Therefore, field studies are needed to verify whether parasitoids that emerge from SIT eggs can maintain similar survival rates under natural conditions. However, it should be noted that in these preliminary tests, both male and female parasitoids were kept virgin and naïve, which may not fully reflect the field conditions where mating typically occurs. While some studies on egg parasitoids have found no significant difference in female longevity between virgin and mated individuals (Nechols et al., 1989), mating can affect lifespan in some species, particularly in males (Liu et al., 2025). Therefore, future studies should investigate the longevity and reproductive performance of mated parasitoids emerged from SIT eggs under more ecologically realistic conditions.

From the perspective of augmentative biological control, in areas where *G. aetherium* is already present, such as California (Talamas et al., 2021) and Chile (Rojas-Gálvez et al., 2021), a possible strategy would be to increase adventive populations and provide conditions that may favor their establishment. In this regard, the sex ratio analysis yielded favorable results, showing that the numbers of males and females emerging from SIT eggs were comparable to those emerging from control eggs. This suggests that egg sterility does not affect parasitoid sex determination. Preliminary tests were conducted to assess whether parasitoid females emerged from SIT eggs and could mate and reproduce using eggs as a substrate. Furthermore, 80% of the emerged females were able to mate and parasitize other eggs, from which progeny subsequently emerged. These preliminary findings require additional replicates and appropriate controls before firm conclusions can be drawn, as offspring fertility is a crucial trait for population maintenance and for understanding whether the system may support increases in the natural enemies of the target pest.

Risk assessment is a necessary prerequisite for classical or augmentative biological control. In this context, sentinel eggs could support the implementation of a monitoring system for released parasitoids. Cristofaro et al. (2022) introduced the concept of "sterile sentinel eggs". Sterility can be achieved through different methods, such as direct irradiation of eggs (Sforza et al., unpublished data), refrigeration, or, as shown in the present study, by irradiating males to obtain SIT eggs. In parasitization tests with *Trissolcus japonicus* (Hymenoptera: Scelionidae), a native parasitoid of *H. halys*, SIT eggs proved to be a more valuable substrate than eggs obtained using the other two approaches (Roselli et al., 2023). This may be because SIT eggs are less physiologically altered than those directly exposed to gamma irradiation or refrigeration. The latter treatments may affect compounds on the egg surface, such as kairomones, which are important for short-range host location by parasitoids (Malek et al., 2021; Scala et al., 2022; Martel and Sforza, 2021). In the case of *G. aetherium*, similar conditions seem to apply: Martel et al. (2019) showed that *G. aetherium* rejected most eggs frozen at -80 °C for 2–3 days. In the few cases where parasitization occurred, the parasitoid completed its development from larva to adult stage. This suggests that the *B. hilaris* embryo may not need to be alive to host and nourish the developing parasitoid. Accordingly, SIT eggs, which mostly contain non-viable embryos, may still be attractive. The deployment, retrieval, and subsequent analysis of SIT eggs in areas subject to biological control could support the monitoring of field-established parasitoids.

In addition, the use of long-lasting SIT sentinel eggs may help researchers identify both native and adventive oophagous parasitoids in areas invaded by the alien pest. Their extended suitability as a parasitizable substrate would also offer a logistical advantage during transport from the rearing site to the irradiation facility and then to the release area. Finally, the sterile nature of these eggs may reduce the risk of introducing new harmful insects, as the hatching rate of eggs produced by mating between a healthy female and a male irradiated at 80 Gy is approximately 7.4% (Cristofaro et al., 2022). This residual fertility indicates a potential limitation of SIT as a stand-alone strategy, particularly in programs that require high overflooding ratios. In the present study, however, SIT is not proposed as an independent technique but as part of an integrated approach with classical biological control. In this context, even partially sterile eggs may still contribute to supporting parasitoid development while reducing host population growth.

Notably, higher irradiation doses, such as 100 Gy, which lead to near-complete sterility, have been previously shown to negatively affect male competitiveness. Irradiated males exhibit impaired vibrational communication (Peccerillo *et al.*, 2023) and altered mating behavior (Mainardi et al., 2025), reducing their ability to compete with fertile males. Therefore, the residual fertility observed at 80 Gy may represent a biologically relevant compromise, allowing sufficient male competitiveness while reducing egg viability. This trade-off between sterility and performance is a recognized constraint in SIT programs and should be carefully considered when optimizing irradiation protocols. Alternative approaches, such as direct irradiation of eggs to obtain fully sterile substrates, may further improve the system and could be explored in future studies. These considerations underline the need to evaluate the combined approach under actual field conditions.

It is worth mentioning that in this study, only apparent parasitization, in which adult parasitoids emerged from exposed eggs, was considered valid. From a biological perspective, however, not all parasitized eggs necessarily allow the host to complete embryonic development, as in cases of non-apparent parasitization. Future studies on this phenomenon could improve our understanding of parasitoid population dynamics under field release scenarios and contribute to a more accurate and realistic risk assessment. In no-choice experiments, no *B. hilaris* nymphs emerged from SIT eggs, even though they were not completely sterile (Cristofaro et al., 2022). This also occurred when no parasitoids emerged from the 10 exposed host eggs, indicating that parasitization was below 100%. This suggests that the actual number of parasitized eggs may have been higher than that estimated. Although not all parasitized eggs provide a viable substrate for complete larval development, they inhibit host development (Martel et al., 2019).

Although this study yielded many promising findings, several aspects require further investigation. Although the current results are consistent with the compatibility between SIT and CBC, additional laboratory and field studies are needed to assess whether potential synergies occur

under natural conditions. Field validation is particularly important for better predicting parasitoid behavior and fitness in the natural environment, and vital parameters should be evaluated in relation to the climatic and ecological conditions of the possible release area. The same applies to egg quality, which is also influenced by environmental pressures, such as predation and desiccation, which may reduce its availability as a substrate for parasitoid reproduction. In this regard, confined areas such as Mediterranean islands where *B. hilaris* is already present, including Pantelleria and Malta, may represent suitable locations for releasing sterile sentinel eggs and testing the potential synergy between the sterile insect technique and biological control within an AW-IPM program.

## Author contribution

CP, CEM, MC, GA and RFHS designed the study. CP, NR and CEM performed the experiments. AP supported the rearing and the irradiation process. CP analysed the obtained data. CP and CEM wrote the first draft of the manuscript. All authors contributed to the final draft and approved the manuscript.

## CRediT authorship contribution statement

**Chiara Peccerillo:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Chiara Elvira Mainardi:** Visualization, Investigation, Data curation, Conceptualization. **Nathalie Ramualde:** Writing – review & editing, Methodology, Conceptualization. **Alessandra Paolini:** Writing – review & editing, Visualization, Methodology. **Massimo Cristofaro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Conceptualization. **Gianfranco Anfora:** Writing – review & editing, Validation, Supervision. **René F.H. Sforza:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

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

## Acknowledgments

The authors would like to thank the team of the Biotechnology and Biological Control Agency (Rome, Italy) and Alessia Cemmi and Ilaria Di Sarcina from the Calliope irradiation facility for the support.

## References

Sforza, R.F.H., Peccerillo, C., Mainardi, C.E., Cemmi, A. & Cristofaro, M. in prep. A preliminary evaluation of the use of *Bagrada hilaris* (Hemiptera: Pentatomidae) irradiated eggs by the parasitoid *Gryon aetherium* (Hymenoptera: Scelionidae).

Aartsma, Y., Bianchi, F.J.J.A., van der Werf, W., Poelman, E.H., 2021. Finding an egg in a haystack: variation in chemical cue use by egg parasitoids of herbivorous insects. Curr. Opin. Insect Sci. 42, 70–76. https://doi.org/10.1016/j.cois.2020.10.006.

Baccaro, S., Cemmi, A., Di Sarcina, I. & Ferrara, G. 2019. Gamma irradiation Calliope facility at ENEA – Casaccia Research Centre (Rome, Italy) ENEA Technical Report RT/2019/4/ENEA, ISSN/0393-3016.

Bundy, C.S., Grasswitz, T.R., Sutherland, C., 2012. First Report of the Invasive Stink Bug *Bagrada hilaris* (Burmeister) (Heteroptera: Pentatomidae) from New Mexico, with Notes on its Biology. Southwest. Entomolog. 37 (3), 411–414. https://doi.org/10.3958/059.037.0317.

Carapezza, A., 1981. Gli Eterotteri dell'Isola di Pantelleria (Insecta, Heteroptera). Il Naturalista Siciliano S. 4 (5), 73–91.

Carpintero, D.L., Quiroga, V.N., Celentano, E. & Holgado, M.G. 2021. Primer registro de *Bagrada hilaris* (Burmeister, 1835)(Hemiptera: Pentatomidae) para la república Argentina. https://ri.conicet.gov.ar/handle/11336/208755.

Carvajal, M.A., Alaniz, A.J., Núñez-Hidalgo, I., González-Césped, C., 2019. Spatial global assessment of the pest *Bagrada hilaris* (Burmeister) (Heteroptera: Pentatomidae): current and future scenarios. Pest Manag. Sci. 75 (3), 809–820. https://doi.org/10.1002/ps.5183.

Chiappini, E., Mazzi, D., Giorgini, M., Conti, E., 2021. Chemical communication in host location by egg parasitoids: a review of the role of kairomones and infochemicals. J. Insect Behav. 34, 1–17.

Colazza, S., Guarino, S., Peri, E., 2004. *Bagrada hilaris* (Burmeister) (Heteroptera: Pentatomidae) a pest of capper in the island of Pantelleria [Capparis spinosa L.; Sicily]. Informatore Fitopatologico (Italy) 54 (12).

Colazza, S., Cusumano, A., Wajnberg, E., 2010. Activity of parasitoids in response to chemical cues from their hosts. In: de Moraes, R.G., Silva, M.C.P.F., Picanço, M.C. (Eds.), Semiochemicals in Integrated Pest Management. Springer, Dordrecht, pp. 1–25. https://doi.org/10.1007/978-90-481-3368-3_1.

Conti, E., Avila, G., Barratt, B., Cingolani, F., Colazza, S., Guarino, S., Hoelmer, K., et al., 2021. Biological control of invasive stink bugs: review of global state and future prospects. Entomol. Exp. Appl. 169 (1), 28–51. https://doi.org/10.1111/eea.12967.

R Core, T. 2023. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing. [Cited 2 February 2023]. https://www.R-project.org/.

Cornelius, M.L., Herlihy, M.V., Vinyard, B.T., Weber, D.C., Greenstone, M.H., 2021. Parasitism and predation on sentinel egg masses of three stink bug species (Hemiptera: Pentatomidae) in native and exotic ornamental landscapes. J. Econ. Entomol. 114 (2), 590–596.

Cristofaro, M., Sforza, R.F.H., Roselli, G., Paolini, A., Cemmi, A., Musmeci, S., Anfora, G., Mazzoni, V., Grodowitz, M., 2022. Effects of Gamma Irradiation on the Fecundity, Fertility, and Longevity of the Invasive Stink Bug Pest *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae). Insects 13 (9), 787. https://doi.org/10.3390/insects13090787.

Deep, K., Kumar, A., Singh, D.P., Yadav, P.R., 2014. Study Of The Ecological Factors Affecting On Food Preference Experiment And Population Dynamics Of *Bagrada Cruciferarum* Krik. J. Exp. Zool. India 17 (1), 257–260.

Dinno, A., 2015. Nonparametric Pairwise Multiple Comparisons in Independent Groups using Dunn's Test. The Stata Journal: Promoting communications on statistics and Stata 15 (1), 292–300. https://doi.org/10.1177/1536867X1501500117.

Falagiarda, M., Carnio, V., Chiesa, S.G., Pignalosa, A., Anfora, G., Angeli, G., Ioriatti, C., Mazzoni, V., Schmidt, S., Zapponi, L., 2023. Factors influencing short-term parasitoid establishment and efficacy for the biological control of *Halyomorpha halys* with the samurai wasp *Trissolcus japonicus*. Pest Manag. Sci. 79 (7), 2397–2414. https://doi.org/10.1002/ps.7423.

Faúndez, E.I., 2018. From Agricultural to Household Pest: The Case of the Painted Bug *Bagrada hilaris* (Burmeister) (Heteroptera: Pentatomidae) in Chile. J. Med. Entomol. 55 (5), 1365–1368. https://doi.org/10.1093/jme/tjy091.

Gard, B., Bout, A., Pierre, P., 2022. Release strategies of *Trissolcus basalis* (Scelionidae) in protected crops against *Nezara viridula* (Pentatomidae): less is more. Crop Prot. 161, 106069. https://doi.org/10.1016/j.cropro.2022.106069.

Guarino, S., Peri, E., Colazza, S., Luchi, N., Michelozzi, M., Loreto, F., 2017. Impact of the invasive painted bug *Bagrada hilaris* on physiological traits of its host Brassica oleracea var botrytis. Arthropod Plant Interact. 11 (5), 649–658. https://doi.org/10.1007/s11829-017-9516-6.

Hendrichs, J., Bloem, K., Hoch, G., Carpenter, J.E., Greany, P., Robinson, A.S., 2009. Improving the cost-effectiveness, trade and safety of biological control for agricultural insect pests using nuclear techniques. Biocontrol Sci. Tech. 19 (sup1), 3–22. https://doi.org/10.1080/09583150902985620.

Horrocks, K.J., Welsh, T., Carpenter, J.E., Suckling, D.M., 2020. Egg Sterilisation of Irradiated *Nezara viridula* (Hemiptera: Pentatomidae). Insects 11 (9), 564. https://doi.org/10.3390/insects11090564.

Huang, T.I., Reed, D.A., Perring, T.M., Palumbo, J.C., 2014. Feeding damage by *Bagrada hilaris* (Hemiptera: Pentatomidae) and impact on growth and chlorophyll content of Brassicaceous plant species. Arthropod Plant Interact. 8 (2), 89–100. https://doi.org/10.1007/s11829-014-9289-0.

Kavita, D., Arvind, K., Singh, D.P., Yadav, R., 2014. Studies of the ecological parameter, site of oviposition, population dynamics and seasonal cycle of *Bagrada cruciferarum* on *Brassica compestris*. Journal of Experimental Zoology, India 17 (1), 331–336.

Klassen, W., Curtis, C.F., Hendrichs, J., 2021. History of the sterile insect technique. In: *Sterile insect technique*. CRC Press, pp. 1–44.

Knipling, E.F., 1955. Possibilities of Insect Control or Eradication Through the Use of Sexually Sterile Males1. J. Econ. Entomol. 48 (4), 459–462. https://doi.org/10.1093/jee/48.4.459.

Lance, D.R., McInnis, D.O., 2021. Biological basis of the sterile insect technique. Sterile Insect Technique 113–142.

Liu, Y., Zhang, Y., Wang, X., Chen, X., 2025. Courtship is a major factor of mating-shortened male lifespan in an egg parasitoid *Anastatus disparis*. Ecol. Evol. 15 (8), e71932. https://doi.org/10.1002/ece3.71932.

Mahmood, R., Jones, W.A., Bajwa, B.E., Rashid, K., 2015. Egg Parasitoids from Pakistan as Possible Classical Biological Control Agents of the Invasive Pest *Bagrada hilaris* (Heteroptera: Pentatomidae). Journal of Entomological Science 50 (2), 147–149. https://doi.org/10.18474/JES14-28.1.

Mainardi, C.E., Peccerillo, C., Musmeci, S., Paolini, A., Sforza, R.F., Cemmi, A., Cristofaro, M., 2025. Sterile but sexy: Assessing the mating competitiveness of irradiated *Bagrada hilaris* males for the development of a Sterile Insect Technique. Insects 16 (4), 391.

Malek, R., Kaser, J.M., Anfora, G., Ciolli, M., Khrimian, A., Weber, D.C., Hoelmer, K.A., 2021. *Trissolcus japonicus* foraging behavior: Implications for host preference and classical biological control. Biol. Control 161, 104700. https://doi.org/10.1016/j.biocontrol.2021.104700.

Martel, G., Sforza, R.F.H., 2021. Catch me if you can: novel foraging behavior of an egg parasitoid, *Gryon gonikopalense*, against the stinkbug pest, *Bagrada hilaris*. J. Pest. Sci. 94 (4), 1161–1169. https://doi.org/10.1007/s10340-020-01325-4.

Martel, G., Augé, M., Talamas, E., Roche, M., Smith, L., Sforza, R.F.H., 2019. First laboratory evaluation of *Gryon gonikopalense* (Hymenoptera: Scelionidae), as potential biological control agent of *Bagrada hilaris* (Hemiptera: Pentatomidae). Biol. Control 135, 48–56.

McIntosh, H., Lowenstein, D.M., Wiman, N.G., Wong, J.S., Lee, J.C., 2019. Parasitism of frozen *Halyomorpha halys* eggs by *Trissolcus japonicus*: applications for rearing and experimentation. Biocontrol Sci. Tech. 29 (5), 478–493. https://doi.org/10.1093/jee/toaa329.

Morrill, W., Almazan, L.P., 1990. Effect of host plant species and age of rice bug (Hemiptera: Alydidae) eggs on parasitism by *Gryon nixoni* (Hymenoptera: Scelionidae). J. Entomol. Sci. 25 (3), 450–452.

Nechols, B.N., Jones, A.L., Simmons, G.A., 1989. Effects of host deprivation and host source on the reproductive biology and longevity of *Gryon pennsylvanicum* (Hymenoptera: Scelionidae). Ann. Entomol. Soc. Am. 82 (3), 382–387. https://doi.org/10.1093/aesa/82.3.382.

Noda, T. 1993. Ovipositional strategy of *Gryon japonicum* . *Nogyo Kankyo Gijutsu Kenkyusho Hokoku = Bulletin of the National Institute of Agro-Environmental Sciences*, 1–51.

Oliveira, R.C., Ikuno, P.H.P., Pratissoli, D., Carvalho, J.R., Hoback, W.W., Salamina, B.A. Z., 2024. Biological characteristics and thermal requirements of *Telenomus podisi* and *Trissolcus basalis* (Hymenoptera: Scelionidae) in fresh and cryopreserved eggs of *Euschistus heros* and *Nezara viridula* (Hemiptera: Pentatomidae). Agronomy 14, 170. https://doi.org/10.3390/agronomy14010170.

Olson, D.L., Nechols, J.R., 1995. Effects of Squash Leaf Trichome Exudates and Honey on Adult Feeding, Survival, and Fecundity of the Squash Bug (Heteroptera: Coreidae) Egg Parasitoid *Gryon pennsylvanicum* (Hymenoptera: Scelionidae). Environ. Entomol. 24 (2), 454–458. https://doi.org/10.1093/ee/24.2.454.

Palumbo, J.C., Perring, T.M., Millar, J.G., Reed, D.A., 2016. Biology, ecology, and management of an invasive stink bug, *Bagrada hilaris*, in North America. Annu. Rev. Entomol. 61, 453–473. https://doi.org/10.1146/annurev-ento-010715-023843.

Peri, E., Benelli, G., Canale, A., Curto, G., Conti, B., Raspi, A., Wajnberg, E., 2006. Effect of host kairomones and oviposition experience on the foraging behavior of the egg parasitoid *Trissolcus basalis*. Biol. Control 36 (3), 235–243. https://doi.org/10.1016/j.biocontrol.2005.08.008.

Reed, D.A., Palumbo, J.C., Perring, T.M., May, C., 2013. *Bagrada hilaris* (Hemiptera: Pentatomidae), An Invasive Stink Bug Attacking Cole Crops in the Southwestern United States. Journal of Integrated Pest Management 4 (3), 1–7. https://doi.org/10.1603/IPM13007.

Rocha, L.D., Redaelli, L., Jr, M., M.d.s., 2006. Oviposition behaviour of *Gryon gallardoi* (Hymenoptera, Scelionidae) on eggs of different ages of *Spartocera dentiventris* (Hemiptera, Coreidae). Iheringia. Série Zoologia 96 (3), 277–282. https://doi.org/10.1590/S0073-47212006000300001.

Rojas-Gálvez, N.R., Talamas, E., Albornoz, M.V., Flores, M.F., Barros-Parada, W., Bout, A., 2021. *Gryon aetherium* Talamas (Hymenoptera, Scelionidae): Parasitoid of *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae) Adventive in Chile. J. Hymenopt. Res. 87, 493–501. https://doi.org/10.3897/jhr.87.75363.

Romeis, J., Shanower, T.G., Madhuri, K., 2000. Biology and field performance of *Gryon clavigrallae* (Hymenoptera: Scelionidae), an egg parasitoid of *Clavigralla* spp. (Hemiptera: Coreidae) in India. Bull. Entomol. Res. 90 (3), 253–263. https://doi.org/10.1017/S0007485300000377.

Roselli, G., Anfora, G., Sasso, R., Zapponi, L., Musmeci, S., Cemmi, A., Suckling, D.M., et al., 2023. Combining Irradiation and Biological Control against Brown Marmorated Stink Bug: Are Sterile Eggs a Suitable Substrate for the Egg Parasitoid *Trissolcus japonicus*? Insects 14 (7), 654.

Sabbatini-Peverieri, G., Dieckhoff, C., Giovannini, L., Marianelli, L., Roversi, P.F., Hoelmer, K., 2020. Rearing *Trissolcus japonicus* and *Trissolcus mitsukurii* for biological control of *Halyomorpha halys*. Insects 11, 787. https://doi.org/10.3390/insects11110787.

Sánchez-Peña, S.R., 2014. First Record in Mexico of the Invasive Stink Bug *Bagrada hilaris* , on Cultivated Crucifers in Saltillo. Southwest. Entomolog. 39 (2), 375–377. https://doi.org/10.3958/059.039.0219.

Scala, M., Fouani, J.M., Zapponi, L., Mazzoni, V., Wells, K.E., Biondi, A., Anfora, G., 2022. Attraction of egg parasitoids Trissolcus mitsukurii and Trissolcus japonicus to the chemical cues of Halyomorpha halys and Nezara viridula. Insects 13 (5), 439.

Shea, K., Possingham, H.P., 2000. Optimal release strategies for biological control agents: an application of stochastic dynamic programming to population management. J. Appl. Ecol. 37 (1), 77–86. https://doi.org/10.1046/j.1365-2664.2000.00467.x.

Simaz, O., Michaelson, J., Wilson, J.K., Talamas, E., Gut, L., Pote, J., Szűcs, M., 2023. Field releases of the exotic parasitoid *Trissolcus japonicus* (Hymenoptera: Scelionidae) and survey of native parasitoids attacking *Halyomorpha halys* (Hemiptera: Pentatomidae) in Michigan. Environ. Entomol. 52 (6), 998–1007. https://doi.org/10.1093/ee/nvad102.

Stack, J.P., Kenerley, C.M., Pettit, R.E. 2020. Application of biological control agents. In: Biocontrol of Plant Diseases, pp. 43–54.

Stahl, J.M., Babendreier, D., Marazzi, C., Caruso, S., Costi, E., Maistrello, L., Haye, T., 2019. Can *Anastatus bifasciatus* be used for augmentative biological control of the brown marmorated stink bug in fruit orchards? Insects 10 (4), 108. https://doi.org/10.3390/insects10040108.

Talamas, E.J., Bremer, J.S., Moore, M.R., Bon, M.-C., Lahey, Z., Roberts, C.G., Combee, L. A., et al., 2021. A maximalist approach to the systematics of a biological control agent: *Gryon aetherium* Talamas, sp. nov. (Hymenoptera, Scelionidae). J. Hymenopt. Res. 87, 323–480. https://doi.org/10.3897/jhr.87.72842.

Taylor, M.E., Bundy, C.S., McPherson, J.E., 2014. Unusual Ovipositional Behavior of the Stink Bug *Bagrada hilaris* (Hemiptera: Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 107 (4), 872–877. https://doi.org/10.1603/AN14029.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., et al., 2019. Welcome to the Tidyverse. Journal of Open Source Software 4 (43), 1686. https://doi.org/10.21105/joss.01686.