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# Sterile but competitive: gamma irradiation of *Bagrada hilaris* males at the Calliope facility for Sterile Insect Technique (SIT) adoption

RT/2026/4/ENEA

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ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES, ENERGY AND SUSTAINABLE ECONOMIC DEVELOPMENT

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## Sterile but competitive: gamma irradiation of *Bagrada hilaris* males at the Calliope facility for Sterile Insect Technique (SIT) adoption

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***Abstract***

*Bagrada hilaris (Hemiptera: Pentatomidae) is a pest species causing severe damage to a wide range of crops, especially in the family of Brassicaceae. In recent years, this insect native to Asia and Africa has expanded its distribution into Europe, establishing populations first on the island of Malta and subsequently on Pantelleria, where it has become a key pest of caper cultivation. Currently, management of this species relies almost entirely on the application of synthetic insecticides which raise concerns about environmental and health impacts. Among sustainable and non-toxic alternatives, the Sterile Insect Technique (SIT) represents a highly promising approach. SIT involves sterilizing males of the target species through gamma irradiation. The sterilized males are then released into the field, where they mate with wild females, progressively reducing the reproductive potential of the pest population. In this study, we compared the sexual behavior of irradiated and non-irradiated males to determine whether sterile males retain sufficient competitiveness for SIT applications. After identifying the optimal irradiation dose to induce male sterility, we evaluated mating performance under different experimental conditions. Our results demonstrate that gamma irradiation does not impair male reproductive behavior at the tested doses. In fact, irradiated males outperformed non-irradiated controls across several behavioral parameters. These findings indicate that SIT could serve as an effective and sustainable strategy for the management of B. hilaris, reducing the use of chemicals and promoting environmentally sound integrated pest management practices.*

***Keywords:*** *Sterile Insect Technique; gamma irradiation; biological control; insect pest; stink bug.*

**Riassunto**

La cimice Bagrada hilaris (Hemiptera: Pentatomidae) è una specie nota per i gravi danni causati a diverse colture, in particolare quelle appartenenti alle Brassicaceae. Negli ultimi anni, questo insetto di origine asiatica e africana ha raggiunto l'Europa, insediandosi prima sull'isola di Malta e successivamente sull'isola di Pantelleria, dove si è stabilito sulle piante di cappero, diventando uno dei principali fitofagi di questa coltura. Attualmente la sua gestione si basa sull'impiego di insetticidi il cui uso solleva molte preoccupazioni per gli impatti ambientali e sanitari. Tra le alternative sostenibili, la Tecnica dell'Insetto Sterile (SIT) rappresenta un approccio particolarmente promettente. La SIT prevede la sterilizzazione dei maschi della specie bersaglio tramite l'irraggiamento con raggi gamma e il loro rilascio in campo affinché si accoppino con le femmine selvatiche, determinando la deposizione di uova sterili e riducendo così la popolazione bersaglio. In questo studio è stato valutato il comportamento sessuale dei maschi irraggiati rispetto ai maschi non irraggiati, al fine di verificare se gli individui sterilizzati mantengano una competitività sufficiente per l'applicazione della SIT. Dopo aver identificato la dose ottimale di irraggiamento per ottenere maschi sterili, sono state analizzate le prestazioni di accoppiamento in diverse condizioni sperimentali. I risultati mostrano che l'irraggiamento non compromette il comportamento riproduttivo dei maschi alle dosi testate; al contrario, per diversi parametri comportamentali gli individui irraggiati hanno evidenziato performance superiori rispetto ai controlli. Queste evidenze indicano che la SIT potrebbe rivelarsi una strategia efficace e sostenibile per il controllo di B. hilaris, contribuendo alla riduzione dell'uso di pesticidi e promuovendo pratiche di gestione integrate e a basso impatto ambientale.

**Parole chiave:** Tecnica Insetto Sterile; irraggiamento gamma; controllo biologico; insetto nocivo; cimice asiatica.

**Sommario**

## 1. Introduction

*Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), native to Eastern Africa, the Middle East, and South Asia [1,2], is now considered one of the most problematic invasive alien species affecting vegetable crops. In recent years, this multivoltine species has rapidly emerged as a major pest of Brassicaceae [3], causing significant economic losses, particularly in vulnerable agricultural systems such as small-scale and low-input farming, organic cropping systems, and insular or geographically isolated agroecosystems [4]. The geographical expansion of this species initially involved the American continent, with reports from several U.S. states [3–5] and Mexico [6,7]. In Europe, *B. hilaris* has been initially recorded in Malta and, more recently, on the island of Pantelleria (Italy) [8], where it has established at high densities on caper (*Capparis spinosa* L.) crops, including the locally cultivated "Cappero di Pantelleria", a product with Protected Geographical Indication (PGI) status, becoming one of the island's key pests.

Damage is associated with the characteristic "lacerate and flush" feeding behavior, in which the insect pierces plant tissues and injects phytotoxic saliva, causing necrosis, deformation, and injury to apical meristems [9,10]. This feeding behavior compromises plant growth and reduces productivity, an effect further exacerbated by the gregarious behavior of nymphs, which can accelerate crop collapse [11].

Current control strategies primarily rely on chemical insecticides [12], which raises concerns about environmental and health impacts. Their effectiveness is often limited due to the presence of soil-dwelling juvenile stages, the high mobility of adults, the potential development of resistance, and the impact on non-target species all highlight the need for more sustainable and selective management approaches [13].

In this context, the Sterile Insect Technique (SIT) represents a promising environmentally friendly control option for *B. hilaris*. SIT involves the mass-rearing of males and their sterilization through exposure to ionizing radiation, with the aim of progressively reducing the fertility of wild populations (Figure 1) [14–16].

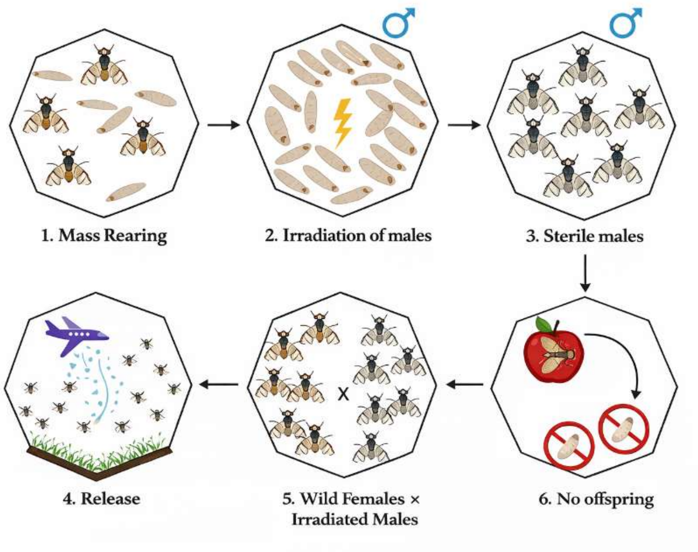


*Figure 1. Stages of the Sterile Insect Technique using the example of fruit flies* (Diptera). *Edited with BioRender.com.*

The technique has been successfully applied in several control programs against Diptera and Lepidoptera [17], and in recent years has been explored for use against phytophagous hemipterans such as *Halyomorpha halys* [18–20] and *Nezara viridula* [21–23]. Although the release of adult hemipterans may pose a risk of additional crop damage, several studies have indicated that SIT can achieve effective population suppression under specific ecological or geographical conditions [19].

Available information on irradiation effects in *B. hilaris* indicates that doses of 64 Gy induce approximately 90% sterility, whereas complete sterility is achieved at 100 Gy [24]. However, the development of reliable operational protocols requires identifying the optimal dose that maximizes sterility while ensuring adequate longevity and competitiveness of irradiated males. Because the efficacy of SIT depends on the ability of treated males to compete with wild males [16], it is essential to understand how irradiation affects reproductive behavior, including the potential physiological impairments associated with radiation exposure [25].

This study provides an experimental framework for evaluating the reproductive performance of *B. hilaris* males exposed to three irradiation doses (60, 80, and 100 Gy) compared with that of non-irradiated individuals. After identifying the optimal dose in terms of induced sterility and maintenance of male competitiveness, the insects were monitored over a three-day period to assess potential changes in behavioral activity over time.

These experiments enhanced our understanding of the reproductive dynamics of *B. hilaris* under controlled conditions, specifically evaluating the performance of irradiated males in mating and sterility transmission. The goal is to contribute to the development of the basic knowledge about biological and behavioral response to irradiation required for designing future SIT programs to eradicate or control this species. Sustainable control methods, such as SIT, are strongly advisable in protected areas, as in the case of Pantelleria island, where *B. hilaris* represents a major concern for PGI caper production.

*Figure 2.* Bagrada hilaris *feeding on a caper fruit in Pantelleria. Photo by C. E. Mainardi.*

## 2. Materials and Methods

### 2.1. Insect collection and rearing

During the summer and autumn of 2023, individuals of *Bagrada hilaris* (primarily adults, but also late-instar nymphs, fourth and fifth instars) were collected on infested caper plants at the Cooperativa Agricola Produttori Capperi on the island of Pantelleria (Italy) (GPS coordinates: 36°46′ 23″ N, 11°57′ 41″ E) (Figure 3). The number of specimens collected varied according to the season, ranging from approximately 100 (summer) to 1000 (autumn) individuals per sampling event. Collections were performed using an entomological aspirator, which proved to be more effective during the hottest hours of the day (from late morning to early afternoon).

Collected individuals were transported in cardboard tubes (4.1 × 11.9 cm) to the quarantine facility of the Edmund Mach Foundation (San Michele all'Adige, TN, Italy) where they were placed in cubic sleeve cloth cages (60 × 60 × 60 cm, BugDorm, Taichung City, Taiwan), inside four larger cages (120 × 60 × 60 cm, BugDorm, Taiwan). As oviposition sites, three Petri dishes (9 cm diameter) per cage containing around 3 mm layer of fine sand (granulometry= 200 μm) were used.

Rearing conditions were kept constant, with a temperature of 26–22 °C (day-night), relative humidity (RH) of 50-60%, and a 14L:10D photoperiod. As a food source, *Brassica oleracea* L. *var. gemmifera* (Brussels sprouts) was provided and replaced with fresh material three times per week. At the same frequency, cages were cleaned to ensure the hygienic and nutritional quality of the rearing substrate.

*Figure 3. Left: Satellite image of the caper crop at Via del Cappero 11, Pantelleria (Italy). Google Earth. Right: Insect collection in Pantelleria. Photo by C. Peccerillo.*

## 2.2. Irradiation procedure

Newly emerged fifth-instar *Bagrada hilaris* nymphs were collected from the laboratory colony, using 50 mL Corning Falcon™ tubes. Individuals were then transferred to 5 cm diameter Petri dishes and kept isolated until adult emergence. This procedure allowed the separation of sexes and the correct identification of males and females based on the sexual dimorphism [4], while ensuring the virginity of all adults used in the experiments.

Groups of 20 newly emerged males were placed in 9 cm Petri dishes and shipped from the Edmund Mach Foundation (San Michele all'Adige, TN, Italy) to Rome (Italy) by DHL, following a five-layer packaging protocol designed to maintain the integrity of the samples during transport.

Upon arrival, the insects were irradiated at the Calliope Gamma Irradiation Facility at ENEA Casaccia Research Center (Rome, Italy) [26]. The individuals were exposed to three radiation doses: 60, 80, and 100 Gy. The dose rate during irradiation treatments was about 175 Gy/h (3 Gy/min) referred to water.

Non-irradiated males and females used as controls were maintained under the same environmental conditions as those of the treated individuals. At the end of the irradiation process, all samples were repacked and returned to the quarantine facility of the Edmund Mach Foundation using the same shipping protocol.

### 2.2.1 Gamma Irradiation at the Calliope Facility

Calliope Gamma Irradiation Facility at ENEA Casaccia Research Center (Rome, Italy) is a pool-type facility equipped with a $^{60}$Co radioisotopic source array housed within a large-volume, shielded cell measuring 7.0 × 6.0 × 3.9 $m^3$ [26].

The source rack consists of 25 $^{60}$Co source rods, arranged in a planar geometry with a total active area of about 41 × 90 cm. The maximum licensed activity of the facility is $3.7 \times 10^{15}$ Bq (100 kCi). By positioning the samples at different distances from the source rack, a wide range of dose rates can be obtained; currently the maximum available dose rate is approximately 4.5 kGy/h (January 2026).

A steel platform installed above the pool, equipped with a central opening that permits the vertical movement of the source rack, enables sample positioning close to the source, thus allowing irradiation at high dose rate values. The facility is also equipped with dedicated diagnostic and remote monitoring systems for continuous control and verification of irradiation parameters.

Calliope also supports irradiation under controlled environmental conditions, allowing experiments to be performed at different temperatures and atmospheric compositions. The facility includes an in-house dosimetric characterization laboratory equipped with different dosimetric systems.

For this study, *B. hilaris* samples were positioned in dedicated sample holders within the irradiation chamber to ensure a minimal dose heterogeneity across replicates.

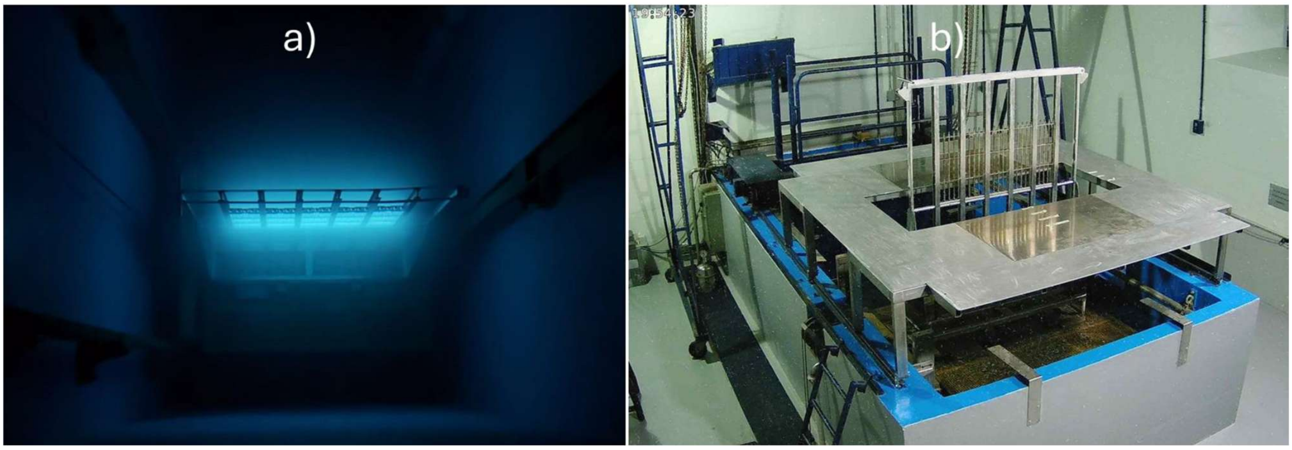


*Figure 4. Calliope source rack at the bottom of the storage pool and b) source rack within the irradiation cell (picture acquired by remote camera).*

## 3. Evaluation of sexual performance at three different doses

The reproductive performance of *Bagrada hilaris* males was evaluated by comparing individuals irradiated at three different doses (60, 80, and 100 Gy) with non-irradiated males used as control. All adults used in the experiments were virgins and belonged to the same age cohort. Behavioral tests were conducted under no-choice conditions, in which a single non-irradiated female was confined with a single irradiated male. All experiments were initiated the day after irradiation exposure.

### 3.1. Experimental Setup

For each treatment, one male–female pair was placed inside a 500 mL transparent glass jar covered with a 680 µm white polyester mesh. A 5 cm diameter Petri dish cap filled with fine sand was provided as an oviposition site, and each pair was supplied with one Brussels sprout (*Brassica oleracea* L.var. *gemmifera*) as food.

Tests were conducted under controlled laboratory conditions: 26–22 ℃ (day-night), 14L:10D photoperiod, 50-60% RH, and an illuminance of approximately 800 lx.

Each jar contained one non-irradiated virgin female and one irradiated male (either 60, 80, or 100 Gy). Twenty replicates were established for each dose. The control consisted of 20 replicates in which a non-irradiated male was paired with a non-irradiated female.

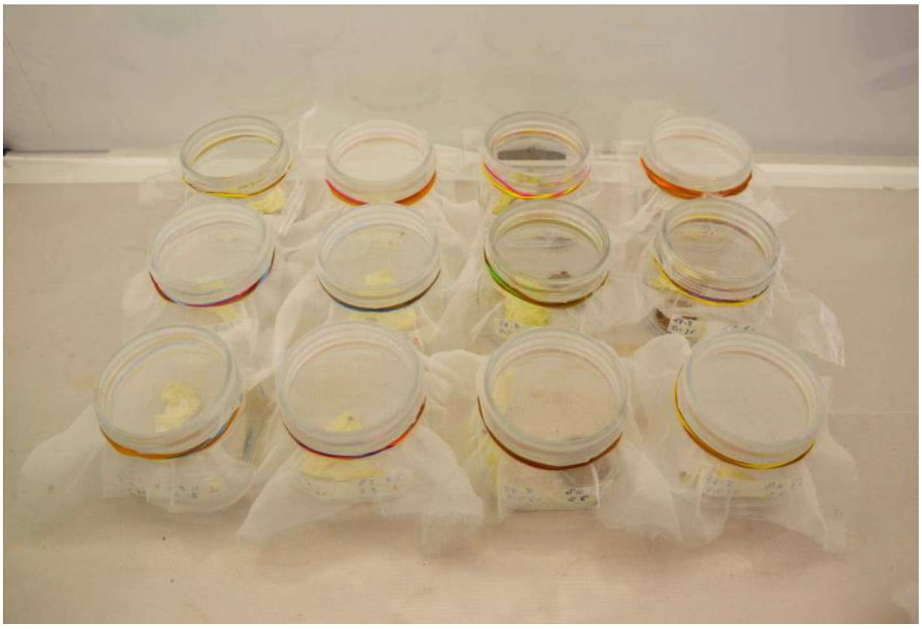

*Figure 5. Glass jars containing pairs of individuals. Photo by C.E. Mainardi*

Individuals were observed for 18 consecutive hours, and the presence or absence of copulation was recorded every 15 min. The 18 h observation period was selected as the most appropriate for a preliminary study, considering that copulation in *B. hilaris* may last several hours [27]. Observations began at 10:00 a.m. and ended at 4:00 a.m., a time chosen to include morning, afternoon, evening, and night-time activity. During the dark phase, a red headlamp (GearLight S500 LED, Walpole, MA, USA) was used to minimize disturbance.

To assess the sexual performance of irradiated males, the following parameters were measured: (i) the time elapsed before the first copulation, (ii) the number of copulation events recorded during observations, (iii) the proportion of time spent in copula (mating rate), and (iv) the duration of the first copulation. These indicators were selected to capture complementary aspects of male mating performance and competitiveness, providing an integrated view of how irradiation affects reproductive behavior.

### 3.2. Data analysis

Statistical analyses were performed considering irradiation dose (0, 60, 80, and 100 Gy) and experimental block as explanatory variables. Mating success (success/failure) was analyzed using a generalized linear mixed model (GLMM) with a binomial distribution, including block as a random effect. Latency to first copulation and duration of the first copulation were treated as count-type variables and analyzed using generalized linear models (GLMs) with a negative binomial distribution. The total number of copulation events per pair was analyzed using a GLM with a Poisson distribution. Statistical significance was assessed at the 95% confidence level.

### 3.3. Results and discussion

Mating occurrence in *Bagrada hilaris* was broadly distributed throughout daylight hours, with a marked reduction during the night. At the two lower irradiation doses (60 and 80 Gy), males exhibited mating frequencies comparable to those of non-irradiated individuals, indicating that reproductive behavior remained largely preserved at these doses (Table 1). Males irradiated at 80 Gy showed a mating rate of 73.3 ± 3.1%, which was higher than that of the control (60.1 ± 3.5%), although the difference was not statistically significant (Table 1). Notably, the time lapse before the first mating at 80 Gy was significantly shorter than that in non-irradiated males (1.21 ± 0.55 vs. 4.67 ± 2.21; p = 0.039), suggesting enhanced sexual readiness. A similar reduction in latency was observed at 60 Gy, although without statistical significance.

In contrast, exposure to the highest dose (100 Gy) resulted in a clear decline in male mating performance. The mating rate was significantly lower than that of the control (14.2 ± 2.5% vs. 21.7 ± 2.9%; p = 0.0419), and males exhibited increased latency to the first copulation (Table 1). Although neither copulation duration nor the total number of mating events differed significantly among doses, females tended to engage in longer copulations with irradiated males, particularly those exposed to 80 Gy (Table 1).

*Table 1. Means and standard errors of the analyzed response variables (mating rate, time lapse before mating, duration of first mating, and number of mating events per couple) of* Bagrada hilaris *in relation to irradiation dose and experimental block. Pairs consisted of one non-irradiated virgin female and one irradiated male (at 60, 80, and 100 Gy). Corresponding controls consisted of one non-irradiated virgin female and one non-irradiated male. Time lapse before first mating and duration of first mating are expressed as the number of observations (obs. n.). Values significantly different from the control are indicated by asterisks, according to the applied model.*

| Experimental Block | Dose (Gy) | Mating Rate (%) | Time Lapse Before First Mating (obs. n.) | First Mating Duration (obs. n.) | N. of Mating Events (n.) |
|---|---|---|---|---|---|
| 1 | 0 | 47.5 ± 3.5 | 9.33 ± 4.09 | 46.20 ± 6.24 | 1.25 ± 0.22 |
| | 60 | 64.5 ± 3.5 | 7.06 ± 3.77 | 49.94 ± 4.78 | 1.40 ± 0.24 |
| 2 | 0 | 60.1 ± 3.5 | 4.67 ± 2.21 | 43.67 ± 7.65 | 1.10 ± 0.19 |
| | 80 | 73.3 ± 3.1 | 1.31 * ± 0.55 | 57.56 ± 5.64 | 1.16 ± 0.21 |
| 3 | 0 | 21.7 ± 2.9 | 16.64 ± 4.40 | 28.55 ± 7.78 | 0.55 ± 0.11 |
| | 100 | 14.2 * ± 2.5 | 26.00 ± 10.59 | 34.33 ± 8.80 | 0.30 ± 0.11 |

Taking together, these findings highlight 80 Gy as the most suitable dose for Sterile Insect Technique (SIT) applications. This dose provides the best compromise between high sterility (≈95%), adequate longevity (32.5 ± 1.4 days; [24]), and preserved reproductive competitiveness, as demonstrated by the high mating rates and reduced copulation latency. Although 60 Gy also induces high sterility (≈90%), the higher behavioral performance observed at 80 Gy compared to the control makes it the preferred choice for SIT-based strategies, where rapid mate location, successful courtship, and sustained copulation are essential for competitive success against wild males.

The detrimental effects observed at 100 Gy are consistent with previous reports [28], which documented alterations in male vibrational signals, specifically reduced peak frequencies, resulting in impaired mate attraction.

Overall, the combined evidence clearly identifies 80 Gy as the optimal irradiation dose for subsequent studies and the most promising for developing an SIT program targeting *B. hilaris*.

## 4. Study of sexual behavior at the optimal dose (80 Gy)

Following the preliminary experiment, differences in mating success between irradiated males (80 Gy) and non-irradiated males were assessed under both no-choice and choice conditions. In addition, feeding activity was monitored to examine possible behavioral alterations associated with irradiation.

### 4.1. Experimental Setup

Fifth-instar *Bagrada hilaris* nymphs were collected from the laboratory colony using an entomological aspirator and transferred individually into 5 cm diameter Petri dishes, where they were maintained until adult emergence. Upon emergence, adults were sexed based on sexual dimorphism and kept virgin. Groups of ten virgin males were then placed in 9 cm Petri dishes and irradiated at 80 Gy at the Calliope Gamma Facility (ENEA Casaccia, Rome, Italy), following the same procedure used in the preliminary tests. To avoid introducing confounding effects related to transport stress, non-irradiated control males underwent the same logistical route, although without exposure to radiation.

#### 4.1.1. No-choice test

In the no-choice test, one non-irradiated female and one male irradiated at 80 Gy were placed in a 500 mL clear glass jar covered with 680 µm white polyester mesh. Each pair was provided with a single Brussels sprout as food. Control pairs consisted of one non-irradiated male and one non-irradiated female.

For each treatment (irradiated and control), 100 replicates were performed. Tests were conducted under controlled laboratory conditions: 26–22 °C (day-night), 50-60% RH, 14L:10D photoperiod, and ~800 lx illumination. Observations began the day after irradiation. Each pair was monitored for three consecutive days, from 10 a.m. to 4 p.m. The time slot was chosen based on the finding of Huang et al., who reported that *B. hilaris* females exhibit peak sexual activity between 10 a.m. and 4 p.m. [9].

The following behavioral variables were recorded hourly:

- presence/absence of mating;
- presence/absence of feeding.

### 4.1.2. Choice test

The same experimental design was applied to choice test. In this case, each jar contained:

- one non-irradiated female;
- one non-irradiated male;
- one male irradiated at 80 Gy.

Two types of controls were included:

1. Positive control: one female with two non-irradiated males.
2. Negative control: one female with two irradiated males (80 Gy).

For each treatment and each control, 100 replicates were performed. Mating and feeding activity were recorded using the same protocol as in the no-choice test (hourly observations from 10 a.m. to 4 p.m. for three consecutive days).

#### 4.1.2.1. Male Marking

To determine which male copulated with the female, males were marked on the pronotum using water-based acrylic markers (Uniposca PC-3M, Mitsubishi Pencil Co., Tokyo) (Figure 6). A total of 50 tests were performed marking the irradiated male in yellow and the non-irradiated male in white, and an additional 50 tests were performed with reversed colors. In the controls, both males were marked to assess any potential effect of marking on behavior.


*Figure 6.* Bagrada hilaris *male marked on the pronotum. Scale bar = 1 mm. Photo by C.E. Mainardi.*

### 4.2. Data analysis

Data from the no-choice and choice tests were analyzed using generalized estimating equations (GEE) for repeated measures, implemented in the *geepack* package in R. A marginal modeling approach was preferred over mixed-effects models because the structure of the dataset could have led to biased estimates under random-effects specifications. The correlation structure was selected according to parsimony criteria using the QIC. All experiments were carried out in five temporal blocks, for a total of

100 replicates per treatment.

In the no-choice test, GEE models with a binomial distribution and logit link function were applied to three response variables: mating (yes/no), male feeding (yes/no), and female feeding (yes/no). The fixed effects included treatment (irradiated versus non-irradiated male) and day of observation. A full factorial design (treatment × day) was adopted for the analysis of mating behavior. Feeding patterns of males and females were examined both separately, using a matrix structure to allow direct comparison of feeding responses between treatments. Potential nonlinear temporal trends were evaluated using the *poly* function in R, and pairwise comparisons between factor levels were performed with the *contrast* function from the *contrast* package.

In the choice test, the same GEE framework was applied to distinguish between copulations involving the irradiated male and those involving the non-irradiated male. A matrix structure was assigned to the binary response to model the two mating alternatives simultaneously. The intercept was set to zero, allowing the model to directly estimate deviations from the expected 50% mating probability between the two males. The day of the experiment was included as a fixed effect. The same model structure was applied to the positive control (two non-irradiated males) and the negative control (two irradiated males) to verify the consistency and robustness of the experimental design.

A separate GEE model was employed to analyze the overall percentage of time spent mating, with treatment group (test, positive control, negative control) and day included as fixed effects. For feeding behavior, three additional GEE models were fitted to compare the relevant pairwise combinations within each triad using treatment and day as fixed effects and applying the *contrast* function for post-hoc comparisons.

### 4.3. Results and discussion

The second experiment confirmed and extended the findings of the preliminary test, providing a detailed evaluation of the mating competitiveness of males irradiated at 80 Gy through both no-choice and choice tests conducted over three consecutive days.

#### 4.3.1 No-choice test

Under no-choice conditions, where each female was individually paired with either an irradiated or a non-irradiated male, irradiation at 80 Gy did not negatively affect mating behavior.

On the contrary, females spent significantly more time in copula with irradiated males (40.0 ± 1.1%) than with non-irradiated males (30.5 ± 1.0%), as supported by the GEE analysis (Wald = 10.12, $p = 0.00146$).

Mating behavior exhibited a dynamic trend across the three days (Figure 7):

-Day 1: irradiated males showed substantially higher mating values (38.5 ± 1.9%) than controls (21.5 ± 1.5%; $Z = 3.18$, $p = 0.0015$).

-Day 2: the mating rate of irradiated males increased further (47.5 ± 1.9%), remaining significantly higher than that of non-irradiated males (31.1 ± 1.8%; $Z = 2.18$, $p = 0.0083$).

-Day 3: mating activity decreased in irradiated males (37.0 ± 1.9%) and aligned with the control group (39.2 ± 1.9%); the decline from Day 2 was significant ($Z = 3.48$, $p < 0.001$).

The GEE analysis indicated significant effects of treatment ($p = 0.02714$), day ($p = 0.00034$), and their interaction (p = 0.00062), confirming that irradiation enhances mating behavior particularly during the first two days.

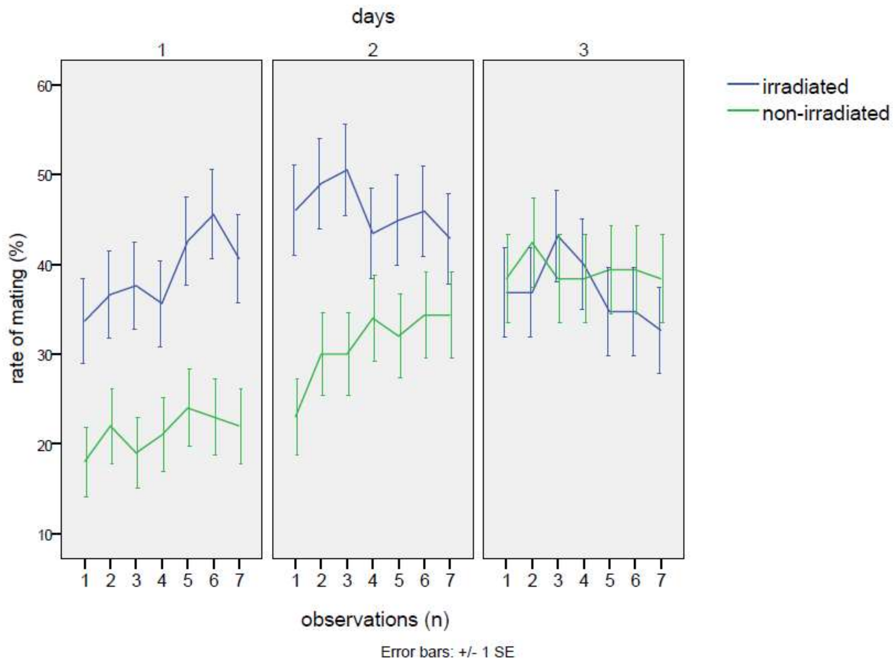


*Figure 7. No-choice test: mating trends measured as the percentage of time spent in mating during the three days of the experiment in the two crosses with the irradiated male and the non-irradiated male of* Bagrada hilaris*. The blue line represents the irradiated male, the green line the non-irradiated male*

Feeding activity showed no irradiation effects in either males or females. Irradiated and non-irradiated males displayed similar proportions of feeding time (Figure 8). The only significant factor was gender, with females feeding more than males (Wald = 22.24, $p = 2.4 \times 10^{-6}$), although this difference diminished on Day 3 (coeff = −0.0466, p = 0.025). Overall, irradiation at 80 Gy does not alter feeding behavior, indicating that physiological functions associated with feeding remain unaffected.

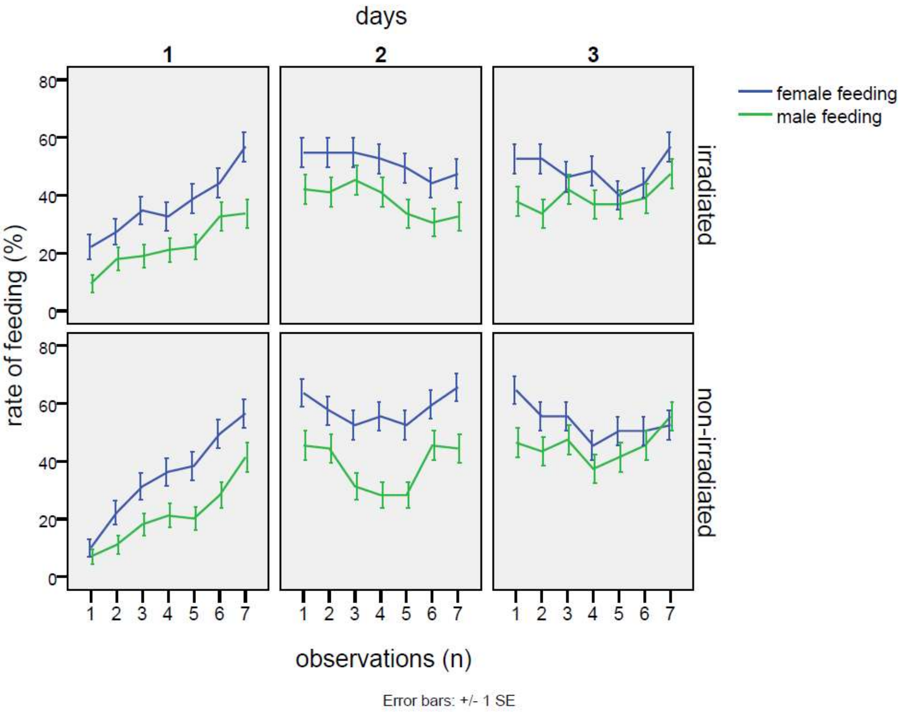


*Figure 8. No-choice test: feeding trends measured as the percentage of time spent in feeding during the three days of the experiment in the two crosses with the irradiated male and non-irradiated male of* Bagrada hilaris. *The blue lines indicate the feeding activity of the female, the green lines indicate that of the male (graph above: irradiated male; graph below: non-irradiated male), under no-choice conditions.*

### 4.3.2. Choice test

In the choice test (one female with one irradiated and one non-irradiated male), marking of individuals did not alter behavioral responses, confirming the safety and neutrality of the marking procedure.

Once mating occurred, females rarely changed partners, as indicated by the strong negative correlation between the copulation times of the two males (r = −0.608***), reflecting the typical monopolization pattern observed in stink bugs. The most relevant outcome is that irradiated males achieved a higher mating success. The mean mating rate was higher for irradiated males (32.2 ± 1.1%) than for non-irradiated males (17.4 ± 0.9%), a difference confirmed by the GEE model (Wald = 9.17, p = 0.027)(Table 2).

*Table 2. Choice test: mean percentage of mating occurrence on the first, second and third day of experiment. The test experiment with the irradiated male and the non-irradiated male in the cage is also compared with the positive control (both males non-irradiated) and negative control (both males irradiated). In the test experiment the male "1" is the irradiated one. Total mean values are also shown. Values followed from different letters are significantly different at p=0.05, as calculated by contrast analysis within the GiLM GEE model. Comparisons are made among the days of the experiment within each treatment. Capital letters refer to the overall comparisons among treatments. Significant differences between male "1" and "2" are marked by asterisks. Levels of significance are reported in the table according to the conventional notation by asterisks: no symbols, p > 0.05; p ≤ 0.05 *; p ≤ 0.01 **.*

| Male Treatment | Day | Cages (*n*) | Total Rate of Mating (%) | ±s.e. | Rate of Mating (Male 1 *) (%) | ±s.e. | Rate of Mating (Male 2 *) (%) | ±s.e. |
|---|---|---|---|---|---|---|---|---|
| Irradiated/ non-irradiated * male1 = irradiated * male2 = non-irradiated | 1 | 79 | 23.7 a | 1.8 | 17.4 a | 1.6 ** | 6.3 a | 1.0 |
| | 2 | | 52.3 b | 2.1 | 35.6 b | 2.0 ** | 16.6 b | 1.6 |
| | 3 | | 72.7 c | 1.9 | 43.6 b | 2.1 | 29.1 c | 1.9 |
| | Total mean | | 49.6 A | 1.2 | 32.2 A | 1.1 * | 17.4 A | 0.9 |
| Both non-irradiated | 1 | 85 | 26.5 a | 1.8 | 10.4 a | 1.3 | 16.0 a | 1.5 |
| | 2 | | 55.7 b | 2.0 | 26.7 b | 1.8 | 29.1 b | 1.9 |
| | 3 | | 70.5 c | 1.9 | 38.7 c | 2.0 | 31.8 b | 1.9 |
| | Total mean | | 50.8 A | 1.2 | 25.2 A | 1.0 | 25.6 B | 1.0 |
| Both irradiated | 1 | 91 | 52.0 a | 2.0 | 25.3 a | 1.7 | 27.8 a | 1.8 |
| | 2 | | 70.8 b | 1.8 | 36.4 b | 1.9 | 34.5 ab | 1.9 |
| | 3 | | 80.0 b | 1.6 | 41.8 b | 2.0 | 38.1 b | 1.9 |
| | Total mean | | 67.9 B | 1.1 | 34.4 A | 1.1 | 33.5 B | 1.1 |

Irradiated males also increased their mating values across the three days (17.4 ± 1.6%; 35.6 ± 2.0%; 43.6 ± 1.2%), while non-irradiated males showed consistently lower levels, suggesting that competition may further stimulate the behavioral performance of irradiated males (Figure 9). An additional noteworthy result emerged from the negative control (two irradiated males), where the total mating rate (67.9 ± 1.1%) exceeded that of the positive control (two non-irradiated males: 50.8 ± 1.2%). This further supports the conclusion that irradiation at 80 Gy does not reduce, and may even enhance, male competitiveness (Figure 9).

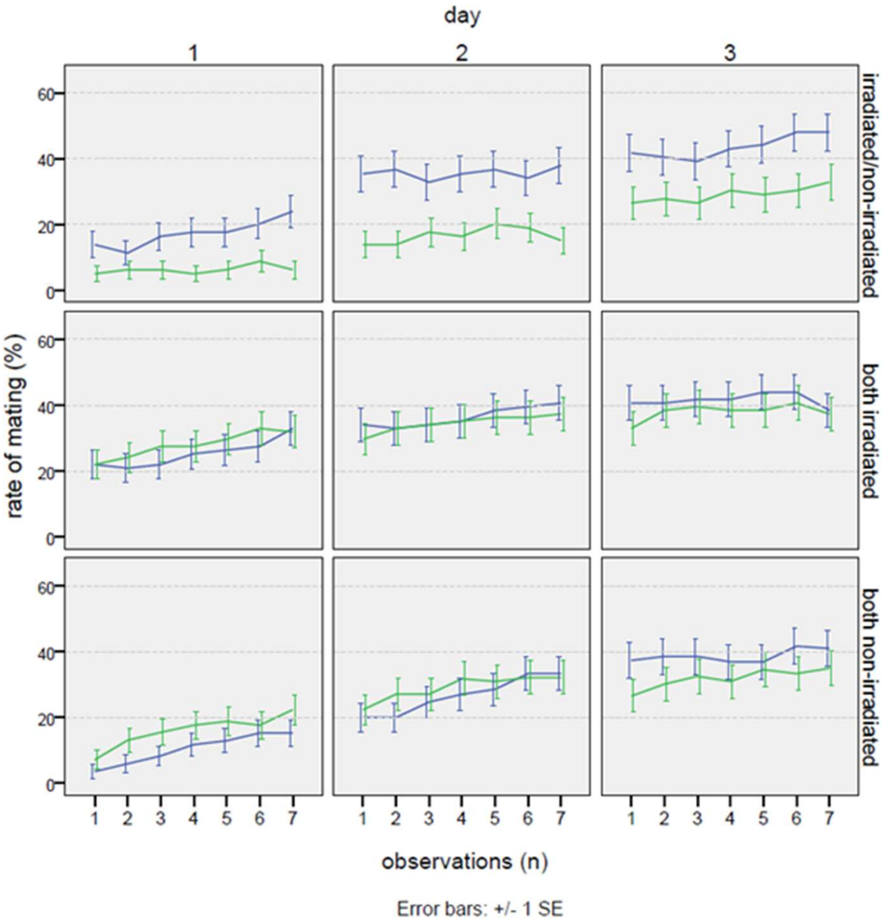


*Figure 9. Choice test: mating occurrence trends expressed as percentage of time, under choice conditions (one non-irradiated female with one non-irradiated male and one irradiated male) in* Bagrada hilaris. *The blue lines represent the irradiated male (or male 1 in the positive and negative controls) and the green lines the non-irradiated one (or male 2 in the positive and negative controls).*

As in the no-choice tests, no differences were observed between irradiated and non-irradiated males in feeding activity (Figure 10). Feeding patterns tended to track those of the female, suggesting coordinated behavior within the group and confirming that irradiation does not impair feeding.

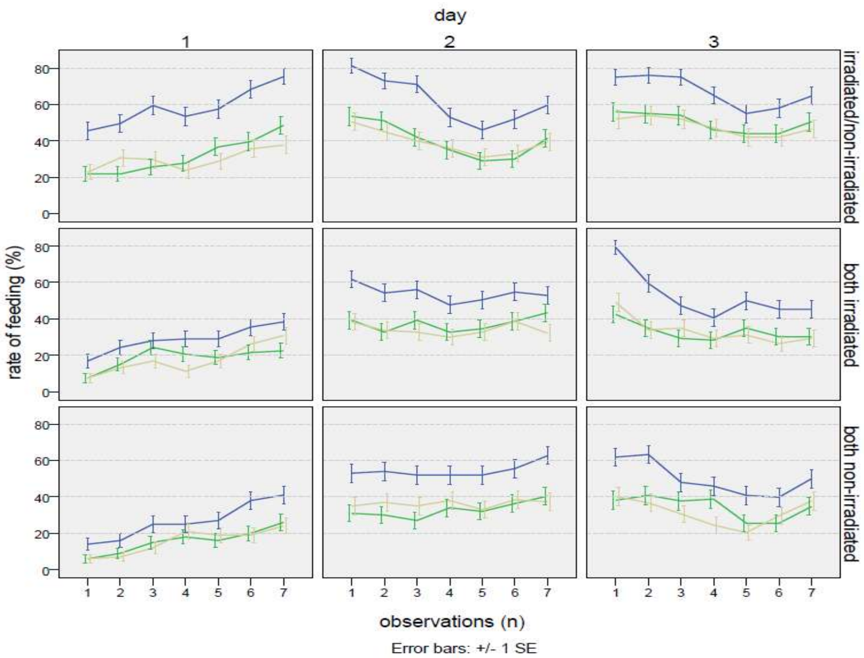


*Figure 10. Choice test: feeding occurrence trends expressed as percentage of time, under choice conditions (one female with one non-irradiated male and one irradiated male) in Bagrada hilaris. The blue line represents the females, the green line the irradiated male (or male "1" In the controls) and the grey line the non-irradiated male (or male "2" in the positive and negative controls).*

The overall results clearly indicate that an irradiation dose of 80 Gy represents the best compromise between achieving high induced sterility (≈95%) and maintaining adequate sexual competitiveness. At this dose, males not only retain their ability to mate successfully, but in several cases, even outperform non-irradiated males. This aspect is crucial for the SIT, which requires released males to remain competitive over time, effectively locate wild females, and perform the courtship behaviors necessary to secure copulation.

In the choice tests, the presence of a competing male appeared to enhance the behavioral performance of irradiated males, which progressively increased the time spent mating from day 1 to day 3. This contrasts with the slight decline observed under no-choice conditions. Therefore, the social environment plays a key role in modulating copulation duration, which in *B. hilaris* may serve as a form of mate guarding [5, 29]: prolonged copulation prevents further insemination by rival males and increases the likelihood of paternity for the first male to mate [30,31].

Irradiation may also stimulate hypercompensatory behavioral responses. As reported by *Peccerillo et al.* [28], irradiated males exhibit more frequent abdominal contractions during copulation, a behavior that may make them more stimulating and therefore more competitive against non-irradiated males. Supporting this interpretation, in the negative control (one female with two irradiated males), the total mating rate exceeded that observed in the positive control (two non-irradiated males), suggesting that under competitive pressure, irradiated males can match or even surpass the performance of fertile males.

Regarding feeding behavior, females of *B. hilaris* spent more time feeding than males, both irradiated and non-irradiated, particularly during the first two days. This difference is likely associated with the metabolic demands of vitellogenesis. Importantly, no differences were detected in feeding activity between the irradiated and non-irradiated males. This finding is highly encouraging for SIT applications: since crop damage is attributable primarily to females, the release of sterile males should result in minimal impact on host plants [32].

Overall, the results indicate that irradiation at 80 Gy does not compromise the biological quality of *B. hilaris* males and may, in some cases, enhance their reproductive performance. Consequently, the application of SIT to *B. hilaris* has emerged as a feasible and potentially effective strategy within an integrated pest management (IPM) framework. The effectiveness of this technique could be further strengthened by its implementation in geographically restricted areas, such as the island of Pantelleria, and by its integration with classical biological control measures.

## Conclusions

This study provides the first comprehensive assessment of the behavioral and reproductive responses of *Bagrada hilaris* males exposed to different gamma irradiation doses, identifying 80 Gy as the optimal dose for the Sterile Insect Technique (SIT) application. At this dose, males achieved high levels of induced sterility (≈95%) while fully retaining their sexual competitiveness. In both no-choice and choice conditions, irradiated males performed as well as or, in several cases, better than non-irradiated individuals, displaying a rapid initiation of copulation, prolonged mating duration, and a stable mating performance across multiple days.

Although these results strongly support the feasibility of SIT against *B. hilaris*, they were obtained under controlled laboratory conditions. Therefore, further research is needed to validate these findings under semi-field and open-field conditions and to refine key operational parameters, such as mass-rearing strategies and the overflooding ratio required to suppress wild populations. Moreover, assessing the robustness of sterile male competitiveness in more complex and variable environments will be crucial to ensure the long-term sustainability and cost-effectiveness of the technique. The ecological characteristics of Pantelleria island, namely insularity and limited risk of reinvasion, make it an ideal candidate for future pilot trials. These features provide a contained environment to evaluate the real-world performance of SIT and obtain important results for expanding the approach to other geographic areas where Bagrada, or other Pentatomidae, are invasive. Taken together, the evidence presented here demonstrates that SIT can be realistically integrated into a broader Integrated Pest Management (IPM) framework for *B. hilaris*. In particular, SIT could be combined with classical biological control agents, such as the egg parasitoid *Gryon aetherium*, which has been proposed as a highly specialized and promising biocontrol agent against *B. hilaris* [33,34]. When implemented in geographically well-defined areas, this integrated approach has the potential to become a sustainable, species-specific tool for the long-term suppression or eradication of this invasive pest species.

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