Physiological host range of *Trissolcus mitsukurii*, a candidate biological control agent of *Halyomorpha halys* in Europe

Lucrezia Giovannini1 · Giuseppino Sabbatini-Peverieri1 · Leonardo Marianelli1 · Gabriele Rondoni2 · Eric Conti2 · Pio Federico Roversi1

Received: 24 March 2021 / Revised: 20 July 2021 / Accepted: 22 July 2021 / Published online: 5 August 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
The invasive stink bug *Halyomorpha halys*, native to East Asia, is a severe agricultural pest of worldwide importance, and chemical insecticides are largely sprayed for its control. Negative impact and failures of chemical pest management led to consider classical biological control as one of the most promising methods in a long-term perspective. The Asian egg parasitoid *Trissolcus japonicus* is the main candidate biocontrol agent of *H. halys*, but more recently a second species, *Trissolcus mitsukurii*, which shares the area of origin with *H. halys*, has drawn special attention after adventive populations were found in Europe. Despite its recent detection, intentional release or redistribution are bound to approval of national petitions, which requires detailed risk analyses. With the aim of providing a contribution for the future development of a risk assessment for release purposes, here we present the first study on the physiological (fundamental) host range of *T. mitsukurii* in Europe. Tests conducted on different hemipterans common to Southern Europe, using three different experimental designs, revealed a oligophagous host range, limited to Pentatomoidea and comparable with the host range displayed by *T. japonicus* under similar laboratory conditions. In addition to its coevolved host *H. halys*, *T. mitsukurii* successfully parasitized the majority of tested pentatomid species and one scutellerid, although with highly variable emergence rates. Notably high parasitization rates were detected on *H. halys*, *Acrosternum heegeri* and *Dolycoris baccarum*. Host egg sizes positively affected parasitoid size and female egg load. Although *T. mitsukurii* might be a promising biological control agent of *H. halys* in Europe, further studies (e.g., chemical ecology, field parasitism) are needed for assessment of non-target risks.

Keywords Classical biological control · Non-target risk assessment · Brown marmorated stink bug · Pentatomidae · Scelionidae · Egg parasitoid

Key message
- *Trissolcus mitsukurii* is a potential biological control agent of *Halyomorpha halys*
- Adventive populations of this egg parasitoid were found recently in Europe parasitizing *H. halys*
- Risk analysis is mandatory for authorization of intentional release for biological control
- *T. mitsukurii* displayed an oligophagous host range within Pentatomoidea

Introduction
The Brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera, Pentatomidae), is a polyphagous invasive pest native of East Asia, currently present in several countries of the Americas and Europe, where it causes severe damage to many agricultural crops (Leskey and Nielsen 2018). The low efficacy and high environmental impact of insecticide treatments, combined with the lack of effective native natural enemies, led to consider classical biological control with egg parasitoids a promising method for long-term
management of *H. halys* in the invaded areas (Abram et al. 2017). Although recent reviews indicated limitations in the success of classical biological control of stink bugs, research in this field needs to highlight any potential approach to find effective pest control solutions (Abram et al. 2020; Conti et al. 2021). These solutions could emerge from the integration of classical biological control with techniques targeting different instars of the pest (Abram et al. 2020), plant resistance (Rondoni et al. 2018), exploitation of plant volatiles to improve parasitoid efficacy (Rondoni et al. 2017), manipulation of stink bug behavior and parasitoid conservation (reviewed by Conti et al. 2021).

The employment of an exotic organism as candidate biological control agent, including redistribution of adventive populations, is strictly regulated by national and international rules (van Lenteren et al. 2006a; EPPO 2014; FAO-IPPC 2017; European Directives 92/43/CEE and 2002/89/CE; Italian DPR 102/2019). Therefore, the approval of a petition for release of a biological control agent requires a thorough risk assessment, which provides detailed information on the biological and ecological traits of the agent, first of all its host range (Hunt et al. 2008). Other aspects should also be considered for risk analysis, including dispersal ability, interspecific competition and hybridization. Laboratory investigations are the first step for non-target risk analysis of the exotic natural enemy and allow to explore its physiological host range. The physiological host range is the range of species a natural enemy can parasitize in the laboratory, and is the foundation on which non-target risk assessment is build up, according to stepwise testing with increasing levels of complexity, including behavioral and chemical ecology assays (Van Lenteren et al. 2006b). Such studies are necessary for moving from a physiological host range outcome to an ecological host range perspective, which allows to prevent or minimize the risk of negative impact caused by a voluntary human intervention in the ecosystem (van Lenteren et al. 2006b).

The Asian egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) is considered, so far, the most valid candidate for biological control of *H. halys* (Zhang et al. 2017; Conti et al. 2021). Its host range, outside the area of origin, was investigated in the USA, Europe and New Zealand. In these different areas, *T. japonicas*, although exhibiting a general oligophagous behavior, has shown a greater preference toward *H. halys* (Hedstrom et al. 2017; Botch and Delfosse 2018; Charles et al. 2019; Lara et al. 2019; Haye et al. 2019; Sabbatini-Peverieri et al., 2021). Adventive populations of *T. japonicus* were discovered recently in Europe and the USA (Talamas et al. 2015; Sabbatini-Peverieri et al. 2018; Stahl et al. 2019). Surprisingly, a second Asian egg parasitoid of *H. halys*, *Trissolcus mitsukurii* (Ashmead) (Hymenoptera, Scelionidae), was also found in Italy, as the first record outside its native range (Sabbatini-Peverieri et al. 2018). However, while *T. japonicus* ability to attack *H. halys* is well-recognized, little is known so far about the preferences and life traits of *T. mitsukurii* (Zhang et al. 2017; Arakawa and Namura 2002; Arakawa et al. 2004). This parasitoid species is known mainly from Japan, where it has been recorded parasitizing in the field eggs of about ten species of Pentatomidae (Yasumatsu and Watanabe 1964; Hokyo et al. 1966; Ryu and Hirashima 1984; Arakawa and Namura 2002). Additionally, in laboratory test conducted in Japan, *T. mitsukurii* was able to parasitize a different pentatomid species in the genus *Plautia* (Arakawa and Namura 2002). Therefore, *T. mitsukurii* seems to be an oligophagous egg parasitoid. In Japan, it was previously reported as one of the most active natural enemies of *Nezara viridula* L. (Hemiptera, Pentatomidae) (Kiritani and Hokyo 1962; Hokyo and Kiritani 1963), although more recent data from the same country reported this parasitoid as commonly associated with *H. halys* (Arakawa and Namura 2002; Arakawa et al. 2004). Instead, the high parasitization rate reported in China for *T. mitsukurii* on *H. halys* (Chu et al. 1997), due to an original misidentification, should be assigned to *T. japonicus* as stated by Yang et al. (2009).

Recent field surveys on adventive *T. mitsukurii* populations in Northern Italy showed high rates of parasitism of *H. halys* eggs, suggesting that this egg parasitoid could be a valid candidate for biological control of the invasive stink bug in addition to *T. japonicus* (Benvenuto et al. 2020; Scaccini et al. 2020; Zapponi et al. 2020). Additionally, field data from Northern Italy suggest low ability of *T. mitsukurii* to attack non-target pentatomid species, *i.e.*, *Dolycoris baccarum* (L.), *Palomena prasina* (L.) and *N. viridula* (Zapponi et al. 2021). However, this is a first and partial observation, conditioned by the status of *T. mitsukurii* populations in the field and the distribution of its potential hosts in the environment. For a clear perspective of the parasitoid host range based on field data, years of observations would be necessary after the natural spread of this species in the area and the establishment of interspecific host-parasitoid relationships.

Thus, because the host range of *T. mitsukurii* outside its original area remains unclear, this should be clarified to initiate the first step of a risk analysis workplan, which is necessary before considering the implementation of this egg parasitoid as a biological control agent (van Lenteren et al. 2006a, b). The present work is the first contribution to the knowledge of the physiological host range of *T. mitsukurii* in the invaded area, conducted comparing the parasitoid performance on the target pest *H. halys* and on non-target Hemipteran species that are common in Europe.
Material and methods

Field sampling, insect collection and rearing

Halyomorpha halys adults were collected in Northern Italy in 2020, both in overwintering sites and in the field during the growing season. A colony was given rise from about 2,500 overwintering specimens and replenished monthly with field collected specimens. Instead, adults of non-target Hemiptera species were collected on various herbaceous plants, shrubs and trees of fallow fields, hedgerows, bushes and orchards during the 2020 growing season, in H. halys habitats and surrounding areas of North and Central Italy. Non-target Hemiptera species were chosen based on the potential host range of the parasitoid and the information mined from the literature, according to the centrifugal phylogenetic approach (Kuhlmann and Mason 2003; Kulmann et al. 2006). Beside H. halys, the following non-target herbivorous Hemiptera species were investigated: the pentatomids Acrosternum heegeri (L.), Aelia acuminata (L.), Ancyrosoma leucogrammnes (Gmelin), Carpocoris pucicus (Poda), D. baccarum, Eurydema ventralis Kolenati, Eurydema oleracea (L.), Graphosoma italicum (Muller), N. viridula, P. prasina, Piezodorus lituratus (F.), and Sciocoris sp., the scutellerid Eurygaster maura (L.), and the coreid Gonocerus juniperi Herrich-Schäffer. Two predator species, Arma custos (Fabricius) (Hemiptera, Pentatomidae) and Rhynocoris iracundus (Poda) (Hemiptera, Reduviidae), were also considered (see Table 1). Number of non-target collected specimens in the field where highly variable, from few specimens up to several dozens, depending on species abundance in the field, collection opportunity and collection method, i.e., sweep net or hand picking on herbaceous plants and trees, or occasionally on other substrates (Tab. 1).

Laboratory colonies were established and maintained using rearing cages (BugDorm 4F4545, Insect MegaView Science Co. Ltd., Taichung, Taiwan) in rearing rooms at 26 ± 1 °C and 16:8 L:D. For the polyphagous phytophagous species selected for the present study, a variety of plants was offered as a bouquet of food sources: seeds (Arachis hypogaea), fresh vegetables (Daucus carota, Phaseolus vulgaris), fresh fruits (Malus domestica, Actinidia delicosa). Instead, more specific substrates were used to rear adults of the oligophagous species. Eurydema species were reared on Brassica oleracea (cauliflower cut in pieces and potted plants) and Capparis spinosa (twigs), G. lineatum was reared on Foeniculum vulgare (potted plants and seeds), while wheat ears were offered to E. maura. Food was purchased from local markets. Plants were self-produced and were used when at least 10 cm tall. The predator species were reared on Acanthoscelides obtectus Say (Coleoptera, Chrysomelidae) adults, N. viridula nymphs and Tenebrio

Table 1 List of the European Hemiptera tested for Trissolcus mitsukurii physiological host suitability, compared to the exotic Halyomorpha halys, and relative egg hatching rates in the rearing facility (n.a. = not assessed)

| Target and non-target species | Family, subfamily, tribe | Egg mass hatching rate in the rearing facility |
|------------------------------|-------------------------|-----------------------------------------------|
| Acrosternum heegeri Fieber    | Pentatomidae, Pentatominae, Pentatomini | n.a. (94.0%*) |
| Aelia acuminata (L.)          | Pentatomidae, Pentatominae, Aelini        | n.a                                          |
| Ancyrosoma leucogrammnes (Gmelin) | Pentatomidae, Podopinae, Graphosomatini | n.a                                          |
| Arma custos (F.)              | Pentatomidae, Asopinae, Asopini           | 77.67% |
| Carcopolis pucicus (Poda)     | Pentatomidae, Pentatominae, Carcoporini   | 95.7% (76.61%*) |
| Dolycoris baccarum (L.)       | Pentatomidae, Pentatominae, Carcoporini   | 88.6% (88.0%*) |
| Eurydema oleracea (L.)        | Pentatomidae, Pentatominae, Strachiini    | n.a                                          |
| Eurydema ventralis Kolenati   | Pentatomidae, Pentatominae, Strachiini    | 93.1% (96.67%*) |
| Graphosma italicum (Muller)   | Pentatomidae, Pentatominae, Graphosomatini | 83.3% |
| Halyomorpha halys (Stål)      | Pentatomidae, Pentatominae, Cappaeini     | 93.35% (90.39%*) |
| Nezara viridula L             | Pentatomidae, Pentatominae, Pentatomini   | 92.34% (85.58%*) |
| Palomena prasina (L.)         | Pentatomidae, Pentatominae, Carcoporini   | n.a. (97.56%*) |
| Piezodorus lituratus (F.)     | Pentatomidae, Pentatominae, Piezodorini   | n.a. (88.0%*) |
| Sciocoris sp. Wollaston       | Pentatomidae, Pentatominae, Sciocorini    | n.a                                          |
| Eurygaster maura (L.)         | Scutelleridae, Eurygastria, Eurygastrini  | 90.15% |
| Gonocerus juniperi Herrich-Schäffer | Coreidae, Coreinae, Gonocerini             | n.a. (100%*) |
| Rhynocoris iracundus (Poda)   | Reduviidae, Harpactorinae, Harpactorini   | n.a. (96.67%*) |

Arma custos and R. iracundus are predators, whereas all other species are herbivores

*Data from Sabbatini Peverieri et al. (2021) using the same rearing condition are reported within parentheses
molitor L. (Coleoptera, Tenebrionidae) pupae. For all species, food and water (provided with wet cotton) were replenished three times per week. Paper towels were hung inside the rearing cages as oviposition substrates, and eggs were collected daily. As a measure of standard quality of laboratory hemipteran rearing, the viability of eggs was occasionally recorded (Tab. 1). At this scope, scopes of egg masses produced by H. halys and non-target species were reared in climatic chamber (HPP750, Memmert GmbH + Co. KG, Schwabach, Germany) at 26 ± 1 °C, 60 ± 5% RH and 16:8 L/D up to egg hatching.

Trissolcus mitsukurii was obtained from about 60 parasitized H. halys egg masses collected in 2018 and 2019 in H. halys infested sites of Northern Italy, the sites where the adventive populations of the egg parasitoid were initially recorded (see Sabatini-Peverieri et al. 2018). These egg masses were reared in the laboratory, and the emerged T. mitsukurii individuals (about 500 specimens) were used to start a laboratory colony. Adults of the egg parasitoid were fed with pure honey droplets. Periodically, fresh egg masses (< 24 h old) of H. halys were offered as host for parasitization and juvenile development. The permanent colony of the egg parasitoid was established and maintained in climatic chamber at 26 ± 1 °C, 60 ± 5% RH and 16:8 L/D; such conditions were considered as standards for the present work and were used for all experiments. Adults of T. mitsukurii and parasitized egg masses were housed in glass tubes (15 cm length, 2 cm diam), closed on both ends by a plastic net (250 μm mesh). Establishment and maintenance of the egg parasitoid colony and all experiments (see below) were conducted under quarantine conditions at CREA facilities. Quarantine laboratories are authorized for studies on exotic beneficial organisms in the framework of H. halys biological control program (MiPAAF, DG/DISR/ DISR05/0013647–19/04/2018 and SFR Regione Toscana 203340 – 12/04/2018).

### No-choice black box tests

A total of three different experimental conditions were considered for the no-choice black box tests. In the first and second experiment, host egg masses were handled as little as possible to avoid the introduction of external contaminants, and any adjustment of size (egg number per mass) was avoided, as to simulate the natural number of host eggs, per egg batch, that female parasitoids would likely encounter in the field (Hedstrom et al. 2017; Botch and Delfosse 2018). For G. juniperi, which lays individual eggs, ten eggs were aligned and glued (using non-toxic clear school Elmer’s® glue, Newell Office Brands) on a cardboard (15 mm × 80 mm), each egg distanced 5 mm from the other. For the tests, one egg mass (or a row of ten single eggs) of a given host species was placed in a glass tube (15 cm length, 2 cm diam.), and one parasitoid female, 7-d old, was introduced for two different exposure times. Specifically, in the first experiment, females were kept for 24 h and a small drop of honey was added on the glass internal surface as food source. In the second experiment, the exposure time was reduced to 2 h and no food was provided.

In the third type of black box no-choice experiment, only one egg was provided to the female for a total time of 2 h. In this experimental condition, a small glass vial (7 cm length, 0.5 cm diam.) was used and closed with a cotton ball to prevent the parasitoid from escaping. This assay is an extremely simplified exposure condition that has been previously shown to provide reliable results for no-choice tests with egg parasitoids (Sabbatini-Peverieri et al. 2021). Tested eggs were glued (Elmer’s® glue) on the cardboard to permit a sufficient stability.

In all no-choice experiments, only T. mitsukurii females originating from H. halys eggs were tested, and only fresh eggs (< 24 h old) of H. halys and non-target species were employed. Before use, eggs and egg masses were inspected under a stereomicroscope to assess their suitability for the tests. In case of, e.g., non-characteristic color of the eggs, unusual egg arrangement in the batch, unswollen eggs in the mass, or unusual low number of eggs per mass (according to known natural egg amount per egg mass of tested species), the egg or the egg mass was discarded. Parasitoids were removed at the end of the allotted time (24 h or 2 h). Eggs were maintained in the glass vials (single eggs) or tubes (egg masses) and were reared in climatic chamber at standard conditions until nymph hatching or parasitoid emergence. Eggs that did not hatch nor produced a parasitoid after three weeks from tests, were classified as “dead eggs.” These were dissected and inspected under a stereomicroscope, and the content was eventually classified as dead parasitoid (pupae or pharate adult), dead Hemiptera nymph, or undetermined content. Emerged adult parasitoids were counted and sexed.

### Effect of host egg size on parasitoid size and egg load

The effects of host eggs on the parasitoid size and egg load was evaluated using T. mitsukurii females emerged from H. halys and non-target hosts of the no-choice black box tests. For this purpose, the volume (mm$^3$) of eggs of host species was calculated by the parabolic barrel formula $V = \pi H (3r^2 + 4Rr + 8R^2) / 15$, where “$H$” is the egg height, “$r$” is the radius of the operculum, and “$R$” is the radius at the center of the egg (Abram et al. 2016; Botch and Delfosse 2018). The head and thorax width and the length of the hind tibia were measured from females emerged from H. halys and non-target hosts. For this purpose, insects were dissected under a stereomicroscope and body parts were measured using Nikon SMZ25 and NIS-Elements software (Nikon.
Corporation, Tokyo, Japan). Additionally, parasitoid females from *H. halys* and non-target host eggs were reared individually for 7 days at standard conditions in climatic chamber. At the seventh day of rearing, the females were dissected and the egg load in their ovary was counted after staining with 1% toluidine bleu (Sabbatini-Peverieri et al. 2020).

**Paired choice black-box tests using parasitoids reared on different hosts**

In a first paired-choice experiment, seven-day-old *T. mitsukurii* females reared on *H. halys* eggs were singly used in dual-choice tests in Petri dishes (9 cm diam.). A female was allowed to exploit two fresh egg masses (< 24 h), one in dual-choice tests in Petri dishes (9 cm diam.). A female eggs were singly used on *T. mitsukurii* females reared on different hosts.

Paired choice black-box tests using parasitoids reared on different hosts

In a first paired-choice experiment, seven-day-old *T. mitsukurii* females reared on *H. halys* eggs were singly used in dual-choice tests in Petri dishes (9 cm diam.). A female was allowed to exploit two fresh egg masses (< 24 h), one of *H. halys* and one of a non-target species (*n = 12*). *Arma custos* or *D. baccarum* were chosen as non-target species because of the high parasitization rates that were recorded in the no-choice tests (over 50% of successful parasitization). *Arma custos* was also chosen as this is a key species for the safeguarding approach in risk analysis. The number of eggs (Table 2) of tested egg masses was not adjusted in order to maintain the similar egg numbers that *T. mitsukurii* would encounter in the field (Haye et al. 2019), with the only exception of the egg masses of *N. viridula*, too large compared to the maximal egg load of *T. mitsukurii* in the ovaries (see Sabbatini-Peverieri et al. 2020). The two egg masses were placed at the bottom of the Petri dish and at the opposite sides of the arena. The position of the egg species was inverted at each replicate. The parasitoid female was placed at the center of the arena and tested for a 2 h time span in climatic chamber at standard condition. Such reduced time of egg mass exposure was adopted because in the previous no-choice experiment, the parasitoid females displayed to parasitize host egg masses within this short time interval, and because this would limit the opportunity for multiple parasitization, which is likely to occur in a 24-h exposure test (Haye et al. 2019). At the end of the exposure, the females were removed from the arena and the egg masses were reared in the climatic chamber at standard condition until the eggs hatched or the parasitoids emerged. Eggs that did not hatch nor produced a parasitoid after three weeks from the experiment were dissected to determine the contents as described previously. A second paired-choice experiment was conducted to evaluate possible effects of the rearing host on host preferences by *T. mitsukurii*. For this purpose, different colonies of *T. mitsukurii* were established and maintained on egg masses of the non-target species, *D. baccarum* or *A. custos*, with the same method used when reared on *H. halys* as host. The same experimental design as described above was applied using *T. mitsukurii* parasitoid females originated from host eggs of either *D. baccarum* or *A. custos*. When females did not make a choice, i.e., they failed to oviposit at least in one of the two offered egg masses, the replicate was excluded from the analysis.

**Statistical analysis**

In no-choice tests with host egg masses exposure for 24 and 2 h, the percentages of hatched Hemiptera eggs, successfully parasitized eggs (emergence of the parasitoid) and dead eggs were analyzed by Kruskall–Wallis and Dunn’s multiple comparison post hoc test using *H. halys* as control species. Data were analyzed only for the species showing at least five replicates (although the entire dataset was reported). Pair-wise comparison among species in single host egg exposure for 2 h was performed using Chi-square test with Yates’ correction. Pooled data on parasitoid female dimensions (head width, thorax width, hind tibia length) were analyzed with Pearson correlation coefficient. The functional relationship between the egg size of host species, emerged parasitoid female size and ovaries egg load was analyzed through linear regression model. In choice test, the percentages of hatched Hemiptera eggs, successfully parasitized eggs (emergence of the parasitoids) and dead eggs were compared using the Mann–Whitney *U* test. Ovarian egg load between *H. halys* and non-target species were compared with Kruskall–Wallis and Dunn’s multiple comparison post hoc test. Statistics were performed using Graphpad Prism 8.

**Results**

**No-choice black box tests**

When Hemiptera egg masses were exposed to *T. mitsukurii* for 24 h, most eggs were suitable or partially suitable for parasitoid development, with significantly different emergence rates from the diverse tested species (*χ² = 112.7; P < 0.0001*) (Table 2). *Trissolcus mitsukurii* was able to parasiitize and develop in 12 pentatomid species out of the 14 that were tested (85.71% of tested species), and in the only tested scutellerid, *E. maura*, although with highly variable success. Conversely, it was not able to exploit the remaining pentatomids *A. leucogrammes* and *E. ventralis*, the coreid *G. juniperi* and the reduviid *R. iracundus* (Table 2). The emergence rate of *T. mitsukurii* from *H. halys* egg masses was very high, close to 100%, and not significantly different compared to emergence from the pentatomids *A. heegeri*, *A. custos*, *C. pudicus*, *D. baccarum*, *G. italicus*, *P. prasina*, *P. literatus*, *Sciocoris* sp. (eight out of 13 tested non-target pentatomid species) and the scutellerid *E. maura* (Table 2; see Table S1 for statistics). Instead, emergences were significantly lower, compared to *H. halys*, when eggs of *Aelia acuminata*, *E. oleracea* and *N. viridula* were tested (Table 2;
see Table S1 for statistics). Sex ratios were strongly female biased in most of the species tested (Table 2).

The percentages of Hemiptera eggs that hatched after exposure to *T. mitsukurii* were significantly different ($\chi^2 = 80.04; P < 0.0001$), and hatching rates were partially complementary to parasitoid emergence rates. Almost none of the eggs of *H. halys*, *A. heegeri*, *D. baccarum* and *P. prasina* hatched, while hatching percentages were relevant for the other pentatomid species, although only *A. leucogrammtes* and *E. ventralis* showed significantly higher rates compared to *H. halys*. The percentages of dead eggs (no hatching and no parasitoid emergence) were significantly different among the tested species ($\chi^2 = 73.50; P < 0.0001$). Dead eggs were present in all Hemiptera species except *H. halys* and *P. prasina* (but only one egg mass of the latter could be tested), and percentages were notably

| Table 2 Physiological suitability of Hemiptera eggs for *Trissolcus mitsukurii* in 24 h black-box tests |
|---------------------------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Species (replicates)            | No. of eggs/egg mass (mean ± SE) | % of eggs with parasitoid emergence/egg mass (mean ± SE) | % of females (range min–max) | % of hatched eggs/egg mass (mean ± SE) | % of dead eggs (mean ± SE) | No. of parasitized egg masses/with parasitoid emergence/total no. of eggs exposed | Total no. of dead eggs |
|---------------------------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|
| *Halyomorpha halys* (12)        | 27.42 ± 0.29  | 93.40 ± 0.60   | 85.18–96.55    | 0 ± 0.00       | 0 ± 0.00       | 12/12/329       | 0   0   0         |
| *Acrosternum heegeri* (12)      | 13.25 ± 0.46  | 68.32 ± 1.24   | 76.92–92.85    | 0 ± 0.00       | 0 ± 0.49       | 12/12/159       | 0   0   1 (adult) |
| *Aelia acamnita* (12)           | 11.83 ± 0.24  | 7.20 ± 3.57*** | 60.00–100.00   | 25.00 ± 13.06  | 67.80 ± 12.26*** | 7/4/142         | 93  0  13 (all adults) |
| *Ancylosoma leucoogrammes* (10) | 11.50 ± 0.45  | 0 ± 0.00***    | –              | 90.00 ± 10.00*** | 10.00 ± 10.00   | 1/10/115        | 3   0   9 (all adults) |
| *Arma custos* (12)              | 14.33 ± 0.63  | 66.32 ± 12.51  | 83.33–93.33    | 25.00 ± 13.06  | 8.67 ± 5.05    | 9/9/172         | 0   0   14 (all adults) |
| *Carpoecoris pudicus* (12)      | 14.08 ± 0.87  | 75.00 ± 13.06  | 87.55–94.73    | 9.22 ± 7.37    | 15.78 ± 9.95   | 129/169         | 0   0   29 (28 pupae, 1 adult) |
| *Dolycoris baccarum* (12)       | 13.92 ± 0.34  | 98.29 ± 0.89   | 85.71–100.00   | 0.55 ± 0.55    | 0.59 ± 0.59    | 12/12/167       | 1   0   0         |
| *Eurydema oleracea* (10)        | 10.10 ± 0.38  | 21.38 ± 11.09**| 60.00–100.00   | 25.00 ± 13.33  | 55.23 ± 11.50***| 4/4/101         | 42  0  12 (all adults) |
| *Eurydema ventralis* (12)       | 11.83 ± 0.32  | 0 ± 0.00***    | –              | 63.81 ± 11.49**| 36.19 ± 11.49* | 5/0/142         | 12  0  41 (6 pupae, 35 adults) |
| *Graphosoma italicum* (12)      | 14.58 ± 0.45  | 78.57 ± 8.05   | 57.14–93.33    | 15.16 ± 8.07   | 6.28 ± 2.74    | 11/11/175       | 1   0   10 (all adults) |
| *Nezara viridula* (12)          | 28.25 ± 0.97  | 1.90 ± 1.57*** | 100            | 29.04 ± 10.45  | 68.36 ± 10.89***| 4/2/339         | 207 0 22 (all adults) |
| *Palomena prasina* (1)          | 12            | 100** n.a      | 75** n.a       | 0** n.a        | 0** n.a        | 1/1/12          | 0   0   0         |
| *Piezodorus liturus* (5)        | 10.60 ± 1.29* | 68.24 ± 18.39  | 50.00–85.71    | 22.97 ± 19.33  | 8.79 ± 5.77    | 4/4/53          | 0   0   6 (all adults) |
| *Scioecoris sp.* (8)            | 10.38 ± 0.57  | 81.60 ± 12.25  | 75.00–91.66    | 10.94 ± 9.28   | 6.07 ± 3.29    | 8/7/83          | 1   0   5 (all adults) |
| *Eurygaster maculipennis* (12)  | 11.67 ± 0.53  | 87.36 ± 8.32   | 80.00–92.85    | 9.03 ± 8.30    | 3.61 ± 2.51    | 11/11/140       | 6   0   0         |
| *Gonocerus juniperi* (12)       | 10.00 ± 0.00  | 0 ± 0.00***    | –              | 97.50 ± 1.31***| 2.51 ± 1.30    | 0/0/100         | 3   0   0         |
| *Rhyncorhyncha iracundus* (3)   | 32.22 ± 0.88  | 0 ± 0.00**     | –              | 74.33 ± 21.16**| 25.67 ± 21.16**| 0/0/97          | 26  0  0         |

Asterisks within each column indicate significant differences among the non-target species and *Halyomorpha halys* (Kruskal–Wallis followed by Dunn’s multiple comparison test, *P* < 0.05; **P** < 0.01; ***P** < 0.001; n.a. = not assessed) (see Table S1 for statistics)
higher in *A. acuminata*, *E. oleracea*, *E. ventralis* and *N. viridula* than in *H. halys* (Table 2; see Table S1 for statistics). Dead specimens of *T. mitsukurii* (pupae and pharate adults) were detected in eggs of suitable and partially suitable pentatomid host species, with the only exceptions of *H. halys*, *D. baccarum* and *P. prasina*. No dead parasitoid specimens were recorded in eggs of the scutellerid *E. maura*, although several eggs died for unknown reason (undetermined content). While no *T. mitsukurii* emerged from *A. leucogrammes* and *E. ventralis*, dissection of dead eggs revealed the presence of high numbers of dead parasitoid specimens (adults and pupae) in both species. Instead, dissections of dead eggs of the coreid *G. juniperi* and the reduviid *R. iracundus* never reported the presence of dead pupae or adults.

When the Hemiptera egg masses were exposed to *T. mitsukurii* for 2 h, results were similar to the 24 h exposure experiment and the emergence rates were significantly different among the tested species ($\chi^2 = 77.38; P < 0.0001$) (Table 3; see Table S1 for statistics). *Trissolcus mitsukurii* was able to parasitize and develop in six out of the nine pentatomid species that were tested (66.67% of tested species) and in the scutellerid *E. maura* (Table 3). Specifically, the emergence rates of the pentatomids *A. heegeri*, *A. custos*, *C. pudicus*, *D. baccarum*, and *G. italicum* (five out of eight non-target pentatomid species) and the scutellerid *E. maura* were

### Table 3 Physiological suitability of Hemiptera eggs for *Trissolcus mitsukurii* in 2 h black box tests

| Species (replicates) | No. eggs/egg mass (mean ± SE) | % of eggs with parasitoid emergence/egg mass (mean ± SE) | % of females (min.–max.) | % of hatched eggs/egg mass (mean ± SE) | % of dead eggs (mean ± SE) | No. of parasitized egg masses/with parasitoid emergence/total no. of eggs exposed | Total no. of dead eggs | With undetermined content | With dead hemipteran nymphs | With dead parasitoid |
|----------------------|-------------------------------|-------------------------------------------------------|--------------------------|---------------------------------------|---------------------------|----------------------------------------------------------------------------------|-----------------------|------------------------|------------------------|------------------|
| *Halyomorpha halys* (12) | 27.58 ± 0.74               | 81.69 ± 3.41                                          | 91.30–96.55             | 18.31 ± 3.41                          | 0.00                      | 12/12/331                                                                        | 0                     | 0                     | 0                     |                  |
| *Acrosternum heegeri* (12) | 14.50 ± 0.68               | 91.11 ± 8.30                                          | 84.61–93.75             | 8.89 ± 8.30                           | 0.00                      | 11/11/174                                                                        | 0                     | 0                     | 0                     |                  |
| *Aelia acuminata* (12) | 10.92 ± 0.34               | 3.19 ± 2.53*                                          | 33.33–100.00            | 23.41 ± 11.78                         | 73.39 ± 11.46***          | 6/1/131                                                                         | 82                    | 0                     | 18 (all adults)        |                  |
| *Arma custos* (12)       | 15.33 ± 0.49               | 40.52 ± 14.47                                         | 92.30–93.33             | 58.33 ± 14.87                         | 1.15 ± 0.77               | 5/5/184                                                                          | 0                     | 0                     | 2 (all adults)         |                  |
| *Carpocoris pudicus* (12) | 12.33 ± 0.33               | 88.46 ± 8.65                                          | 83.33–92.85             | 8.97 ± 8.30                           | 2.56 ± 2.56               | 11/11/160                                                                        | 0                     | 0                     | 4 (all adults)         |                  |
| *Dolycoris baccarum* (12) | 14.00 ± 0.24               | 83.28 ± 4.41                                          | 50.00–92.85             | 5.40 ± 3.53                           | 11.40 ± 4.26              | 10/10/175                                                                        | 11                    | 0                     | 0                     |                  |
| *Eurydema ventralis* (12) | 11.67 ± 0.19               | 0 ± 0.00**                                            | –                       | 90.66 ± 3.78                          | 9.28 ± 3.77               | 1/0/140                                                                          | 9                     | 0                     | 4 (all adults)         |                  |
| *Graphosoma italicum* (12) | 14.75 ± 0.31               | 77.12 ± 5.84                                          | 57.14–93.33             | 18.87 ± 6.36                          | 4.00 ± 2.72               | 12/12/177                                                                        | 0                     | 0                     | 7 (all adults)         |                  |
| *Nezara viridula* (12)   | 34.25 ± 1.16               | 0 ± 0.00**                                            | –                       | 22.85 ± 10.62                         | 77.15 ± 9.38***           | 5/0/411                                                                          | 260                   | 0                     | 27 (all adults)        |                  |
| *Eurygaster maura* (12)  | 13.58 ± 0.26               | 88.64 ± 8.13                                          | 85.71–93.33             | 8.33 ± 8.33                           | 3.02 ± 1.08               | 11/11/163                                                                        | 0                     | 0                     | 5 (all adults)         |                  |

Asterisks within each column indicate significant differences among the non-target species and *Halyomorpha halys* (Kruskall–Wallis followed by Dunn’s multiple comparison test, *P* < 0.05; **P* < 0.01; ***P* < 0.001) (see Table S1 for statistics)
not significantly different from that of *H. halys* (Table 3; see Table S1 for statistics). Sex ratios were strongly female biased in most of the species tested (Table 3). Hatching rates of Hemiptera eggs were significantly different ($\chi^2 = 46.52, P < 0.0001$) and complementary with parasitoid emergence rates and dead eggs. Percentages of dead eggs were also significantly different ($\chi^2 = 74.06, P < 0.0001$) and consistent with previous data. Dead pupae and adults of the parasitoid were observed in most of the tested species, but not in *H. halys*, *A. heegeri* and *D. baccarum*. Specifically, eggs of *A. acuminata* showed lower parasitization success compared to *H. halys*, with significantly lower parasitoid emergence and higher dead eggs containing dead parasitoids. Similarly, eggs of *N. viridula* showed no parasitoid emergence and significantly higher dead eggs containing dead parasitoids. Instead, a high rate of nymphs emerged from *E. ventralis* eggs and no parasitoid emerged, while some dead parasitoids were found in the eggs (Table 3; see Table S1 for statistics).

When a single Hemiptera egg was exposed to *T. mitsukurii* for 2 h, six out of seven pentatomid species (85.71% of tested species), and the scutellerid *E. maura* were suitable for *T. mitsukurii* development (Table 4). Percentages of parasitoid emergence from eggs of non-target species were significantly lower compared to those from *H. halys* (Table 4; see Table S2 for statistics), except for *P. prasina* where 100% of emergence success was recorded, like in *H. halys*. No parasitoids emerged from eggs of *N. viridula* and no parasitoid pupae or pharate adults were found in dead eggs of this pentatomid species.

**Effect of host egg size on parasitoid size and egg load**

The three-body dimensions measured on *T. mitsukurii* females (head width, thorax width and hind tibia length, see Table S3) revealed to be highly correlated (head width vs. thorax width: $r = 0.96, P < 0.0001$; head width vs. hind tibia length: $r = 0.81, P < 0.0001$; thorax width vs. hind tibia length: $r = 0.81, P < 0.0001$). Within each Hemiptera host species, the female head width was used as main parameter to analyze in the linear regression model as a function of host egg volume. Regression analysis displayed a positive trend between the volume of host eggs and the head width of the emerged parasitoid females ($r^2 = 0.7673, P = 0.0002$) (Fig. 1).

The egg loads (see Table S4) of *T. mitsukurii* females originated from the different Hemiptera host species were significantly different ($\chi^2 = 65.75; P < 0.0001$). Parasitoid females that developed in *H. halys* exhibited a higher number of eggs in their ovaries compared to other non-target pentatomid species (see Table S4 for statistics), except for *C. pudicus* and *A. heegeri* and the scutellerid *E. maura*. Regression analysis displayed a positive trend between host egg volume and the ovarian egg load ($r^2 = 0.8053, P < 0.0001$) (Fig. 2).

**Table 4** Physiological suitability of Hemiptera eggs for *Trissolcus mitsukurii* in 2 h exposure of a single host egg

| Species                      | % of emerged parasitoids | % of hatched eggs | % of dead eggs |
|------------------------------|--------------------------|-------------------|---------------|
|                              |                          |                   |               |
|                              |                          |                   | With undetermined content or a dead hemipteran nymph | Containing pupae or adults |
| *Halyomorpha halys* (31)     | 100                      | 0                 | 0             | 0               |
| *Acrosternum heegeri* (30)   | 46.67***                 | 53.33             | 0             | 0               |
| *Arma custos* (43)           | 30.23***                 | 55.81             | 9.31          | 4.65            |
| *Dolycoris baccarum* (30)    | 20.00***                 | 63.33             | 16.67         | 0               |
| *Graphosoma italicum* (30)   | 26.67***                 | 16.67             | 0             | 56.67           |
| *Nezara viridula* (30)       | 0***                     | 56.67             | 43.33         | 0               |
| *Palomena prasina* (26)      | 100                      | 0                 | 0             | 0               |
| *Eurygaster maura* (34)      | 2.94***                  | 26.47             | 61.77         | 8.82            |

Among brackets number of females tested; asterisks within each column indicate significant differences among the non-target species and *Halyomorpha halys* (Chi-square test with Yates’ correction, ***$P < 0.001$) (see Table S2 for statistics).
vs. A. custos, $U = 38.50, P = 0.1339$). Percentages of dead eggs were not significantly different neither ($H. halys$ vs. $D. baccarum$, $U = 58, P = 0.3811$; $H. halys$ vs. $A. custos$, $U = 45.50, P = 0.3342$) (Fig. 3). A lower percentage of nymphs hatched from $H. halys$ compared to $A. custos$ eggs ($U = 30, P = 0.0434$), while similar percentages emerged from $H. halys$ and $D. baccarum$ eggs ($U = 65, P = 0.6924$).

When the parasitoid was reared on a non-target host ($D. baccarum$ or $A. custos$), the emergence of the progeny of $T. mitsukurii$ females showed no significant differences in the case of $H. halys$ vs. $D. baccarum$ ($U = 72, P > 0.9999$) and in that of $H. halys$ vs. $A. custos$ ($U = 30.50, P = 0.1214$) (Fig. 4). Additionally, no differences were observed in the percentage of dead eggs ($H. halys$ vs. $D. baccarum$, $U = 57, P = 0.8444$; $H. halys$ vs. $A. custos$, $U = 28, P = 0.0927$) nor in the percentage of hatched nymphs ($H. halys$ vs. $D. baccarum$, $U = 65, P = 0.6924$; $H. halys$ vs. $A. custos$, $U = 37.50, P = 0.3541$).
Discussion

The egg parasitoid *T. mitsukurii* has been poorly investigated till now as a candidate biological control agent of *H. halys*, especially when compared to other egg parasitoid species like *T. japonicus* and the non-coevolved *Anastatus bifasciatus* (Geoffroy) (Hymenoptera, Eupelmidae), on which a large literature is available from China, Europe, USA and New Zealand. Field data from Japan indicate for *T. mitsukurii* an oligophagous behavior (Yasumatsu and Watanabe 1964; Hokyo et al., 1966; Ryu and Hirashima, 1984; Arakawa and Namura 2002), and data from the invaded areas in Europe (Northern Italy) evidenced the ability of this parasitoid to locate and successfully parasitize *H. halys* egg masses in the field (Sabbatini-Peverieri et al. 2018; Benvenuto et al. 2020; Scaccini et al. 2020; Zapponi et al. 2020). Furthermore, laboratory studies confirm that the reproductive biology of this parasitoid on *H. halys* is similar to that of *T. japonicus* on the same host (Sabbatini-Peverieri et al. 2020).

In our experiments under laboratory conditions and 24 h of egg mass exposure, *T. mitsukurii* displayed the ability to parasitize and develop successfully in eight (61.54%) out of 13 tested non-target pentatomid species, with no significant differences compared to the coevolved host *H. halys*, while...
additional three species were partially suitable although at a significantly lower level compared to H. halys. These results were largely confirmed when H. halys and non-target species were exposed to the parasitoid only for 2 h, as T. mitsukurii successfully parasitized five (62.50%) out of eight non-target pentatomid species with similar rates than when parasitizing H. halys. Additionally, the scutellerid E. maura was successfully parasitized in both the 24 h and 2 h tests, whereas the coreid G. juniperi and the revid R. iracundus were not. This can be at least partially explained if we consider that scutellerids belong to the same superfamily of pentatomids, i.e., Pentatomoidea. In previous laboratory investigations, E. maura was revealed to be physiologically suitable also for T. japonicus (Haye et al. 2019). However, because scutellerids were never found as hosts of neither T. mitsukurii nor T. japonicus in the field, it is possible to hypothesize that other ecological factors, e.g., habitat preferences, interspecific competition or oviposition periods, may limit the access to this host. Laboratory host range of T. mitsukurii can be considered similar to that of T. japonicus, which showed comparable results in previous host specificity tests on European non-target species, as 13 pentatomids were suitable for parasitoid development (Haye et al. 2019; Sabbatini-Peverieri et al. 2021).

The highly variable rates of T. mitsukurii emergence from non-target eggs, both in the 24 h and 2 h exposure experiments, could be discussed in terms of host egg recognition/acceptance and suitability, based on data on parasitoid emergence, egg eclosion, egg death and the presence of dead parasitoids in eggs. Thus, H. halys, A. heegeri and D. baccarum appear to be easily recognized by T. mitsukurii and highly suitable hosts for this parasitoid under laboratory conditions, as emergence rates were always very high, egg hatching low, and almost no dead eggs were recorded. Similar results were also observed in P. prasina, although this species was tested with only one egg mass due to the poor rearing success in the laboratory. Most of the other non-target species seemed to be less suitable, although statistically not different from H. halys, because of the high presence of dead eggs, often containing a dead parasitoid pupa or a pharate adult (A. custos, C. pudicus, G. italicum, P. lituratus, Scioecoris sp.). Still, rates of successful parasitism of this species were rather high. High rates of parasitization successes under laboratory conditions were observed also for T. japonicus in China on H. halys and other pentatomid species (Zhang et al., 2017), while other studies in the USA and New Zealand reported lower rates both on the target, H. halys, and no targets, probably due to the different physiological status of tested females (Hedstrom et al. 2017; Botch and Delfosse 2018; Charles et al. 2019; Lara et al. 2019). In our study, a few non-target pentatomid hosts showed significantly lower T. mitsukurii emergence rates compared to emergence from H. halys. This was due either to low recognition and parasitization, as indicated by high egg hatching rates (A. leucogrammes), or to low suitability to parasitoid development, as indicated by the high rates of dead eggs (A. acuminata, E. oleracea, N. viridula), or to both reasons (E. ventralis). The occurrence of dead T. mitsukurii pupae or adults in several Hemiptera host eggs further reflects a low physiological suitability. Remarkably, dead parasitoids were found also in the eggs of pentatomid species that did not allow any parasitoid adult emergence, i.e., E. ventralis and A. leucogrammes. In the field, such cases might act as an evolutionary trap, as was stated for T. japonicus in similar cases, and unsuitable biochemical contentment of hemipteran eggs can here play a key role (Abram et al. 2014; Haye et al. 2019).

Surprisingly, as discussed above, the eggs of N. viridula showed a very low suitability rate for T. mitsukurii. This is a rather unexpected result because, although not coevolved due to the different geographical origin, N. viridula was addressed in the past as a main host for T. mitsukurii in its area of origin (Kiritani and Hokyo 1962; Hokyo and Kiritani 1963; Hokyo et al. 1966; Arakawa and Namura 2002; Arakawa et al. 2004). In our experiment, a very low parasitoid emergence rate and a high rate of dead eggs was detected, especially if compared with the high hatching rate observed in unexposed N. viridula egg masses. Moreover, in only about one tenth of the dead eggs, it was possible to find dead parasitoid pupae or adults, whereas the egg content could not be determined in the other cases. We can hypothesize that parasitization occurred also in these cases, but the parasitoid failed to survive already during the first instars, further suggesting that N. viridula eggs are not suitable for larval development of T. mitsukurii. A similar result was observed in T. japonicus, as the parasitizing females caused 100% mortality in N. viridula eggs, but no emergence of adults was observed, nor parasitoid presence could be clearly identified from egg dissection (Haye et al. 2019). The low suitability of N. viridula to T. mitsukurii deserves further investigations.

The extremely simplified experiment conducted using a single host egg, exposed to T. mitsukurii for 2 h, showed that among the seven pentatomid and one scutellerid species tested, most of them were detected as physiologically suitable, failing only in the case of N. viridula, which confirms its extremely low physiological suitability for T. mitsukurii. Remarkably, in such kind of test, only H. halys and P. prasina allowed 100% parasitism success by T. mitsukurii, while parasitoid emergence from all other non-target host species was significantly lower. Comparing the three methods of host egg exposure to females of T. mitsukurii adopted in the present study (exposure of egg masses for 24 h and for 2 h and exposure of single eggs for 2 h) and considering only the Hemiptera species that were tested in all three experiments, the 2 h test with single egg
exposure failed to detect host physiological suitability in one case, the less suitable host species *N. viridula*. Additionally, a high number of hatched eggs was observed in all species except *H. halys* and *P. prasina*. These results indicate that a single host egg was not easily recognized and probed by the parasitoid. Therefore, by exposing a single egg, the degree of host acceptance and physiological suitability might be more difficult to define since the effect of clustered eggs is not taken into account. However, the use of single host egg exposure was already adopted for host specificity tests with *T. japonicus* and the output of physiological host suitability range was comparable with the results from experiments conducted using egg mass exposed for 24 h using similar host species (Haye et al. 2019; Sabbatini-Peverieri et al. 2021). This simple method of exposure might permit to filter a first list of suitable non-target species, which can be later investigated using more complex procedures, thus optimizing resources and time.

In future tests, the time of egg exposure to parasitoids can be reduced (depending on the size of arena and on complexity of tests) as a few hours are adequate rather than a 24-h experimental design. Here, considering outputs of the present work, three hours might act as optimal exposure time. The reduction in time might be a relevant factor in both choice and no-choice tests since this would increase parasitoid selectivity by reducing the number of encounters with egg masses, thus preventing host acceptance as a consequence of multiple contacts (Haye et al., 2019). Under field conditions, a first rejection of a potential host species might turn out in the parasitoid searching for more suitable hosts, while this is not permitted in the constrained environments under laboratory studies (Botch and Delfosse 2018; Boyle et al. 2020).

In our experiments, we also aimed at comparing host parasitism by *T. mitsukurii* in black-box choice tests under 2 h exposure and the effect of rearing host, using *H. halys* vs. either one of two suitable non-target species, *D. baccarum* and *A. custos*. No significant differences in host parasitism by *T. mitsukurii* females were observed, and the host species used to rear *T. mitsukurii* females did not play a significant role in host choice. However, behavioral investigations focusing on the parasitoid first choice might be useful to reveal possible preferences that cannot be evaluated in 2-h black box tests. Unlike *T. mitsukurii*, *T. japonicus* reared on *H. halys* was shown to prefer *H. halys* in two-choice tests conducted in small arenas, and partially also in more complex systems using large cages and plants as ovipositing substrates (Hedstrom et al. 2017; Botch and Delfosse 2018; Haye et al. 2019). On the other side, preferences seemed to vanish when *T. japonicus* was reared on non-target hosts. Although choice tests in simplified experimental designs are fundamental for a first understanding of the likelihood of parasitization, more complex systems are required for a higher certainty of results.

The last experiment was conducted to evaluate the effect of host egg size on the size and egg load of emerged *T. mitsukurii*. The results indicated that host egg volume significantly affected the size of emerging adult females and the number of the eggs in their ovaries, i.e., larger host eggs produced larger females with higher egg loads, confirming previous findings by Arakawa and Namura (2004). Since many non-target host species are smaller in size than *H. halys*, it can be assumed that a population of *T. mitsukurii* originating from non-target species results in females with lower reproductive ability. This was already speculated for *T. japonicus* by Botch and Delfosse (2018) and observed successively in laboratory tests (Sabbatini-Peverieri et al. 2021).

Summarizing results of the present study, *T. mitsukurii* displayed in laboratory tests no specific host specialization, showing capability to successfully parasitize most of tested Pentatomoidea species, including *A. custos*, an Asopinae predator, as was previously assessed also for *T. japonicus* (Haye et al. 2019). However, while in our laboratory experiments, *T. mitsukurii* showed similar host preference toward more than one pentatomid species, *T. japonicus* appeared to prefer its coevolved host *H. halys* both in laboratory and field conditions (Milnes and Beers 2019; Botch and Delfosse 2018). It is well-known that testing conditions in the simplified laboratory environment can be misleading in non-target risk analysis; therefore, the results must be carefully evaluated (Haye et al. 2019). Thus, more complex systems are needed to better simulate field environmental factors, and experiments with *T. mitsukurii* should adopt multidisciplinary and integrated approaches. These were considered in the case of *T. japonicus*, and the large literature on laboratory host range tests evidenced, on a global scale, that the physiological host range of this parasitoid embraces more than two dozens of species among tested pentatomids (Zhang et al. 2017; Hedstrom et al. 2017; Botch and Delfosse 2018; Charles et al. a. 2019; Haye et al. 2019; Lara et al. 2019). Meanwhile, by means of olfactometer bioassays to test volatiles from hosts and host-induced plant volatiles, a higher level of species selection might emerge, as chemical ecology experiments allow to predict what cues, from which host species, the parasitoids are able to exploit in the field during host location (Bertoldi et al. 2019).

Considering the topical interest of classical biological control of *H. halys*, new field data on host preference by *T. mitsukurii* in Europe are expected to appear soon in the literature. Combined with the information we provide here and with further studies involving olfactory cues from host and host plants, and this will help understand the actual host range of *T. mitsukurii* in its novel area of establishment, allowing evaluation of the possible risks associated with its use as a biocontrol agent of *H. halys*. 
Authors’ contributions

LG, GSP and LM conceived and designed research. LG, GSP, LM, GR, EC, PFR collected material in the field. GL and GSP conducted experiments. PFR involved in funding acquisition, resources and supervision. LG, GSP and GR involved in data analysis. LG and GSP involved in data curation and writing original draft. LG, GSP, GR and EC involved in writing final version. LG, GSP, LM, GR, EC and PFR involved in review and editing. All authors read and approved the manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01415-x.

Acknowledgements We are grateful to Iris Bernardinelli, Giorgio Malossini and Luca Benvenuto of ERSA Friuli Venezia Giulia, and to Giacomo Vaccari and Stefano Caruso of the Consorzio Fitosanitario Modena for the help in the field collection of H. halys in infested sites in northern Italy. We thank Riccardo Frosinini, Andrea Rocchini, Paolo Toccafondi, Tiziano Fabbricatore and Luca Madonni for assistance in H. halys and non-target species rearing in the laboratory. This work was supported by the Ministero per le Politiche Agricole, Alimentari e Forestali (MiPAAF), projects “Salvaolivi” (DM 0033437 21/12/2017) and “Proteggo 1.3” (MIPAAF DISRV 0155972 6/04/2021).

Funding This research was founded by the Ministero delle Politiche Agrarie Alimentari e Forestali.

Availability of data and material Available under request to the corresponding author.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no conflict of interest.

Ethics approval This article does not contain any studies with human participants or vertebrates performed by any of the authors.

References

Abram PK, Gariepy TD, Boivin G, Brodeur J (2014) An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol Invasions 16:1387–1395
Abram PK, Parent JP, Brodeur J, Boivin J (2016) Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life-history and behavioural traits. Biol J Linn Soc 117:620–632
Abram PK, Hoelmer KA, Acebes-Doria A et al (2017) Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North America and Europe. J Pest Sci 90:1009–1020
Abram PK, Mills NJ, Beers EH (2020) Review: classical biological control of invasive stink bugs with egg parasitoids—What does success look like? Pest Manag Sci 76:1980–1992
Arakawa R, Miura M, Fujita M (2004) Effects of host species on the body size, fecundity, and longevity of Trissolcus mitsukurii (Hymenoptera: Scelionidae), a solitary egg parasitoid of stink bugs. Appl Entomol Zool 39:177–181
Arakawa R, Namura Y, (2002) Effects of temperature on development of three Trissolcus spp. (Hymenoptera: Scelionidae), egg parasitoids of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae). Entomol Sci 5:215–218
Benvenuto L, Bernardinelli I, Malossini G, Sabbatini Peverieri G, Marianelli L, Roversi PF (2020) Monitoraggio di ovatrici parassitizzate di Halyomorpha halys in un actuindieto biologico in Friuli Venezia Giulia nel biennio 2018–2019. Atti Giornate Fitopatologiche 1:161–166
Bertoldi V, Rondoni G, Brodeur J, Conti E (2019) An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host. Front Physiol 10:746. https://doi.org/10.3389/fphys.2019.00746
Botch PS, Delfosse ES (2018) Host-acceptance behavior of Trissolcus japonicus (Hymenoptera: Scelionidae) reared on the invasive Halyomorpha halys (Heteroptera: Pentatomidae) and nontarget species. Environ Entomol 47:403–411
Boyle SM, Weber DC, Hough-Goldstein J, Hoelmer KA (2020) Host kairomones influence searching behavior of Trissolcus japonicus (Hymenoptera: Scelionidae), a parasitoid of Halyomorpha halys (Heteroptera: Pentatomidae). Environ Entomol 49(1):15–20. https://doi.org/10.1093/ee/nvz155
Charles JG, Avila GA, Hoelmer KA, Hunt S, Gardner-Gee R, MacDonald F, Davis V (2019) Experimental assessment of the biosafety of Trissolcus japonicus in New Zealand, prior to the anticipated arrival of the invasive pest Halyomorpha halys. Biocontrol 64:367–379. https://doi.org/10.1007/s10526-019-09949-x
Chu F-J, Zhou Z-F, Li R-P, Liu X-C (1997) Study on control and observation of the bionomics characteristics of Halyomorpha picea Fabricius. J Agric Univ Hebei 20:12–17
Conti E, Avila G, Barratt B, Cingolani F, Colazza S, Guarino S, Hoelmer K, Laumann RA, Maistrello L, Martel G et al (2021) Biological control of invasive stink bugs: review of global state and future prospects. Ent Exp Appl 169(1):28–51
EPPO (2014) PM 6/2 (3) Import and release of non-indigenous biological control agents. Bulletin OEPP/EPPO Bulletin 44(3):320–329
FAO-IPPC (2017) ISPM 3 Guidelines for the export, shipment, import and release of non-indigenous biological control agents and other beneficial organisms. www.ippc.int
Haye T, Moraglio ST, Stahl J, Visentin S, de Gregorio T, Taverna L (2019) Fundamental host range of Trissolcus japonicus in Europe. J Pest Sci 93:171–182. https://doi.org/10.1007/s10526-019-09112-3
Hedstrom C, Lowenstein D, Andrews H, Bui B, Wiman N (2017) Pentatomid host suitability and the discovery of introduced populations of Trissolcus japonicus in Oregon. J Pest Sci 90:1169–1179
Hokyo N, Kiritan K, Nakasui F, Shiga M (1966) Comparative biology of the two scelionid egg parasites of Nezara viridula L. (Hemiptera: Pentatomidae). Jpn J Appl Entomol Z 1:94–102
Hokyo N, Kiritan K (1963) Two species of egg parasites as contemporaneous mortality factors in the egg population of the southern green stink bug, Nezara viridula. Jpn J Appl Entomol Z 7:214–226
Hunt E, Kuhlmann U, Sheppard A, Qin TK, Barratt BIP, Harrison L, Mason PG, Parker D, Flanders RV, Goolsby J (2008) Review of invertebrate biological control regulation in Australia, New Zealand, Canada and the USA: recommendations for a harmonized European system. J Appl Entomol 132:89–123
Kiritan K, Hokyo N (1962) Studies on the life table of the southern green stink bug, Nezara viridula. Jpn J Appl Entomol Z 6:124–140
Kuhlmann U, Mason PG (2003) Use of field host range surveys for selecting candidate non-target species for physiological host specificity testing of entomopathogenic biological control agents. In: 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA, January 14–18, 2002. USDA Forest Service FHTET-03-05, pp. 370–377.
Stahl J, Tortorici F, Pontini M, Bon MC, Hoelmer K, Marazzi C, Tavella L, Haye T (2019) First discovery of adventive populations of Trissolcus japonicus (Ashmead) in Europe. J Pest Sci 92:371–379. doi: https://doi.org/10.1007/s10344-018-1061-2

Talamas EJ, Herlihy MV, Dieckhoff C, Hoelmer KA, Buffington ML, Bon M-C, Weber DC (2015) Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J Hym Res 43:119–128. https://doi.org/10.3897/JHR.43.4661

van Lenteren JC, Bale J, Bigler F, Hokkanen HMT, Loomans AJM (2006a) Assessing risks of releasing exotic biological control agents of arthropod pests. Annu Rev Entomol 51:609–634

van Lenteren JC, Cock MJW, Hoffmeister TS, Sands DPA (2006b) Host specificity in arthropod biological control, methods for testing and interpretation of the data. In: Bigler F, Babendreier D, Kuhlmann U (eds) Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CAB International, pp. 38–63.

Yang Z-Q, Yao Y-X, Qiu L-F, Li Z-X (2009) A new species of Trissolcus (Hymenoptera: Scelionidae) parasitizing eggs of Halyomorpha halys (Heteroptera: Pentatomidae) in China with comments on its biology. Ann Entomol Soc Am 102:39–47

Yasumatsu K, Watanabe C (1964) A tentative catalogue of insect natural enemies of injurious insects in Japan—part 1. Parasite-Predator Host Catalogue. Fukuoka, Japan: Entomological Laboratory, Faculty of Agriculture Kyushu University

Zapponi L, Bon MC, Fonui AM, Anfora G, Schmidt S, Falangardi M (2020) Assemblage of the egg parasitoids of the invasive stink bug Halyomorpha halys: insights on plant host associations. Insects. https://doi.org/10.3390/insects11090588

Zapponi L, Tortorici F, Anfora G, Bardella S, Bariselli M, Benvenuto L, Bernardielli I, Butturini A, Caruso S, Colla R, Costi E, Dall P, Di Bella E, Falangardi M, Giovanni L, Haye T, Maistrello L, Malossini G, Marazzi C, Mariani L, Mele A, Michelon L, Moraglio ST, Pozzebon A, Preti M, Salvetti M, Scaccini D, Schmidt S, Szalatnay D, Tavella L, Tommasini MG, Vaccari G, Zandigiacomo P, Zappanni-Peverieri G (2021) Assessing the distribution of exotic egg parasitoids of Halyomorpha halys in Europe with a large-scale monitoring program. Insects 12(4):316. https://doi.org/10.3390/insects12040316

Zhang J, Zhang F, Garepy T, Mason P, Gillespie D, Talamas E, Haye T (2017) Seasonal parasitism and host specificity of Trissolcus japonicus in northern China. J Pest Sci 90:1127–1141

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.