

## Comparative life tables of *Trissolcus japonicus* and *Trissolcus mitsukurii*, egg parasitoids of *Halyomorpha halys*

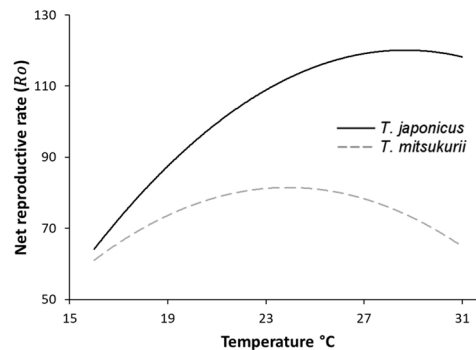
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### HIGHLIGHTS

- *Trissolcus japonicus* has a greater longevity than *T. mitsukurii*.
- *Trissolcus japonicus* has a higher net reproductive rate than *T. mitsukurii* at 26 and 31 °C.
- The intrinsic rate of increase of *T. mitsukurii* at 21 °C is higher compared to *T. japonicus*.
- *Trissolcus mitsukurii* has a higher fitness at a lower range of temperatures than *T. japonicus*.
- *Trissolcus japonicus* demonstrated higher performance as Biological Control Agent.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Keywords:

Classical biological control  
Invasive species  
Egg parasitoids  
Development rate  
Thermal biology

### ABSTRACT

*Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) are Asian egg parasitoids that are considered important natural enemies of the brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). These species are expanding their geographic distribution outside their native area, which suggests investigating their adaptability to environmental conditions. In a laboratory experiment, we investigated the life table parameters of these two egg parasitoid species under four constant temperatures, i.e., 16, 21, 26 and 31 °C. Results showed that the longevity of *T. japonicus* was higher than that of *T. mitsukurii* at all temperatures considered. The net reproductive rate ( $R_0$ ) of *T. japonicus* was higher than that of *T. mitsukurii* at 26 and 31 °C. However, the intrinsic rate of increase ( $r_m$ ) at 26 and 31 °C was similar between the two species, and at 21 °C, *T. mitsukurii* exhibited a higher intrinsic and finite rate of increase. The two species complete their development at 16 °C, but with the lowest performances. Results suggest a higher increase of *T. mitsukurii* population when mean temperatures are equal to 21 °C, while the population increase of *T. japonicus* is expected to be favoured at higher temperatures.

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## 1. Introduction

Knowledge of the effects of temperatures on natural enemy biodemography is important for understanding host-parasitoid dynamics and impact on pest regulation (Bellows et al., 1992; Carey, 2001). Thus, life table construction is a milestone in evaluating natural enemies population performances on economically important pests (Van Driesche and Bellows, 1996).

In the past two decades, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) has become an important pest worldwide. Native to Asia, it spread to many countries following human pathways; it was first recorded in Pennsylvania in 1996 (Hoebeke and Carter, 2003), then it has spread throughout the United States, Canada (Fogain and Graff, 2011; Leskey and Nielsen, 2018) and South America (Faúndez and Rider, 2017). In Europe, *H. halys* was first detected in Switzerland in 2007 (Wermelinger et al., 2008), and later, it spread to almost all European countries, where the dynamics of infestation are established on a landscape scale (Laterza et al., 2023; Park et al., 2023; Tamburini et al., 2023).

*Halyomorpha halys* is a highly polyphagous insect, feeding on more than 170 species of crop and non-crop plants (Leskey and Nielsen, 2018). Like other heteropterans, *H. halys* is a piercing-sucking insect that can feed on different plant organs (Lee et al., 2013; Scaccini and Pozzebon, 2021) but prefers fruit structures. Crop plants mostly damaged are fruit crops such as apple, pear, peach, kiwifruit and cherry (Fornasiero et al., 2023; Francati et al., 2021; Leskey and Nielsen, 2018; Maistrello et al., 2017; Moore et al., 2019), but also nut crops such as hazelnut, almond, walnut and pistachio (Bosco et al., 2018; Stahl et al., 2021, 2020) and open field crops such as maize and soybean (Cissel et al., 2015; Nielsen et al., 2011).

Chemical control against *H. halys* relies mainly on broad-spectrum insecticides, even though they are ineffective in controlling this pest (Kuhar and Kamminga, 2017). Among sustainable strategies for control of this pest, biological control based on Scelionidae egg parasitoids is seen as the most promising (Gutierrez et al., 2023; Lee et al., 2013). Scelionid wasps are specialised in egg parasitism of insects: they have high searching abilities and reproductive rates, positive host density responsiveness, and usually synchrony with the host (Orr, 1988). Two scelionid egg parasitoids were considered the most effective natural enemies of *H. halys* in Asia, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae). In Asia, the most common biological control agent of *H. halys* is *T. japonicus*, which showed 50–80 % of parasitism both in China and Japan (Avila et al., 2021; Kamiyama et al., 2021; Zhang et al., 2017). While *T. japonicus* is reported to be present both in China and Japan, *T. mitsukurii* has been recorded to parasitise *H. halys* in Japan, with performances comparable to those of *T. japonicus* (Kamiyama et al., 2021; Lee et al., 2013). Outside its native range, *T. japonicus* was first detected in North America in 2014 (Talamas et al., 2015) and in Canada in 2017 (Garipey and Talamas, 2019). In Europe, *T. japonicus* was detected in Switzerland in 2017 (Stahl et al., 2019), in Italy in 2018 (Sabbatini Peverieri et al., 2018), and in Germany in 2020 (Dieckhoff et al., 2021). Instead, adventive populations of *T. mitsukurii* were found in Italy from 2016 (Scaccini et al., 2020) and later on in Slovenia (Rot et al., 2021) and France (Bout et al., 2021). In invaded areas, both parasitoids are expanding their geographic range and increasing their impact on the host (Abram et al., 2019; Hedstrom et al., 2017; Kaser et al., 2018; Mele et al., 2022; Zapponi et al., 2021). However, hyperparasitoids that develop on primary parasitoid species are adding complexity to the host-parasitoid system (Giovannini et al., 2021a; Mele et al., 2021; Moraglio et al., 2020; Sabbatini Peverieri et al., 2019; Scaccini et al., 2020).

Both *T. japonicus* and *T. mitsukurii* have been considered for classical and augmentative biological control strategies. Indeed, many aspects of the biology (Malek et al., 2021; McIntosh et al., 2020; Sabbatini Peverieri et al., 2020; Scala et al., 2022) and the non-target effects of

these two species have recently been investigated (Giovannini et al., 2021b; Haye et al., 2020; Hedstrom et al., 2017; Rondoni et al., 2022). However, temperature-related development of these two egg parasitoid species has not been studied in detail. In this work, *T. japonicus* and *T. mitsukurii* were reared in laboratory conditions using *H. halys* eggs as host and under four constant temperatures to investigate the life-history traits of these two important biological control agents.

## 2. Materials and methods

### 2.1. Insect rearing

Laboratory colonies of *H. halys*, *T. japonicus* and *T. mitsukurii* were established in 2020 from field-collected insects in Veneto region, northeastern Italy. Stink bugs were reared at  $26 \pm 2$  °C,  $60 \pm 10$  % RH, and 16:8 (L:D) photoperiod in insect cages ( $30 \times 30 \times 50$  cm) with green bean plants (*Phaseolus vulgaris*), and sunflower seeds (*Helianthus annuus*), apple (*Malus domestica*) and kiwi (*Actinidia chinensis*) fruits added as additional food sources. Water was provided through a wetted cotton ball. Egg masses were collected and preserved at 6 °C for no more than one month before being used in this study (Bittau et al., 2021; Wong et al., 2021). *Trissolcus* colonies were maintained on *H. halys* egg masses and reared at  $26 \pm 1$  °C,  $60 \pm 10$  % RH, and photoperiod 16:8 (L:D).

### 2.2. Experimental design

To run the experiment, 40 newly-emerged (<1 day-old) couples of each parasitoid species were isolated. Each couple was placed in a centrifuge tube, with a netted cap for ventilation and honey provided as a food source, renewed every other day. Parasitoid couples were placed at 16, 21, 26, or  $31 \pm 1$  °C in four different climatic chambers with a relative humidity of  $60 \pm 10$  % and photoperiod 16:8 (L:D). Ten couples of *T. mitsukurii* and ten of *T. japonicus* were placed in each chamber. A single fresh *H. halys* egg mass was supplied daily to each couple until the death of the female parasitoid. Parasitoids were left ovipositing for 24 h and then parasitised egg masses were moved to another centrifuge tube, labelled and kept in the same climatic chamber where they were parasitised. Parasitoid couples and parasitised egg masses were observed daily to check parasitoid emergence and survivorship.

Adult parasitoids from parasitised *H. halys* egg masses were counted and sexed. Moreover, egg dissection was performed under a stereomicroscope (Stemi 508, Carl Zeiss Microscopy GmbH, Jena, Germany) to count parasitoids that possibly failed to develop to the adult stage.

### 2.3. Data analysis

Female and male survivorships were compared with Kaplan-Meier Survival Analysis with Log-rank test by using LIFETEST procedure of SAS software ver. 9.4 (SAS Institute Inc., 2016). The mean number of eggs (i.e., the sum of emerged parasitoids and parasitoids that failed to emerge) produced by each female, the mean number of eggs per day, the progeny sex ratio and the progeny survival ratio were analysed with a two-way ANOVA and Tukey post-hoc test ( $\alpha = 0.05$ ), with temperature, parasitoid species and their interaction considered as independent variables. To run the ANOVA, the GLM procedure of SAS software ver. 9.4 (SAS Institute Inc., 2016) was used, and ANOVA assumptions were checked before analysis. To follow normality assumptions, progeny sex ratio and progeny survival ratio were angular transformed (i.e., the arcsine of the square root of the ratio) before analysis. Male and female development times from egg to emergence of the adult were analysed with a generalized linear model ( $\alpha = 0.05$ ) using GENMOD procedure of SAS software ver. 9.4 (SAS Institute Inc.).

The development time required from egg to adult was used to understand the upper and lower thermal limits. The relationship between development rate (1/days) and temperature were fitted with both a

**Table 1**  
Definitions and formulae for life table parameters (Maia et al., 2000).

| Parameter | Definition   | Formula                                  |
|-----------|--|--|
| $x$       | Age interval in days   |  |
| $m_x$     | Age-specific fecundity<br>The average offspring females per female in age $x$                                      |  |
| $l_x$     | Age-specific survival<br>Proportion of females surviving to start of the age $x$                                   |  |
| $R_0$     | Net reproductive rate<br>The mean offspring females per female, during the entire oviposition period               | $\sum l_x \times m_x$                    |
| $r_m$     | Intrinsic rate of increase<br>Rate of natural increase in closed population  | $\sum e^{-rx} \times l_x \times m_x = 1$ |
| $\lambda$ | Finite rate of increase<br>Rate of increase per unit time per individual   | $e^{r_m}$                                |
| $T$       | Generation time<br>Mean time span between the birth of individuals of a generation and that of the next generation | $\frac{\ln(R_0)}{r_m}$                   |
| $DT$      | Doubling time<br>Time span necessary for doubling the initial population   | $\frac{\ln(2)}{r_m}$                     |

linear (Campbell et al., 1974) and non-linear model (Lactin et al., 1995), since at optimum temperature ranges (as the case of our study), the linear relationship best fits development rate (Ikemoto and Takai, 2000), but cannot estimate the upper thermal limit. To run the models, the “devRate” package (Rebaudo et al., 2018) implemented in R version 4.1.2 was used (R Core Team, 2021).

The following fecundity data were used in the evaluation of life table parameters: net reproductive rate ( $R_0$ ), intrinsic rate of increase ( $r_m$ ), finite rate of increase ( $\lambda$ ), mean generation time ( $T$ ) and doubling time ( $DT$ ) (Carey, 1993; Maia et al., 2000). Parameter symbols, formulae and definitions are summarized in Table 1 and follow Maia et al. (2000). Life table parameters were estimated with the Jackknife method proposed by Meyer et al. (1986), and multiple comparisons between temperatures

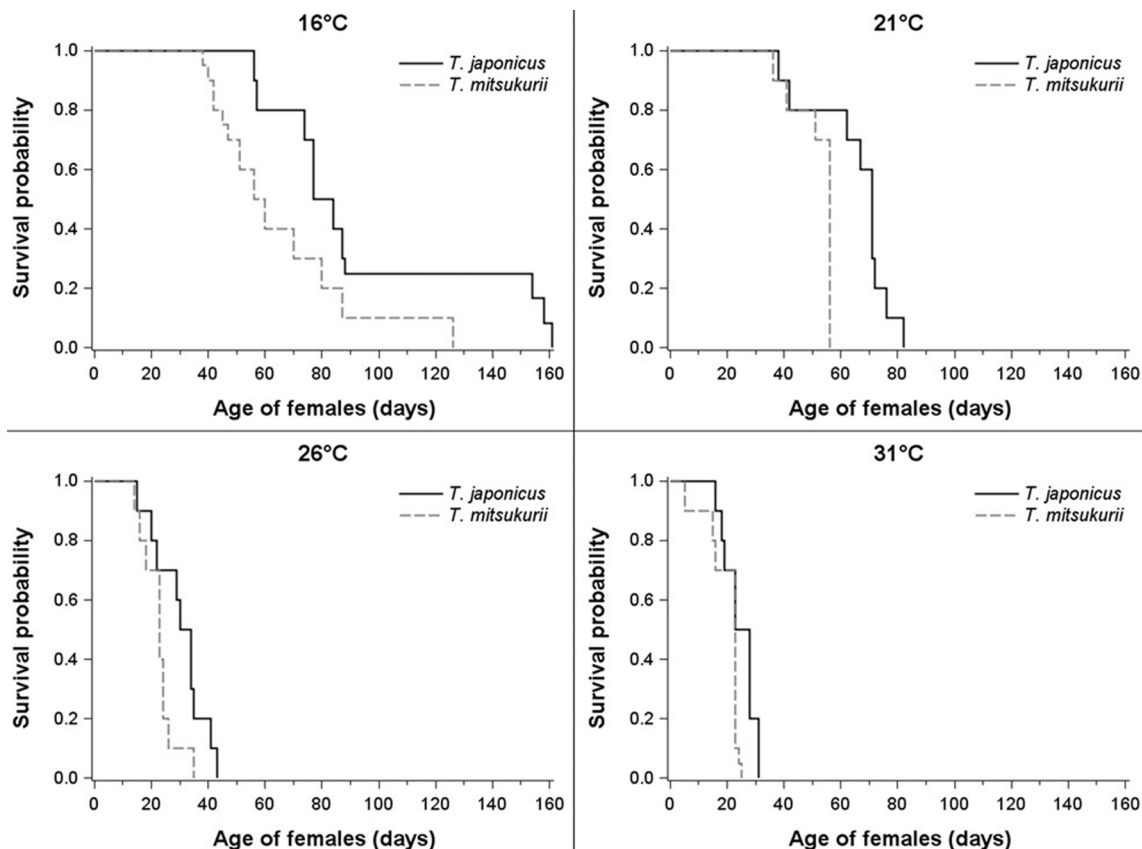
and parasitoid species were done with a two-tailed  $t$  test ( $\alpha = 0.05$ ) using Jackknife estimates of their respective variances. Analyses were run with the SAS program developed by Maia et al. (2000) using SAS software ver. 9.4 (SAS Institute Inc., 2016).

**3. Results**

The lifespan of *T. japonicus* and *T. mitsukurii* varied among the four different temperatures and decreased along with temperature for both males (*T. japonicus*:  $\chi^2 = 46.8432$ ,  $df = 3$ ,  $P < 0.0001$ ; *T. mitsukurii*:  $\chi^2 = 49.0932$ ,  $df = 3$ ,  $P < 0.0001$ ) and females (*T. japonicus*:  $\chi^2 = 106.5664$ ,  $df = 3$ ,  $P < 0.0001$ ; *T. mitsukurii*:  $\chi^2 = 93.5094$ ,  $df = 3$ ,  $P < 0.0001$ ). Females lived the longest at 16 °C (*T. japonicus*: 161 days; *T. mitsukurii*: 126 days) and the shortest at 31 °C (*T. japonicus*: 31 days; *T. mitsukurii*: 25 days) (Fig. 1). The longevity of males was shorter than females at all the tested temperatures, and male longevity decreased with increasing temperature being the shortest at 31 °C (*T. japonicus*: 21 days; *T. mitsukurii*: 15 days) and highest at 16 °C (*T. japonicus*: 137 days; *T. mitsukurii*: 120 days). Females of *T. japonicus* had a higher survival

**Table 2**  
Results of the Log-rank test on the differences between *T. japonicus* and *T. mitsukurii* males and females survival at 16, 21, 26 and 31 °C.

| Temperature | Sex | $\chi^2$ | df | P value  |
|-------------|-----|----------|----|----------|
| 16 °C       | F   | 7.8188   | 1  | 0.0052   |
|             | M   | 1.0016   | 1  | 0.3169   |
| 21 °C       | F   | 16.9255  | 1  | < 0.0001 |
|             | M   | 7.2944   | 1  | 0.0069   |
| 26 °C       | F   | 7.9769   | 1  | 0.0047   |
|             | M   | 6.3414   | 1  | 0.0118   |
| 31 °C       | F   | 7.9790   | 1  | 0.0047   |
|             | M   | 6.4954   | 1  | 0.0108   |



**Fig. 1.** Kaplan-Meier survival curves of females of *T. japonicus* and *T. mitsukurii* at four constant temperatures.

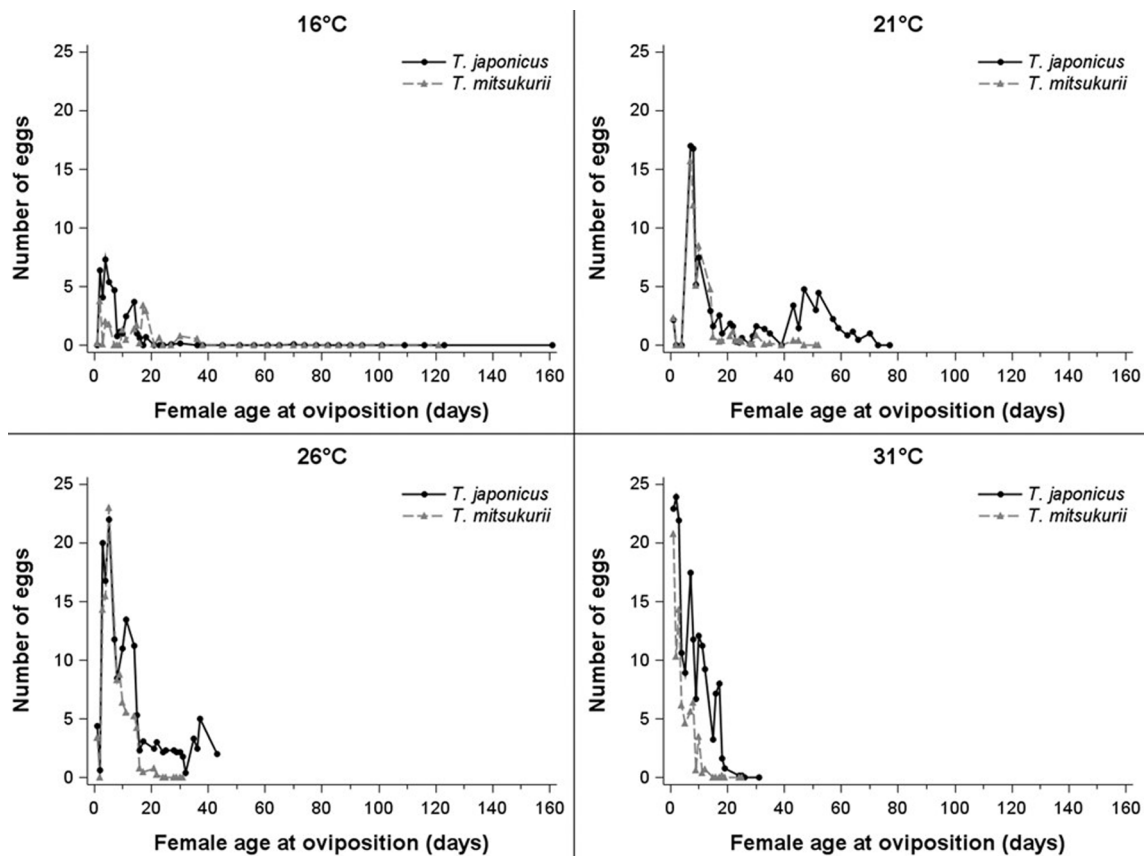


Fig. 2. Average number of eggs laid by a female of *T. japonicus* and *T. mitsukurii* at four constant temperatures.

Table 3

Mean ( $\pm$ S.E.) number of eggs per female, number of eggs per female per day, progeny sex ratio and progeny survival of *T. japonicus* and *T. mitsukurii* under four constant temperatures. Different letters indicate significant differences at Tukey test on least square means ( $\alpha = 0.05$ ).

| Temperature | Specie               | N. of eggs per female |    | N. of eggs per female per day |    | Mean sex ratio (f:m) |   | Mean progeny survival |   |
|-------------|----------------------|-----------------------|----|-------------------------------|----|----------------------|---|-----------------------|---|
| 16 °C       | <i>T. japonicus</i>  | 37.10 $\pm$ 3.41      | d  | 1.75 $\pm$ 0.16               | e  | 0.76 $\pm$ 0.03      | a | 0.74 $\pm$ 0.06       | b |
|             | <i>T. mitsukurii</i> | 26.20 $\pm$ 4.19      | d  | 1.19 $\pm$ 0.18               | e  | 0.82 $\pm$ 0.04      |   | 0.62 $\pm$ 0.07       |   |
| 21 °C       | <i>T. japonicus</i>  | 83.50 $\pm$ 4.81      | bc | 3.43 $\pm$ 0.53               | d  | 0.78 $\pm$ 0.04      | a | 0.90 $\pm$ 0.02       | a |
|             | <i>T. mitsukurii</i> | 68.60 $\pm$ 5.48      | c  | 2.36 $\pm$ 0.27               | d  | 0.82 $\pm$ 0.03      |   | 0.94 $\pm$ 0.03       |   |
| 26 °C       | <i>T. japonicus</i>  | 145.90 $\pm$ 8.96     | a  | 7.50 $\pm$ 0.41               | b  | 0.63 $\pm$ 0.04      | b | 0.90 $\pm$ 0.01       | a |
|             | <i>T. mitsukurii</i> | 95.20 $\pm$ 6.64      | b  | 6.30 $\pm$ 0.47               | bc | 0.54 $\pm$ 0.04      |   | 0.90 $\pm$ 0.02       |   |
| 31 °C       | <i>T. japonicus</i>  | 165.30 $\pm$ 11.34    | a  | 11.36 $\pm$ 0.86              | a  | 0.62 $\pm$ 0.05      | b | 0.91 $\pm$ 0.02       | a |
|             | <i>T. mitsukurii</i> | 73.50 $\pm$ 5.70      | bc | 5.10 $\pm$ 0.43               | c  | 0.72 $\pm$ 0.03      |   | 0.85 $\pm$ 0.02       |   |

probability compared to *T. mitsukurii* at all temperatures tested (Table 2; Fig. 1). Males of *T. japonicus* had a higher survival probability than *T. mitsukurii* at 21, 26 and 31 °C, while the survival probability at 16 °C did not differ between the two species (Table 2; Fig. S1).

In total, 1748 egg masses were observed to assess parasitoid fecundity. Both *T. japonicus* and *T. mitsukurii* started laying eggs from the first day of their lifespan, and the number eggs laid tended to be highest during the first 1–5 days of the oviposition period (Fig. 2). For all temperatures tested, the daily fecundity of both parasitoid species decreased with increasing age of the females (Fig. 2). The mean number of eggs produced by a female during the total lifespan was different among temperatures and between the two species ( $F_{3,71} = 12.87$ ,  $P < 0.0001$ ; Table S1). The total number of eggs laid throughout the lifespan of *T. japonicus* increased with the increase in temperature, being higher at 31 °C with respect to 26, 21 and 16 °C (Table 3). Instead, *T. mitsukurii* laid more eggs at 26 °C with respect to 31, 21 and 16 °C (Table 3). At 31 and 26 °C, *T. japonicus* laid more eggs in the total lifespan compared to *T. mitsukurii*, while no significant differences between the two species were observed at 21 and 16 °C (Table 3). The mean number of eggs laid

per day by *T. japonicus* was higher compared to those of *T. mitsukurii* at 31 °C, while no significant differences between the two species were observed at 26, 21 and 16 °C ( $F_{3,71} = 16.28$ ,  $P < 0.0001$ ; Tables 3 and S1). The average offspring sex ratio was 68:32 (female:male) for *T. japonicus* and 72:28 (female:male) for *T. mitsukurii*. The sex ratio differed at different temperatures ( $F_{3,70} = 11.22$ ,  $P < 0.0001$ ): at 16 and 21 °C, female emergence was higher than that at 26 and 31 °C (Table 3). No differences were found between the two parasitoid species nor the interaction between species and temperature (Table S1). The mean progeny survival was lower at 16 °C than at 21, 26 and 31 °C ( $F_{3,71} = 12.89$ ,  $P < 0.0001$ ; Table 3), and no differences emerged between the two *Trissolcus* species nor the interaction between parasitoid species and temperature (Table S1).

Life table parameters are shown in Table 4. The net reproductive rate ( $R_0$ ) of *T. japonicus* increased from 16 to 26 °C and slightly decreased at 31 °C, while that of *T. mitsukurii* increased from 16 to 21 °C and then decreased with the increase in temperature. The intrinsic ( $r_m$ ) and finite rate ( $\lambda$ ) of increase of both species rose with the increase in temperature, instead the mean generation time ( $T$ ) and doubling time ( $DT$ ) decreased

**Table 4**

Life table parameters (mean ± S.E.) of *T. japonicus* and *T. mitsukurii* under four constant temperatures. Different letters indicate significant differences at two-tailed *t* test ( $\alpha = 0.05$ ). Capital letters refer to significant differences between the two species, while lowercase letters refer to significant differences among temperatures considering the same species.

| Species                     | Parameter                     | 16 °C                         | 21 °C           | 26 °C            | 31 °C             |                 |
|-----------------------------|-------------------------------|-------------------------------|-----------------|------------------|-------------------|-----------------|
| <i>Trissolcus japonicus</i> | Ro(female/female)             | 65.38 ± 7.24c                 | 95.96 ± 9.74 bA | 121.24 ± 6.25 aA | 116.99 ± 7.34 abA |                 |
|                             | $r_m$ (female/female/day)     | 0.15 ± 0.03c                  | 0.35 ± 0.03 cB  | 0.85 ± 0.04 bA   | 1.49 ± 0.08 aA    |                 |
|                             | $\lambda$ (female/female/day) | 1.07 ± 0.09 d                 | 1.41 ± 0.04 cB  | 2.35 ± 0.09 bA   | 4.40 ± 0.35 aA    |                 |
|                             | T(days)                       | 17.14 ± 1.27 a                | 13.12 ± 1.14 bA | 5.60 ± 0.25 cA   | 3.20 ± 0.20 dA    |                 |
|                             | DT(days)                      | 2.68 ± 0.24 a                 | 1.99 ± 0.15 bA  | 0.81 ± 0.04 cA   | 0.47 ± 0.02 dA    |                 |
|                             | <i>Trissolcus mitsukurii</i>  | Ro(female/female)             | 58.65 ± 6.21c   | 86.00 ± 6.56 aA  | 72.77 ± 7.27 abB  | 67.32 ± 4.61 bB |
|                             |                               | $r_m$ (female/female/day)     | 0.18 ± 0.01 d   | 0.45 ± 0.03 cA   | 0.83 ± 0.05 bA    | 1.51 ± 0.07 aA  |
|                             |                               | $\lambda$ (female/female/day) | 1.06 ± 0.07 d   | 1.56 ± 0.05 cA   | 2.28 ± 0.11 bA    | 4.49 ± 0.35 aA  |
|                             |                               | T(days)                       | 16.22 ± 1.12 a  | 9.84 ± 0.61 bB   | 5.13 ± 0.30 cA    | 2.79 ± 0.13 dA  |
|                             |                               | DT(days)                      | 2.34 ± 0.33 a   | 1.53 ± 0.08 bB   | 0.84 ± 0.05 cA    | 0.46 ± 0.02 dA  |

**Table 5**

Mean (±S.E.) number of days required for the development of females and males of *T. japonicus* and *T. mitsukurii* under four constant temperatures. Different letters indicate significant differences at  $\chi^2$  test on least square means ( $\alpha = 0.05$ ).

| Sex    | Temperature          | Species              | Developmental time (days) |   |
|--------|----------------------|----------------------|---------------------------|---|
| Female | 16 °C                | <i>T. japonicus</i>  | 33.10 ± 0.05              | b |
|        |                      | <i>T. mitsukurii</i> | 33.90 ± 0.10              | a |
|        | 21 °C                | <i>T. japonicus</i>  | 23.25 ± 0.10              | d |
|        |                      | <i>T. mitsukurii</i> | 23.90 ± 0.12              | c |
|        | 26 °C                | <i>T. japonicus</i>  | 11.90 ± 0.14              | f |
|        |                      | <i>T. mitsukurii</i> | 12.40 ± 0.11              | e |
| 31 °C  | <i>T. japonicus</i>  | 7.78 ± 0.10          | g                         |   |
|        | <i>T. mitsukurii</i> | 8.10 ± 0.12          | g                         |   |
| Male   | 16 °C                | <i>T. japonicus</i>  | 31.10 ± 0.10              | a |
|        |                      | <i>T. mitsukurii</i> | 31.35 ± 0.15              | a |
|        | 21 °C                | <i>T. japonicus</i>  | 22.00 ± 0                 | b |
|        |                      | <i>T. mitsukurii</i> | 22.25 ± 0.10              | b |
|        | 26 °C                | <i>T. japonicus</i>  | 11.01 ± 0.05              | c |
|        |                      | <i>T. mitsukurii</i> | 11.25 ± 0.12              | c |
| 31 °C  | <i>T. japonicus</i>  | 7.36 ± 0.10          | d                         |   |
|        | <i>T. mitsukurii</i> | 7.25 ± 0.10          | d                         |   |

with the increase in temperature. The net reproductive rate (*Ro*) at 26 and 31 °C was higher for *T. japonicus* with respect to *T. mitsukurii*, while no differences between species were found at the other temperature. At 21 °C, the intrinsic ( $r_m$ ) and the finite rate of increase ( $\lambda$ ) were significantly higher for *T. mitsukurii* respect to that of *T. japonicus*, and these parameters resulted similar between species at the other temperatures. Furthermore, the mean generation time (*T*) and doubling time (*DT*) at 21 °C were significantly higher for *T. japonicus* compared to *T. mitsukurii*. No significant differences emerged between the two species at 16 °C.

The time for development from egg to adult emergence was different between temperatures, species and sex of the progeny ( $\chi^2 = 83.64$ , *df* = 6, *P* < 0.0001; Table S2). The developmental time of both species decreased with increasing temperature (Table 5). Generally, the males of both species emerged one to two days before females independent of temperature (Table 5). Males' developmental time did not differ between *T. japonicus* and *T. mitsukurii*, while females' development time varied between species, with females of *T. mitsukurii* emerging later than

*T. japonicus* at 16, 21 and 26 °C (no differences were observed at 31 °C; Table 5).

Linear regression models showed the lower temperature threshold for development as  $T_0 = 12.6$  °C for *T. japonicus* ( $y = 0.0070809x - 0.0890064$ ;  $R^2 = 0.71$ ) and  $T_0 = 11.9$  °C for *T. mitsukurii* ( $y = 0.0061545x - 0.0731682$ ;  $R^2 = 0.56$ ; Fig. 3). The Lactin-2 model estimated the upper temperature threshold for development as  $T_m = 35.2$  °C ( $R^2 = 0.71$ ) for *T. japonicus* and  $T_m = 33.5$  °C ( $R^2 = 0.74$ ) for *T. mitsukurii* (Fig. 3).

#### 4. Discussion

The developmental biology of *T. japonicus* and *T. mitsukurii* emerging from *H. halys* egg masses suggest different efficacy as biological control agents when facing different temperatures. Understanding the optimal temperature for parasitoid development and reproduction is crucial in studying host-parasitoid interactions, as it enables the implementation of effective biological control programmes in different geographic and climatic areas and also in a climate change scenarios (Bellows et al., 1992; Carey, 2001; Furlong and Zalucki, 2017; Jeffs and Lewis, 2013; Klapwijk et al., 2010; Tortorici et al., 2023; Van Driesche and Bellows, 1996). Results show a higher survivorship of males and females of *T. japonicus* at all the tested temperatures. Moreover, females of *T. japonicus* lived longer as compared to other *Trissolcus* species such as *T. semistriatus* (Nees von Esenbeck), *T. brochymenae* (Ashmead), *T. basalis* (Wollaston), *T. teretis* (Johnson), *T. urichi* (Crawford) and *T. simoni* (Mayr) (Kivan and Kilic, 2006, 2005; Laumann et al., 2008). However, it should be pointed out that different host egg dimensions influence parasitoid body size and thus their longevity (Arakawa et al., 2004; Bezemer et al., 2005; Jervis et al., 2007).

The sex ratio was female biased for both parasitoid species as already reported in studies on *T. japonicus*, *T. mitsukurii*, and other *Trissolcus* species (James and Warren, 1991; Kivan and Kilic, 2006, 2005; Laumann et al., 2008; Sabbatini Peverieri et al., 2020). For both parasitoid species, more males emerged at 26 and 31 °C than at 16 and 21 °C, indicating that a high temperature can influence sex allocation in parasitoid wasps (Kivan and Kilic, 2006; Moiroux et al., 2014). In our experiment, the sex ratio of both species was lower with respect to natural sex ratio observed by many authors (Avila et al., 2021; Kamiyama et al., 2021; Mele et al., 2022; Zhang et al., 2017) but also with respect to other laboratory experiments (Sabbatini Peverieri et al., 2020), possibly due to repeated oviposition that gradually led to a reduction in the female:male sex ratio (Jervis et al., 2007).

As already observed for other *Trissolcus* species, the progeny survival of both *T. japonicus* and *T. mitsukurii* is reduced with temperatures lower than 21 °C (James and Warren, 1991; Kivan and Kilic, 2005, 2006). *Trissolcus japonicus* females demonstrated a higher fecundity with respect to *T. mitsukurii* at higher temperature. The number of eggs laid by *T. japonicus* increased with increasing temperature, while *T. mitsukurii* showed a different response to the temperature. The fecundity of *T. mitsukurii* increased from 16 to 26 °C but decreases at 31 °C, possibly because an increase in metabolic cost due to temperature could limit the fecundity of this species (Ellers et al., 2001; Mills, 1981). The optimum temperature range for oogenesis and oviposition generally varies between species and beyond this range insects cannot sustain both for an extended period (Jervis et al., 2007). The fecundity increases gradually with increasing temperature until it reaches a maximum level, above which the egg production falls down steeply due to an increase in metabolic costs (Bursell, 1974). According to the results obtained here, *T. mitsukurii* reached maximum fecundity between 26 and 31 °C, while the maximum for *T. japonicus* seemed to be greater than or equal to 31 °C, suggesting better performances of the latter at higher temperatures.

Considering the results on the net reproductive rate, the growth rate of *T. japonicus* population is expected to be higher compared to *T. mitsukurii* during the summer period when average temperature

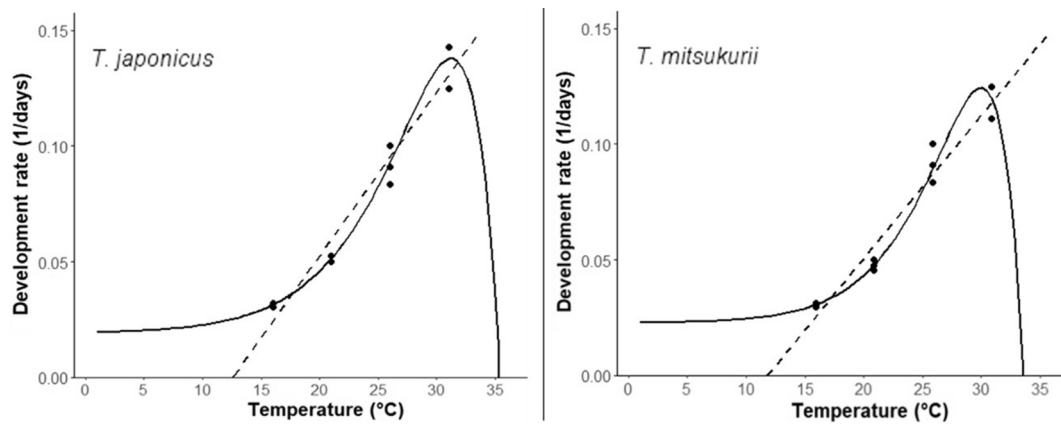


Fig. 3. Relationship between development rate (1/days) of *T. japonicus* and *T. mitsukurii* (egg to adult emergence) over temperature, described by the linear model and the Lactin-2 model.

ranges between 26 and 31 °C, while the differences for the population growth rate of the two species are expected to be low at lower temperature. Even though *T. japonicus* laid more eggs at 21 °C with respect to *T. mitsukurii*, the intrinsic rate of increase and the finite rate of increase of *T. mitsukurii* were higher than those of *T. japonicus*. This is probably due to a higher sex ratio for *T. mitsukurii* at 21 °C with respect to *T. japonicus* that counterbalanced the advantage given by the high fecundity. Also, the mean generation time and doubling time of *T. japonicus* were significantly higher than those of *T. mitsukurii*. These results suggest that when the average temperature is 21 °C, *T. mitsukurii* populations can grow faster than *T. japonicus* ones. However, the higher fecundity expressed at 26 and 31 °C by *T. japonicus* revealed a potentially higher capacity of parasitism at higher temperatures.

Even though the intrinsic rate of increase of a parasitoid population is considered a realistic predictor of the parasitoid effectiveness (Birch, 1948), this should be coupled with the functional response based on searching efficiency, attack rate, and also phenological synchrony with the host (Hassell, 2000). All these aspects should be taken into consideration when comparing the performances of the two species (Hochberg and Holt, 1999; Kidd and Jervis, 2007). Moreover, the interspecific competition between *T. japonicus* and *T. mitsukurii* in parasitizing *H. halys* could vary according to the environmental conditions, since temperature changes could affect the outcome of the competition (Costi et al., 2022; Giovannini et al., 2022; Hance et al., 2007; Hassell, 2000). The higher intrinsic rate of increase of *T. mitsukurii* at lower temperature may indicate a faster population increase early in the season. A differential adaptation to temperature condition is also highlighted by the developmental thresholds. The minimum threshold for development of *Trissolcus mitsukurii* is about 1 °C lower than *T. japonicus*. On the contrary the maximum threshold for development is about 2 °C higher for *T. japonicus* than *T. mitsukurii*. All these aspects should be taken into account for the development of biological control programmes in invaded areas. In Italy, *H. halys* is known to develop two generations annually and starts to oviposit when mean temperatures are between 20 °C and 23 °C (Costi et al., 2017). Before the oviposition period of *H. halys*, *T. mitsukurii* likely parasitizes pentatomid species other than *H. halys* (e.g., *Nezara vididula*, *Eurydema* spp., *Dolycoris baccarum*, *Raphigaster nebulosa*, *Palomena prasina*) (Angeli et al., 2021), since it has a broader host range compared to *T. japonicus* (Giovannini et al., 2021b; Sabbatini-Peverieri et al., 2021). All in all, a *T. mitsukurii* population may have a faster increase with respect to *T. japonicus* at the beginning of the oviposition period of the *H. halys* overwintered generation, but *T. japonicus* population is expected to grow at higher levels than *T. mitsukurii* during the summer period, when *H. halys* lays more eggs (Costi et al., 2017).

Environmental factors play a key role in the distribution of the two parasitoid species in invaded areas. According to our results,

*T. mitsukurii* has higher performances at a lower temperature range compared to *T. japonicus* confirming the model predictions by Tortorici et al. (2023) that mapped a trend of *T. mitsukurii* to prefer higher latitudes with cooler climate compared to *T. japonicus*. Therefore, the coexistence of the two species in invaded areas depends on climate factors but also on searching efficiency because the first *Trissolcus* that reaches and exploits the egg mass is the one with the highest reproductive success (Costi et al., 2022; Giovannini et al., 2022). Ideally, the coexistence could provide better control since the abundance of one or the other species could vary along with the growing season according to the climate. However, when parasitoids attack the same host stage, replacement is more probable and mainly driven by parasitoids population abundance and their searching efficiency (Hassell, 2000; Pedersen and Mills, 2004).

The impact of the increase in average temperatures due to climate warming is expected to be stronger on higher trophic levels, since the upper thermal limit of *H. halys* is 36.5 °C, which is actually higher than those of *T. japonicus* (35.2 °C) and *T. mitsukurii* (33.5 °C) (Agosta et al., 2018; Hance et al., 2007; Karl and Trenberth, 2003; Monticelli et al., 2021). The impact of increasing temperature is expected to be higher on *T. mitsukurii* than the other species. Moreover, divergence between thermal preferences of the host and those of parasitoids can lead to a temporal or geographical disruption of the synchronization, increasing the risk of pest outbreaks (Hance et al., 2007; Monticelli et al., 2021; Tougeron and Tena, 2019). For this reason, the analysis of thermal preferences of biological control agents and how host-parasitoid systems react to changes in temperature can support the selection of biological control agents.

## 5. Conclusions

Biological control of *H. halys* with egg parasitoids is currently considered the most promising long-term solution against this pest. Exotic egg parasitoids are currently the most effective natural enemies for biological control of *H. halys* in the invaded areas. The two parasitoids considered here coexist in both native and introduced areas, but some differences in habitat suitability emerged using a modelling approach (Tortorici et al., 2023). The present study shed light on these differences by investigating life table parameters of *T. japonicus* and *T. mitsukurii* under different temperatures. *Trissolcus japonicus* demonstrated higher performances as biological control agent with respect to *T. mitsukurii*. However, *T. mitsukurii* resulted as being better adapted to low temperature regimes. The results obtained here can be useful to predict the outcome of biological control programmes in locations with different thermal traits.

## Funding

This work was supported by Regione Veneto – U.O. Fitosanitario – Direzione Agroambiente, Programmazione e Gestione ittica e faunistico-venatoria. The activities were performed in the framework of the program authorized and funded by Veneto region (DGR n. 611 19 May 2020) and the Italian biocontrol program against *H. halys* (decree of the Italian Ministry of the Environment, Land and Sea n.0042967 of 09/06/2020). This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN0000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

## CRediT authorship contribution statement

**Alberto Mele:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Dinvesh Sai Avaniagadda:** Data curation, Investigation. **Enrico Ceccato:** Investigation, Data curation. **Gabriel Bamidele Olawuyi:** Data curation, Investigation. **Filippo Simoni:** Data curation, Investigation, Methodology. **Carlo Duso:** Conceptualization.  **Davide Scaccini:** Writing – review & editing, Methodology, Conceptualization. **Alberto Pozzebon:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

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

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2024.105548>.

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