Development and thermal activity thresholds of European mirid predatory bugs

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ABSTRACT

Generalist predators belonging to Dicyphini (Hemiptera: Miridae) play an important role in pest control in vegetable crops. Temperature is one the most important factors affecting their efficacy as biological control agents (BCAs) and a better understanding of temperature effects can help to select the best performing species for certain climatic conditions. In this study we assessed the thermal requirements of six dicyphine species: Dicyphus bolivari (2 different strains), Dicyphus eckerleini, Dicyphus errans, Dicyphus flavoviridis, Nesidiocoris tenuis and Macrolophus pygmaeus. Two experimental methods were used: one static, by recording the developmental times at six temperatures (15–40 °C) and one dynamic, by determining low and high temperature thresholds for movement. Based on the results of both methods we identified two groups: D. tenuis, M. pygmaeus and D. bolivari showed the best performance at high temperatures and the species D. errans, D. eckerleini and D. flavoviridis were most active at low temperatures. Dicyphus bolivari and N. tenuis were the only species able to reach adulthood at the constant temperature of 35 °C. At low temperatures, D. eckerleini and D. errans were the only species still able to walk below 0 °C.

The species less vulnerable for lower temperatures were more vulnerable for higher temperatures and vice-versa. Among the tested species, the larger sized species seem to be better adapted to lower temperature and the smaller sized species better to higher temperatures. Females and males in all species differed in their cold and heat tolerance. Males were in general better adapted to higher temperatures and females better adapted to lower temperatures.
1. Introduction

In the Mediterranean area, several species of generalist predators belonging to the tribe Dicyphini (Hemiptera: Miridae) play a significant role in reducing pest populations in outdoor and protected vegetable crops (Ingegno et al., 2009; Perdikis et al., 2011; Messelinik et al., 2015; van Lenteren, 2012; Bouagga et al., 2018; Leman et al., 2019). They can prey on a wide range of crop pests such as aphids, spider mites, thrips, whiteflies, lepidopterans (Albajes and Alomar, 1999; Perdikis and Lykouressis, 2000; Voigt et al., 2006; Ingegno et al., 2008, 2009, 2017a). Moreover, Dicyphini are currently among the most effective predators against the invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Urbaneja et al., 2009, 2012; Ingegno et al., 2013, 2019; Zappalà et al., 2013; Ferracini et al., 2019). Due to their effectiveness, some of these mirid bugs are produced and commercialized worldwide for pest control, such as *Macrolophus pygmaeus* (Rambur) and *Nesidocoris tenuis* (Reuter) (van Lenteren, 2012).

Dicyphine species are true omnivores, and prefer hairy plants because of morphological and behavioural adjustments (Voigt et al., 2007; Ingegno et al., 2008, 2011, 2016). The presence of insect carrion entrapped by glandular trichomes might also attract them to some of these plants, as observed for other predatory bugs (Krimmel and Pearse, 2013). During spring, they move from wild host plants used as winter refuges to agricultural crops (Garbarra et al., 2004; Balzan and Moonen, 2014; Balzan, 2017). Their crop colonizing ability and their predatory efficacy are affected by several factors, such as the presence and abundance of host plants surrounding the crop, the suitability of the prey and host plant as food source, and the climatic conditions.

In Europe, many endemic dicyphine species can be considered as potential biological control agents (BCAs) (Messelinik et al., 2015; Ingegno et al., 2017a, 2019; Biondi et al., 2018). The species richness of this tribe may offer opportunities to select the most suitable candidates for biological pest control in specific crops or at certain climatic conditions. Some greenhouse crops for example in northern Europe are grown during winter at low temperatures, which can reduce the activity or survival of some species of natural enemies (Hatherly et al., 2005, 2008; Hughes et al., 2009). The same situation occurs in the Mediterranean area where extreme hot temperatures during summer can hamper the performance of natural enemies (Thomson et al., 2010; Aguilar-Fenollosa and Jacas, 2014). Moreover, the rate and the duration of insect development strongly depend upon the temperature to which the insects are exposed (Perdikis and Lykouressis, 2000, 2002; Saleh and Sengonca, 2001, 2003; Sanchez et al., 2009). The temperature below which no measurable development occurs is the threshold of temperatures in which insects can survive there is a more restricted applicable climate chambers (70% RH, 16:8 h L:D). A newly emerged adult was sexed, weighed with an analytical balance (Mettler Toledo AE100) and length was measured by chill coma recovery (Ayrinhac et al., 2004; Macdonald et al., 2004; Castañeda et al., 2005) and motility across a range of temperatures (Allen, 2010; Hughes et al., 2010). A method for rapid non-lethal dynamic measurement of thermal tolerance in small insects has been developed by using a temperature-controlled block in which multiple individuals can be observed without direct handling or disturbance with camera recording for retrospective analysis (Hazell et al., 2008). The thermal thresholds for activity observed with this technique can be a good indication for heat or cold tolerance.

In this study, we determined the developmental time at different temperatures (static method) and the thermal thresholds for motility (dynamic method) of the following six species: *Dicyphus bolivari* Lindberg (formerly described as *Dicyphus marocanus* Wagner (Sanchez and Cassis, 2018)), *Dicyphus eckerlei* Wagner, *Dicyphus errans* (Wolff), *Dicyphus flavoviridis* Tamamini, *M. pygmaeus* and *N. tenuis*. These species showed potential as BCAs for pest control in vegetable crops and some are already commercialized for augmentative biological control. Therefore, information on their thermal requirements will be useful to select species in relation to climatic conditions.

2. Methodology

2.1. Insect collection and rearing

Colonies of *D. eckerlei* were started from individuals collected in Hungary on *Geranium* spp. This species was reared on small tobacco plants *Nicotiana tabacum* L. in transparent plastic cylinder cages (H 27 cm, Ø 25 cm; JET 107 PM, Jokey plastic GmbH, Sohland, Germany) with the drilled lid covered with a fine net mesh. Colonies of *D. errans* and *D. flavoviridis* originated from individuals collected in the Alpine valleys in Piedmont (NW Italy) on various herbaceous plants. These two latter species were reared on *Solanium nigrum* L. and tobacco plants, respectively, in net cages (60 × 60 × 90 cm; Kweekkooi, Vermandel, The Netherlands). Two *D. bolivari* strains were collected in two Spanish regions, namely in Valencia area (V) on tomato and in Barcelona area (B) on *Solanium luteum* Mill. *Macrolophus pygmaeus* and *N. tenuis* were provided by Koppert B.V. (Berkel en Rodenrijs, The Netherlands) from populations originally coming from South France and Spain, respectively. Colonies of *D. bolivari*, *M. pygmaeus* and *N. tenuis* were reared on pods of the flat bean *Phaseolus vulgaris* L. in transparent plastic cylinder cages (H 27 cm, Ø 25 cm; JET 107 PM, Jokey plastic GmbH, Sohland, Germany) with the drilled lid covered with a fine net mesh. All dicyphine rearrings were supplied with eggs of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) mixed with dehydrated and decapsules cysts of *Artemia* sp. (Anostraca: Artemiidae) (Entofood, Koppert B.V. Berkel en Rodenrijs, The Netherlands) and kept at 25 °C, 70% RH, 16:8 h L:D.

2.2. Development at different temperatures (static method)

The developmental times at six constant temperatures (15, 20, 25, 30, 35, 40 °C) were assessed for all the tested species (*D. eckerlei*, *D. errans*, *D. flavoviridis*, two *D. bolivari* strains, *M. pygmaeus* and *N. tenuis*) in respective climate chambers (70% RH, 16:8 h L:D). A newly emerged nymph (< 1 day old) was placed in a single plastic medicine cup (H 40 mm, Ømin 25 mm, Ømax 30 mm), closed with a drilled cap covered with a fine mesh. In each container, a gerbera leaf disk (Ø 25 mm) was provided with E. kuehniella eggs on a 15 mm high Gypsum plaster layer wetted with water to maintain humidity. Nymphs were checked every day removing the exuviae. Leaf disks, provided with food, were replaced every 2 days. Emerged adults were sexed, weighed with an analytical balance (Mettler Toledo AE100) and length was measured using a biocular microscope. Fifteen replicates were performed for each species/strain and temperature combination.

2.3. Tolerance at different temperatures (dynamic method)

For recording the thermal thresholds for activity for both low and...
Table 1

Duration (mean ± SE) of the nymphal instars (I; II; III; IV; V) of six mirid predatory species (Dicyphus bolivari – B: Barcelona strain, V: Valencia strain, D. eckerleini, D. errans, D. flavoviridis, Macrolophus pygmaeus, Nesidiocoris tenuis), at six temperatures (15, 20, 25, 30, 35, 40 °C). For each instar and temperature (15, 20, 25 °C) means in column followed by different letters are significantly different (Bonferroni, P < 0.05).

| Species / T°C | 15 °C | 20 °C | 25 °C | 30 °C | 35 °C | 40 °C |
|---------------|-------|-------|-------|-------|-------|-------|
| Dicyphus bolivari B | 12.46 ± 0.53b | 6.00 ± 0.52ab | 4.79 ± 0.52 | 3.00 ± 0.47a | 3.80 ± 0.42 | 8.08 ± 0.54a |
| Dicyphus bolivari V | 8.14 ± 0.52a | 6.36 ± 0.52ab | 4.36 ± 0.52a | 4.06 ± 0.48a | 3.47 ± 0.46 | 2.00 ± 0.00 |
| Dicyphus eckerleini | 8.80 ± 0.50a | 4.00 ± 0.50a | 3.79 ± 0.52a | 2.73 ± 0.50a | 3.67 ± 0.41 | – |
| Dicyphus errans | 9.43 ± 0.52a | 5.00 ± 0.52ab | 3.59 ± 0.47a | 2.60 ± 0.50a | 3.18 ± 0.76 | – |
| Dicyphus flavoviridis | 14.62 ± 0.53b | 5.60 ± 0.50ab | 4.30 ± 0.52a | 6.60 ± 0.50b | 5.50 ± 1.50 | – |
| Macrolophus pygmaeus | 8.09 ± 0.58a | 5.79 ± 0.52ab | 2.88 ± 0.48a | 3.47 ± 0.50a | 2.40 ± 0.21 | 2.00 ± 0.00 |
| Nesidiocoris tenuis | 18.50 ± 0.52c | 6.40 ± 0.50b | 4.15 ± 0.53a | 3.13 ± 0.48a | 3.44 ± 0.20 | 2.00 ± 0.00 |

| Species / T°C | 15 °C | 20 °C | 25 °C | 30 °C | 35 °C | 40 °C |
|---------------|-------|-------|-------|-------|-------|-------|
| Dicyphus bolivari B | 9.42 ± 0.53b | 5.08 ± 0.53bc | 3.00 ± 0.53a | 3.08 ± 0.51ab | 2.00 ± 0.00 | – |
| Dicyphus bolivari V | 8.58 ± 0.53b | 5.00 ± 0.58bc | 2.93 ± 0.49a | 2.00 ± 0.46a | – | – |
| Dicyphus eckerleini | 6.36 ± 0.49a | 4.08 ± 0.51abc | 2.92 ± 0.51a | 2.36 ± 0.49a | 4.00 ± 1.53 | – |
| Dicyphus errans | 6.00 ± 0.53a | 3.55 ± 0.55abc | 4.24 ± 0.45a | 1.92 ± 0.51a | – | – |
| Dicyphus flavoviridis | 8.73 ± 0.55b | 5.57 ± 0.49c | 4.25 ± 0.51 | 4.25 ± 0.49b | – | – |
| Macrolophus pygmaeus | 4.89 ± 0.61a | 3.25 ± 0.53ab | 3.07 ± 0.53a | 2.08 ± 0.51a | – | – |
| Nesidiocoris tenuis | 10.46 ± 0.52b | 2.27 ± 0.47a | 2.31 ± 0.63a | 1.50 ± 0.46a | 1.61 ± 0.20 | – |

| Species / T°C | 15 °C | 20 °C | 25 °C | 30 °C | 35 °C | 40 °C |
|---------------|-------|-------|-------|-------|-------|-------|
| Dicyphus bolivari B | 12.67 ± 0.82cd | 8.64 ± 0.85bc | 5.13 ± 0.82b | 7.00 ± 2.83a | 3.00 | – |
| Dicyphus bolivari V | 13.58 ± 0.82 | 8.87 ± 0.85 | 5.00 ± 0.85bc | 4.21 ± 0.76a | 5.33 ± 0.63 | – |
| Dicyphus eckerleini | 8.73 ± 0.55 | 4.90 ± 0.54c | 4.34 ± 0.51 | 3.57 ± 0.49b | – | – |
| Dicyphus errans | 6.60 ± 0.52 | 2.00 ± 0.00 | 1.50 ± 0.00 | 1.50 ± 0.00 | 1.50 ± 0.00 | – |
| Dicyphus flavoviridis | 16.80 ± 0.89 | 14.67 ± 0.94 | 10.10 ± 0.76 | 6.38 ± 0.54 | 6.38 ± 0.54 | – |
| Macrolophus pygmaeus | 12.00 ± 0.94 | 14.67 ± 0.94 | 14.67 ± 0.94 | 14.67 ± 0.94 | 14.67 ± 0.94 | – |
| Nesidiocoris tenuis | 6.25 ± 0.99 | 4.30 ± 0.85 | 3.12 ± 0.78 | 2.50 ± 0.67 | 2.50 ± 0.67 | – |
1.0 °C min\(^{-1}\), and it was further lowered from 10 °C to –8°C at a rate of 0.5 °C min\(^{-1}\). For each species/strain, 10 males and 10 females divided in groups of five individuals were tested.

Microscope. Temperatures at which a visible physiological response (such as paralysis, spasm, trembles, etc.) was evident were recorded as key temperatures (KTs). For each species/strain, 10 males and 10 females divided in groups of five individuals were tested.

The arena was recorded through the glass with a digital video-camera (F25-ME, Julabo, USA Inc.) by pumping heated or cooled fluid through channels bored into a thermostated aluminium block (130 × 60 × 20 mm). Insects were placed in a circular arena (Ø 25 mm, 75 mm high) in the middle of this block. The whole block was covered with a layer of double glass and placed in a block of extruded polystyrene foam for insulation (Styrodur ®).

To check the chill tolerance, the temperature was reduced from the laboratory temperature (20 °C) until 30 °C at an increasing rate of 0.25 °C min\(^{-1}\). We used the method developed by Hazell et al. (2008).

Temperature was controlled with a refrigerating/heating circulation (F25-ME, Julabo, USA Inc.) by pumping heated or cooled fluid across it to prevent condensation below 0 °C.

To check the heat tolerance, the temperature was increased from the laboratory temperature (20 °C) until 30 °C at an initial increasing rate of 0.25 °C min\(^{-1}\), and it was further increased from 30 °C to 48 °C at a rate of 0.5 °C min\(^{-1}\). Temperature was then decreased back to 20 °C at a rate of 1.0 °C min\(^{-1}\). The total experimental running time was about 2 h, and pictures were taken every minute, resulting in an total of 120 pictures for each run.

Analyses of the developmental time was only based on temperatures that allowed at least one individual per species/strain to reach adulthood. The survival rate in each developmental stage was referred to the initial number of individuals used in the trial, and differences in survivorship were analysed by a General Linear Model (GLM) with a binomial distribution and logit link function. Temperature was considered as a fixed factor in order to avoid a linear or defined relationship between temperature and insect development. To compare the duration of developmental time within developmental stages among species and temperatures, a two-way analysis of variance (ANOVA) was performed, since data were normally distributed. Length and weight of adults emerged in the developmental trials were compared by performing multivariate ANOVA with temperature, sex and species as fixed factors and considering all the interactions among them. Then, within each species body weight and length were analysed by performing a one-way ANOVA and differences among developmental temperatures were separated with Tukey’s tests (P < 0.05).

Differences in temperature tolerance, both for chill and heat, among species were investigated with Generalized Linear Mixed Models (GLMMs) with a normal distribution and an identity link function, and an unstructured covariance matrix. Species and sex were analysed as fixed effects, KT as a repeated measure. Sex × species, sex × KT, and species × KT interactions were also analysed. Models including species × KT interactions were also analysed. Models including

### Table 2

| Species               | T °C | 1st     | 2nd     | 3rd     | 4th     | 5th     |
|-----------------------|-----|---------|---------|---------|---------|---------|
| Dicyphus bolivari B   | 15  | 86.7 ± 8.77 | 86.7 ± 8.77 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 20  | 93.3 ± 6.44 | 86.7 ± 8.77 | 80.0 ± 10.32 | 80.0 ± 10.32 | 73.3 ± 11.41 |
|                       | 25  | 93.3 ± 6.44 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 76.5 ± 10.28 | 35.3 ± 11.59 | 5.9 ± 5.7 * |
| Dicyphus bolivari V   | 15  | 93.3 ± 6.44 | 86.7 ± 8.77 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 20  | 93.3 ± 6.44 | 73.3 ± 11.41 | 66.7 ± 12.17 | 60.0 ± 12.64 | 53.3 ± 12.88 |
|                       | 25  | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 81.3 ± 9.75 | 18.8 ± 9.75 |
| Dicyphus eckerleini   | 15  | 100.0 ± 0   | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 |
|                       | 20  | 100.0 ± 0   | 93.3 ± 6.44 | 86.7 ± 8.77 | 86.7 ± 8.77 | 80.0 ± 10.32 |
|                       | 25  | 93.3 ± 6.44 | 93.3 ± 6.44 | 86.7 ± 8.77 | 86.7 ± 8.77 | 80.0 ± 10.32 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 81.3 ± 9.75 | 18.8 ± 9.75 |
| Dicyphus errans       | 15  | 93.3 ± 6.44 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 20  | 93.3 ± 6.44 | 73.3 ± 11.41 | 73.3 ± 11.41 | 73.3 ± 11.41 | 66.7 ± 12.17 |
|                       | 25  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 88.2 ± 7.81 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 88.2 ± 7.81 | 88.2 ± 7.81 |
| Dicyphus flavoviridis | 15  | 86.7 ± 8.77 | 73.3 ± 11.41 | 73.3 ± 11.41 | 66.7 ± 12.17 | 66.7 ± 12.17 |
|                       | 20  | 100.0 ± 0   | 100.0 ± 0   | 93.3 ± 6.44 | 93.3 ± 6.44 | 93.3 ± 6.44 |
|                       | 25  | 93.3 ± 6.44 | 93.3 ± 6.44 | 86.7 ± 8.77 | 86.7 ± 8.77 | 86.7 ± 8.77 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   |
| Macrolophus pygmaeus  | 15  | 73.3 ± 11.41 | 66.7 ± 12.17 | 60.0 ± 12.64 | 60.0 ± 12.64 | 60.0 ± 12.64 |
|                       | 20  | 93.3 ± 6.44 | 80.0 ± 10.32 | 80.0 ± 10.32 | 73.3 ± 11.41 | 73.3 ± 11.41 |
|                       | 25  | 100.0 ± 0   | 100.0 ± 0   | 81.3 ± 9.75 | 75.0 ± 10.82 | 68.7 ± 11.58 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 86.7 ± 8.77 | 80.0 ± 10.32 |
| Nesidiocoris tenuis   | 15  | 93.3 ± 6.44 | 93.3 ± 6.44 | 86.7 ± 8.77 | 86.7 ± 8.77 | 86.7 ± 8.77 |
|                       | 20  | 80.0 ± 10.32 | 86.7 ± 8.77 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 25  | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 | 80.0 ± 10.32 |
|                       | 30  | 100.0 ± 0   | 100.0 ± 0   | 100.0 ± 0   | 87.5 ± 8.26 | 87.5 ± 8.26 |
different predictors were compared by sequentially subtracting the predictors from the full model and, subsequently, by calculating the Akaike Information Criterion (AIC) (Akaike, 1978) and selecting the best model. Differences among treatments were considered at a 5% level of confidence, using pairwise Bonferroni protected t-tests. All statistical analyses were performed with the statistical package SPSS 24 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Development at different temperatures (static method)

None of the tested species/strain was able to develop until adulthood at 40 °C (Table 1). At 35 °C, only strain B of D. bolivari and N. tenuis reached adulthood. Dicyphus eckerleini reached the 4th juvenile instar, but all other species could stopped the development at the 2nd or 3rd instar (Table 1).

Among temperatures that allowed at least one individual per species to reach adulthood, the survival rate did not differ in the first four instars, but it was different in the 5th instar (df = 3; χ² = 12.615; P = 0.006) as well as the interaction between species × temperature (df = 18; χ² = 34.181; P = 0.012). In particular, at 30 °C, both D. bolivari strains showed a higher mortality in the last juvenile instar compared to the other species and temperatures (Table 2).

At 15, 20, 25 and 30 °C, significant differences in the duration of each nymphal instar were observed among species, temperatures, and in the interaction between species × temperature, except in the 2nd juvenile instar (Table 1). No significant differences in the total developmental duration, from 1st instar to adult, were found between sexes (df = 1; F = 1.054; P = 0.306), in the interactions species × sex (df = 6; F = 1.916; P = 0.079) and sex × temperature (df = 3; F = 1.216; P = 0.304). On the contrary, there were significant differences among species (df = 6; F = 118.402; P < 0.001), temperatures (df = 3; F = 2167.165; P < 0.001), and in the interaction between species × temperature (df = 18; F = 10.096; P < 0.001). The longest developmental time was observed at 15 °C, for D. flavoviridis, while the shortest development time was observed at 30 °C for N. tenuis. (Fig. 1).

Significant differences in adult length were found among species (df = 6; F = 131.251; P < 0.001), sexes (df = 1; F = 4.059; P = 0.045), temperatures (df = 1; F = 4.276; P = 0.006), and in the interactions between species × sex (df = 6; F = 5.527; P < 0.001) and species × temperature (df = 18; F = 2.593; P = 0.001), while no differences were observed in the interactions between sex × temperature (df = 3; F = 0.617; P = 0.605) and species × sex × temperature (df = 16; F = 1.040; P = 0.415). Within each species, differences in body length across temperatures were recorded for D. bolivari B (df = 3; F = 3.196; P = 0.037), D. bolivari V (df = 3; F = 5.987; P = 0.002), D. eckerleini (df = 3; F = 3.436; P = 0.026), D. flavoviridis (df = 3; F = 2.972; P = 0.043), and N. tenuis (df = 3; F = 3.694; P = 0.019) (Fig. 2).

Significant differences in adult weight were recorded among species (df = 6; F = 154.312; P < 0.001), sexes (df = 1; F = 213.217; P < 0.001), temperatures (df = 3; F = 10.055; P < 0.001), and in the interactions between species × temperature (df = 3; F = 2.469; P = 0.063) and sex × species × temperature (df = 16; F = 0.874; P = 0.600). Within each species, differences in body weight among temperatures were observed for D. bolivari B (df = 3; F = 3.946; P = 0.017), D. bolivari V (df = 3; F = 4.055; P = 0.015), and D. flavoviridis (df = 3; F = 12.249; P < 0.001) (Fig. 3).

3.2. Tolerance at different temperatures (dynamic method)

Mirids responded to changes in temperature with an increase or decrease in walking speed. Then, when a critical temperature was reached, the insect lost locomotory function (critical thermal temperature). A further temperature increase or decrease made the insect completely immobile, i.e. the temperature at which there was the last subtle movements (coma temperature). By reversing the temperature trend from low temperatures, a recover of subtle motility (coma recovery) and of locomotory function (activity recovery) could occur.

3.2.1. Chill tolerance

From video recording, four KTIs were recorded to evaluate the cold tolerance for each individual: KT1-stop walking (critical thermal minimum, CTₘₜ₁), KT2-stop moving (chill coma level), KT3-start moving (chill coma recovery), KT4-start walking (activity recovery).

A GLMM with all fixed factors included gave the best maximum likelihood (AIC = 2,528.041), and significant differences among all the fixed effects (df = 38, 590; F = 162.808; P < 0.001).

Significant differences in low temperature tolerance were found between sexes (df = 1, 590; F = 14.571; P < 0.001), and in the interaction between sex × KT (df = 3, 590; F = 3.456; P = 0.016). In all the comparisons, critical temperatures were lower for females than males, except for KT1 (df = 590; t = 0.216; P = 0.829). In fact, females tolerated lower mean temperatures than males (i.e. KT2: females, -4.7 ± 0.1 °C; males, -4.1 ± 0.1 °C; df = 590; t = 4.693; P < 0.001) and recovered at lower mean temperatures from the chill coma (i.e. KT3: females, 1.3 ± 0.3 °C; males, 2.3 ± 0.3 °C; df = 1, 590; t = 2.563; P = 0.011). Moreover, females showed an earlier activity recovery (i.e. KT4: females, 7.7 ± 0.4 °C; males, 9.3 ± 0.3 °C; df = 1, 590; t = 3.250; P = 0.001). The interaction between sex × species was significantly different (df = 6, 590; F = 6.771; P < 0.001), in particular, for D. bolivari V (df = 1, 590; t = –3.556; P < 0.001), M. pygmaeus (df = 1, 590; t = –4.598; P < 0.001) and N. tenuis (df = 1, 590; t = –4.656; P < 0.001), in which females tolerated lower temperatures than males. In the interaction between species × KT, significant differences were found at all temperatures (df = 18, 590; F = 16.722; P < 0.001).

Dicyphus eckerleini and D. errans showed the lowest KT1, while M. pygmaeus, N. tenuis and D. bolivari V reached KT1 earlier (df = 6; 590; F = 49.941; P < 0.001). Indeed, M. pygmaeus was the first species that reached KT2, while D. eckerleini, D. errans and D. flavoviridis were the last ones (df = 6, 590; F = 63.225; P < 0.001). Moreover, M. pygmaeus was one of the last species that recovered from the chill coma together with both D. bolivari strains and N. tenuis (KT3: df = 6, 590; F = 4.061; P = 0.001). For the full activity recovery, N. tenuis followed by D. errans needed higher temperatures compared to the other tested species (KT4: df = 6, 590; F = 11.925; P < 0.001) (Table 3).

3.2.2. Heat tolerance

Fig. 3.) From video recording, two KT6s were recorded to evaluate the heat tolerance for each individual: KT5-stop walking (CTₘₜ₅), KT6-stop moving (hot coma level); no recovery was possible since all the individuals died. A GLMM with all fixed factors included gave the best maximum likelihood (AIC = 2,528.041), and significant differences among all the fixed effects (df = 22, 278; F = 20.050.774; P < 0.001).

Significant differences in high temperature tolerance were found between sexes (df = 1, 278; F = 9.484; P = 0.002), and in the interaction between sex × KT (df = 1, 278; F = 12.813; P < 0.001). KT6 was significantly higher for males than females, whereas there were no significant differences for KT5 (females: 43.6 ± 0.2 °C; males: 43.7 ± 0.2 °C; df = 278; t = 0.509; P = 0.611). Males tolerated higher temperatures than females with a mean CTₘₜ₆ of 47.3 ± 0.1 °C and 46.5 ± 0.1 °C, respectively (df = 278; T = 5.744; P < 0.001). The interaction between sex × species was significantly different (df = 6, 278; F = 4.538; P < 0.001), in particular for D. bolivari B.
(df = 278; t = 2.450; P < 0.015) and \textit{M. pygmaeus} (df = 278; t = 4.870; P < 0.001) in which the recorded KTs were higher for males than females. In the interaction between species × KT, significant differences were also found (df = 6, 278; F = 13.871; P < 0.001).

\textit{Dicyphus eckerleini} and \textit{D. errans} showed a lower KT5 compared to \textit{D. bolivari} strains, \textit{M. pygmaeus} and \textit{N. tenuis} (df = 6, 278; F = 9.351; P < 0.001) (Table 3). Also \textit{D. flavoviridis} showed a lower tolerance to high temperature compared to both \textit{D. bolivari} strains and \textit{M. pygmaeus}. Regarding the hot coma temperature, differences were found between \textit{N. tenuis} and \textit{D. bolivari} strains, \textit{M. pygmaeus} and \textit{D. eckerleini} (KT6: df = 6, 278; F = 6.014; P < 0.001) (Table 3).

4. Discussion

Previous studies on insect thermal biology focused mainly on direct

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_1}
\caption{Nymphal developmental duration (days) at six temperatures (T) (15, 20, 25, 30, 35, 40 °C) of six mirid predatory species: \textit{Dicyphus bolivari} (Barcelona and Valencia strain), \textit{D. eckerleini}, \textit{D. errans}, \textit{D. flavoviridis}, \textit{Macrolophus pygmaeus}, \textit{Nesidiocoris tenuis}. For each temperature, bars followed by different letters are significantly different (Bonferroni, P < 0.05).}
\end{figure}
lethal effects of low temperatures and on overwintering strategies. However, temperature can influence other species-specific thermal thresholds, such as development, walking or flying skills reducing or preventing movement. Climatic conditions close to these thresholds can indirectly increase mortality, because of the inability of species to find food resources or to escape from predation or parasitism (Renault et al., 2002; Turnock and Fields, 2005; Pitts and Wall, 2006; Hatherly et al., 2008). In fact, as the temperature decreased or increased until a limit threshold (i.e. KT1, KT5), we observed that the individuals lost the ability to move their limbs in a coordinated manner and were therefore unable to walk. Since in all the trials, no recovery from the hot coma was possible, this threshold, unlike chill coma, is more likely an irreversible state leading to mortality (Hazell et al., 2010; Hughes et al., 2010). Consequently, the study of sublethal thermal tolerance traits can help to determine the potential efficacy of a BCA across a range of temperatures (Hughes et al., 2010).

In temperate zones, Honek (1996) reported a significant trend for decreasing average lower temperature threshold with increasing geographical latitude based on literature data of 335 insect species. Therefore, the presence of different dicyphine species on tomato crops, depending on geographic zone, can probably be explained by a different tolerance to lower and higher temperatures. The knowledge of these thermal limits can help in the selection of the most suitable BCAs in relation to climatic conditions. Based on our results, we can separate...
two main groups of species, one more adapted to a temperate climate (D. errans, D. eckerleini and D. flavoviridis) and the other more adapted to a warmer climate (D. bolivari, M. pygmaeus and N. tenius). Our data shows a clear relationship between the minimum and maximum thermal thresholds. The species less vulnerable to lower temperatures were more vulnerable to higher temperatures and the opposite; species more vulnerable to lower temperatures were less vulnerable to high temperatures. These findings confirm the general theory that each species of insect can only develop over a limited range of temperatures, the so-called thermal window (Dixon et al., 2009).

In all treatments, D. eckerleini and D. errans were the only species having the CTmin (stop walking) at temperatures below 0 °C. Both species showed similar developmental spans and were able to tolerate lower temperature compared to the other species, suggesting that they can be considered as suitable BCAs in cropping areas with cooler temperatures. This is consistent with the latitudinal distribution of D. errans, a species that is widespread throughout Europe (except in Finland, Latvia, Lithuania, northern Ireland, northern Russia and Slovakia) and western Asia (Kerzhner, 1999). Similarly, D. eckerleini is spread in southeastern Europe and in the Middle East (Kerzhner, 1999) and also tolerates low temperatures, maybe because of more extreme winter conditions in eastern Europe than western Europe. Also D. flavoviridis showed a good cold resistance (KT1 approx 1 °C), but at the same time a long developmental time at 15 °C (about 2 months). This is probably related to its bigger body size compared to the other tested species. This species is reported in Italy, France and Switzerland, and is mainly spread in wet piedmont environments (Kerzhner, 1999; Ingegno et al., 2008; Matoçq and Streito, 2013). Moreover, females are brachypteran so they are not able to cover long distance in their life span (Tamanini, 1949). This characteristic could be usefully exploited for a spot release to prevent natural escaping in the crop surroundings especially in new areas. Establishment of non-native generalist species outside the glasshouse may have undesirable effects on native populations, either by competition or predation (Hatherly et al., 2005; van Lenteren et al., 2006). However, this risk should not be overstated because the establishment of most mirids strongly depends on the presence of suitable host plants in the agroecosystem. At the moment, little is known about the actual predatory effectiveness of these more cold tolerant species, since predation trials were performed only for D. errans so far (Ingegno et al., 2013, 2017b, 2019; Ingegno et al., 2017a). Therefore, further research is needed to know the potential of D. eckerleini and D. flavoviridis for their possible use as BCAs in temperate areas.

Our experiments showed that the three species D. bolivari, M. pygmaeus and N. tenius were able to tolerate higher temperatures than the other three tested species, which makes them suitable candidates as BCAs in crops under warmer temperatures. Native to the Palearctic ecozone, M. pygmaeus is distributed from Finland to Algeria, the Azores Islands and Tajikistan (Sanchez et al., 2012), and is abundant in the Mediterranean region depending on geographic area, season and crop cycle (Hamdi and Bonato, 2014). Also N. tenius is a common species in Mediterranean region but it has its northern limit in North Italy; moreover, it is spread also in Africa, Central America, South Asia and Oceania, and it is reported in many crops mainly from localities with a mild climate (Sanchez et al., 2009). Recently, this species also established unintentionally in many Dutch tomato greenhouses (GM, personal observation). Dicyphus bolivari has been reported in Morocco and in Spain where it was found preying on T. absoluta (Abbas et al., 2014). Despite its distribution, Pérez-Hedo and Urbaneja (2014) suggested that the optimal temperature range of D. bolivari is below that of the commercialized species M. pygmaeus and N. tenius. This is in contrast with our data that showed no significant differences in the CTmax for both D. bolivari strains and in the CTmin only for D. bolivari V compared to the other two species mentioned above. We also observed that D. bolivari B and N. tenius were the only species able to reach adulthood at the constant temperature of 35 °C. However, the mortality at 30 °C was higher for both D. bolivari strains than the other species and temperatures, which might limit their performance under hot climatic conditions.

Both D. bolivari strains showed similar duration in their nymphal development, which was for most temperatures longer compared to the other tested species, except for D. flavoviridis. Our data on development times at 25 °C are generally consistent with what obtained in previous studies (Perdikis and Lykouressis, 2000; Urbaneja et al., 2005; Sanchez et al., 2009; Ingegno et al., 2011, 2017b, 2019; Abbas et al., 2014; Mollá et al., 2014), with exception of D. flavoviridis and D. eckerleini, which were here investigated for the first time. A longer juvenile developmental time may increase the total prey consumption per individual. Indeed, Mollá et al. (2014) reported three-fold predation values on the total number of E. kuehniella eggs preyed by nymphal instars of D. bolivari compared to those consumed by N. tenius and M. pygmaeus. However, it remains to be clarified whether this trait is positive, because a longer generation time also results in a slower numerical response, which may be detrimental for biological control in the short term (Abrams and Matsuda, 1996; Symondson et al., 2002). So far, only a few studies have been performed on D. bolivari biology and behaviour (Abbas et al., 2014; Pérez-Hedo and Urbaneja, 2014; Ingegno et al., 2019), although more information might actually be available in the literature because of a possible misidentification with the species Dicyphus tamarini Wagner (J.A. Sanchez, personal communications).

In the chill tolerance experiment, N. tenius showed a significantly higher activity recovery temperature (about 13 °C) compared to the other species. This result could explain its low chill tolerance reported by other authors who observed its inability to survive through winter in cold climates (Hughes et al., 2009). By using the same refrigerating/heating circulating module to investigate the thermal activity threshold of N. tenius, Hughes et al. (2010) registered different KTs. The main differences were in KT2 and KT4, which in our experiment were lower and higher, respectively (i.e. ΔKT2: –4 °C; ΔKT4: +3 °C), while the heat KTs (i.e. KTS and KTb) were similar (Table 3). Beside the origin of the mirid populations and their previous acclimation, this could be due to a more stressful biologic consequence caused by heat. In fact, often the
hot comma temperature and the upper lethal limit do not differ significantly (Hazell et al., 2010; Hughes et al., 2010). The chill comma temperature registered by Hughes et al. (2010) for *N. tenuis* was higher than all the KT2 registered in our experiments, in which *M. pygmaeus* showed the highest KT2. Despite the different trend curves applied in the two studies, this record suggests a minor cold resistance for *M. pygmaeus* compared to the other tested species. Nevertheless, in NW Italy during the cold season, nymphs can be found overwintering with adults (Ingegno et al., 2009), underlying the establishment potential of these mirid predators. Moreover, it has to be considered that experimental conditions were extremely forced while, in environmental conditions, temperature fluctuations are less extreme concerning intensity and duration, allowing a more likely successful establishment.

Our results showed interestingly that in all species females and males differed in their cold and heat tolerance. Males and females were more vulnerable to cold and to heat, respectively. This difference in thermal tolerance might be explained by different size between males and females. The effect of sex on insect cold hardness has been often ignored but can play an important role in the supercooling ability of an organism (Renault et al., 2002). Carrillo et al. (2004) found no difference in the supercooling points between males and females of *Diamesa mendotae* Muttikowski (Diptera: Chironomidae). On the contrary, highly significant differences between sexes in the supercooling point temperature distributions were found in *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae): males were characterized by lower supercooling point temperatures than females (Salin et al., 2000). Although the differences in body size and in fat content or metabolism (i.e. females have different nutritional needs than males (Stockhoff, 1993; Mira, 2000; Telang et al., 2001; Moreau et al., 2003; Colinet et al., 2007)) are evident, the exact mechanisms behind thermal requirements remain unclear. Whether body size can be a good indicator for thermal tolerance might be interesting for further studies. Larger sized and smaller sized species in this study seemed to be better adapted to lower and higher temperatures, respectively. Moreover, manipulation of rearing temperatures can be worth to explore since it can influence both phenotypic characteristics, such as size and efficiency of BCAs as reported for generalist parasitoids of the genus *Aphidius* (Hymenoptera: Braconidae) (Ismail et al., 2014; Jerbi-Elayed et al., 2015a, 2015b).

We think that our study can provide useful basic information to predict which mirid predatory species can be suitable in relation to environmental conditions, especially in glasshouses where thermal settings are known and controlled during the crop season. Besides thermal thresholds for activity, the choice of the best mirid predator should obviously include also other criteria such as predatory efficacy, developmental time, prey and plant preference. In fact, host plants may influence the behaviour of both prey and predator, and their use as companion plants or in crop consociation can play a fundamental role depending on the geographical region (Sanchez, 2008; Arnó et al., 2010; Castañé et al., 2011; Biondi et al., 2016; Naselli et al., 2016). In conclusion, the thermal threshold studies for activity of arthropod natural enemies are a useful tool to get a rapid improvement of their suitability as BCAs for certain climatic conditions in addition to population dynamic experiments.

**CRediT authorship contribution statement**

**Barbara L. Ingegno**: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft.

**Gerben J. Messelink**: Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition. **Ada Leman**: Methodology, Investigation. **Dario Sacco**: Formal analysis. **Luciana Travella**: Conceptualization, Methodology, Writing - review & editing.

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