



## Development and thermal activity thresholds of European mirid predatory bugs

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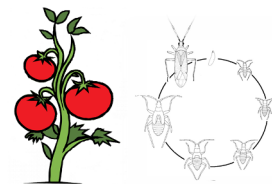
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### GRAPHICAL ABSTRACT

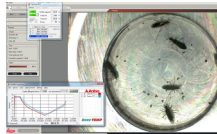
#### AIM:

to assess the thermal limits of 6 potential BCAs (Heteroptera: Miridae)



#### METHODS:

1 static at 6 temperatures (15° to 40°C)  
1 dynamic (ascending or descending T°)



#### RESULTS:

3 species more adapt for temperate climate  
3 species more adapt for warm climate



### ARTICLE INFO

#### Keywords:

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*Dicyphus bolivari*  
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*Macrolophus pygmaeus*  
*Nesidiocoris tenuis*

### ABSTRACT

Generalist predators belonging to Dicyphini (Hemiptera: Miridae) play an important role in pest control in vegetable crops. Temperature is one of 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: *N. 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.

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## 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; Messelink 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* (Meyrich) (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 *Nesidiocoris 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 (Gabarra 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) (Messelink 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 development (Campbell et al., 1974). In studies of thermal tolerance, particular behaviours are linked to physiological limits to temperature. Although the critical thermal maximum ( $CT_{max}$ ) may occur at different temperatures in relation to the species, the behavioural response (i.e. the onset of spasms) is the same across a diversity of taxa, representing an excellent index and standard for evaluating the thermal requirements and physiology of an organism.

To determine thermal tolerance, two major methods are commonly used: (1) the static method, which measures the time to death at constant test temperatures, and (2) the dynamic method, which increases or decreases test temperatures until an end point is reached (Lutterschmidt and Hutchison, 1997). However, within the range of temperatures in which insects can survive there is a more restricted zone in which insects are capable of normal locomotory function (Mellanby, 1939). In temperate regions, where conditions are rarely severe enough to cause direct mortality, these movement thresholds can be an indication for activity at lower or higher temperatures, which is very important for selecting natural enemies for various climatic conditions (Hughes et al., 2010). Traits often examined include non-lethal measurements such as chill coma temperature (Mellanby, 1939; Colhoun, 1960; Gaston and Chown, 1999), 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 maroccanus* Wagner (Sanchez and Cassis, 2018)], *Dicyphus eckerleini* Wagner, *Dicyphus errans* (Wolff), *Dicyphus flavoviridis* Tamanini, *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. eckerleini* 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 *Solanum 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 *Solanum 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 rearings were supplied with eggs of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) mixed with dehydrated and decapsuled 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 development times at six constant temperatures (15, 20, 25, 30, 35, 40 °C) were assessed for all the tested species (*D. eckerleini*, *D. errans*, *D. flavoviridis*, two *D. bolivari* strains, *M. pygmaeus* and *N. tenuis*) in apposite 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, Ø<sub>min</sub> 25 mm, Ø<sub>max</sub> 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 by 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, *D. eckertleini*, *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, 30 °C) means in column followed by different letters are significantly different (Bonferroni, P < 0.05).

Instar	1st						2nd					
	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
<i>Dicyphus bolivari</i> B	12.46 ± 0.53b	6.00 ± 0.52 ab	4.79 ± 0.52 ab	3.00 ± 0.47 a	3.80 ± 0.42	2.00 ± 0.42	8.08 ± 0.54 a	3.54 ± 0.54 a	2.50 ± 0.56 a	2.88 ± 0.47 a	4.50 ± 0.50	-
<i>Dicyphus bolivari</i> V	8.14 ± 0.52 a	6.36 ± 0.52b	4.36 ± 0.52b	4.06 ± 0.48 a	3.47 ± 0.46	2.00 ± 0.00	9.23 ± 0.54 a	3.64 ± 0.54 a	3.50 ± 0.52 a	3.75 ± 0.48 a	5.33 ± 0.88	-
<i>Dicyphus eckertleini</i>	8.80 ± 0.50 a	4.00 ± 0.50 a	3.79 ± 0.52 a	2.73 ± 0.50 a	3.67 ± 0.41	-	8.07 ± 0.52 a	3.43 ± 0.52 a	2.93 ± 0.52 a	2.40 ± 0.50 a	3.00 ± 0.73	-
<i>Dicyphus errans</i>	9.43 ± 0.52 a	5.00 ± 0.52 ab	3.59 ± 0.47 a	2.60 ± 0.50 a	3.18 ± 0.76	-	7.50 ± 0.56 a	6.64 ± 0.58b	2.59 ± 0.47 a	2.64 ± 0.52 a	2.50 ± 0.50	-
<i>Dicyphus flavoviridis</i>	14.62 ± 0.53b	5.60 ± 0.50 ab	4.30 ± 0.52 a	6.60 ± 0.50b	5.50 ± 1.50	-	7.45 ± 0.58 a	4.73 ± 0.50 ab	3.25 ± 0.54 a	2.33 ± 0.50 a	-	-
<i>Macrolophus pygmaeus</i>	8.09 ± 0.58 a	5.79 ± 0.52 ab	2.88 ± 0.48 a	3.47 ± 0.50 a	2.40 ± 0.21	2.00 ± 0.00	7.10 ± 0.61 a	4.08 ± 0.56 a	2.46 ± 0.54 a	2.47 ± 0.50 a	2.00 ± 0.00	-
<i>Nesidiocoris tenuis</i>	18.50 ± 0.52c	6.40 ± 0.50b	4.15 ± 0.53 a	3.13 ± 0.48 a	3.44 ± 0.20	2.00 ± 0.00	9.29 ± 0.52 a	4.53 ± 0.5 ab	1.62 ± 0.54 a	1.75 ± 0.48 a	1.79 ± 0.11	-
Instar	3rd						4th					
Species / T°C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
<i>Dicyphus bolivari</i> B	9.42 ± 0.53b	5.08 ± 0.53 bc	3.00 ± 0.53 a	3.08 ± 0.51 ab	2.00 ± 0.00	-	11.08 ± 0.56b	4.58 ± 0.56 ab	3.17 ± 0.56 abc	2.50 ± 0.56 abc	2.50 ± 0.79 a	2.00
<i>Dicyphus bolivari</i> V	8.58 ± 0.53b	5.00 ± 0.58 bc	2.93 ± 0.49 a	2.00 ± 0.46 a	-	-	10.67 ± 0.56b	5.33 ± 0.65 bc	4.29 ± 0.52 bc	3.23 ± 0.54 a	-	-
<i>Dicyphus eckertleini</i>	6.36 ± 0.49 a	4.08 ± 0.51 abc	2.92 ± 0.51 a	2.36 ± 0.49 a	4.00 ± 1.53	-	10.92 ± 0.54b	5.46 ± 0.54 bc	2.15 ± 0.54 ab	3.08 ± 0.54 a	-	-
<i>Dicyphus errans</i>	6.00 ± 0.53 a	3.55 ± 0.55 abc	4.24 ± 0.45 a	1.92 ± 0.51 a	-	-	6.92 ± 0.56 a	3.73 ± 0.58 ab	3.53 ± 0.47 abc	2.36 ± 0.58 a	-	-
<i>Dicyphus flavoviridis</i>	8.73 ± 0.55b	5.57 ± 0.49c	4.25 ± 0.51 a	4.57 ± 0.49b	-	-	11.30 ± 0.61b	6.93 ± 0.52c	4.66 ± 0.54c	6.38 ± 0.54b	-	-
<i>Macrolophus pygmaeus</i>	4.89 ± 0.61 a	3.25 ± 0.53 ab	3.25 ± 0.53 a	2.08 ± 0.51 a	-	-	10.56 ± 0.65b	4.82 ± 0.58 abc	3.27 ± 0.58 abc	3.08 ± 0.56 a	-	-
<i>Nesidiocoris tenuis</i>	10.46 ± 0.51b	2.27 ± 0.47 a	2.31 ± 0.51 a	1.50 ± 0.46 a	1.63 ± 0.20	-	9.00 ± 0.61 ab	2.47 ± 0.50 a	2.00 ± 0.54 a	1.25 ± 0.48 a	1.60 ± 0.27	-
Instar	5th						Statistics					
Species / T°C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	source / instar	1st	2nd	3rd	4th	5th
<i>Dicyphus bolivari</i> B	12.67 ± 0.82cd	8.64 ± 0.85c	5.33 ± 0.82b	7.00 ± 2.83 a	3.00	-	species (df = 6)	F = 27.167;	F = 1.838;	F = 10.04;	F = 18.752;	F = 34.036;
<i>Dicyphus bolivari</i> V	13.58 ± 0.82cd	8.37 ± 1.00 bc	4.21 ± 0.76 ab	5.33 ± 1.63 a	-	-	T	P < 0.001	P = 0.091	P < 0.001	P < 0.001	P < 0.001
<i>Dicyphus eckertleini</i>	8.92 ± 0.82 abc	8.91 ± 0.85c	4.23 ± 0.78 ab	3.33 ± 0.94 a	-	-	F = 339.360;	F = 159.338;	F = 138.648;	F = 221.932;	F = 101.742;	F = 101.742;
<i>Dicyphus errans</i>	12.17 ± 0.82 bc	5.90 ± 0.89 bc	3.07 ± 0.73 ab	3.70 ± 0.89 a	-	-	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
<i>Dicyphus flavoviridis</i>	16.80 ± 0.89 d	14.67 ± 0.94 d	10.10 ± 0.78c	1.85 ± 0.78 a	-	-	species * T (df = 3)	F = 14.743;	F = 2.459;	F = 5.551;	F = 2.661;	F = 6.511;
<i>Macrolophus pygmaeus</i>	12.00 ± 0.94cd	4.36 ± 0.85 ab	5.55 ± 0.85b	2.00 ± 0.82 a	-	-	species * T (df = 18)	P < 0.001	P = 0.001	P < 0.001	P < 0.001	P < 0.001
<i>Nesidiocoris tenuis</i>	6.20 ± 0.89 a	2.20 ± 0.73 a	1.69 ± 0.78 a	1.38 ± 0.71 a	1.36 ± 0.20	-						

**Table 2**

Survival rate (mean  $\pm$  SE) of nymphal instars of six mirid predatory species (*Dicyphus bolivari* - B: Barcelona strain, V: Valencia strain, *D. eckerleini*, *D. errans*, *D. flavoviridis*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*), at four temperatures (15, 20, 25, 30 °C). Values in columns followed by an asterisk are significantly different (Bonferroni,  $P < 0.05$ ).

Species	T °C	Instar				
		1st	2nd	3rd	4th	5th
<i>Dicyphus bolivari</i> B	15	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	20	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	73.3 $\pm$ 11.41
	25	93.3 $\pm$ 6.44	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	76.5 $\pm$ 10.28	35.3 $\pm$ 11.59	5.9 $\pm$ 5.7 *
<i>Dicyphus bolivari</i> V	15	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	20	93.3 $\pm$ 6.44	73.3 $\pm$ 11.41	66.7 $\pm$ 12.17	60.0 $\pm$ 12.64	53.3 $\pm$ 12.88
	25	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	100.0 $\pm$ 0	81.3 $\pm$ 9.75	18.8 $\pm$ 9.75 *
<i>Dicyphus eckerleini</i>	15	100.0 $\pm$ 0	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32
	20	100.0 $\pm$ 0	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77	73.3 $\pm$ 11.41
	25	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	60.0 $\pm$ 12.64
<i>Dicyphus errans</i>	15	93.3 $\pm$ 6.44	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	20	93.3 $\pm$ 6.44	73.3 $\pm$ 11.41	73.3 $\pm$ 11.41	73.3 $\pm$ 11.41	66.7 $\pm$ 12.17
	25	100.0 $\pm$ 0	100.0 $\pm$ 0	100.0 $\pm$ 0	100.0 $\pm$ 0	88.2 $\pm$ 7.81
	30	100.0 $\pm$ 0	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	73.3 $\pm$ 11.41	66.7 $\pm$ 12.17
<i>Dicyphus flavoviridis</i>	15	86.7 $\pm$ 8.77	73.3 $\pm$ 11.41	73.3 $\pm$ 11.41	66.7 $\pm$ 12.17	66.7 $\pm$ 12.17
	20	100.0 $\pm$ 0	100.0 $\pm$ 0	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	60.0 $\pm$ 12.64
	25	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	86.7 $\pm$ 8.77
<i>Macrolophus pygmaeus</i>	15	73.3 $\pm$ 11.41	66.7 $\pm$ 12.17	60.0 $\pm$ 12.64	60.0 $\pm$ 12.64	60.0 $\pm$ 12.64
	20	93.3 $\pm$ 6.44	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	73.3 $\pm$ 11.41	73.3 $\pm$ 11.41
	25	100.0 $\pm$ 0	81.3 $\pm$ 9.75	75.0 $\pm$ 10.82	68.7 $\pm$ 11.58	68.8 $\pm$ 11.58
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
<i>Nesidiocoris tenuis</i>	15	93.3 $\pm$ 6.44	93.3 $\pm$ 6.44	86.7 $\pm$ 8.77	66.7 $\pm$ 12.17	66.7 $\pm$ 12.17
	20	80.0 $\pm$ 10.32	86.7 $\pm$ 8.77	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	25	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32	80.0 $\pm$ 10.32
	30	100.0 $\pm$ 0	100.0 $\pm$ 0	100.0 $\pm$ 0	100.0 $\pm$ 0	87.5 $\pm$ 8.26

high temperatures, we used the method developed by Hazell et al. (2008). Temperature was controlled with a refrigerating/heating circulator (F25-ME, Julabo, USA Inc.) by pumping heated or cooled fluid through channels bored into a thermostated aluminium block (130  $\times$  60  $\times$  20 mm). Insects were placed in a circular arena ( $\varnothing$  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®, BASF). Insect behaviour in the arena was recorded through the glass with a digital video-camera (Leica DFC425, GmbH, Wetzlar, Germany) connected to a stereomicroscope. 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.

#### 2.4. Chill tolerance

To check the chill tolerance, the temperature was reduced from the rearing temperature (25 °C) to 10 °C at an initial decreasing rate of 1.0 °C min<sup>-1</sup>, and it was further lowered from 10 °C to -8 °C at a rate of 0.20 °C min<sup>-1</sup>. Temperature was then increased back to 10 °C at a rate of 0.25 °C min<sup>-1</sup> and to 25 °C at a rate of 1.0 °C min<sup>-1</sup>. The total experimental running time was about 3 h, and pictures were taken every minute, resulting in a total of 180 pictures for each run. A fan positioned on the upper surface of the cover provided a continuous air flow across it to prevent condensation below 0 °C.

#### 2.5. Heat tolerance

To check the heat tolerance, the temperature was increased from laboratory temperature (20 °C) until 30 °C at an initial increasing rate of

0.5 °C min<sup>-1</sup>, and it was further increased from 30 °C to 48 °C at a rate of 0.25 °C min<sup>-1</sup>. Temperature was then decreased back to 20 °C at a rate of 1.0 °C min<sup>-1</sup>. 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.

#### 2.6. Statistical analyses

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  $\times$  species, sex  $\times$  KT, species  $\times$  KT interactions were also analysed. Models including

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 stop 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 ( $df = 3$ ;  $\chi^2 = 12.615$ ;  $P = 0.006$ ) as well as the interaction between species  $\times$  temperature ( $df = 18$ ;  $\chi^2 = 34.181$ ;  $P = 0.012$ ). 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  $\times$  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  $\times$  sex ( $df = 6$ ;  $F = 1.916$ ;  $P = 0.079$ ) and sex  $\times$  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  $\times$  temperature ( $df = 18$ ;  $F = 10.096$ ;  $P < 0.001$ ). The longest developmental time was observed at 15° for *D. flavoviridis* recorded, while the shortest development time was observed at 30° 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  $\times$  sex ( $df = 6$ ;  $F = 5.277$ ;  $P < 0.001$ ) and species  $\times$  temperature ( $df = 18$ ;  $F = 2.593$ ;  $P = 0.001$ ), while no differences were observed in the interactions between sex  $\times$  temperature ( $df = 3$ ;  $F = 0.617$ ;  $P = 0.605$ ) and species  $\times$  sex  $\times$  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 ( $df = 6$ ;  $F = 152.031$ ;  $P < 0.001$ ), sexes ( $df = 1$ ;  $F = 213.217$ ;  $P < 0.001$ ), temperatures ( $df = 3$ ;  $F = 10.055$ ;  $P < 0.001$ ), and in the interactions between species  $\times$  temperature ( $df = 18$ ;  $F = 2.957$ ;  $P < 0.001$ ) and species  $\times$  sex ( $df = 6$ ;  $F = 3.887$ ;  $P = 0.001$ ), while there were no differences in the interactions between sex  $\times$  temperature ( $df = 3$ ;  $F = 2.469$ ;  $P = 0.063$ ) and sex  $\times$  species  $\times$  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 KT's were recorded to evaluate the cold tolerance for each individual: KT1-stop walking (critical thermal minimum,  $CT_{min}$ ), 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 ( $df = 38, 590$ ;  $F = 162.808$ ;  $P < 0.001$ ).

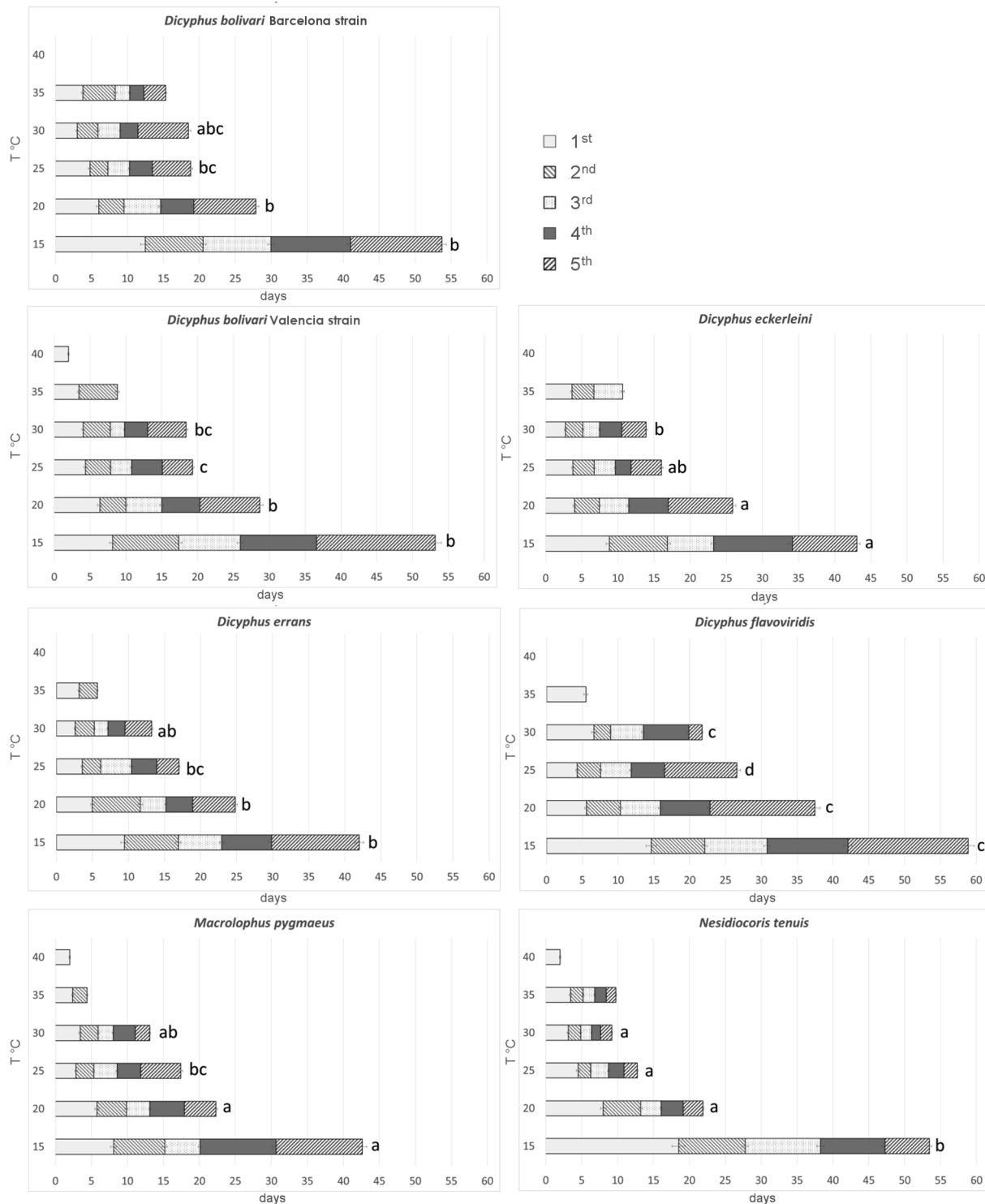
Significant differences in low temperature tolerance were found between sexes ( $df = 1, 590$ ;  $F = 14.571$ ;  $P < 0.001$ ), and in the interaction between sex  $\times$  KT ( $df = 3, 590$ ;  $F = 3.456$ ;  $P = 0.016$ ). In all the comparisons, critical temperatures were lower for females than males, except for KT1 ( $df = 590$ ;  $t = 0.216$ ;  $P = 0.829$ ). In fact, females tolerated lower mean temperatures than males (i.e. KT2: females,  $-4.7 \pm 0.1$  °C; males,  $-4.1 \pm 0.1$  °C;  $df = 590$ ;  $t = 4.693$ ;  $P < 0.001$ ) and recovered at lower mean temperatures from the chill coma (i.e. KT3: females,  $1.3 \pm 0.3$  °C; males,  $2.3 \pm 0.3$  °C;  $df = 1, 590$ ;  $t = 2.563$ ;  $P = 0.011$ ). Moreover, females showed an earlier activity recovery (i.e. KT4: females,  $7.7 \pm 0.4$  °C; males,  $9.3 \pm 0.3$  °C;  $df = 1, 590$ ;  $t = 3.250$ ;  $P = 0.001$ ). The interaction between sex  $\times$  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  $\times$  KT, significant differences were found at all temperatures ( $df = 18, 590$ ;  $F = 16.672$ ;  $P < 0.001$ ).

*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

From video recording, two KT's were recorded to evaluate the heat tolerance for each individual: KT5-stop walking ( $CT_{max}$ ), 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  $\times$  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:  $43.6 \pm 0.2$  °C; males:  $43.7 \pm 0.2$  °C;  $df = 278$ ;  $t = 0.509$ ;  $P = 0.611$ ). Males tolerated higher temperature than females with a mean  $CT_{max}$  of  $47.3 \pm 0.1$  °C and  $46.5 \pm 0.1$  °C, respectively ( $df = 278$ ;  $T = 5.744$ ;  $P < 0.001$ ). The interaction between sex  $\times$  species were significantly different ( $df = 6, 278$ ;  $F = 4.538$ ;  $P < 0.001$ ), in particular for *D. bolivari* B



**Fig. 1.** Nymphal developmental duration (days) at six temperatures (T) (15, 20, 25, 30, 35, 40 °C) of six mirid predatory species: *Dicyphus bolivari* (Barcelona and Valencia strain), *D. eckerleini*, *D. errans*, *D. flavoviridis*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*. For each temperature, bars followed by different letters are significantly different (Bonferroni,  $P < 0.05$ ).

( $df = 278$ ;  $t = 2.450$ ;  $P < 0.015$ ) and *M. pygmaeus* ( $df = 278$ ;  $t = 4.870$ ;  $P < 0.001$ ) in which the recorded KT5 were higher for males than females. In the interaction between species  $\times$  KT, significant differences were also found ( $df = 6, 278$ ;  $F = 13.871$ ;  $P < 0.001$ ).

*Dicyphus eckerleini* and *D. errans* showed a lower KT5 compared to *D. bolivari* strains, *M. pygmaeus* and *N. tenuis* ( $df = 6, 278$ ;  $F = 9.351$ ;  $P < 0.001$ ) (Table 3). Also *D. flavoviridis* showed a lower tolerance to

high temperature compared to both *D. bolivari* strains and *M. pygmaeus*. Regarding the hot coma temperature, differences were found between *N. tenuis* and *D. bolivari* strains, *M. pygmaeus* and *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

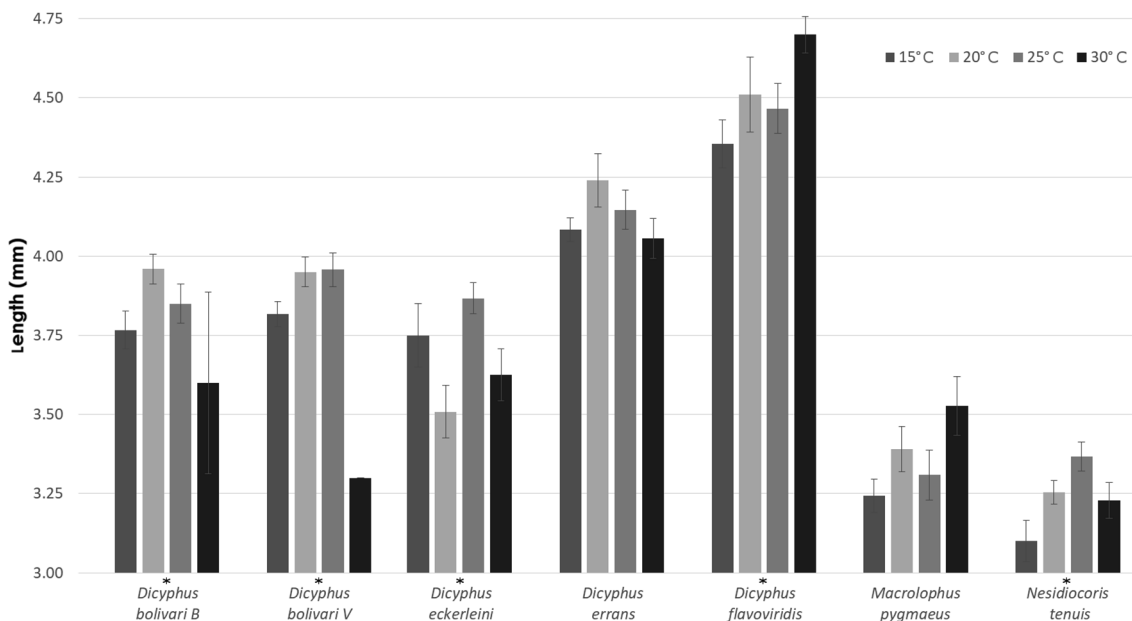


Fig. 2. Mean length (mm) ± SE of adults (females and males) of six mirid predatory species (*Dicyphus bolivari* - B: Barcelona strain; V: Valencia strain, *D. eckerleini*, *D. errans*, *D. flavoviridis*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*), emerged in the developmental trials. \* indicates that there are significant differences within the species (Tukey, P < 0.05).

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

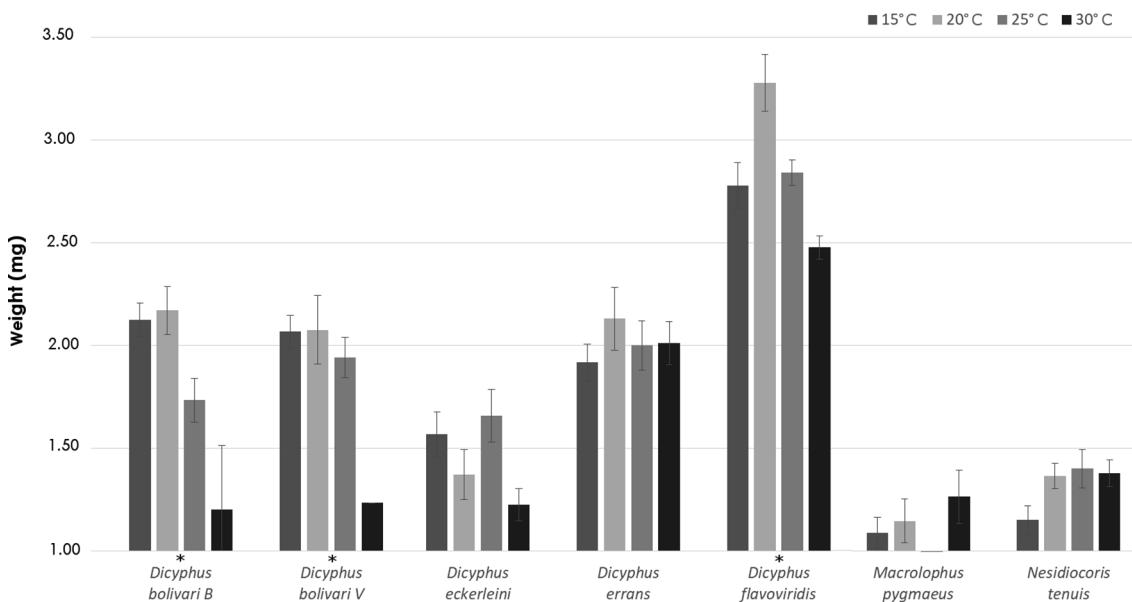


Fig. 3. Mean weight (g) ± SE of adults (females and males) of six mirid predatory species (*Dicyphus bolivari* - B: Barcelona strain; V: Valencia strain, *D. eckerleini*, *D. errans*, *D. flavoviridis*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*), emerged in the developmental trials. \* indicates that there are significant differences within the species (Tukey, P < 0.05).

**Table 3**

Key temperatures (KTs) (mean  $\pm$  SE) of six mirid predatory species tested in chill and heat tolerance experiments. KT1: critical thermal minimum (stop walking); KT2: chill coma level (stop moving); KT3: chill coma recovery (start moving); KT4: activity recovery (start walking); KT5: critical thermal maximum (stop walking); KT6: hot coma level (stop moving). Means in columns followed by different letters are significantly different (Bonferroni,  $P < 0.05$ ).

Species	Chill tolerance experiments				Heat tolerance experiments	
	KT1	KT2	KT3	KT4	KT5	KT6
<i>Dicyphus bolivari</i> B	2.12 $\pm$ 0.40b	-4.62 $\pm$ 0.17b	2.61 $\pm$ 0.46b	8.55 $\pm$ 0.58a	44.07 $\pm$ 0.28c	47.38 $\pm$ 0.19b
<i>Dicyphus bolivari</i> V	4.39 $\pm$ 0.44c	-4.59 $\pm$ 0.19b	2.16 $\pm$ 0.52b	7.95 $\pm$ 0.65a	44.53 $\pm$ 0.29c	47.02 $\pm$ 0.20b
<i>Dicyphus eckerleini</i>	-2.61 $\pm$ 0.43 a	-5.54 $\pm$ 0.19a	-0.08 $\pm$ 0.51a	6.25 $\pm$ 0.64a	42.71 $\pm$ 0.29a	47.50 $\pm$ 0.19c
<i>Dicyphus errans</i>	-2.83 $\pm$ 0.43a	-5.61 $\pm$ 0.19a	1.91 $\pm$ 0.51a	10.04 $\pm$ 0.64b	42.62 $\pm$ 0.28a	46.76 $\pm$ 0.19abc
<i>Dicyphus flavoviridis</i>	0.97 $\pm$ 0.42b	-5.08 $\pm$ 0.18ab	0.90 $\pm$ 0.50a	6.64 $\pm$ 0.62a	42.94 $\pm$ 0.27ab	46.62 $\pm$ 0.18ab
<i>Macrolophus pygmaeus</i>	3.89 $\pm$ 0.45c	-1.22 $\pm$ 0.19c	2.34 $\pm$ 0.52b	7.36 $\pm$ 0.66a	44.69 $\pm$ 0.29c	47.00 $\pm$ 0.20b
<i>Nesidiocoris tenuis</i>	4.15 $\pm$ 0.44c	-4.07 $\pm$ 0.19b	2.65 $\pm$ 0.52b	12.67 $\pm$ 0.65c	43.99 $\pm$ 0.29bc	46.11 $\pm$ 0.19a

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. tenuis*). 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  $CT_{min}$  (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; Matocq 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 overestimated 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. tenuis* 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 Palaearctic 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. tenuis* 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. tenuis*. This is in contrast with our data that showed no significant differences in the  $CT_{max}$  for both *D. bolivari* strains and in the  $CT_{min}$  only for *D. bolivari* V compared to the other two species mentioned above. We also observed that *D. bolivari* B and *N. tenuis* 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. tenuis* 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 tamaninii* Wagner (J.A. Sanchez, personal communications).

In the chill tolerance experiment, *N. tenuis* 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 circulator model to investigate the thermal activity threshold of *N. tenuis*, Hughes et al. (2010) registered different KT's. The main differences were in KT2 and KT4, which in our experiment were lower and higher, respectively (i.e.  $\Delta KT2$ : -4°C;  $\Delta KT4$ : +3°C), while the heat KT's (i.e. KT5 and KT6) were similar (Table 3). Beside the origin of the mirid populations and their previous acclimatation, this could be due to a more stressful biologic consequence caused by heat. In fact, often the



hot coma temperature and the upper lethal limit do not differ significantly (Hazell et al., 2010; Hughes et al., 2010). The chill coma temperature registered by Hughes et al. (2010) for *N. tenuis* was higher than all the KT2 registered in our experiment, 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* Muttkowski (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 in pest control as reported for *D. errans* (Ingegno et al., 2017c). Some dicyphines are stenophagous or even plant specific, while others are polyphagous, and some can cause damage by feeding on plants (i.e. *N. tenuis*), producing necrotic rings in vegetative parts and flower abortion 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 impression 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**

**Tavella:** Conceptualization, Methodology, Writing - review & editing.

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