

# Effect of simulated heat waves on the behaviour of two mirid predators

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## Abstract

Extreme climatic events, including heat waves with high-temperature peaks during the day, are expected to occur more often in many parts of the world due to climate change and may exert negative effects on existing biological control strategies. To assess the effects of high-temperature peaks on two commonly used, and naturally co-occurring mirid predators, adults and nymphs of *Nesidiocoris tenuis* and *Macrolophus pygmaeus* were exposed either to constant 25°C or daily mean temperatures of 25°C with cycles rising up to 30 or 40°C. Preferred location of the two mirid species on different strata of tomato plants was investigated when they were exposed to the different temperature regimens alone or combined. Activity of mirids under the different temperature regimens was continuously monitored for 48 h using a Drosophila Activity Monitor system. Finally, the efficacy of the mirids preying on *Tuta absoluta* eggs was measured at the different temperature regimens in a Petri dish assay. Heat waves reaching 30 and 40°C in some instances affected the location of the mirids either when they were on the plant alone or under competition conditions. Locomotory activity of *M. pygmaeus* in the 40°C treatment was strongly reduced, whereas it remained high in *N. tenuis*. Effects of temperature peaks on prey consumption were visible in *M. pygmaeus* nymphs, *N. tenuis* females and males of both species. We suggest that the different responses of the two species to high-temperature peaks may reduce their competition and support sustained control when both species are present simultaneously. Moreover, both species were found to be less susceptible to heat peaks when compared to previously reported results for their prey *T. absoluta*.

## KEYWORDS

activity, biological control, high temperature, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*, *Tuta absoluta*

## 1 | INTRODUCTION

Climate change does not only cause higher average temperatures but also leads to a higher frequency and magnitude of extreme climatic events, such as heat waves (Fischer & Schär, 2010;

Seneviratne et al., 2014). Because the thermal performance curve (TPC) of insects usually has a distinct shape with an accelerating increase up to an optimum followed by a steep decline, many insects increase in their performance at moderately elevated temperatures (Colinet et al., 2015; Stoks et al., 2017). It is thus predicted that in the

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non-tropical regions of the world, elevated temperatures lead to an increased number of generations as well as higher activity and metabolism of herbivorous insects and thus an increase in crop losses (Deutsch et al., 2018; Skendžić et al., 2021). However, once the thermal optimum is passed, temperatures quickly reach critical limits for development and survival of insects and therefore maximum temperatures experienced during short-term hot weather events can be more detrimental (Colinet et al., 2015; Stoks et al., 2017; Zhu et al., 2019). Those temperature extremes may lead to decreased energetic efficiency, to altered and potentially costly behaviour, such as shelter seeking and fasting, to decreased and ceased locomotion, and finally to sterility and death due to the denaturation of proteins (Abram et al., 2017; Colinet et al., 2015; Hughes et al., 2010; Ingegno et al., 2021; Sentis et al., 2012).

In an agricultural context, effects on pest control can be negative, when parasitoids and predators are stronger affected by altered climatic conditions than their hosts or prey (Sentis et al., 2013; Skendžić et al., 2021). For biological control, it is therefore important to choose the appropriate agents that can thrive under the thermal conditions that they find in the field or greenhouse environment. Most recently mirid predators have gained increased interest as biological control agents, in particular against the invasive tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) (Urbaneja et al., 2012; van Lenteren et al., 2020) despite the fact that they are also known to feed on the plant (Chinchilla-Ramírez et al., 2021; Gillespie & McGregor, 2000; Sanchez, 2008). In Europe, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (both Heteroptera: Miridae) are commercially available for augmentative release against this pest as well as against whiteflies, aphids, thrips, and spider mites (Ingegno & Tavella, 2019; Pérez-Hedo et al., 2021). *Macrolophus pygmaeus* is generally used in cooler climates, whereas *N. tenuis* is used in the Mediterranean region, based on the species' original distribution, and their thermal requirements (Arnó et al., 2010; Messelink et al., 2015; Sanchez et al., 2009, 2012), although damage to the crop plants precludes the use of *N. tenuis* in certain areas, seasons and crop types. Both predator species develop optimally between 27 and 30°C. While above 31°C excessive mortality in *M. pygmaeus* has been observed, *N. tenuis* has a high survival at 32°C and can even complete its development at 35°C; the latter is not possible for *M. pygmaeus* (Ingegno & Tavella, 2019; Martínez-García et al., 2016, 2017). Both species also co-occur in parts of their natural range, for example, in Spain and Greece, on common hosts (i.e. solanaceous plants) and exert control on whiteflies and the tomato leafminer simultaneously (Arnó et al., 2006; Dumont et al., 2021; Ingegno & Tavella, 2019; Lampropoulos et al., 2013). Perdakis et al. (2014) have observed that *N. tenuis* usually occupies the upper part of the plant, whereas *M. pygmaeus* preferably forages and oviposits on the middle and lower part. This limits competition and intraguild predation between the two species, which becomes visible at high density and when food is scarce (Dumont et al., 2021; Michaelides et al., 2018; Moreno-Ripoll et al., 2012). It is not clear, however, how this pattern would be influenced by high temperatures that might alter the

behaviour of the mirids. We thus assessed how the two species respond to elevated temperatures during the diel cycle and how these temperatures would affect their spatial distribution on the plant, their locomotion activity, and their prey consumption in Petri dishes. We hypothesized that the preferred locations of the mirids on the plant would be altered by the high-temperature peaks, that the thermophilous *N. tenuis* would be less affected by the elevated temperatures, and that interactions between the two species would change under high-temperature conditions.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental set up

All experiments were conducted in climate cabinets (Panasonic MLR 352 H-PE, Labtech Services, Villmergen, Switzerland) to ensure controlled conditions and to comply with Swiss regulations concerning the handling of non-native species.

### 2.2 | Plant material

Seeds from tomato *Solanum lycopersicum* L. var. "Rentita" and tobacco *Nicotiana tabacum* L. (Kiepenkerl, Everswinkel, Germany) were used. Seedlings were individually grown in root ball trays (5 cm dia. × 7 cm) filled with organic substrate enriched with 3 kg/m<sup>3</sup> long-term fertilizer (Manna Cote 4M Wilhelm Haug GmbH, Ammerbuch, Germany). After 21 days, the plants were individually transferred to 0.5-L pots provided with organic substrate and weekly fertilized with 100 mL liquid fertilizer (0.2%) Manna (Wilhelm Haug GmbH). The plants were kept in a greenhouse at 25 ± 1°C, 60 ± 10% relative humidity (RH), 16:8 light: dark (L:D) and irrigated every other day.

### 2.3 | Insect rearing

The rearing of *T. absoluta* was established in the laboratory from individuals obtained by Andermatt Biocontrol AG (Switzerland). The stock colony was maintained on fresh tomato plants inside mesh cages (47.5 × 47.5 × 47.5 cm) (BugDorm-4F4545). Larvae were placed on fresh tomato branches supported by a floral foam on a plate. New branches were added every other day and the floral foams were supplied with water regularly. When the pupal period started, this procedure was stopped until emergence of adults. Adults were collected and transferred to a new cage with fresh plants. The rearing was kept in a walk-in climate cabin at 22 ± 1°C, 70 ± 10% RH and 16:8 L:D. Uniform cohorts of *T. absoluta* eggs obtained during 24–48 h were used for the experiments.

*Nesidiocoris tenuis* was obtained from Agrobio S.L. (Spain) and *Macrolophus pygmaeus* was obtained from Andermatt Biocontrol AG (Switzerland). To rear the predator stock colony, the specimens were

kept on tobacco plants inside mesh cages (BugDorm, see above) and feed ad libitum with eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae; AGROLINE Bioprotect, Switzerland). To control the age of mirids, they were transferred to a new cage with the same conditions, once the emergence of adults occurred. The rearing of mirids was kept in a climate-controlled cabin at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, 16:8hL:D.

## 2.4 | Temperature regimens

To assess the response of the predators to heat waves, we used three temperature regimens as follows: (1)  $25^\circ\text{C}$  constant, (2)  $25^\circ\text{C}$  average with a peak of  $30^\circ\text{C}$ , and (3)  $25^\circ\text{C}$  average with a peak of  $40^\circ\text{C}$  (Figure 1). The baseline temperature was chosen to approximate average greenhouse temperatures during the growing season in Switzerland (Janique Koller, Agroscope, personal communication) and to allow for sufficient amplitude of temperature peaks without killing the insects. Light intensity and temperature were increased stepwise. For regimens 2 and 3 with fluctuating temperature, the temperature peak that was held for 2h coincided with the highest light intensity. For all temperature regimens, the average relative humidity  $65 \pm 10\%$  and the photoperiod 16:8L:D. Temperature programs were run within climate cabinets (see above) and actual conditions experienced by the mirids during the assay were recorded using dataloggers (Ecolog TH1, Elpro-Buchs AG, Buchs, Switzerland).

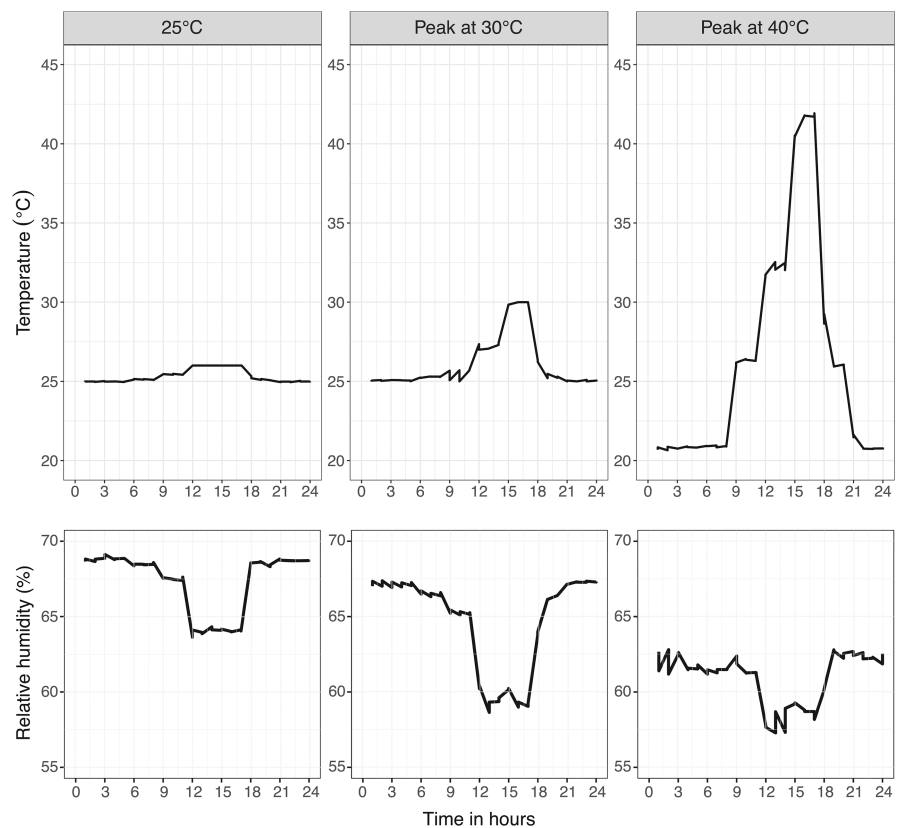
## 2.5 | Bioassays

### 2.5.1 | Predator response to high temperatures

#### Location on plants

We assessed the location of predators on whole plants under the three temperature regimens. One tomato plant with 10 fully developed composite leaves and 25–30cm height was introduced into a cage (BugDorm, see above) with about 100–200 adult *T. absoluta* of mixed sexes for 24h prior to the experiment, to allow for oviposition. As young females lay 25 or more eggs per day (Silva et al., 2015) at least 1250 eggs were assumed to be present on each plant as food source for the mirids. One plant with eggs was used per experimental unit.

Adults and nymphs from 3rd to 5th instar of *N. tenuis* (Nt) and *M. pygmaeus* (Mp) were released into each cage as follows: (1) ten nymphs of (Mp), (2) ten nymphs of (Nt), (3) ten adults of (Mp), (4) ten adults of (Nt), and (5) ten adults each of both species (Mp+Nt). Adults were not older than 7 days and of mixed sex with either a 1:1 or slightly female biased sex ratio. As nymphs of the two species could not be reliably distinguished in the chosen setup on the plant, no treatment with mixed nymphs was considered. The plants were divided in upper, middle and lower strata, each having 8–10cm of height depending on the original plant size. After 48h, the location of the mirids present per plant strata was recorded, as well as the number of dead individuals. This was done visually through the transparent plastic front of the bugdorm and without manipulating



**FIGURE 1** Temperature regimens and humidity as measured by dataloggers during the experiment in which constant ( $25^\circ\text{C}$ ) and fluctuating temperatures (with either a peak at 30 or  $40^\circ\text{C}$ ) were employed to generate heat waves. Light conditions during the experiment were as follows: (1) dark period from 21:00 to 05:00h, (2) intermediate light period from 06:00h to 11:00h and 18:00h to 20:00h, and (3) intense light period from 12:00h to 17:00h.

the plant during the morning in a period with baseline temperature. Ten replicates per treatment were assessed.

## Locomotory activity

To test the hypothesis that the two mirid species differ in their response to temperature and in the threshold temperatures for heat damage, the activity of individual predators was measured using a *Drosophila* Activity Monitor (DAM) system (TriKinetics, Waltham, MA). This system registers how many times each individual crosses an infrared light beam within a glass tube. Each crossing is counted, i.e., a higher activity of the insect becomes visible as a higher count.

Male and female adults less than 7 days old and nymphs from 3rd to 5th instar of *N. tenuis* and *M. pygmaeus* were assessed under the three temperature regimens. Glass tubes of 5 mm diameter were supplied with a water-agar solution and eggs of *E. kuehniella* on one end, in order to supply food resources for keeping the predators alive during the experiment. The tubes were covered with small rubber stopper at the other end. A single predator was placed into each tube and all tubes were placed into the monitor units of the DAM system subsequently. Two monitors with each 32 activity tubes were connected in the system. The data records were done in 5-min intervals during 48 h. The light and dark periods recognized by the DAM system during the experiment were the same as programmed in the climatic chambers (16:8 L:D).

The following experimental treatments were set up: (1) female Mp, (2) female Nt, (3) male Mp, (4) male Nt, (5) nymph Mp, (6) nymph Nt. After 48 h the survival of mirids was assessed. All individuals were included in the mortality analysis (initial sample size of 55–80 individuals per treatment) but only those individuals that survived the full experimental period were included into the activity analysis, (final sample size of 28–62).

### 2.5.2 | Temperature modulating biocontrol efficacy

In the efficacy experiments, females, males, and nymphs of *N. tenuis* and *M. pygmaeus* were individually kept in 5-mL plastic tubes to starve for 1 h before release into the experimental arena. Up to 7-day-old adults of both predators were used in the experiments. A Petri dish (7 cm diameter) served as experimental arena containing tomato leaflets placed on a solid water agar solution. 100 fresh (<24 h old) eggs of *T. absoluta* were placed on the tomato leaflet using a fine brush. A single predator was released into each dish in front of the prey and the Petri dishes were covered with a film of PVC paper. Several small holes were pinched into the PVC paper using a fine needle to ensure air circulation. After 24 h, the predation rate was estimated by counting the number of intact eggs under a stereomicroscope (Leica MZ 125 10X). The quantity of the eggs was chosen according to Michaelides et al. (2018), who reported that *N. tenuis* and *M. pygmaeus* are able to prey between 70 and 90 eggs at maximum within 24 h at 25°C. To determine how varying temperature

modulates their biocontrol efficacy we selected a density with excess eggs for both mirid species.

The following experimental treatments were set up: (1) female Mp, (2) female Nt, (3) male Mp, (4) male Nt, (5) nymph Mp, (6) nymph Nt. Adults were not older than 7 days and nymphs were in the 4th or 5th instar. For each treatment, 20 replicates were considered, no mortality occurred during the 24 h experimental period.

## 2.6 | Statistical analyses

Mortality of mirids during the on-plant experiment on predator location was modelled separately for each species and separately for nymphs and adults, since nymphs were only kept alone but not mixed. Generalized linear models (GLM) with binomial distribution were employed to assess the effects of the fixed factors temperature and – only for adult mirids alone/mixed as well as their interaction on the binary outcome of survival (dead/alive). Non-significant interaction terms ( $p > 0.05$ ) were excluded from the final models. An analysis of deviance was conducted for all models followed by Dunn post-hoc tests. To assess the preferred location of mirids on the plants, the number of surviving individuals per stratum was counted at the end of the 48 h experimental period. The location of individuals at the different plant strata was analysed separately for *N. tenuis* and *M. pygmaeus* adults and nymphs alone and compared across temperature regimens using contingency tables. Also with contingency tables, the location of adult individuals of the two species and at different temperature regimens was analysed separately to assess the effect of alone/mixed on their preferred location. To account for the twofold use of data, the significance threshold was corrected to  $p = .025$ .

Mortality of mirids in the experiment on locomotory activity was modelled separately for each species using generalized linear models (GLM) with binomial distribution to assess the effects of the fixed factors temperature and – only for adult mirids sex/stage as well as their interaction on the binary outcome of survival (dead/alive). An analysis of deviance was conducted for all models followed by Dunn post-hoc tests. Movement of mirids was analysed as total count during the 48 h experimental period. To account for unequal variances, GLMs with negative binomial distribution and log link function were used to analyse the effect of the fixed factors sex/stage and temperature as well as their interaction on the counts of movement for each species separately. Differences between day- and nighttime activity were analysed by comparing the mean number of movement-counts during the 8 h of total darkness (21:00 to 5:00) and the 5 h of highest light intensity (12:00 to 17:00) with Wilcoxon signed rank tests for each species, stage/sex and temperature regimen.

To assess predation, the number of predated eggs was counted over the full experimental period of 24 h. To account for unequal variances, GLMs with negative binomial distribution and log link function were used to analyse the effect of the fixed factors sex/stage and temperature as well as their interaction on the number

of predated eggs for each species separately. Due to significant interaction effects separate models were run for females, males and nymphs, followed by Tukey's post hoc tests adjusted for a family of 3 estimates to analyse the effect of temperature.

All statistical analyses using generalized linear models were performed in R version 4.0.2 (R Core Team, 2020) contingency analyses and Wilcoxon signed rank tests were conducted with SPSS version 26 (IBM, 2019). All raw data are stored in a public repository (Duarte Martinez et al., 2023).

### 3 | RESULTS

#### 3.1 | Location on plants

Across all temperature treatments and sex/stages,  $28 \pm 19\%$  (mean  $\pm$  SE) of *M. pygmaeus* and  $26 \pm 19\%$  of *N. tenuis* died during the experimental period of 48h (Table 1). In *M. pygmaeus* adults kept alone and in nymphs, temperature regimen had a significant influence on mortality. In both cases, lower mortality was observed in individuals exposed to peaks of 30°C compared to those at constant 25°C. Furthermore, significantly higher mortality of adults was observed in the mixed-treatment as compared to the treatment in which adults were placed on the plant alone ( $\chi^2 = 6.3515$ ;  $df = 2$ ,  $p = 0.012$ ). In adult *N. tenuis* no effect of temperature regimen on mortality was visible. However, nymphs of *N. tenuis* were significantly affected by temperature regimen with individuals at 25°C having a significantly higher mortality compared to those under regimens with heat peaks of 30 and 40°C. A significant higher mortality for adult individuals in the mixed treatment was observed ( $\chi^2 = 4.8598$ ;  $df = 1$ ,  $p = 0.027$ ) compared to the alone treatment.

When present on the plant alone, no significant effect of temperature regimen on location was detected in adult *M. pygmaeus*, but in all temperature regimens most individuals were found at the middle strata (Figure 2a). Location of *M. pygmaeus* nymphs was significantly affected by temperature regimen ( $\chi^2 = 13.521$ ;  $df = 4$ ,  $p = 0.009$ ). As indicated by the means and confidence intervals nymphs preferred

the upper and middle stratum at 25 and 30°C, whereas no preference was visible at 40°C (Figure 2c). Temperature regimen significantly affected the distribution of *N. tenuis* adults ( $\chi^2 = 16.041$ ;  $df = 4$ ,  $p = 0.003$ ) and nymphs ( $\chi^2 = 32.800$ ;  $df = 4$ ,  $p < 0.001$ ). As indicated by the means and confidence intervals most adults were found on the highest plant stratum at all temperature regimens (Figure 2a), while in nymphs this preference was only visible at 30 and 40°C (Figure 2c). When adults of both species were mixed (as compared to adults of the individual species alone), this had a significant effect on the distribution of *N. tenuis* at 25°C ( $\chi^2 = 29.938$ ;  $df = 4$ ,  $p < 0.001$ ) and on *M. pygmaeus* at 40°C ( $\chi^2 = 10.090$ ;  $df = 4$ ,  $p = 0.006$ ). No clear preference for any stratum was visible for both species at 25 and 30°C, whereas at 40°C *N. tenuis* preferred the high plant stratum (Figure 2b).

#### 3.2 | Locomotory activity

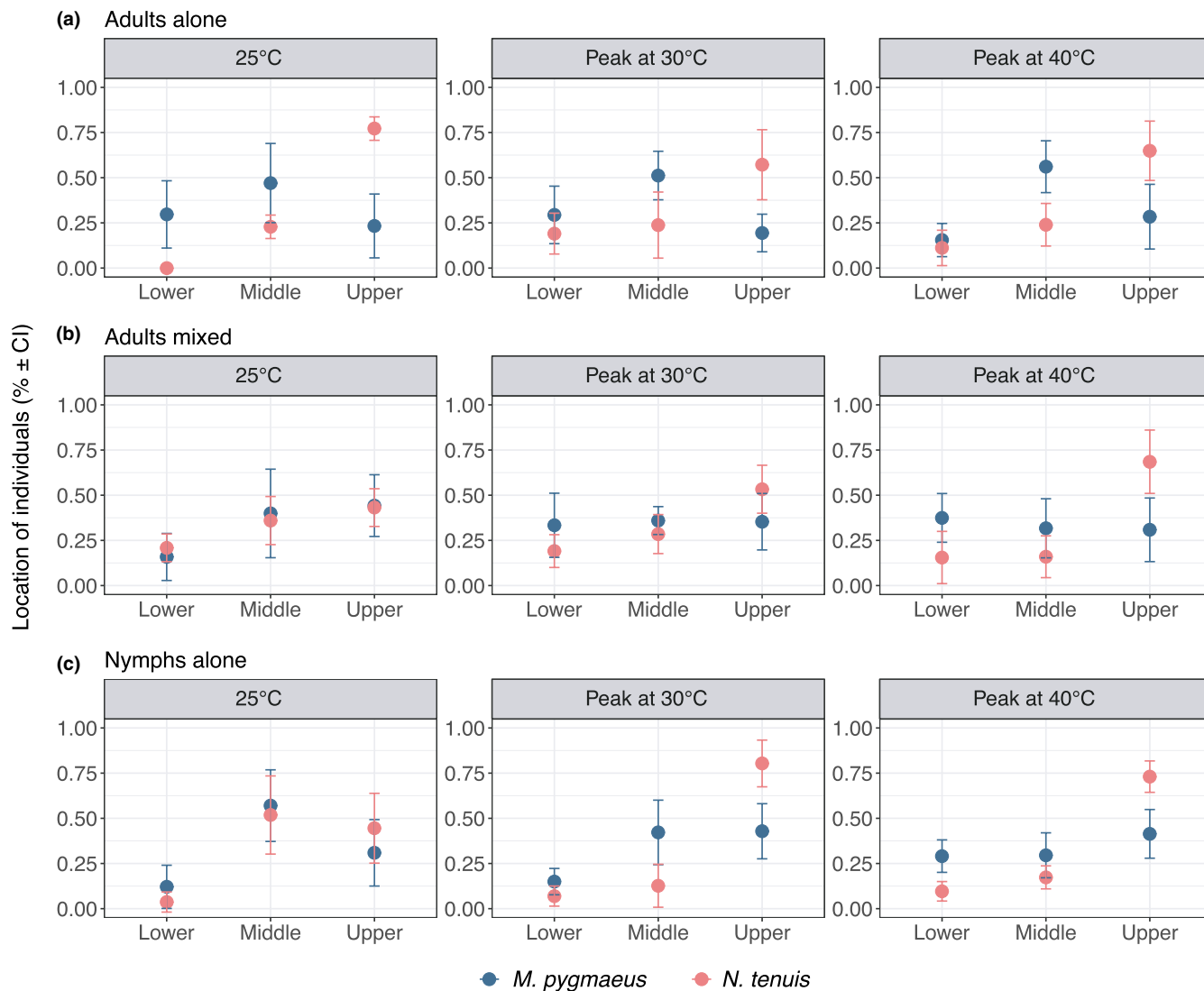
The mortality during the locomotory assay was lower in *M. pygmaeus* ( $12 \pm 6\%$ ; mean  $\pm$  SE) across all temperature treatments and sex/stages compared to *N. tenuis* ( $29 \pm 4\%$ ) with the highest mortality recorded in male *N. tenuis* at 30°C (50%) (Table 2). In *M. pygmaeus* the mortality was significantly affected by temperature regimen ( $\chi^2 = 12.1377$ ;  $df = 2$ ,  $p < 0.001$ ) but not by sex/stage or the interaction of sex/stage with temperature. No significant differences were detected during multiple comparisons of the temperature regimens (Dunn test). In *N. tenuis* mortality was significantly affected by sex/stage ( $\chi^2 = 31.2940$ ;  $df = 2$ ,  $p < 0.001$ ) as well as by the interaction of sex/stage and temperature regimen ( $\chi^2 = 7.8481$ ;  $df = 4$ ,  $p = 0.020$ ) but not by temperature regimen. Again, no significant differences were detected during multiple comparisons of males, females and nymphs (Dunn test).

In the overall models for *M. pygmaeus*, the locomotory activity differed significantly for the factors sex/stage ( $\chi^2 = 87.174$ ,  $df = 2$ ,  $p < 0.001$ ) and temperature regimen ( $\chi^2 = 94.901$ ,  $df = 2$ ,  $p < 0.001$ ) and a significant interaction between both factors was observed ( $\chi^2 = 28.222$ ,  $df = 4$ ,  $p < 0.001$ ) (Figure 3). When separated by sex/

TABLE 1 Mortality (no. of individuals) of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* during the experiment on location of mirids per plant stratum at different temperature regimens. Values followed by different letters are significantly different (Dunn test  $p < 0.05$ ).

		Mortality [mean $\pm$ SE]			$\chi^2$	p
		25	30	40		
<i>Macrolophus pygmaeus</i>						
Nymphs	Alone	2.90 $\pm$ 0.57 a	0.70 $\pm$ 0.28 b	1.10 $\pm$ 0.50 ab	8.9044	<b>0.003</b>
Adults	Alone	3.30 $\pm$ 0.45 a	2.90 $\pm$ 0.26 b	2.40 $\pm$ 0.40 ab	4.1268	<b>0.042</b>
	Mixed	4.90 $\pm$ 0.43	3.00 $\pm$ 0.51	3.60 $\pm$ 0.49		
<i>Nesidiocoris tenuis</i>						
Nymphs	Alone	4.00 $\pm$ 0.53 a	1.40 $\pm$ 0.43 b	1.80 $\pm$ 0.49 b	9.6236	<b>0.002</b>
Adults	Alone	2.00 $\pm$ 0.32	3.00 $\pm$ 0.35	2.00 $\pm$ 0.53	1.6248	0.2043
	Mixed	3.20 $\pm$ 0.70	2.00 $\pm$ 0.47	4.20 $\pm$ 0.24		

Note: Bold values for p indicate statistical significance ( $p < 0.05$ ).



**FIGURE 2** Location of mirids per plant stratum at different temperature regimens. Adults and nymphs of *Macrolophus pygmaeus* (Mp) and *Nesidiocoris tenuis* (Nt) were assessed either alone or mixed (only for adults) ( $n = 10$  per treatment). Records were taken 48 h after the predators were released on tomato plants. Location preference was estimated using the proportion of surviving individuals per stratum and the corresponding confidence interval.

stage, temperature regimen had a significant effect on *M. pygmaeus* female ( $\chi^2 = 96.293$ ,  $df = 2$ ,  $p < 0.001$ ), male ( $\chi^2 = 76.658$ ,  $df = 2$ ,  $p < 0.001$ ) and nymph ( $\chi^2 = 45.754$ ,  $df = 2$ ,  $p < 0.001$ ) activity (Figure 3). In all three groups, activity was significantly reduced at 40°C compared to the activity at 25 and 30°C. For nymphs, activity was highest at 30°C and intermediate at 25°C.

Similarly, for *N. tenuis*, the locomotory activity differed significantly among sex/stage (i.e. female, male, and nymphs) ( $\chi^2 = 52.211$ ,  $df = 2$ ,  $p < 0.001$ ). There was a significant interaction between the factors sex/stage and temperature ( $\chi^2 = 17.669$ ,  $df = 4$ ,  $p = 0.001$ ) but no overall significant effect of temperature alone. An influence of temperature on activity of *N. tenuis* was not visible in adults but only in nymphs ( $\chi^2 = 28.059$ ,  $df = 2$ ,  $p < 0.001$ ) (Figure 3). Nymphs were most active at 30°C compared to 25 and 40°C.

In the course of the observation period, locomotory activity of females, males and nymphs of *M. pygmaeus* demonstrated a diurnal

pattern (Figure 4), whereas no such clear pattern was visible for *N. tenuis* (Figure 5). Significantly higher nighttime activity was detected for *M. pygmaeus* nymphs at 30°C, for females at 25 and 30°C and for males at all temperature regimens (Table 3). In *N. tenuis* higher nighttime activity was only visible in nymphs at 30°C, and males at 25 and 30°C. Females displayed higher daytime activity at 40°C (Table 3).

### 3.3 | Temperature modulating biocontrol efficacy

Overall, *N. tenuis* consumed more eggs within the 24-h experimental period than *M. pygmaeus* with the highest average of 94 eggs consumed by individual females at 25°C (Figure 6). The full models demonstrated that for both species the number of eggs consumed differed significantly among sex/stage (*N. tenuis*:  $\chi^2 = 12.906$ ,  $df = 2$ ,  $p = 0.002$ ; *M. pygmaeus*:  $\chi^2 = 8.202$ ,  $df = 2$ ,  $p = 0.017$ ). The interaction

between the factors sex/stage and temperature was also significant for both species (*N. tenuis*:  $\chi^2=17.0$ ,  $df=4$ ,  $p=0.002$ ; *M. pygmaeus*:  $\chi^2=24.497$ ,  $df=4$ ,  $p<0.001$ ).

When separated by sex/stage the prey consumption by female *M. pygmaeus* was not significantly different among temperature regimens. For males, temperature had a significant effect ( $\chi^2=17.404$ ,  $df=2$ ,  $p<0.001$ ), efficacy at 30°C was significantly decreased in comparison with the consumption at 25 and 40°C. Nymph efficacy was also affected by temperature ( $\chi^2=8.682$ ,  $df=2$ ,  $p=0.0130$ ), with a significant decrease at 40°C compared to 25°C.

For *N. tenuis* females a significant effect of temperature on prey consumption was visible ( $\chi^2=12.393$ ,  $df=2$ ,  $p=0.002$ ), where prey consumption decreased significantly at 30 and 40°C. Also, for males, temperature had a significant effect ( $\chi^2=6.352$ ,  $df=2$ ,  $p=0.0417$ ) as consumption was significantly reduced at 30°C in comparison with

**TABLE 2** Mortality (%) of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* during the experiment on locomotion activity at different temperature regimens.

	Mortality [%]		
	25	30	40
<i>Macrolophus pygmaeus</i>			
Nymphs	3.13	7.81	21.88
Females	13.85	7.46	23.44
Males	6.94	11.67	10.94
<i>Nesidiocoris tenuis</i>			
Nymphs	9.09	12.50	27.85
Females	31.25	27.59	26.39
Males	35.00	50.00	45.16

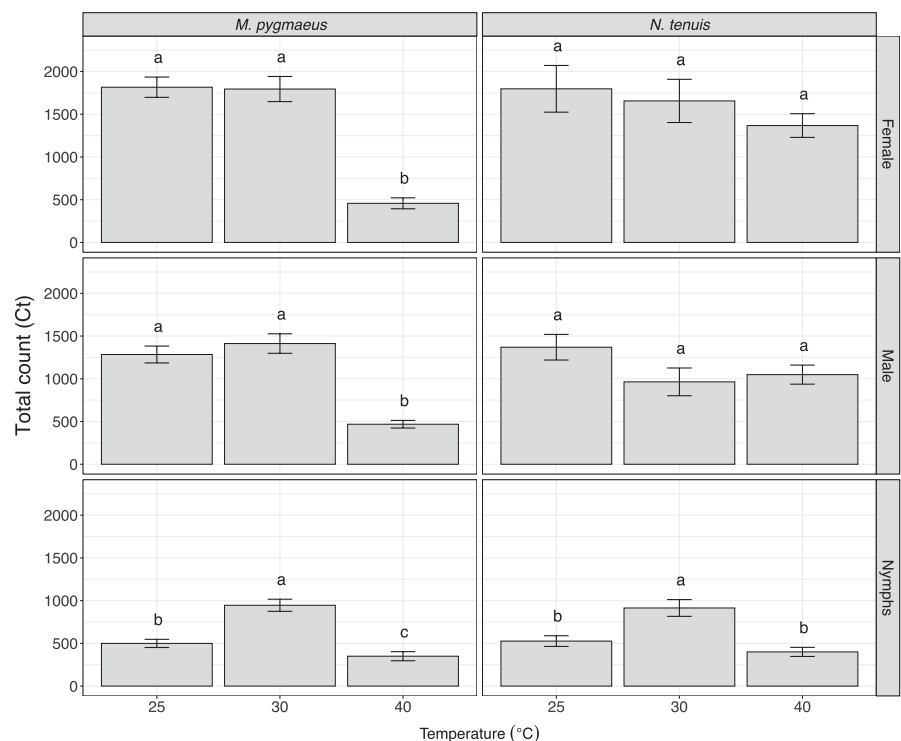
the 40°C treatment. No significant differences were observed for nymphs.

## 4 | DISCUSSION

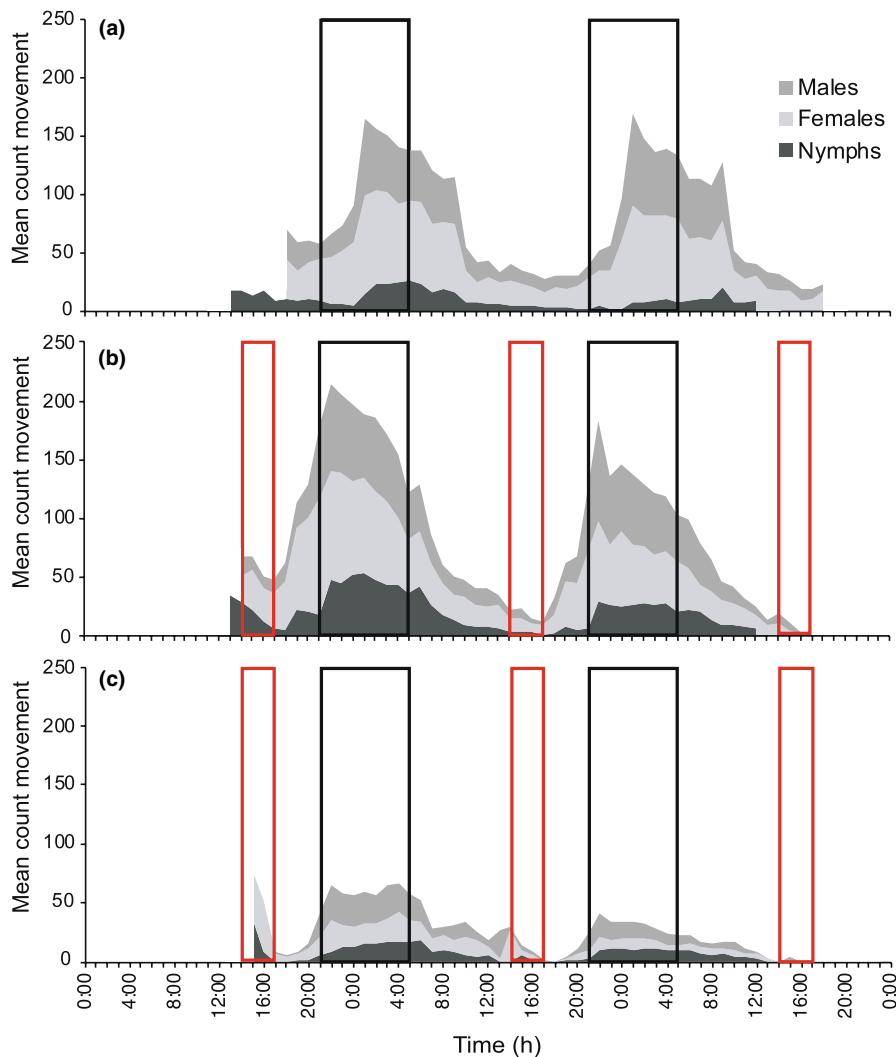
Our results revealed that daily heat peaks compared to comparable (i.e. same daily mean) constant temperature regimens affected the on-plant location, locomotion and predation activity of some of the stages of the mirid predators *M. pygmaeus* and *N. tenuis*.

When ten individuals of one species were present on a plant, temperature significantly affected mortality of *M. pygmaeus* adults and nymphs as well as of *N. tenuis* nymphs. In all cases, mortality at 25°C was higher than at a regimen with a temperature peak at 30°C, indicating that the mirids actually benefitted from the fluctuating intermediate temperature, while no clear negative effect of the 40°C regimen was found. It is known that fluctuating temperatures within the permissive range generally improve insect performance (Colinet et al., 2015). This is based on the unequal form of the thermal performance curve as well as other factors such as adjustments in gene expression (Liefing et al., 2017). As 30°C is still a permissive temperature for both mirids, their survival might have been enhanced by the temperature fluctuation compared to the constant 25°C.

When kept alone, the two species differed in their distribution under most temperature regimens, with *N. tenuis* preferring the upper plant parts and *M. pygmaeus* more commonly found on the central part. This general pattern was unaffected by the heat peaks for adult *M. pygmaeus*. In case of *N. tenuis*, the preference for the upper strata was prevalent under all temperature regimens despite the fact that the overall distribution was affected by temperature.



**FIGURE 3** Locomotory activity of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* at different temperature regimens during 48h. Columns followed by a different letter show significant differences between treatments (GLMs with negative binomial distribution, followed by Tukey's post hoc tests adjusted for multiple comparisons,  $p \leq 0.05$ ).



**FIGURE 4** Locomotory dynamic of *Macrolophus pygmaeus* under different temperature regimens during 48 h: a: at 25°C constant, b: with 30°C heat peaks and c: with a 40°C heat peaks. Activity is shown as the number of times individuals crossed a light beam in the activity monitor. Male individuals are shown in medium grey, females in light grey and nymphs in dark. Time periods with highest intensity heat peak are shown as red squares, dark periods as black squares. Only those periods are shown where at least 50% of individuals were monitored ( $n \geq 27$ ).

That *N. tenuis* prefers the upper strata of the plant has been previously reported at 24–25°C (Konan et al., 2021; Perdakis et al., 2014). Nymphs of both species responded stronger to temperature regimens with nymphs of *M. pygmaeus* showing no more preference for the upper plant part at 40°C and nymphs of *N. tenuis* showing no preference for this stratum at 25°C.

Similar effects were visible when ten adults of both species were present on the plant simultaneously, with *M. pygmaeus* being affected at 40°C and *N. tenuis* at 25°C. It is possible that at 25°C the increased competition lead *N. tenuis* to move to the less preferred parts of the plant as females of this species have the highest egg consumption at 25°C and food limitation may have occurred at the density of 20 mirids per plant either due to a limited quantity or unequal distribution of *T. absoluta* eggs on the plant. On the other hand, mobility of male and female *M. pygmaeus* at 40°C is impaired and therefore may have reduced their competitive ability at this temperature. Since in the mixed treatment the number of individuals was twice as high as in the single treatments, intraspecific and interspecific competition cannot be disentangled.

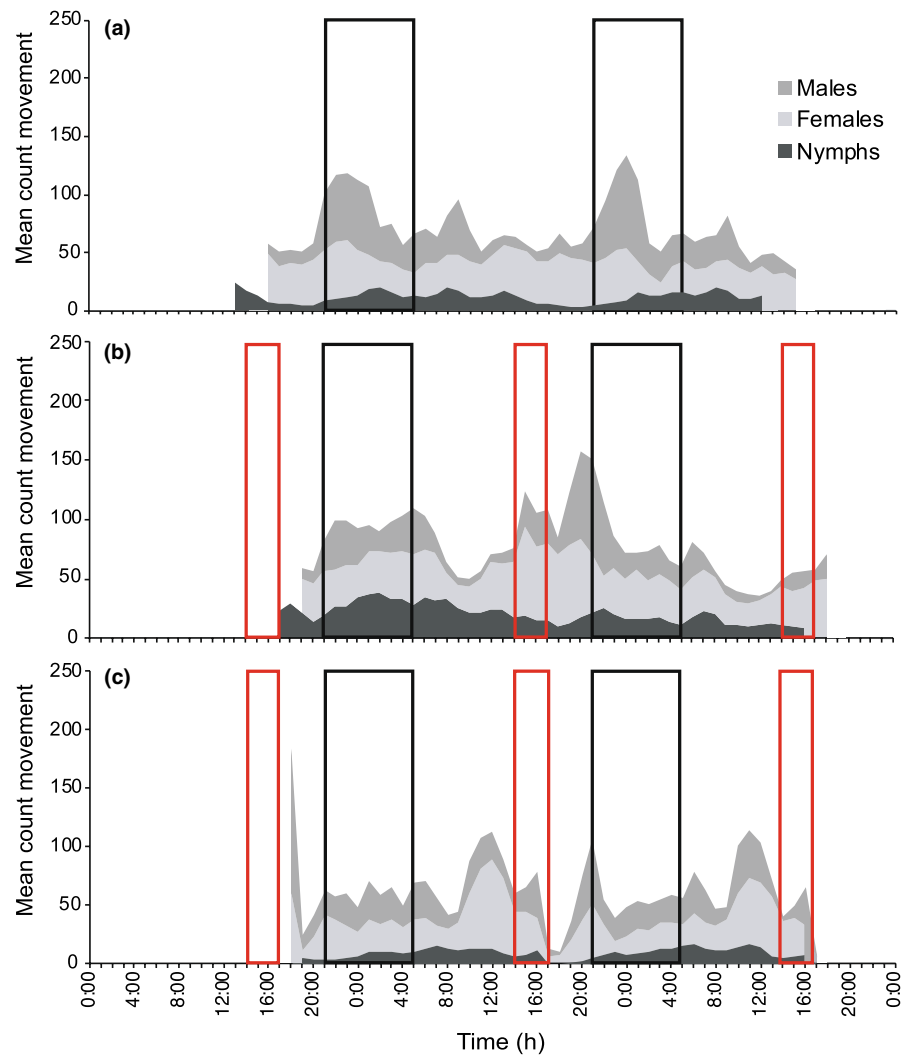
The occurrence of extreme climatic events can have strong effects on arthropods, mainly in open fragmented habitats,

characteristic for highly managed landscapes (Gols et al., 2021). Thus, the availability of microclimatic refuges is of vital importance (Thakur et al., 2020). For example, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) seek out places with optimal temperature on apple leaves (Caillon et al., 2014). Similarly, aphids are more likely to drop from plants under heat stress, which was suggested to be a form of behavioural thermoregulation to reach cooler microclimates (Ma & Ma, 2012).

Within the temperate range, activity in ectothermic insects generally increases with temperature (Abram et al., 2017). Accordingly, mirids faced with higher temperatures respond with higher activity (i.e. faster walking speed) compared to lower activity at cold temperatures (Hughes et al., 2010; Ingegno et al., 2021). However, once a critical threshold is passed, locomotion becomes impaired rapidly, up to a status of heat coma, where insects can no longer escape from harmful situations (González-Tokman et al., 2020; Gunderson & Stillman, 2015; Ingegno et al., 2021). In the experiment measuring locomotory activity, temperature peaks had no significant effect on *N. tenuis* adults and little effect on nymphs, whereas all stages of *M. pygmaeus* were significantly impaired in their locomotion in the regimen with a peak at 40°C. Therefore, it can be assumed that



**FIGURE 5** Locomotory dynamic of *Nesidiocoris tenuis* under different temperature regimens during 48 h. a: at 25°C constant, b: with 30°C heat peaks and c: with a 40°C heat peaks. Activity is shown as the number of times individuals crossed a light beam in the activity monitor. Male individuals are shown in medium grey, females in light grey and nymphs in dark. Time periods with highest intensity heat peak are shown as red squares, dark periods as black squares. Only those periods are shown where at least 50% of individuals were monitored ( $n \geq 19$ ).



the thermal activity threshold is reached for *M. pygmaeus* at 40°C whereas it is not for *N. tenuis* adults. In contrast, Ingegno et al. (2021) found no significant difference in the critical thermal maxima (i.e. when individuals stop walking) between the two species. When comparing the upper temperature threshold for *N. tenuis* with its prey *T. urticae*, the mirids were not as tolerant to heat and lost their ability of coordinated movement at 43.5°C significantly below the upper thermal limits of *T. urticae* at 47.3°C (Hughes et al., 2010). For comparison, the upper thermal limits for *T. absoluta* have been estimated to be between 34.6 and 37.3°C (De Campos et al., 2020; Krechmer & Foerster, 2015; Martins et al., 2016). Therefore, both mirids should be able to attack the pest under high-temperature conditions. In addition, females of both mirid species were able to consume more than 70% of the prey offered during 24 h at the 40°C temperature regimen.

At 30°C, all stages of *M. pygmaeus* demonstrated a significantly higher night- than daytime activity. This pattern was also visible in females at 25°C and in males at 25 and 40°C. Similarly, Perdikis et al. (2004) found higher predation in this species in the dark phase and particularly pronounced at 30°C (constant) compared to 20 and 25°C. However, peaks of 40°C seem to have impaired *M. pygmaeus*

permanently in our study, as locomotion activity remained low during night and mortality was increased. While heat coma and immediate death in this species are reported to occur at temperatures above 47°C (Ingegno et al., 2021), accumulated damage during repeated exposures to stressful conditions seem to have impaired the species at temperatures below the above-mentioned threshold. In *N. tenuis*, higher nighttime activity was visible in nymphs at 30°C and males at 25°C while females did not display this pattern at all, but a higher daytime activity at 40°C. However, mortality, in particular in *N. tenuis* adults, was relatively high. It is possible that, compared to *M. pygmaeus* or *N. tenuis* nymphs *N. tenuis* adults were less able to deal with the artificial conditions in the glass vials and that their higher resource requirements due to continued activity at the high temperatures could not be met by the limited number of eggs that were available in the agar in the glass vials.

Heat peaks affected voracity of the different sexes and stages of *M. pygmaeus* and *N. tenuis* differently, as it was visible in the strong interaction effects for the factors sex/stage and temperature for both species. Female *M. pygmaeus* did not differ in their prey consumption under different temperature regimens, whereas female *N. tenuis* consumed the provided prey almost completely

	N	Movement counts [mean ± SE]		z	p	
		Dark	Light			
<i>Macrolophus pygmaeus</i>						
Nymphs	25	62	10.66 ± 1.24	11.03 ± 1.47	-0.747	0.455
	30	59	34.67 ± 2.79	13.51 ± 1.71	-6.318	<0.001
	40	50	11.63 ± 2.13	5.87 ± 0.95	-1.892	0.058
Females	25	56	58.25 ± 4.06	18.26 ± 2.14	-6.354	<0.001
	30	62	67.65 ± 5.51	14.64 ± 1.92	-6.846	<0.001
	40	49	13.85 ± 2.92	11.64 ± 1.35	0.214	0.831
Males	25	67	40.15 ± 3.15	11.60 ± 1.50	-6.971	<0.001
	30	53	60.38 ± 4.97	8.34 ± 1.15	-6.334	<0.001
	40	57	19.65 ± 2.41	5.51 ± 0.94	-5.363	<0.001
<i>Nesidiocoris tenuis</i>						
Nymphs	25	50	12.28 ± 1.36	13.13 ± 1.86	-0.502	0.616
	30	56	25.02 ± 2.46	15.52 ± 2.29	-4.005	<0.001
	40	57	8.47 ± 1.24	8.65 ± 1.36	-0.218	0.827
Females	25	55	32.72 ± 4.10	40.65 ± 7.14	0.884	0.377
	30	42	35.80 ± 5.69	40.24 ± 7.44	0.319	0.75
	40	53	25.20 ± 2.83	45.91 ± 5.72	3.829	<0.001
Males	25	52	46.70 ± 5.60	10.30 ± 2.17	-6.238	<0.001
	30	28	31.26 ± 6.45	12.59 ± 2.86	-2.459	0.014
	40	34	25.08 ± 3.37	21.18 ± 3.24	-0.675	0.499

Note: Bold values for *p* indicate statistical significance ( $p < 0.05$ ).

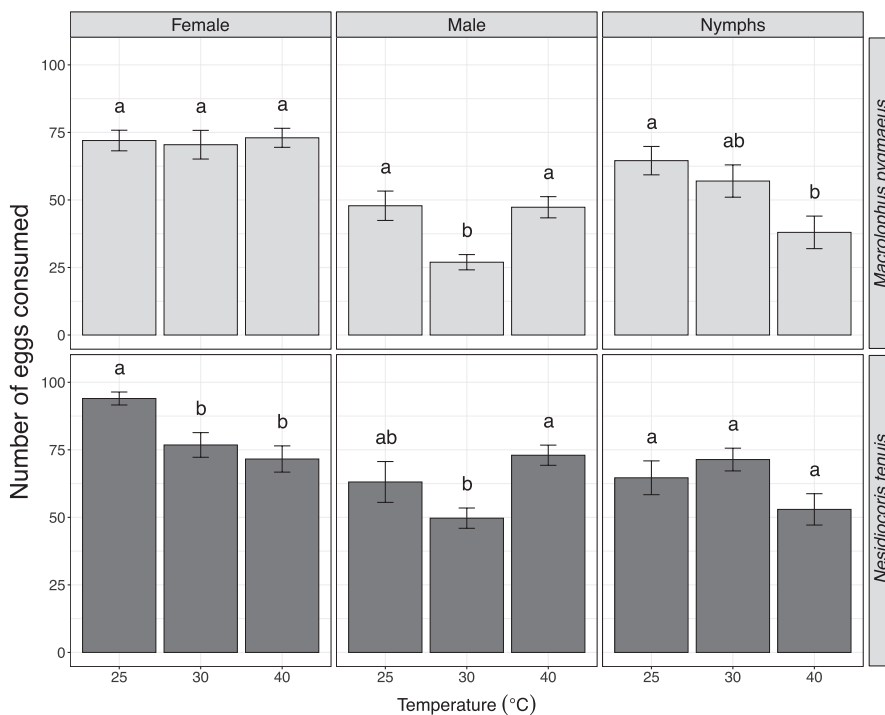


FIGURE 6 Predatory efficacy of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* under different temperature regimes during 24 h. Columns followed by a different letter show significant differences between treatments (GLMs with negative binomial distribution and log link function, followed by Tukey's post hoc tests adjusted,  $p \leq 0.05$ ).

at constant 25°C and decreased in their voracity towards a level similar to *M. pygmaeus* at 40°C heat peaks. Interestingly males of both species consumed the least prey at intermediate conditions of 30°C heat peaks. Feeding decisions in omnivorous predators

are complex and the plant context needs to be considered when interpreting observations (Gillespie & McGregor, 2000). Mirids use plants not only as food (Castañé et al., 2011; Pérez-Hedo & Urbaneja, 2016) but also to satisfy their needs for water (Gillespie

& McGregor, 2000). How any shift from zoophagy to phytophagy under changing temperature regimens might have affected our results has not been investigated.

Across the three different experiments only *M. pygmaeus* nymphs were consistently affected by one of the regimens (i.e. the 40°C regimen). It seems that this high temperature impairs nymphs of this species strongly. No further consistent effects of temperature regimens across experiments were detected for adult *M. pygmaeus* or both stages of *N. tenuis*. While in the case of *M. pygmaeus*, temperature affected female activity but not efficacy, *N. tenuis* female efficacy but not activity was affected. No consistent effects on location were detected either. However, the experiments cannot directly be compared. In the activity tubes, the prey availability was relatively low (i.e., maintenance diet with *E. kuehniella* eggs) and required higher energy expenditure for access, while the period of exposure to high temperatures was longer (48 h). In the efficiency test, the density of available prey was higher and the time of exposure to high temperatures was shorter (24 h). Furthermore, insects on the tomato plants were able to evade unsuitable temperatures to a certain extent by changing their location, whereas they were fully exposed in the tubes and dishes for the other experiments.

Biological processes ranging from growth and development to feeding and dispersal are determined by metabolic processes directly influenced by temperature. Heat peaks thus influence physiology as well as behavioural interactions between species and trophic interactions (Gillespie et al., 2012; Sentis et al., 2013). Sometimes behavioural changes may explain observed ecological changes related to increased temperatures even better than the physiological and metabolic processes alone (Abram et al., 2017). In the case of predators, extreme heat events have been reported to have a strong effect on aspects such as locomotion, biology, niche preference and efficacy (Damien & Tougeron, 2019; González-Tokman et al., 2020; Halsey, 2016; Laws, 2017). In our study, we found strong impacts of the heat peaks on the mirids evident in altered locomotion, diurnal rhythm, preferred location and altered efficacy. It is likely, that some of these effects are buffered under natural conditions when mirids are able to seek out their preferred habitat or have access to various food sources. Yet the observed effects point to abilities to deal with temperature peaks in the two species. Both predatory mirids act against tomato pests in greenhouses and compete for prey when co-occurring in the same habitat (Duarte Martínez et al., 2022). It is possible that in the case of *N. tenuis* and *M. pygmaeus* the competition would be reduced, due to the different preferred location on the plant, different response to temperature peaks and different daytime activity. Indeed, enhanced control of *T. absoluta* due to complementarity of the two predators was suggested (Konan et al., 2021; Lampropoulos et al., 2013) and sustained biocontrol efficiency over 10 weeks was observed when both predators were applied together in greenhouse compartments (Yao et al., 2022). Future studies should investigate if the two species are able to coexist long-term and if their concurrent presence could ultimately also ensure pest control over a wider temperature range, with *M. pygmaeus* dominating at lower, and *N. tenuis* at higher temperatures.

## AUTHOR CONTRIBUTIONS

**Leticia Duarte Martínez:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; visualization; writing – review and editing. **Jörg Romeis:** Conceptualization; funding acquisition; supervision; writing – review and editing. **Jana Collatz:** Conceptualization; formal analysis; methodology; supervision; writing – original draft.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

All raw data are stored in a public repository: <https://doi.org/10.6084/m9.figshare.21988241.v2>.

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