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Temperature-Driven Selection of Predatory Mirid Bugs for Improving Aphid Control in Sweet Pepper Crops

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Abstract: Recent studies have suggested that predatory mirid bugs may be an effective alternative for controlling aphids in sweet pepper greenhouses. This study examined the effect of three constant temperatures (18, 24, and 30 °C) on the life history traits of predatory mirid species *Nesidiocoris tenuis*, *Macrolophus pygmaeus*, and *Dicyphus bolivari*, preying on the aphid *Myzus persicae nicotianae* in sweet pepper plants. As the temperature increased, the survival rate decreased for *M. pygmaeus* and *D. bolivari*, while for *N. tenuis*, it was the opposite, and their survival increased. When considering all the biological traits studied together, the estimated intrinsic rate of increase (r_m) decreased as the temperature increased for *M. pygmaeus* and *D. bolivari*. In contrast, the rate increased as the temperature increased for *N. tenuis*. These results suggest that *M. pygmaeus* and *D. bolivari* are better adapted to lower temperatures than *N. tenuis*, which is more adapted to warm temperatures. The implications of these results for improving the biological control of aphids in sweet pepper greenhouses through the use of mirid bugs are discussed in relation to different temperature regimes.

Keywords: *Myzus persicae nicotianae*; *Nesidiocoris tenuis*; *Macrolophus pygmaeus*; *Dicyphus bolivari*; biological control

1. Introduction

Sweet pepper cultivation in greenhouses is one of the largest crops in which the use of augmentative biological control is more established in the Mediterranean basin [1,2]. Most sweet pepper pests can be controlled by releasing natural enemies, so pesticides are not commonly used. The main pest in sweet pepper cultivation in greenhouses is western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Tripidae), primarily because they act as a vector of the tomato spotted wilt virus (TSWV) [3,4]. Additionally, the sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a significant secondary pest that harms crops by removing sap and degrading fruit quality through the accumulation of sooty mold [5,6].

Two significant milestones have taken place in this crop to deal with these two key pests [7]. Firstly, from the end of the 1990s, the use of the predatory mite *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) and the minute pirate bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) for thrips control became common [8], whereas at the beginning of this decade, the parasitoid *Eretmocerus mundus* Mercet started to be released for whitefly control [6,9,10]. The combined use of *A. cucumeris* and *O. laevigatus* was highly successful in controlling thrips in cropping systems where planting typically occurred in late fall and harvests ended in late summer due to favorable weather conditions for the establishment of both natural enemies [11] and low pest pressures at the beginning of the crop [12]. Nevertheless, in cropping systems where planting occurs in summer and ends in the following spring, this biocontrol approach was not effective, mainly due to the adverse weather conditions of high temperature and low relative humidity [13,14] and the high pest pressure at the beginning of the crop [15]. Furthermore, the use of *E. mundus* significantly

reduced but did not eliminate the need for additional pesticide treatments against *B. tabaci* [16]. This second milestone occurred with the emergence of the biological control market of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) [17]. The replacement of *A. cucumeris* with *A. swirskii* in the biocontrol package for this crop, which is well-adapted to arid conditions [18,19], resulted in the effective control of both *B. tabaci* and *F. occidentalis*, as this phytoseiid is highly effective against both pests under a wide range of climatic conditions [15,20]. Since incorporating *A. swirskii* into the pest control program for sweet pepper, biological control has become widespread in almost all sweet pepper cropping areas (i.e., approximately 8500 ha in Southeastern Spain) [1,7,21,22]. However, managing some secondary pests, such as aphids, still requires selective pesticide applications [1]. Despite the availability of commercial biocontrol agents for common aphid species in sweet pepper, such as *Myzus persicae* (Sulzer), *Myzus persicae nicotianae* Blackman, *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae), they are not always effective enough [23,24]. The need for multiple releases of natural enemies increases the final cost of the biocontrol program for this crop [25–27].

One alternative to improve the biological control of aphids might be the use of predatory mirid bugs (Hemiptera: Miridae), which, due to their zoophytophagous behavior, can establish in a sweet pepper crop before aphid infestations [25,28–30]. Previous studies suggest the positive role that predatory mirids *Nesidiocoris tenuis* (Reuter), *Macrolophus pygmaeus* Rambur, and *Dicyphus bolivari* Lindberg (Hemiptera: Miridae) could have in controlling aphids in sweet pepper plants [25,28,31,32]. Pérez-Hedo et al. (2015) demonstrated that females of the three predatory mirids, *N. tenuis*, *M. pygmaeus*, and *D. bolivari*, actively preyed on *M. persicae* and were strongly attracted to the odor of infested *M. persicae* plants. Furthermore, the three species of mirids could reproduce on aphids and establish themselves on sweet pepper plants at temperatures between 23 and 27 °C, relative humidity of 65 ± 10%, and under natural photoperiods (approximately 14L: 10D), significantly reducing the number of *M. persicae* per leaf. The performance of the three species of mirids in reducing *M. persicae* populations was similar in these experiments [28]. Going further, Bouagga et al. [30] tested the combined use of either *N. tenuis* or *M. pygmaeus* with *A. swirskii* as a potential method for controlling thrips, whiteflies, and aphids in sweet pepper crops. These authors demonstrated that integrating one of these mirids with the already established predator *A. swirskii* could enhance sweet pepper pest management since a satisfactory control of the three pests was accomplished. However, the effectiveness of these predatory mirid bugs may vary depending on temperature, and in sweet pepper crops, different climatic conditions could be found depending on the planting period. The climatic conditions will impact these predatory mirids' establishment, spread, and overall effectiveness. Laboratory experiments were conducted to study the influence of temperature on the predation capacity and life history traits of these three mirid species when feeding on the tobacco aphid, *M. persicae nicotianae* Blackman.

2. Materials and Methods

2.1. Experimental Insects

The red clonal colonies of the tobacco aphid, *M. persicae nicotianae*, were initiated in 2013 with individuals collected in sweet pepper fields located in the Valencia province (Spain). These colonies were maintained on pesticide-free potted sweet pepper plants (var." Lipari," Clause Spain S.A.U., Almería, Spain) and housed in a climatic chamber at 25 ± 2 °C, 60–80% RH and a 16:8 h (L:D) photoperiod at Instituto Valenciano de Investigaciones Agrarias (IVIA). Rearing took place in screened cages (120 × 70 × 125 cm), where groups of six sweet pepper plants (approximately 25 cm high) were introduced weekly.

Dicyphus bolivari individuals were initially collected in Valencia province (Spain) tomato fields. They were then reared on pesticide-free tomato seedlings "Optima" (Seminis Vegetable Seeds, Inc., Almería, Spain) using frozen eggs of the factitious prey *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) as food, while *N. tenuis* and *M. pygmaeus*

adults were obtained from a commercial supplier (NESIBUG® and MYRICAL®; Koppert Biological Systems, S.L., Águilas, Murcia, Spain). Each bottle held about 500 individuals, including both mature nymphs and young adults that were no more than three days old. The specimens were fed a diet of *E. kuehniella* eggs (FJ Calvo, Koppert BS; Personal Communication).

2.2. Predation Capacity

The predatory capacity of female individuals from the three studied species of Miridae was evaluated and compared at three constant temperatures: 30 ± 1 °C, 24 ± 1 °C, and 18 ± 1 °C, representing the thermal regimes found in protected sweet pepper crops. Three plastic cages measuring $60 \times 60 \times 60$ cm (BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan) were each populated with one of the three tested predatory mirid species, with 95–100 adult mirids of each species (2–4 days old). In each cage, the individuals were starved of prey for 24 h before use. Additionally, four non-infested sweet pepper plants without flowers (25 cm high) were introduced in each cage during the prey starvation period to allow the mirids to adapt to sweet pepper plants. None of the three species of mirids exhibited cannibalistic behavior. *Myzus persicae nicotianae*-infested leaves were detached from infested plants of the stock colony. Using a stereoscopic binocular microscope and a small brush, the fourth instar nymphs of *M. persicae nicotianae* were carefully chosen and transferred onto sweet pepper leaves in Petri dishes (9 cm in diameter). The fourth nymphal instars of *M. persicae nicotianae* were readily distinguishable based on their morphology [33]. Each of the three predator species' females was exposed to 20 fourth instar nymphs, with soaked cotton plugs providing water. For each temperature and species, fifteen replicates were carried out. After 24 h, the predators were removed from the arenas, and the number of nymphs consumed was determined.

2.3. Developmental Time and Juvenile Survivorship

Ten adult couples (approximately less than 4 days old) of each predator species were released separately in methacrylate cages ($40 \times 40 \times 40$ cm) with four sweet pepper plants for 24 h. After that, plants bearing predator eggs were removed and cut into small sections, placed on Petri dishes (60 mm in diameter) with filter paper to avoid excess humidity, and were maintained at three studied temperatures 18 °C, 24 °C, and 30 °C. Egg hatching was monitored daily by examining the dishes. Any newly hatched nymphs less than 24 h old were transferred individually into 30 mm Petri dishes. The predators were provided with second to third-instar nymphs of *M. persicae nicotianae*, sweet pepper leaf disks with a diameter of 20 mm, and water-soaked cotton wool to serve as a water source. Around 10–12 nymphs of *M. persicae nicotianae* were added per Petri dish, which always exceeded the daily consumption rate of nymphs for the three tested mirids. The development and survival of the nymphs were observed daily using a stereomicroscope until they reached adulthood or died. The Petri dishes were kept in a climatic chamber at three different temperatures, with a relative humidity of $65 \pm 5\%$ RH and a photoperiod of 14:10 h (L:D).

2.4. Reproductive Parameters

Newly emerged adults of *N. tenuis*, *M. pygmaeus*, and *D. bolivari*, which were reared from nymphs at three different temperatures (18 °C, 24 °C, and 30 °C), were transferred in pairs (one female and one male) to plastic cups (370 cm^3) containing a tender apical sweet pepper flush (approximately 14 cm) with second and third instar nymphs of *M. persicae nicotianae* that were provided as food *ad libitum*. The water in the bottom cup was accessed by pushing the apical sweet pepper flushes through a hole in the inner cups. Following the methodology of Abbas et al. [34], adapted from Sánchez et al. [35], the larger plastic cups were positioned inside smaller ones (230 cm^3) and filled with water. The bigger cups were then secured with a rubber band and covered with a fine mesh.

Every 5 days, pairs of adults were relocated to a fresh plastic cup with fresh *M. persicae nicotianae* nymphs until the female's death. A young male (obtained from the corresponding

immature bioassay) was given to each female to guarantee to mate during her lifetime, and when it perished, it was substituted. Every day, adult mortality was monitored, and every other day, the hatching of eggs laid in the cups by the females was observed. To determine the sex ratio of the offspring, female offspring were reared to adulthood in the same containers and under the same environmental conditions as their birthplace and provided with ad libitum *E. kuehniella* eggs.

2.5. Demographic Growth Indexes

Demographic indices were then calculated and compared for the three studied constant temperatures (18 °C, 24 °C, and 30 °C). Daily survival values and the number of offspring produced by the females were used to construct life tables. The intrinsic rate of increase (r_m; females/female/day), the net reproductive rate (R_0; female eggs/female), and generation time (T; days) following demographic growth parameters were generated [36,37]. The standard error for r_m, R_0 and T was estimated using the Jackknife technique on raw data. This involved calculating the per capita r_m, R_0 and T by omitting one replicate per trial and repeating this process until pseudo-values were obtained for all possible omission cases. The standard error was then computed using the Jackknife formula. [38].

2.6. Statistical Analysis

The effect of the predator species on the predation capacity and life history traits (development time from eggs to adult and per each nymphal stage; lifetime fertility, female longevity; progeny sex ratio, and the three demographical indexes) for each temperature were subjected to a two-way analysis of variance and Tukey's test was used for mean separation ($p < 0.05$).

3. Results

3.1. Predation Capacity

The three species of mirids preyed on *M. persicae nicotianae* at the three tested temperatures (Figure 1). *Nesidiocoris tenuis* preyed on more nymphs of *M. persicae nicotianae* than *M. pygmaeus* and *D. bolivari* ($F_{2,126} = 23.78$, $p < 0.001$), and predation was significantly different between the temperatures ($F_{2,126} = 17.38$, $p < 0.001$). An interaction between the species and temperature was found ($F_{2,126} = 6.28$, $p = 0.0001$). As depicted in Figure 1, the positive response of *N. tenuis* to increasing temperatures was distinct from the other two species.
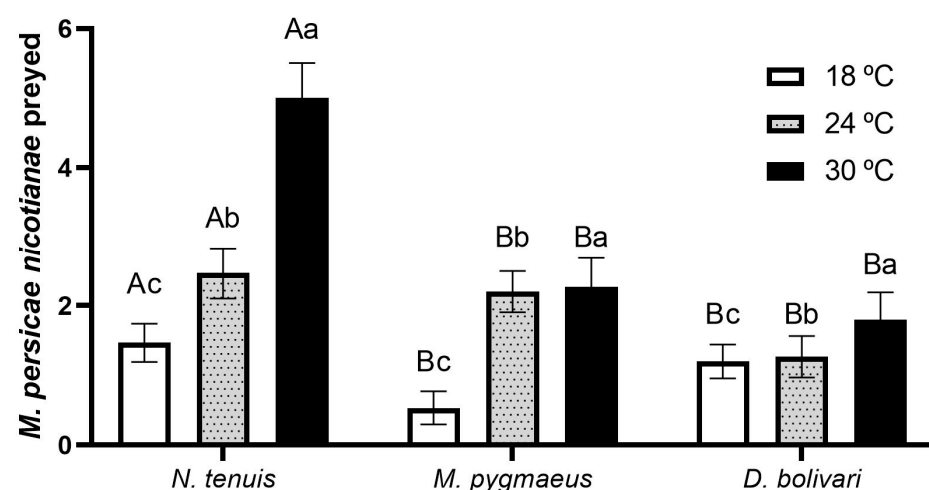
Figure 1. Number (mean ± SE) of fourth instar nymphs of *M. persicae nicotianae* consumed by females of *N. tenuis*, *M. pygmaeus* and *D. bolivari* on sweet pepper leaf discs at 18, 24 and 30 ± 1 °C and 14:10 h L:D. Uppercase letters indicate significant differences between mirid species, while lowercase letters denote differences between temperatures (Two-Way ANOVA; Tukey-test; $p < 0.05$).

3.2. Developmental Time and Survivorship of Immatures

The duration of the egg stage was longer in *D. bolivari* compared to *M. pygmaeus*, and *M. pygmaeus* had a longer duration than *N. tenuis* ($F_{2,467} = 4263$, $p < 0.001$) (Figure 2A). Additionally, significantly different durations were observed among each of the three temperatures studied ($F_{2,467} = 383.4$, $p < 0.001$), with a significant interaction existing between both factors (species and temperature) ($F_{4,467} = 37.56$, $p < 0.001$), indicating that each species responded differently to the temperature.
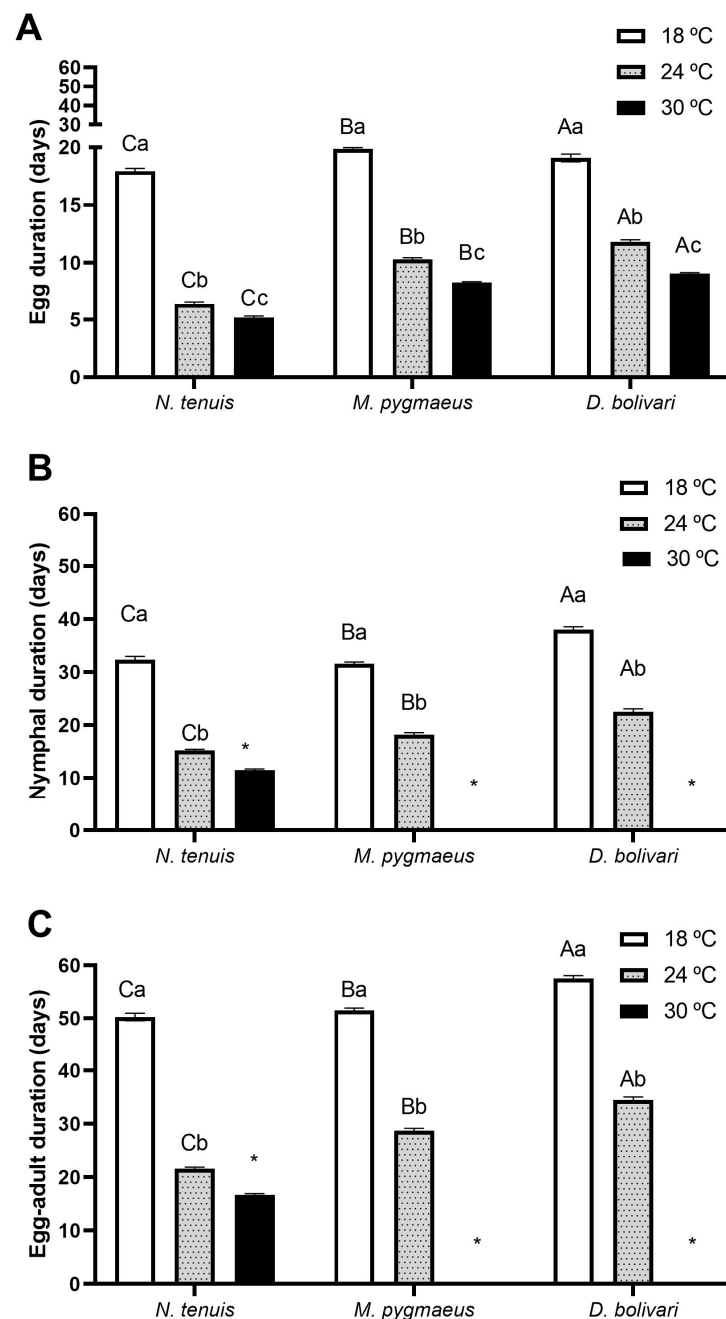
Figure 2. Mean (± SE) duration (days) of egg (**A**), nymphs (**B**) and total development from egg to adult (**C**) of *N. tenuis*, *M. pygmaeus* and *D. bolivari* preying on *M. persicae nicotianae* on sweet pepper leaf discs at 18, 24 and 30 ± 1 °C and 14:10 h L:D. Uppercase letters indicate significant differences between mirid species, while lowercase letters denote differences between temperatures (Two-Way ANOVA; Tukey-test; $p < 0.05$). * At 30 °C, neither *M. pygmaeus* nor *D. bolivari* completed their development, so the two-way ANOVA was only applied to 18 °C and 24 °C.

Development of *M. pygmaeus* and *D. bolivari* only occurred at 18° and 24 °C, whereas *N. tenuis* could develop at the three temperatures tested (Figure 2B,C). The duration of the nymph stage and the total duration from egg to adult varied among the three mirid species, with *D. bolivari* exhibiting the longest duration, *M. pygmaeus* showing an intermediate duration, and *N. tenuis* displaying the shortest duration (Nymphs: $F_{2,213} = 90.49$, $p < 0.001$; Total: $F_{2,213} = 152.2$, $p < 0.001$). Both periods had longer durations at 18 °C compared to 24 °C (Nymphs: $F_{1,213} = 1596$, $p < 0.001$; Total: ($F_{1,213} = 3091$, $p < 0.001$). Additionally, an interaction was observed between both factors (Nymphs: $F_{2,213} = 10.37$, $p < 0.001$; Total: ($F_{2,213} = 21.53$, $p < 0.001$).

The survival of the three predatory mirid bugs at the three temperatures assayed is shown in Figure 3. The temperature significantly influenced the survival of the three mirid species ($F_{2,412} = 32.44$, $p < 0.001$), which also varied among the species ($F_{2,412} = 17.78$, $p < 0.001$). The survival of *M. pygmaeus* and *D. bolivari* was similar, decreasing as the temperature increased. This trend was different for *N. tenuis*, whose survival increased at higher temperatures, resulting in an interaction between the two factors analyzed (species and temperature) ($F_{2,412} = 18.47$, $p < 0.001$).
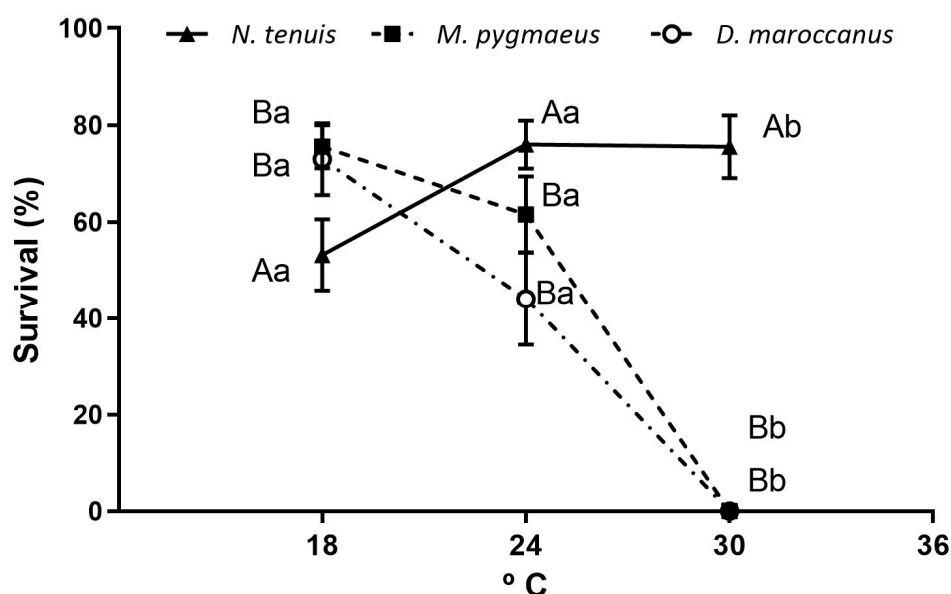
Figure 3. Instar-specific survival (%) of the immature stages of *N. tenuis*, *M. pygmaeus* and *D. bolivari* when preying on *M. persicae nicotianae* on sweet pepper leaf discs at 18, 24 and 30 ± 1 °C and 14:10 h L:D. Uppercase letters indicate significant differences between mirid species, while lowercase letters denote differences between temperatures (Two-Way ANOVA; Tukey-test; $p < 0.05$).

3.3. Reproductive Parameters

The temperature significantly affected lifetime fertility ($F_{1,72} = 16.98$, $p < 0.001$), female longevity ($F_{1,73} = 41.46$, $p < 0.001$), and the progeny sex-ratio ($F_{1,48} = 9.945$, $p = 0.003$) for the three predatory mirid bugs tested (Table 1). Because no nymph of *M. pygmaeus* and *D. bolivari* reached adulthood, reproductive parameters could only be obtained for *N. tenuis* at 30 °C. *Dicyphus bolivari* and *M. pygmaeus* produced significantly more nymphs than *N. tenuis* ($F_{2,72} = 6.253$, $p = 0.003$). Although the progeny of *N. tenuis* at 18 °C was almost negligible, it increased at higher temperatures, in contrast to the decrease observed in *M. pygmaeus* and *D. bolivari*. This resulted in a significant interaction between the two factors under study ($F_{2,72} = 19.0$, $p < 0.001$). Female longevity was higher in *D. bolivari* than in *M. pygmaeus* and *N. tenuis* ($F_{2,73} = 12.58$, $p < 0.001$). An interaction was found for female longevity between the temperature and species ($F_{2,73} = 3.729$, $p = 0.029$). The sex ratio was significantly skewed toward females in the case of *D. bolivari* and *N. tenuis* when compared to *M. pygmaeus* ($F_{2,48} = 14.27$, $p < 0.001$), with no interaction found between the temperature and species ($F_{2,48} = 0.1532$, $p = 0.858$).

Table 1. Mean ($\pm$ SE) lifetime fertility (total offspring produced/female), female longevity (days) and progeny sex ratio (percentage of females/total offspring) of *N. tenuis*, *M. pygmaeus* and *D. bolivari* preying on *M. persicae nicotianae* on sweet pepper leaf discs at 18, 24 and 30 $\pm$ 1 °C and 14:10 h L:D. Uppercase letters indicate significant differences between mirid species, while lowercase letters denote differences between temperatures (Two-Way ANOVA; Tukey-test; $p < 0.05$). * At 30 °C, neither *M. pygmaeus* nor *D. bolivari* completed their development so two-way ANOVA was only applied to 18 °C and 24 °C.

| Temp.	Instar	*N. tenuis*	*M. pygmaeus*	*D. bolivari*
18 °C	Lifetime fertility	0.9 $\pm$ 0.5 Ba (n = 10)	12.2 $\pm$ 1.8 Aa (n = 13)	20.6 $\pm$ 5.3 Aa (n = 10)
	Female longevity	29.4 $\pm$ 4.2 Ba (n = 11)	44.1 $\pm$ 2.6 Ba (n = 13)	75.5 $\pm$ 13.4 Aa (n = 10)
	Progeny sex-ratio	75.0 $\pm$ 25.0 Aa (n = 4)	52.8 $\pm$ 2.1 Ba (n = 12)	93.1 $\pm$ 3.6 Aa (n = 7)
24 °C	Lifetime fertility	7.3 $\pm$ 1.0 Bb (n = 21)	3.2 $\pm$ 0.7 Ab (n = 12)	2.7 $\pm$ 0.7 Ab (n = 10)
	Female longevity	17. 1 $\pm$ 2.0 Bb (n = 21)	17.8 $\pm$ 3.6 Bb (n = 12)	29.3 $\pm$ 3.8 Ab (n = 10)
	Progeny sex-ratio	50.9 $\pm$ 7.0 Ab (n = 16)	42.0 $\pm$ 6.2 Bb (n = 5)	75.8 $\pm$ 9.2 Ab (n = 6)
30 °C	Lifetime fertility	6.4 $\pm$ 1.6 (n = 11)	*	*
	Female longevity	7. 2 $\pm$ 1.3 (n = 11)	*	*
	Progeny sex-ratio	62.0 $\pm$ 0.1 (n = 6)	*	*

Age-specific fertility depended on the age of the female, and this relationship was stronger as temperatures increased (Figure 4). At 18 °C (Figure 4A), the shape of the age-specific fertility curve was very smooth for *D. bolivari*: the rate slowly increased until around 2 nymphs per day 30 days after emergence, which was maintained for almost 40 additional days and then decreased. Similarly, *M. pygmaeus* reached around 3 nymphs per day 10 days after emergence, holding this rate for about 10 extra days, then slowly falling until day 35. The daily fertility for *N. tenuis* was almost nil at this temperature. At 24 °C (Figure 4B), the shape of the age-specific fertility curve changed considerably: it continuously and sharply decreased from the beginning for *N. tenuis* and *M. pygmaeus*, whereas it was smoother for *D. bolivari*. At 30 °C (Figure 4C), *N. tenuis* behaved similarly at 24 °C.

3.4. Demographic Indexes

The generation time (T) significantly decreased as the temperature increased in the three predatory mirid species ($F_{1,72} = 12{,}733$, $p < 0.001$) and was significantly different between the three predatory mirids ($F_{2,72} = 1644$, $p < 0.001$) (Figure 5A). Generation time was higher for *D. bolivari* than for *M. pygmaeus*, and these values for both species were higher than that of *N. tenuis* at 18 and 24 °C, reflecting more accelerated reproduction for *N. tenuis*. Because both *D. bolivari* and *M. pygmaeus* could not successfully develop at 30 °C, generation time was zero, whereas *N. tenuis* was similar to that obtained at 24 °C. The estimated reproductive rate (R_0) was significantly different between temperatures ($F_{1,72} = 2026$, $p < 0.001$) and species ($F_{2,72} = 996.5$, $p < 0.001$) (Figure 5B). For *M. pygmaeus* and *D. bolivari*, R_0 decreased as the temperature increased to reach the value of zero at 30 °C. However, in the case of *N. tenuis*, this reproductive parameter increased from 18 °C to 24 °C and slightly decreased to 30 °C. The estimated intrinsic rates of increase (r_m) were significantly different between temperatures ($F_{1,72} = 72.31$, $p < 0.001$) and species ($F_{2,72} = 318.5$, $p < 0.001$) (Figure 5C). The intrinsic rate of increase decreased as temperatures

increased for *M. pygmaeus* and *D. bolivari,* whereas it increased for *N. tenuis* (Figure 5C). An interaction was found between the temperature and mirid species for the estimated demographic parameters (*T*: $F_{2,72} = 156.5$, $p < 0.001$; R_0: $F_{2,72} = 1678$, $p < 0.001$ and r_m: $F_{2,72} = 5985$, $p < 0.001$).
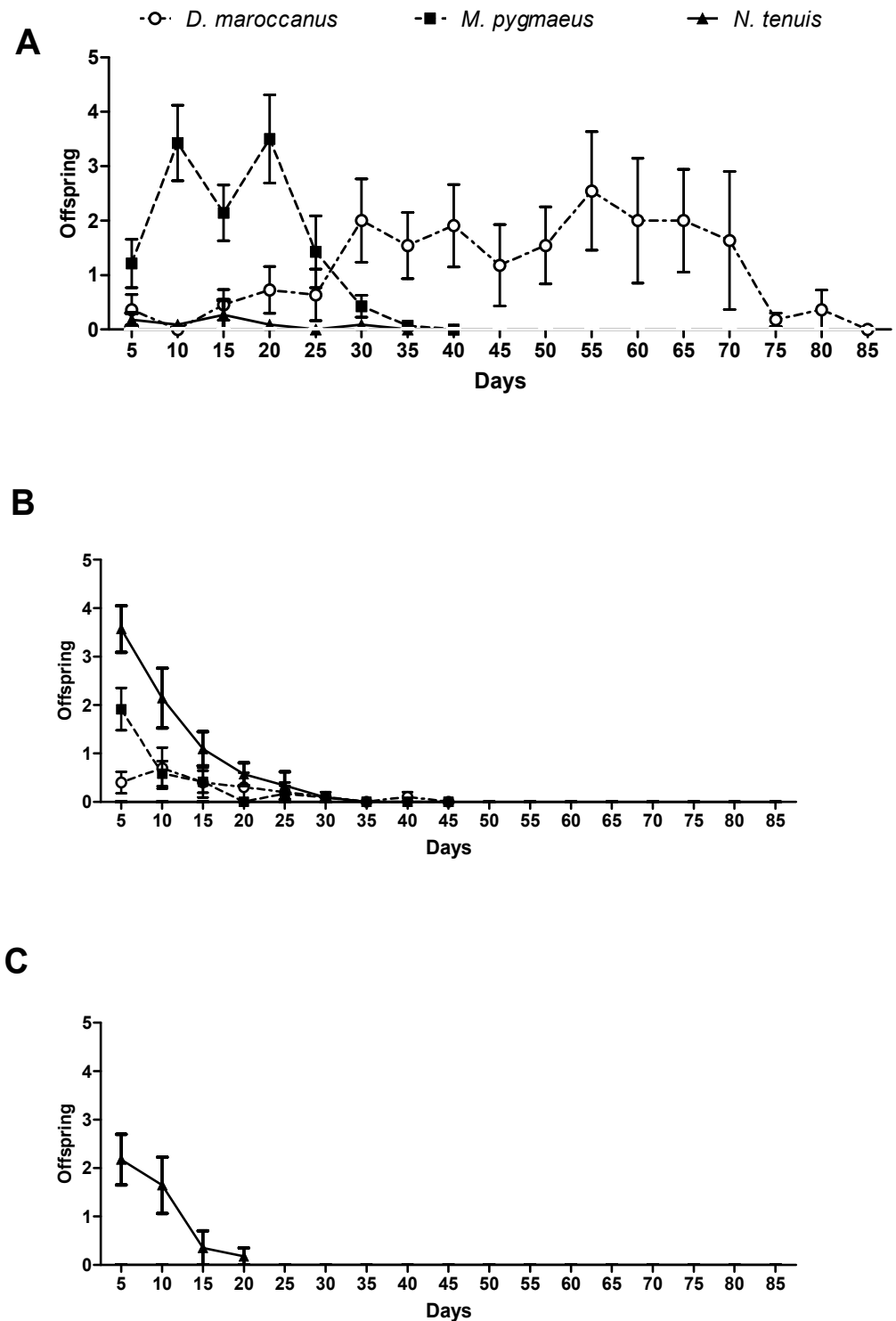
Figure 4. Age-specific fertility (number of N_1 produced/female/day $\pm$ SE) of *N. tenuis*, *M. pygmaeus* and *D. bolivari* preying on *M. persicae nicotianae* on sweet pepper leaf discs at 18 (**A**), 24 (**B**) and 30 $\pm$ 1° C (**C**) and 14:10 h L:D.
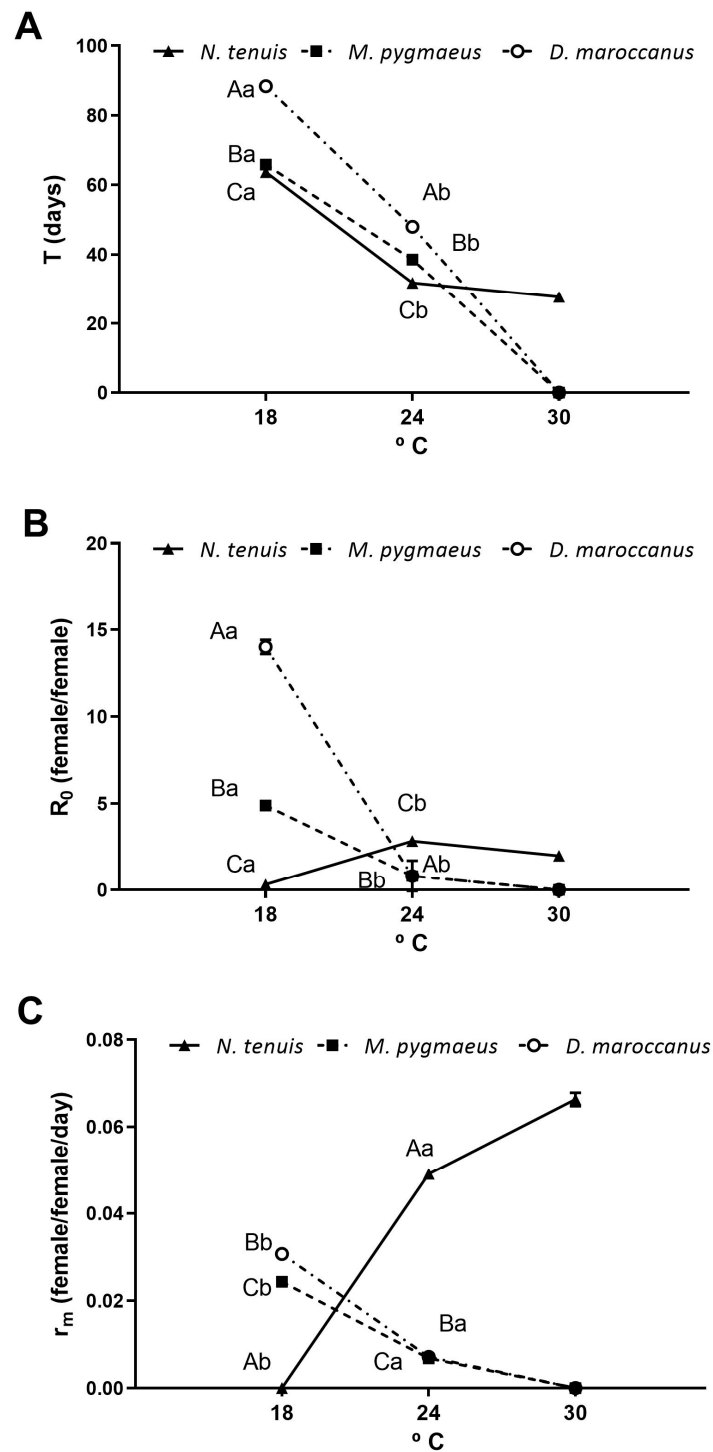
Figure 5. Mean (± SE) generation time T (days) (**A**), net reproductive rate R_0 (females/female) (**B**) and intrinsic rate of increase r_m (females/female/day) (**C**) for *N. tenuis*, *M. pygmaeus* and *D. bolivari* preying on *M. persicae nicotianae* on sweet pepper leaf discs at 18, 24 and 30 ± 1 °C and 14:10 h L:D. Means values accompanied by the same letter are not significantly different (Tukey test; $p > 0.05$). Uppercase letters indicate significant differences between mirid species, while lowercase letters denote differences between temperatures (Two-Way ANOVA; Tukey-test; $p < 0.05$). At 30 °C, neither *M. pygmaeus* nor *D. bolivari* completed their development so two-way ANOVA was only applied to 18 °C and 24 °C.

4. Discussion

In this study, we provide a detailed and comprehensive analysis of the influence of temperature on the biology of three mirids that are susceptible to use against aphids in sweet pepper crops. Our results showed that life history parameters varied considerably among the three predator species and were consistent with many previous studies on the influence of temperature on the three mirid species studied in this work [31,35,39–51]. The difference in temperature sensitivity between *N. tenuis*, *M. pygmaeus* and *D. bolivari* was widely known. Our results confirm that when preying on *M. persicae nicotianae* on sweet pepper, *N. tenuis* was more thermophilic compared to *M. pygmaeus* and *D. bolivari*. Of the three species, *D. bolivari* was the best-adapted species for temperate climates. These differential traits between the two species explain their different geographical distribution [52,53]. As an illustration, in the Iberian Peninsula, *M. pygmaeus* and *D. bolivari* were predominantly located in the northern region, while *N. tenuis* was commonly found in the southern region [53]. This geographical distribution could be associated with the temperature variation from north to south. In addition to their geographical distribution, their adaptation to temperatures was also related to their varying crop presence in regions where these species overlapped. In fact, in the horticultural crops of the Valencian Community (eastern half of the Iberian Peninsula), it is typical for *D. bolivari* to appear and establish itself at the start of the crop when average temperatures are below 20 °C, only to disappear and be replaced by *N. tenuis* in the spring when temperatures consistently exceed 20 °C.

In addition to temperature, intraguild interactions should also be considered in the coexistence of mirid species [54–56]. However, a comprehensive analysis of all potential interactions is necessary when accounting for the factors that may affect them. Apart from temperature, other factors are equally or even more critical in impacting these intraguild interactions, such as the type and availability of prey or crops [54,56–59]. As an example of this complexity, for *N. tenuis* and *D. bolivari*, the main cause of temporal displacement in horticultural open fields of the Valencian Community was *N. tenuis*'s superior competitive ability due to its better adaptation to higher temperatures and ability to deplete food sources for *D. bolivari* [55]. Contrary to this, in a controlled climate such as the Dutch greenhouses, releasing *D. bolivari* from the nursery could regulate unwanted infestations of *N. tenuis*. *Dicyphus bolivari* acts as an intraguild predator, reducing the plant damage caused by *N. tenuis* [60].

Among the previously described factors that can have a greater impact on the biology of mirids is the type of prey they consume [61–63]. Our work demonstrates that *M. persicae nicotianae* is not the most effective prey for development, as its biotic potential is relatively low compared to the results obtained in other studies with different types of prey [35,39,43,48,63,64]. For example, Perdikis and Likouressis [63] observed that *M. pygmaeus,* when feeding on *M. persicae,* had better biological parameters than those obtained in the present study with *M. persicae nicotianae*. Even *M. pygmaeus* could complete its cycle and reproduce at 30°, which was not achieved in our research. Therefore, this seemed to confirm the lower suitability of *M. pygmaeus nicotianae* as prey compared to *M. persicae*. In addition to the already known drawbacks of *M. persicae nicotianae* in comparison to *M. persicae sensu stricto*, such as (i) having evolved an enhanced tolerance to pyridine alkaloid nicotine, a natural insecticide produced by tobacco and other Solanaceae [65], and (ii) showing reduced sensitivity to neonicotinoids due to their chemical relationship with nicotine [66], our results also demonstrated its inferior quality as prey for mirids. Jalali and Michaud [67] already observed that *M. persicae nicotianae* had lower nutritional value for the ladybug *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) when aphids were developed on sweet pepper.

Our results clearly show the effect of temperature on the three mirid species. Temperature can affect the efficacy of predatory mirids in several ways, including (i) their development rate, survival and reproductive success: temperature changes, which can alter the development time and survival of mirids and can also affect their ability to reproduce and control pest populations, and (ii): their activity level: predatory mirids are ectothermic,

meaning their body temperature is dependent on the environment, and therefore, changes in temperature can affect their metabolism and activity levels, leading to changes in their ability to locate and consume prey. The intrinsic rate of development of *D. bolivari* and *M. pygmaeus* decreases as temperature increases, while the opposite occurs with *N. tenuis*. However, another critical parameter that is known for practical use in biological control strategies is its predation potential related to activity level. This work shows how the three species prey on different amounts of aphids in 24 h at the three studied temperatures. *Nesidiocoris tenuis* maintains the highest prey ratios at all three temperatures, especially at 30 °C, doubling the number of prey consumed compared to the other two species. Therefore, although this temperature is a crucial factor and its influence is evident in various studied biological parameters, relying solely on the intrinsic rate of development as a precise parameter from which to infer its predation capacity and potential as a biological control agent may not be accurate. To this end, van Lenteren et al. [68] developed the term "pest kill rate," where all the biological parameters were integrated with the prey capacity. The pest kill rate is the average number of pests killed per day during the lifespan of the natural enemy in question. This kill rate is, therefore, a much more accurate parameter for comparing predators than the intrinsic rate of development. However, the design of our study did not include this possibility, and we did not approach the experimental design by evaluating the parameters necessary to estimate the kill rate; certainly, for future work, this would be the next step: the estimation of the kill rate for predators.

Mirids are not widely used in augmentative biological control programs for pepper crops when managing aphids in the Mediterranean Basin. However, several studies have shown their great potential for controlling this pest. Messelink et al. [69] showed that the preventive release of *M. pygmaeus* and *D. tamaninii* together with supplemental food (eggs of the flour moth *Ephestia kuehniella* Zeller and decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg) controlled the populations of *M. persicae* in sweet peppers. Bouagga et al. [30] demonstrated how using mirids (either *N. tenuis* or *M. pygmaeus*) combined with the predatory mite *A. swirskii* provided control over the three major pests affecting sweet peppers in Southern Europe: thrips, whitefly, and aphids. However, if *O. laevigatus* was used instead of mirids, the control of aphids could not be achieved. Despite these findings, mirids are still not widely utilized in commercial greenhouse sweet pepper cultivation.

5. Conclusions

Temperature is a critical factor in the success of predatory mirids and as biological control agents. Temperature changes can affect various aspects of their biology and behavior, such as their rate of development, activity level, and reproductive success. Based on the findings of this study, *M. pygmaeus* and *D. pygmaeus* appeared to be effective in temperate climates where temperatures were moderate and moderate changes occurred throughout the year. Meanwhile, *N. tenuis* is more suitable for warmer climates with higher temperatures. When selecting a species of mirid for controlling aphid populations, it is essential to consider the environment and temperature of the target area. The results of this study underscore the importance of temperature in the effectiveness of predatory mirids and highlight the need to understand how temperature affects their biology and behavior to develop effective strategies for using them as biological control agents against pests.

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