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Biological traits of the predatory mirid *Macrolophus praeclarus*, a candidate biocontrol agent for the Neotropical region

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Abstract

The predatory mirid *Macrolophus praeclarus* is widely distributed throughout the Americas, and is reported to prey upon several horticultural pest species. However, little is known about its biology, thermal requirements, crop odour preferences, phytophagy, and capability to induce defensive responses in plants. When five temperatures studied (20, 25, 30, 33 and 35°C) were tested and *Ephestia kuehniella* was used as prey, the developmental time from egg to adult on tomato, was longest at 20°C (56.3 d) and shortest at 33°C (22.7 d). The ability of nymphs to develop to adults decreased as the temperature increased, with the highest number of nymphs reaching the adult stage at 20°C (78.0%) and lowest at 35°C (0%). The lower and upper developmental thresholds were estimated at 11.2° and 35.3°C, respectively. The maximum developmental rate occurred at 31.7°C and the thermal constant was 454.0 ± 8.1 degree days. The highest predation rate of *E. kuehniella* eggs was obtained at 30°C. In Y-tube olfactory choice tests, *M. praeclarus* selected tomato, sweet pepper and egg-plant odours more frequently than no plant control treatment. *Macrolophus praeclarus* feeding did not damage tomato plants compared to another zoophytophagous mirid, *Nesidiocoris tenuis*, which caused necrotic rings. The phytophagy of *M. praeclarus* induced defensive responses in tomato plants through the upregulation of the jasmonic acid metabolic pathway. The implications of the findings for using *M. praeclarus* in tomato biological control programmes in the Americas are discussed.

Introduction

Generalist predators contribute to the management of a wide range of agricultural pests, around the world (Symondson *et al.*, 2002; Bouvet *et al.*, 2019). In Europe, several integrated pest management (IPM) programmes, based on the augmentation and conservation of polyphagous predators, have been successfully developed and implemented (Pérez-Hedo and Urbaneja, 2016; van Lenteren *et al.*, 2018, 2020). Releases of predatory mirid bugs (Hemiptera: Miridae) [*Nesidiocoris tenuis* Reuter in Southern Europe and *Macrolophus pygmaeus* (Rambur) in Northern Europe] and conservation of their natural populations have resulted in effective control of key tomato (*Solanum lycopersicum* L.) pests, including the sweet potato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Arnó *et al.*, 2010; Urbaneja *et al.*, 2012; Pérez-Hedo *et al.*, 2017; van Lenteren *et al.*, 2020). However, zoophytophagous mirids can cause damage to crop plants when prey is scarce or they are confined in greenhouses (Pérez-Hedo *et al.*, 2021; Chinchilla-Ramírez *et al.*, 2021).

Following the success achieved with the use of predatory mirids in Europe horticultural crops (van Lenteren *et al.*, 2018, 2020), efforts to find a native species within this group of predators on the American continent have increased (Bueno *et al.*, 2013, 2018; Lellani *et al.*, 2014; Silva *et al.*, 2016; Smith and Krey, 2019; van Lenteren *et al.*, 2019; Pérez-Hedo *et al.*, 2021; Roda *et al.*, 2020). The reduced efficacy of IPM programmes due to insecticide resistance acquired by *B. tabaci* (Stansly and Naranjo, 2010) and the threat of multiple invasive pests, such as *T. absoluta* and the old World bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in North America (Biondi and Desneux, 2019; Tembrock *et al.*, 2019), has compelled this search for management alternatives. Native mirid species may provide similar control as known biological agents with the added advantages of being already

adapted to local conditions, and they would not require quarantine evaluation and release permits (Shipp and Wang, 2006; Calvo *et al.*, 2016; Pérez-Hedo *et al.*, 2021).

Macrolophus praeclarus (Distant) (Hemiptera: Miridae) has been previously reported in Central and South Florida and Puerto Rico, and several tropical countries of the American continent including Brazil, Colombia, Cuba, Dominican Republic, French Guyana, Guatemala, Mexico, Nicaragua, Paraguay, Peru, and Suriname (Carvalho and Rosas, 1965; Carvalho *et al.*, 1977; Cassis, 1984; Chérot *et al.*, 2007; Ferreira and Henry, 2011; Martínez *et al.*, 2014; Chérot and Carpintero, 2016; Serra and van Lenteren, 2020). In these countries, *M. praeclarus* has been found mainly on tobacco (*Nicotiana tabacum* L.) and tomato crops, although it has also been reported on citrus and its associated weeds (Arellano and Vergara, 2016), on non-cultivated plants such as *Martynia annua* L. (Lamiales: Martyniaceae) (Vera and Ayala, 1979) and succulent sesame [(*Uncarina grandidieri* (Baill.) Stapf (Lamiales: Pedaliaceae)] (Roda *et al.*, 2020). Like other mirids, *M. praeclarus* is a polyphagous predator, preying on eggs and early instars of the tobacco budworm *Heliothis virescens* (Fab.) (Lepidoptera: Noctuidae), green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and eggs and early instars of *B. tabaci* (Ayala *et al.*, 1982; Soto and Nakano, 2009; Roda *et al.*, 2020).

Recent studies have shown the ability of *M. praeclarus* to control *B. tabaci* in field cage studies under South Florida spring and fall conditions (Roda *et al.*, 2020). Ten days after infesting tomato plants with 10 adult whiteflies, the release of *M. praeclarus* (1 male and 1 female/plant) reduced the total number of whiteflies on infested tomatoes by close to 100% 6 weeks after adding the mirids. In fact, *M. praeclarus* reduced the number of whitefly nymphs earlier than *N. tenuis*. *Macrolophus praeclarus* was able to increase its population levels in tomato plants, reaching levels around 30 individuals/plant, without causing plant damage. In contrast, *N. tenuis* and *Engytatus modestus* (Distant) (Hemiptera: Miridae) cause damage to tomato plants under the same experimental conditions (Roda *et al.*, 2020). Despite these promising results, the biology of *M. praeclarus* is almost unknown. We characterized the South Florida population of *M. praeclarus*' molecularly (Supplementary Material) and determined *M. praeclarus*' developmental thermal requirements, its predation capacity at five constant temperatures, and its host plant odour preference for tomato, sweet pepper and eggplant. Finally, we evaluated, under controlled conditions, the potential of this species to cause plant damage on tomato and assessed whether its phytophagy can induce defensive plant responses in tomato plants. This information will help to clarify the region and crops where *M. praeclarus* could be used as a biological control agent, to help in the development of mass rearing techniques, and to guide subsequent studies for its field application.

Materials and methods

Plants and insects

Macrolophus praeclarus was collected from ornamental succulent sesame located at Fairchild Botanical Gardens in Miami, Florida, USA. This population of *M. praeclarus* was characterized by molecular methods and the accession number MT154517 was submitted to the GenBank (Supplementary Material). *Nesidiocoris tenuis* was collected from the same plant species but at a private residence in Miami

Beach, Florida. These field collected mirids were used to establish colonies on pesticide-free tomato seedling var. Sweet 'n' Neat Cherry (Bonnie Plants; Home Depot, Fort Myers, FL) at University of Florida, Southwest Florida Research and Education Center (SWFREC), Immokalee, FL. A mix of frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and cysts of the crustacean *Artemia* spp. (Anostraca: Artemiidae) (1:5 w:w) (Entofood®; Koppert B.S.) was used as an artificial source of prey for the mirids in the colonies. Only frozen eggs of *E. kuehniella* (supplied by Koppert B.S.) were used in the experiments described below.

Tomato var. Grand Marshall, sweet pepper (*Capsicum annuum* L.) var. Antebellum and eggplant (*Solanum melongena* L.) var. Black Beauty (Barnett, Parting Plants, Inc. Immokalee, FL) plants were transplanted into individual 0.5-L pots. Plants were not fertilized or treated with pesticides. Colonies and plants were maintained at $25 \pm 1^\circ\text{C}$, 60% humidity and 14:10 h (L:D) photoperiodic conditions in climatic chambers at SWFREC.

Development and survival of *M. praeclarus* at different temperatures

The developmental time from egg to adult and nymphal survival of *M. praeclarus* was studied at five constant temperatures (20, 25, 30, 33 and 35°C) with a 14:10 h photoperiod and $70 \pm 10\%$ relative humidity. *Macrolophus praeclarus* oviposition was synchronized in order to obtain a similarly aged cohort for each temperature. For each experimental temperature, 30 pairs of *M. praeclarus* were introduced inside a plastic cage (60 × 60 × 60 cm, BugDorm-2 insect tents; MegaView Science Co., Ltd.; Taichung, Taiwan) containing two clean tomato plants (~20 cm). After 24 h, the main and secondary stems of the middle-apical part of each of the plants, which contained eggs less than 24 h old, were cut into sections approximately 5 cm long. The stem pieces were placed into Petri dishes (140 mm diameter) and held in Percival incubators (Model I-36LL-1, Percival Scientific Inc. Perry, IA, USA) at each test temperature. A ball of wet cotton was added to maintain humidity inside each Petri dish. The Petri dishes were checked daily for 1st instar nymphs less than 24 h after emergence. The egg developmental time for each nymph was considered as the period from the cohort formation until the detection of the nymph. These nymphs were transferred individually to Petri dishes (45 mm diameter) that contained a 2 cm tomato stem piece, a moistened piece of cotton, and *E. kuehniella* eggs provided *ad libitum*. Daily, the instar of each individual nymph was checked and the tomato stem piece replaced, until the adult stage was recorded or the nymph was found dead. Changes from one nymphal stage to another were recorded when they molted and exuvia was observed.

Temperature thresholds and thermal constant

Total developmental rates (egg-adult) ($y = 1/\text{developmental time}$) were plotted against temperatures and fitted with Lactin model for nonlinear regression (Lactin *et al.*, 1995): $r(T) = e^{\rho T} - e^{(\rho T_{\max} - T_{\max} - T)/\Delta} + \lambda$, where $r(T)$ is the developmental rate at temperature T , T_{\max} is the temperature of maximal developmental rate, ρ , Δ and λ are fitted parameters. The upper developmental threshold and maximal developmental rate were estimated from the regression. Developmental rates for temperatures below T_{\max} were fitted with a linear regression ($y = a + bT$). This was required to estimate the lower developmental threshold ($T = -a/b$) and the heat accumulation in degree-days ($K = 100/b$) required to complete

Table 1. Primers used for quantification of *EF1* (elongation factor-1), *ASR1* (abscisic acid stress ripening protein), *PR1* (pathogenesis-related protein 1) and *PIN2* (JA-regulated defense protein) genes.

Gene	Primer forward (5' → 3')	Primer reverse (5' → 3')
<i>EF1</i>	5-GATTGGTGGTATTGGAAGTGC-3	5-AGCTTCGTGGTGCATCTC-3
<i>ASR1</i>	5-ACACCACCACCACCACCTGT-3	5-GTGTTTGTGTGCATGTTGTGGA-3
<i>PR1</i>	5-CCGTGCAATTGTGGGTGC-3	5-GAGTTGCCAGACTACTTGAGT-3
<i>PIN2</i>	5-GAAAATCGTTAATTTATCCAC-3	5-ACATACAAACTTTCATCTTTA-3

each stage above the threshold (T) (Campbell *et al.*, 1974). The standard error of K was the standard error of the slope (b) divided by the square of the slope.

Predation capacity of *M. praeclarus* at different temperatures

The capacity of female and male *M. praeclarus* to feed on *E. kuehniella* eggs was evaluated in a no-choice experiment under five constant temperatures (20, 25, 30, 33 and 35°C) with a 14:10 h photoperiod and $70 \pm 10\%$ relative humidity. Female and male *M. praeclarus*, randomly selected from the colony, were individually placed inside a 5 ml vial and starved for 8 h before use. Water was provided by placing a piece of saturated cotton in the vial. After the starvation period, individuals were introduced individually into experimental arena, a 45 mm diameter Petri dish with 120 *E. kuehniella* eggs. Eggs were placed on the sticky surface of a post-it note (Post-it® Notes, 3 M, Cynthia, KY, USA). In each experimental arena, a ball of wet cotton was added as a water source for the mirid and to maintain humidity inside the Petri dish. After 24 h, the predators were removed from the arenas and the number of eggs preyed upon was counted for the 5 temperatures using a dissecting microscope (10×). The presence of empty chorions was used as evidence of predation (Mollá *et al.*, 2014). At least 15 replicates were performed for each temperature, except for 33°C where only nine replicates were obtained.

Preference of *M. praeclarus* for sweet pepper, eggplant and tomato odours

A Y-tube olfactometer experiment was conducted to test the olfactory responses of *M. praeclarus* females to sweet pepper, eggplant and tomato. The females were less than 7 days old, presumptively mated, and were starved for 24 h. The Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) consisted of a 2.4-cm-diameter Y-shaped glass tube with a 13.5-cm long base and two arms each 5.75 cm long (Pérez-Hedo and Urbaneja, 2015). Both side arms were connected via high-density polyethylene (HDPE) tubes to two identical glass jars (2.5 l volume). Each jar was connected to an air pump that produced a unidirectional humidified airflow at 150 ml min^{-1} . Three 60-cm T8 LED integrated tubes (EHI48-T822, Energy Harness Corporation (EHC), Cape Coral, FL) were positioned 40 cm above the olfactometer. All Y-tube experiments were conducted in climatic chambers ($23 \pm 2^\circ\text{C}$, $60 \pm 10\%$ RH). The tomato, eggplant and pepper plants, approximately 20 cm in height, were placed in the jars 10 min before adding a single female into the entry of the tube. The mirid was observed until she had walked at least 3 cm up one of the arms or until 15 min had elapsed. Females that did not select a side arm within 15 min were recorded as 'no-choice' and were excluded from data analysis. A minimum of 33 mirids

that selected an odour source were recorded for each pair. After recording responses of five mirids, the Y-tube was washed with soap and water, rinsed with acetone, and allowed to dry for 5 min. The test plants were subsequently switched between the left and right side arms to minimize any spatial effect on choice. One plant was used to test the response of 10 females and they were replaced with new plants for the remaining replicates.

Phytophagy by *M. praeclarus*

Phytophagy by *M. praeclarus* was evaluated by comparing it with *N. tenuis*, a species known to cause damage under these experimental conditions (Pérez-Hedo and Urbaneja, 2016; Chinchilla-Ramírez *et al.*, 2021). The number of necrotic rings caused by mirid feeding were counted as well as the number of withered leaflets produced by 10 adults (five males and five females) on a fully expanded young leaf of the apical part of an entire tomato plant (Urbaneja-Bernat *et al.*, 2019; Chinchilla-Ramírez *et al.*, 2020). The insects used had completed their final molt 3–5 days prior to the start of the experiment. The plants were held in a Percival incubator at 25°C, 60% humidity and 14:10 h (L:D) photoperiod and watered every 2 days. Each leaf was enclosed in a muslin bag (15 × 21 cm) with 10 adults for 5 days without a supplementary food source. The dead individuals were removed daily from muslin bags to prevent necrophagy during this experiment. At the end of the experiment, all remaining insects were counted and total mortality was recorded. Phytophagy was evaluated by counting the number of necrotic rings visible on the petiole and the rachis. The number of withered leaflets per leaf was counted as indicator of wilting. Eight replicates were performed for each species.

Plant defense response induction by *M. praeclarus*

To assess the induction potential of plant defensive responses by the mirid feeding on tomato plants, 20 individuals (sex ratio 1:1) were released into a cage (60 × 60 × 60 cm BugDorm-2 insect tents) containing a single tomato plant (25 cm) and allowed to feed. After 24 h, the mirids were removed and the apical part of the plant was cut and immediately ground in liquid nitrogen. The same amount of material was collected from clean plants that had not been in contact with any arthropod since germination. Six replicates were performed per treatment. For each replicate, 100 mg of the samples were used for RNA extraction to determine the expression levels of genes related to plant defense. Specifically, *ASR1* (marker gene for abscisic acid, ABA), *PIN2* (marker gene for jasmonic acid, JA) and *PR1* (marker gene for the salicylic acid signalling pathway, SA) were quantified. Total RNA was extracted using a plant RNA extraction kit (Omega Bio-Tek Inc., Doraville, GA, USA), which was treated with

Table 2. Mean developmental times (days \pm SE) for eggs and nymphs, nymphal survival (%) and sex ratio (females/total) of *M. praeclarus* when reared on *E. kuehniella* eggs on detached tomato stem pieces at five constant temperatures and 14:10 h L:D.

°C	Egg	N_1	N_2	N_3	N_4	N_5	Total Nymphs	Total Egg-Adult	Survival (%)	Sex ratio (%)
20	25.2 \pm 0.3 n = 41	5.8 \pm 0.1 n = 40	4.8 \pm 0.1 n = 39	4.6 \pm 0.1 n = 38	6.0 \pm 0.1 n = 35	9.9 \pm 0.1 n = 32	31.1 \pm 0.3 n = 32	56.3 \pm 0.5 c	78.0 \pm 7.1	65.6 \pm 8.2
25	12.9 \pm 0.2 n = 45	3.5 \pm 0.1 n = 40	3.1 \pm 0.1 n = 37	2.8 \pm 0.1 n = 36	3.6 \pm 0.1 n = 33	5.1 \pm 0.1 n = 32	18.1 \pm 0.3 n = 32	31.1 \pm 0.3 b	71.1 \pm 7.7	52.0 \pm 8.4
30	9.2 \pm 0.2 n = 49	3.5 \pm 0.1 n = 43	2.4 \pm 0.1 n = 29	2.6 \pm 0.2 n = 27	2.5 \pm 0.1 n = 24	3.0 \pm 0.2 n = 24	14.0 \pm 0.2 n = 24	23.2 \pm 0.2 a	49.0 \pm 9.2	55.0 \pm 8.3
33	8.3 \pm 0.1 n = 36	2.5 \pm 0.2 n = 27	2.6 \pm 0.1 n = 22	2.5 \pm 0.1 n = 19	2.8 \pm 0.2 n = 14	4.2 \pm 0.2 n = 14	14.7 \pm 0.3 n = 14	22.7 \pm 0.3 a	39.0 \pm 8.2	42.9 \pm 13.7
35	8.8 \pm 0.1 n = 28	3.5 \pm 0.2 n = 12	3.33 \pm 0.3 n = 6	-	-	-	-	-	0%	-

Male and female development times were grouped. For total developmental time, data followed by a different letter are significantly different ($P < 0.05$, Tukey test).

DNase-free DNase (Promega Corporation, Madison, Wisconsin, USA) to eliminate genomic DNA contamination. The RT reaction and the SYBR PCR reaction was performed as described by Pérez-Hedo *et al.* (2015). Quantitative PCR was performed using the Smart Cycler II sequence detector (Cepheid, Sunnyvale, CA, USA) with standard PCR conditions. Expression of *EF1* (elongation factor-1) was used as a standard control gene for normalization. The nucleotide sequences of the gene-specific primers are described in table 1.

Data analysis

The data from the olfactory responses were analysed using a chi-squared goodness-of-fit test based on a null model where the odour sources were selected with equal frequency. Predation capacity and developmental time were subjected to two-way analysis of variance to evaluate the effect of factors sex and temperature, using Tukey's test for mean separation in the event of a significant F ($P < 0.05$). To determine whether survival and sex ratio of *M. praeclarus* was affected by temperature, the fitted values of the expected sex ratio and of survival were obtained with a generalized linear model (GLM) with quasibinomial family selected as the appropriate model. The mirid mortality in the phytophagy experiment and the gene expression analyses were analysed using one-tailed Student's t test ($P < 0.05$). All analysis were performed with SPSS software (IBM SPSS, 2004) except the GLM which was conducted with the R freeware statistical package (Version 1.0.143) (RStudio Team, 2015) and the fit of the Lactin nonlinear model using a least-squares method which was established with TableCurve 2D program (Systat Software Inc., San José, California).

Results

Development and survival of *M. praeclarus* at different temperatures

Macrolophus praeclarus completed development from egg to adult at 20, 25, 30 and 33°C (table 2). However, individuals tested at 35°C did not survive beyond the second nymphal instar. The duration of development did not differ between sexes ($F = 0.338$; $df = 1, 102$ $P = 0.562$), and developmental times were grouped (table 2). The developmental time at 30 and 33°C was significantly shorter than that found at 25°C, which was shorter than that at 20°C ($F = 1237.6$; $df = 3, 102$ $P < 0.0001$). No interaction between temperature and sex was found ($F = 0.300$; $df = 3, 102$ $P < 0.825$).

The survival of *M. praeclarus* decreased with increasing temperature (GLM quasibinomial: $\chi^2 = 230.83$; $P < 0.0001$) (fig. S2). At 20°C, 78.0% of the individuals completed their cycle and only 39.0% completed it at 33°C. Although, the sex ratio of *M. praeclarus* was biased towards females at 20°C and towards males at 33°C, there was no significant effect of temperature on the sex ratio of this species (GLM quasibinomial: $\chi^2 = 2.165$; $P = 0.539$).

Temperature thresholds, and thermal constant

Rates of development (y) were fitted with a nonlinear regression [$r(T) = e^{\rho T} - e^{(\rho T_{\max} - T_{\max} - T)/\Delta} + \lambda$] ($\rho = 0.0598 \pm 0.0017$, $\Delta = 1.3151 \pm 0.4528$, $\lambda = -1.4222 \pm 0.0037$; estimate \pm SE)] ($r^2 = 0.9597$; $F = 785.9$; $df = 3, 134$; $P < 0.0001$). The upper

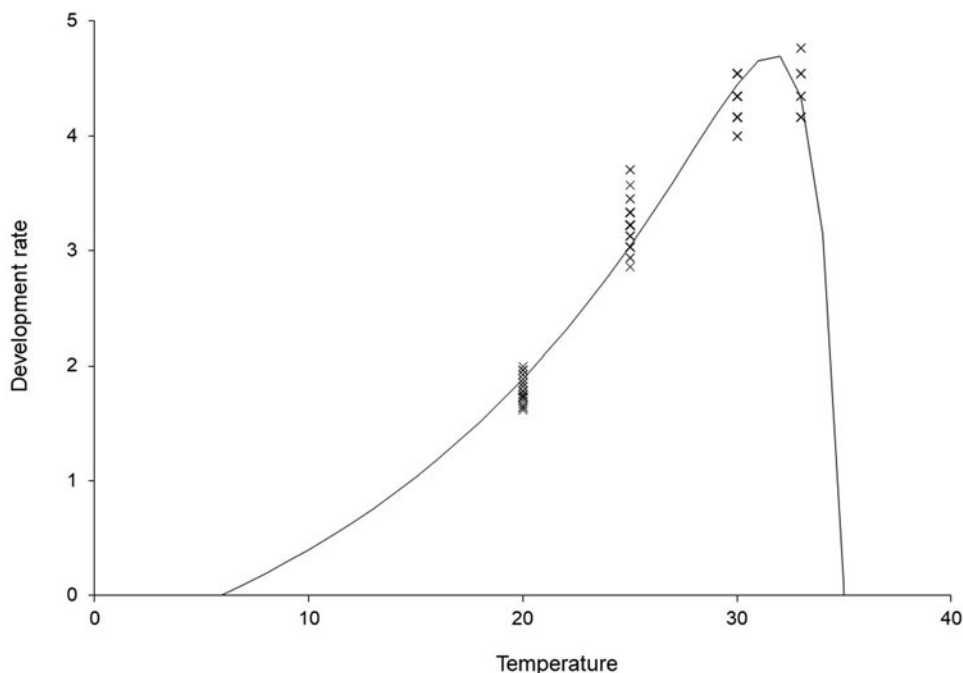


Figure 1. Effect of constant temperatures on developmental rate (d^{-1}) of *M. praeclarus* when reared on *E. kuehniella* on detached tomato stems. Fitted curve: Lactin model (Lactin *et al.*, 1995).

developmental threshold was 35.3°C, and maximal developmental rate occurred at 31.7°C (fig. 1). For estimating lower temperature thresholds, developmental rates observed between 20° and 33°C were further fitted with a linear regression ($y = 0.2204x - 2.4762$; $r^2 = 0.9322$; $F = 1374$; $df = 1, 100$; $P < 0.0001$). The lower developmental threshold estimated from this equation was 11.23°C. Using this estimate of the lower developmental threshold, a thermal constant of 454.0 ± 8.1 degree days (DD) ($n = 88$) was calculated.

Predatory capacity of *M. praeclarus* at different temperatures

Female *M. praeclarus* fed on significantly more *E. kuehniella* eggs than males (68% more, $F = 18.453$; $df = 1, 153$; $P < 0.001$) (fig. 2). For both sexes, the highest predation rate took place at 30°C followed by 25°C and 33°C, which was significantly higher than that obtained at 20°C ($F = 26.909$; $df = 3, 153$; $P < 0.001$). All adults died within 24 h of the experiment at 35°C, hence data of predation was not considered for this temperature. No interaction between sex and temperature was obtained ($F = 1.583$; $df = 3, 153$; $P = 0.196$).

Macrolophus praeclarus response to sweet pepper, eggplant and tomato odours

In the Y-tube olfactometer study, female *M. praeclarus* showed preference for the odours emitted from the three plants over the blank, no plant control treatment ($\chi^2 = 4.829$; $P = 0.0280$; $\chi^2 = 8.758$; $P = 0.0031$; $\chi^2 = 5.121$; $P = 0.0236$ for tomato, sweet pepper and eggplant, respectively) (fig. 3). When *M. praeclarus* females had a choice between tomato and sweet pepper, sweet pepper and eggplant or between tomato and eggplant, they showed no preference ($\chi^2 = 1.882$; $P = 0.1701$; $\chi^2 = 1.400$; $P = 0.2367$; $\chi^2 = 1.324$; $P = 0.2498$, respectively).

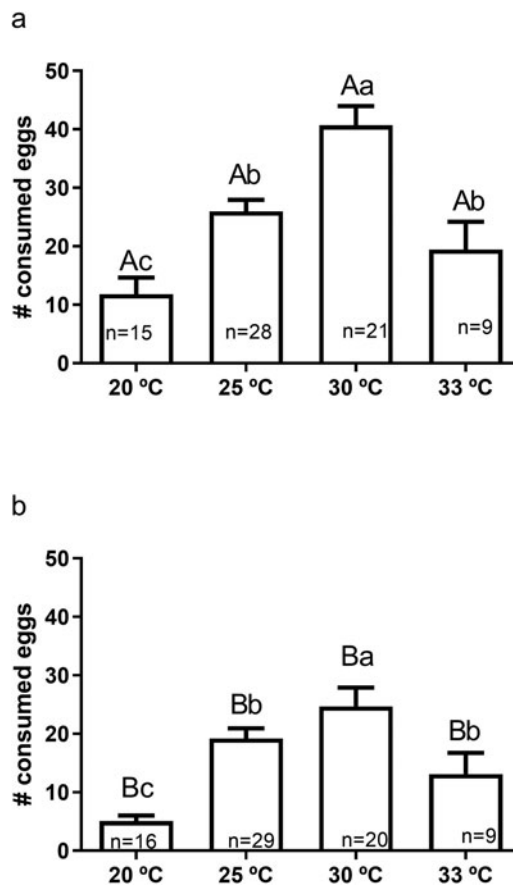


Figure 2. Number (mean \pm SE) of *E. kuehniella* eggs consumed by (a) females and (b) males of *M. praeclarus* at four constant temperatures and 14:10 h L:D. Bars topped by the same upper- and lower-case letters represent means that are not statistically different among temperatures and sexes, respectively (ANOVA $P < 0.05$). n = number of replicates.

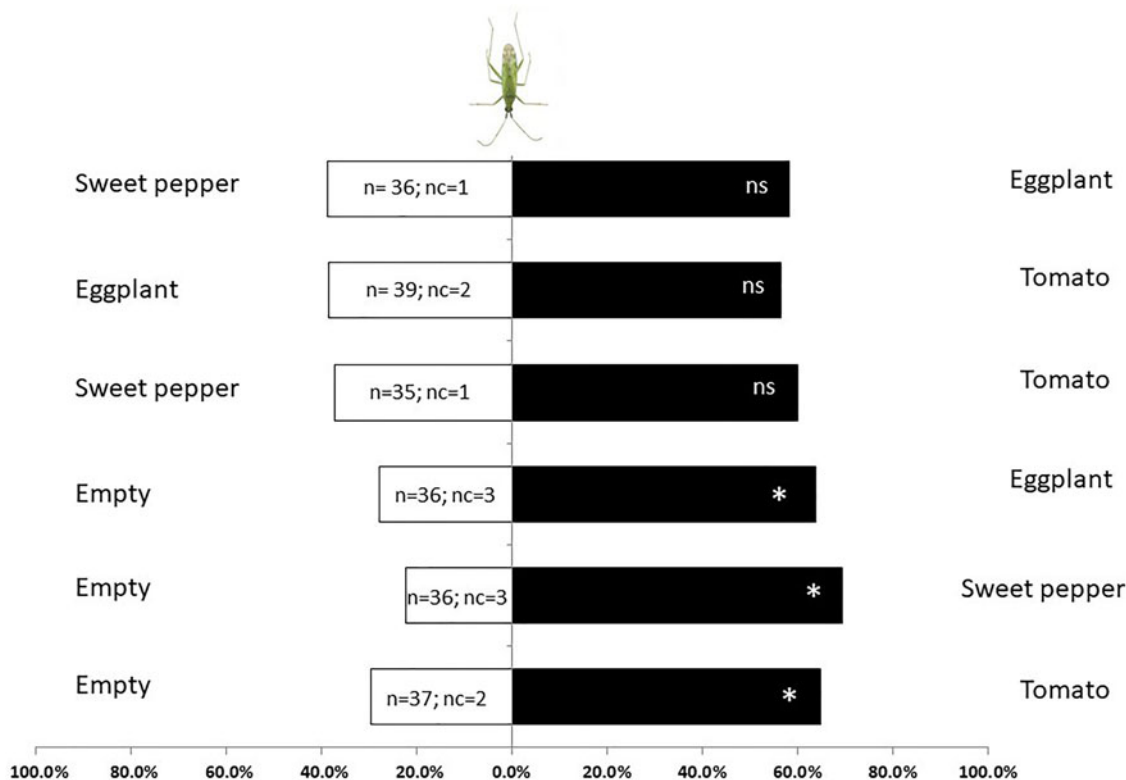


Figure 3. Response of *M. praeclarus* females in a Y-tube olfactometer to the odours of sweet pepper, eggplant and tomato. “n” indicates the number of individuals tested, “nc” indicates the number of individuals that did not make a choice and “n.s.” indicates non-significance. Significant differences based on a χ^2 -test are marked with asterisk ($P < 0.05$).

Phytophagy by *M. praeclarus*

The number of *N. tenuis* that died inside the muslin bags during the phytophagy test was 3.8 ± 0.3 per replicate, which was significantly higher than that of 2.3 ± 0.5 of *M. praeclarus* ($t_{10} = 0.6948$; $P = 0.2515$). Despite more individuals surviving, *M. praeclarus* did not produce necrotic rings, withered leaves, or other signs of visible damage on those tomato plants exposed to its feeding. In contrast, *N. tenuis* caused 3.2 ± 0.5 necrotic rings per replicate and 1.5 ± 0.4 withered leaflets on a fully expanded young leaf of the apical part of an entire tomato plant.

Plant defense response induction by *M. praeclarus*

Transcriptional analysis showed that the phytophagy of *M. praeclarus* on the apical part of tomato plants increased the expression of the *PIN2* gene (JA pathway) ($t_{10} = 1.827$; $P = 0.0488$), suggesting the activation of the related metabolic pathway (fig. 4b). In contrast, the *ASR1* (ABA pathway) and the *PR1* gene (SA pathway) expressions were not altered in *M. praeclarus* punctured plants compared to intact tomato plants ($t_{10} = 0.6948$; $P = 0.2515$ and $t_{10} = 0.2063$; $P = 0.4202$, respectively) (fig. 4a, c).

Discussion

To our knowledge, this is the first study detailing biological traits of the Neotropical zoophytophagous mirid, *M. praeclarus*. *Macrolophus praeclarus* was able to complete its development at four different temperatures (20, 25, 30 and 33°C) tested when supplemented with *E. kuehniella* eggs, but did not develop beyond the second nymphal instar under a constant temperature of 35°C. Our study indicates a low adaptability of *M. praeclarus* to extreme

temperatures. This is known for tropical species, which are exposed to a narrow temperature range. These species are more affected by extreme temperature variations than species adapted to wider temperature regimes found in colder climates (Janzen, 1967; Rosner, 2013). Our results suggest that *M. praeclarus* may not perform well in warm climates. The results indicate *M. praeclarus* would likely be more successful as a biological control agent in temperate climates or in open field conditions, where its optimal temperature range of 25–30°C occurs. However, our results were obtained under constant temperatures, which does not occur under field conditions where temperatures fluctuate throughout the day. In fact, *M. praeclarus* was able to establish and control *B. tabaci* effectively under the tomato growing conditions in South Florida that occur in spring and autumn (Roda *et al.*, 2020). In this study, the mean temperature was in the optimal range for *M. praeclarus* (25–30°C), however, a maximum of more than 35°C and a minimum of less than 15°C had occurred.

The developmental time from egg to adult of *M. praeclarus* at 20°C (56.3 d) was considerably longer than reported for other species of *Macrolophus*. For example, *M. caliginosus* Wagner [syn. *Macrolophus melanotoma* (Costa)] took 47.7 days to develop on the whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), *M. pygmaeus* took the same amount of time to develop on the aphid *M. persicae* and *E. kuehniella*, and *M. basinicornis* (Stal) needed 39.3 d to reach adulthood when reared on *E. kuehniella* eggs (Hart *et al.*, 2002; Perdakis and Lykouressis, 2002; Martínez-García *et al.*, 2017; Bueno *et al.*, 2018). The developmental parameters of mirids vary depending on the quality of the prey consumed (Sylla *et al.*, 2016). The developmental time for *M. praeclarus* may decrease

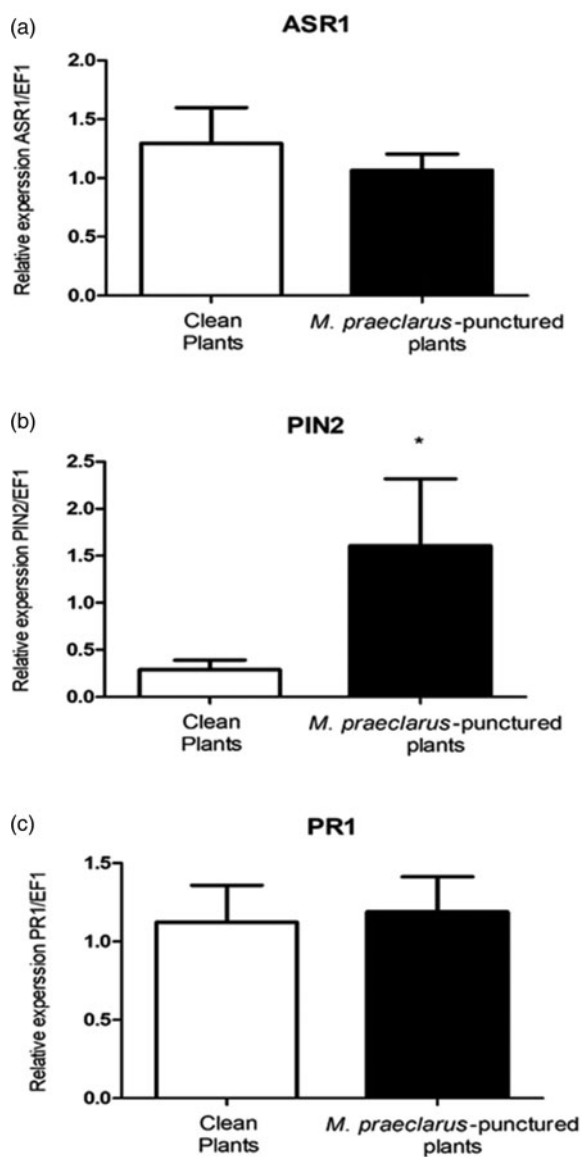


Figure 4. Expression of (a) ASR1, (b) PIN2 and (c) PR1 at the apical part of tomato plants either intact or exposed to the phytophagy of *M. praeclarus*. Transcription levels were normalized to the expression of EF1a measured in the same sample. Data are presented as the means \pm SE of five biological replicates of transcription expression in relation to the housekeeping gene ($n=6$). Asterisks on the bars show significant differences ($P < 0.05$) based on Tukey comparisons.

if more nutritious prey are available. As temperatures increase, the time required for *M. praeclarus* to complete the development from egg to adult was significantly reduced. At temperatures near 30°C, developmental times were comparable to other *Macrolophus* species studied. For example, *Macrolophus praeclarus*' developmental thermal constant of 454 DD is very similar to *M. caliginosus* (435 DD) (Hart *et al.*, 2002), *M. pygmaeus* (455.1 DD) (Perdikis and Lykouressis, 2002) and *M. basicornis* (476.2 DD) (Bueno *et al.*, 2018). However, the lower temperature threshold for *M. praeclarus* (11.2°C) is higher than that of *M. caliginosus* (6.9°C), *M. pygmaeus* (7.2°C on *T. vaporariorum* or 8.7 on *E. kuehniella*) and *M. basicornis* (7.9°C).

We determined that *M. praeclarus* actively fed on *E. kuehniella* eggs. Given that *E. kuehniella* is a high-quality prey for predatory mirids, which is widely used in mass-rearing

(Pérez-Hedo *et al.*, 2021), the predation rates found in this study may serve as a benchmark for establishing the mass-rearing conditions of *M. praeclarus*. Knowing the number of *E. kuehniella* eggs to be used can be not only interesting in mass rearing systems, but also when planning augmentative releases that may require supplementary food. The highest predation rates found in this study (40.7 eggs per female per day at 30°C) are similar to other mirid species when preying upon *E. kuehniella* (Mollá *et al.*, 2014; Urbaneja-Bernat *et al.*, 2015; Bueno *et al.*, 2018). Egg predation, combined with recent studies showed that *M. praeclarus* controlled *B. tabaci* in tomato plants (Roda *et al.*, 2020), suggest the potential of this species as a biological agent. However, further studies are needed to determine whether *M. praeclarus* can control other key pests in horticultural crops, such as the lepidopteran *T. absoluta* and the thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). This point is important since it has been shown that the different species of the *Macrolophus* genus respond differently to crop pests. For example, *M. basicornis* is capable of reproducing successfully on *T. absoluta* eggs (Silva *et al.*, 2016; van Lenteren *et al.*, 2019) while *M. pygmaeus* does not (Mollá *et al.*, 2014; Sylla *et al.*, 2016).

One of the most desired traits when evaluating whether a mirid is a good candidate as a biological control agent is that its phytophagous behaviour does not result in damage to the crop (Pérez-Hedo *et al.*, 2017; van Lenteren *et al.*, 2019; Pérez-Hedo *et al.*, 2021). Roda *et al.* (2020) showed that 30 nymphs per plant of *M. praeclarus* did not damage tomato plants in field cage experiments. Here, under controlled experimental conditions previously used to evaluate plant damage of other mirids species (Chinchilla-Ramírez *et al.*, 2020), we showed that approximately eight *M. praeclarus* adults per leaflet caused no damage to tomatoes whereas approximately six adults of *N. tenuis* per leaflet did cause damage to the plant. These results are very encouraging since augmentative releases of *M. praeclarus* in tomato would likely not cause damage to the tomato plant. Thus, there would not be a need to continuously monitor populations or to control overpopulations as in the case of *N. tenuis* (Pérez-Hedo *et al.*, 2021).

Our results showed that *M. praeclarus* responds to tomato, sweet pepper and eggplant odours. An important aspect to determine their application as biological control agents would be to evaluate the survival rate of *M. praeclarus* has on each of these crops without the availability of prey and their capacity of these crops to retain mirid populations in periods of prey scarcity (Pérez-Hedo *et al.*, 2021). These applied aspects have direct implications for pest control as survival varies significantly between various species of mirids and crops (Perdikis and Lykouressis, 2002; Lucas and Alomar, 2002a, b; Urbaneja *et al.*, 2005; Abbas *et al.*, 2014).

In this study, we showed that the phytophagy of *M. praeclarus* in tomato plants increases the expression of PIN2, a marker gene for jasmonic acid (JA) metabolic pathway. JA activation by other species of mirids, such as *N. tenuis* or *M. pygmaeus*, triggers the emission of HIPV's, in tomato and sweet pepper plants, which resulted in attraction to parasitoids (Pérez-Hedo *et al.*, 2015; Bouagga *et al.*, 2018). The activation of this route can also increase the number of transcripts and protease inhibitors, which coincides with the reduced performance of various pests such as the whitefly *B. tabaci* on sweet pepper or two spotted spider mite on tomato *Tetranychus urticae* Koch (Acari: Tetranychidae) (Pappas *et al.*, 2015; Bouagga *et al.*, 2018; Pérez-Hedo *et al.*, 2018). Furthermore, it has recently been confirmed that activation of this pathway by the phytophagy of

N. tenuis also reduces tomato spotted wilt virus (TSWV) infection in sweet pepper (Bouagga et al., 2020). Further investigations of the effects that defensive activation mediated by *M. praeclarus* may have in tomato plants are warranted.

In summary, our results showed that *M. praeclarus* is well adapted to climatic conditions prevailing in the Neotropical region and provide valuable information for the development of the mass-rearing of this species. With the positive results obtained recently that show the capacity of *M. praeclarus* to establish on tomato plants where it effectively controls *B. tabaci* (Roda et al., 2020), studies should focus on the development of tomato integrated pest management programmes for the Neotropical region of the American continent, based on the release of this zoophytophagous species.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0007485321000067>.

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