Article

Effect of Sex, Age and Temperature on the Functional Response of *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), on Eggs of *Tuta absoluta*

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Effect of Sex, Age and Temperature on the Functional Response of *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), on Eggs of *Tuta absoluta*

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Simple Summary: The predatory insects *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) are important biological control agents used commercially for the control of main pests in greenhouses, such as *Tuta absoluta* Meyrick (Lep.: Gelechiidae) and whiteflies. In this study we investigated the predation efficacy of young and old females and males of the two mirid bugs, *M. pygmaeus* and *N. tenuis* when feeding on eggs of *T. absoluta* on tomato leaves. Young females of *M. pygmaeus* exhibited a higher predation efficiency compared to old ones, whereas males displayed a consistently low efficiency of predation irrespective of their age. Both young females and males of *N. tenuis* displayed a similarly high predation efficiency, although the old females exhibited a higher efficiency of predation compared to their male counterparts, but lower than the efficacy of the *M. pygmaeus* individuals. Our findings indicate that the two predatory species have different functional response characteristics to their prey depending mainly on their sex and age which may influence their efficacy as biological control agents.

Abstract: The predatory mirids *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) are used for the biological control of *Tuta absoluta* Meyrick (Lep.: Gelechiidae) and other pests in tomato greenhouses. The functional response of 1 (young) and 10 (old) days old adult females and males of *M. pygmaeus* and *N. tenuis* on eggs of *T. absoluta*, was determined on tomato at two temperatures (20° and 25°C) and LD 16:8 and found to be Type II. *M. pygmaeus* females exhibited higher predation efficiency, than males at both tested temperatures. Young *M. pygmaeus* females had a higher efficiency than old ones, whereas males had a low efficiency irrespective of age. The predation efficiency of *N. tenuis* was high (but lower than *M. pygmaeus*) in both young females and males, although old females had a higher efficiency than the respective males. Our results show that the two predatory species have different functional response characteristics to their prey depending mainly on sex and age which may affect their role as biological control agents.

Keywords: *M. pygmaeus*; *N. tenuis*; Miridae; *T.absoluta*; functional response; tomato; sex; adult age; temperature; predator

1. Introduction

The dicyphine mirid bugs *Macrolophus pygmaeus* Ramber and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), are endemically present in the Mediterranean basin and are commercialized as beneficial insects in biological pest control systems [1–3]. *M. pygmaeus* has been utilized for nearly three decades, predominantly in augmentative biological control in greenhouses, to regulate such small vegetable crop pests as spider mites, whiteflies, aphids, thrips, and lepidopteran pests (mainly eggs and young larvae) [2–4]. Despite being identified as an actor in biological control later, *N. tenuis* has gained significant attention, due to its great predatory capacity and performance at high temperatures [5,6]. The capacity of the two species as biocontrol agents against *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), has been investigated and highlighted [7,8]. Both *M. pygmaeus* and *N. tenuis* exhibit zoophytophagy, a special type of omnivory, with the ability to feed on both plant tissue and a broad spectrum of arthropods [9]. The presence of both peptidase and amylase in the salivary glands has been associated with this polyphagous feeding capacity [10]. These two species have a proclivity for zoophagy, although they facultatively exhibit phytophagy, which may play a substantial role in their preservation and fitness [9,11–19]. The ability to utilize both trophic sources offers a significant advantage when it comes to the desired early establishment of the predator in the crop before pest infestation proliferates [20]. *M. pygmaeus* has the added ability to complete its life cycle without access to prey, feeding exclusively on the host plant tissue of tomato and a multitude of cultivated vegetable plants [21]. To contrast this, there are few recorded plant species, notably the native to tropical and subtropical regions *Sesasum indicum* Linnaeus (Pedaliaceae) and *Cleome hassleriana* Chod. (Brassicales: Cleomaceae) that are suitable banker plants for the preyless development and oviposition of *N. tenuis* [22,23]. *N. tenuis* is considered to possess a greater voracity than *M. pygmaeus*, and in cases of high population densities may cause economically important plant damage and yield loss, in the form of aborted flowers and fruit, and necrotic rings, due to phytophagy on vegetative and reproductive plant parts [24–27]. As such, the status of *N. tenuis* as pest or beneficial insect has been a point of contention [28,29]. However, in the Mediterranean region, the benefits emanating from its high generalist predation capacity, especially on more than one tomato pests, such as the invasive pest *T. absoluta* and whiteflies, have been argued to outweigh the danger of plant feeding-associated damage [9].

The microlepidodpteran pest, *T. absoluta* is considered one of the most devastating pests of tomato, along with the whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). Native to South America, it has since extended its presence to Europe (beginning with Spain in 2006), Africa (starting with N.Africa, Tunisia and Morocco in 2008), and Asia (starting with Turkey in 2009) [30,31]. The larvae are voracious and feed on all aerial plant parts, in protected galleries within leaves, stems, and fruit. When appropriate control measures aren't implemented, infestation can lead to complete crop failure [31]. Certain characteristics associated with the ecology and the biology of this pest also favour the development of resistance to a broad range of insecticides, rendering control ineffective [32–36]. The inadequacy of chemical control in delivering sufficient crop protection serves as a catalyst for endeavours aimed at developing and implementing alternative control methods, including the utilization of beneficial insects, as integral components of Integrated Pest Management (IPM).

Functional response experiments have been a longstanding tool for evaluating the effectiveness of biocontrol agents in controlling arthropod pests and defining the range of pest densities and conditions within which the biological control agent remains effective. The functional response of a predator to prey density describes the per capita feeding rate of a predator as a function of resource density and is classified according to the three types described by Holling, 1959 [37]. Both Type II and Type III functional responses of predators, are desired characteristics for beneficial arthropods utilized as biological control agents and have been reported for many mirid predators [38–42]. Two parameters, the attack rate coefficient (α) (ability to capture) and the handling time (T_h) (ability to handle and consume prey), are used to provide insight into the functional response of the predator. Especially in the case of females, such foraging performance information is impactful, as it directly controls both the quantity and quality of offspring, thus influencing the establishment of predators, the dynamics of the populations, and the overall predation efficacy [43].

Various functional response experiments have been conducted to elucidate the efficacy of the two species when consuming a multitude of small arthropods, with the results depending on factors, such as host plant, spacial complexity, prey item, prey instar and temperature [40–42,44–50].

The primary objective of this study was to assess the predatory efficacy of the two species, *M. pygmaeus* and *N. tenuis* against eggs of *T. absoluta*. Functional response experiments were conducted at seven predefined densities of synchronous prey *T.absoluta eggs* under laboratory conditions, taking into account the impact of adult sex and predator aging on predation, at two experimental temperatures. Our findings indicate that both young and old males and females of the two predatory species exhibit a Type II functional response at the two experimental temperatures. However, the two species have different functional response characteristics to their prey depending mainly on their sex and age which may influence their role as biological control agents.

2. Materials and Methods

2.1. Insect Colonies

The stock colonies of *M. pygmaeus* and *N. tenuis* were maintained in the laboratory in cylindrical plastic cages (diameter, 25cm, height, 25cm), with fresh bean pods as oviposition substrate and dehydrated *E. kuehniella* Zeller (Lepidoptera: Pyralidae) eggs *ad libitum*, at 25°C and a photoperiod of 16:8 (L:D).

The stock colony of *T. absoluta* was established with adults emerged from heavily infested tomato leaves collected from a commercial plantation in northern Greece. The colony was maintained on tomato plants in tent cages, at 25°C and a photoperiod of 16:8 (L:D).

Predation experiments were conducted at two temperatures (20° and 25°C), a photoperiod of 16:8 (L:D) and 65 ±5% RH. One or 10-days old adult females and males of either *M. pygmaeus* or *N. tenuis* from the laboratory colonies were kept in an empty rearing cage, with access only to water for 24 hours, before their use in the functional response experiments.

2.2. Bioassay

For the functional response experiments, a large number of approximately 300 *T. absoluta* adults were transferred from the laboratory colony to an insect rearing cage (60cmx60cmx60cm) for egg laying. Tomato leaflets of comparable size (approximately 6cm, height and 3cm, width) were excised from tomato plants and their petioles enveloped in dampened cotton wool in plastic vials with water. The vials with the leaflets were maintained for 1 to 3 hours in the ovipositional cage for *T. absoluta* egg laying. Subsequently, the number of eggs laid on both the upper and under side of each leaflet was scored and the complete leaflet served as experimental arena. In functional response experiments the predator's mobility is limited to the controlled experimental arena, whereas in natural agroecosystems, both predator and prey freely navigate. Our arenas were adopted in an attempt to align the experimental conditions more closely with field conditions, where the predator consumes *T. absoluta* eggs deposited on both upper and underside leaf surfaces. The egg densities were selected through preliminary tests, to ensure maximum levels of predation.

The tomato leaflets with the eggs were transferred into individual cages consisting of inverted plastic cups (volume, 500cm^3) with dual openings on opposite sides of their cylindrical walls, covered with fine mesh, to facilitate air circulation. Each cup was sealed with a bottom lid and included a circular aperture at the top, allowing the introduction of the predator individual into the experimental arena before being sealed with a cork.

The experimental setup comprised an individual cage with one leaflet positioned in the vial, with a specific egg density, to which either a previously starved male or female of *M. pygmaeus* or *N. tenuis* was added. After a 24-hour period, the number of eggs consumed by the predator was scored.

2.3. Data Analysis

The mean number of prey consumed was plotted, as a function of prey density, and the data fitted to Rogers and Royamas' random predator equation:

$$N_e = N_0(1 - \exp[\alpha(T_h N_e - T)]), \tag{1}$$

N_e is the number of prey/eggs consumed, N_0 the initial prey density, T_h the handling time, T the duration of the experiment (24 hours) and α the attack rate.

Simplifying the above equation using the "Lambert-W" function *W(x)* [51]:

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$$N_e = N_0 - \frac{W[(aT_h N_0 \exp[\alpha(T_h N_e - T)])]}{aT_h}, \tag{2}$$

The values for the parameters α and T_h were estimated through a non-linear fit of the data to the provided above equation in Python, accounting for the standard deviation of the data. The determination coefficient, R^2, was calculated in addition to the parameters' errors.

The statistical analysis concerning the effect of age, sex and predator species on the prey consumption at each temperature, was quantified through a three-way analysis of variance (ANOVA, type I), accounting for a generalized linear model (GLM) with quasipoisson distribution. The relevant analysis was performed in R (version 4.3.1; R core team 2023-06-16).

3. Results

3.1. Predation of *M. pygmaeus* on Eggs of *T. absoluta*

The functional response data of adult females and males of *M. pygmaeus* on eggs of *T. absoluta* was successfully fitted to Royama (1971) and Rogers (1972) equation and consequently exhibit a Type II functional response (Figure 1, Table 1).

As shown in Table 1, the estimated values of attack rate in 1-day old females were ~0.70 and ~0.51 and those of the handling time ~0.31 and ~0.27 hours, at 20° and 25°C respectively, whereas in males, the respective estimated values of attack rate were lower (~0.09 and ~0.10) and those of the handling time longer (~0.57 and ~0.33 hours).

In 10-day old females the estimated values of attack rate were ~0.33 and ~0.36 and handling time were ~0.28 and ~0.24 hours respectively, at 20° and 25°C, whereas in males the respective values of attack rate were ~0.19 and ~0.08 and those of handling times ~0.61 and ~0.42 hours respectively.

Our results show that females exhibited markedly higher attack rates and shorter handling times, than their male counterparts at both tested temperatures and age groups.

The lowest value of attack rate of females was ~0.33 and the highest ~0.70. The females' shortest handling time was ~0.24 hours and the longest, ~0.31 hours. In comparison, the lowest attack rate value obtained for males was ~0.08 and the highest attack rate, ~0.19, much lower than the values for the females. The shortest handling time values for males were ~0.33 hours and the longest, ~0.61 hours, the latter being almost double the handling time of the females.

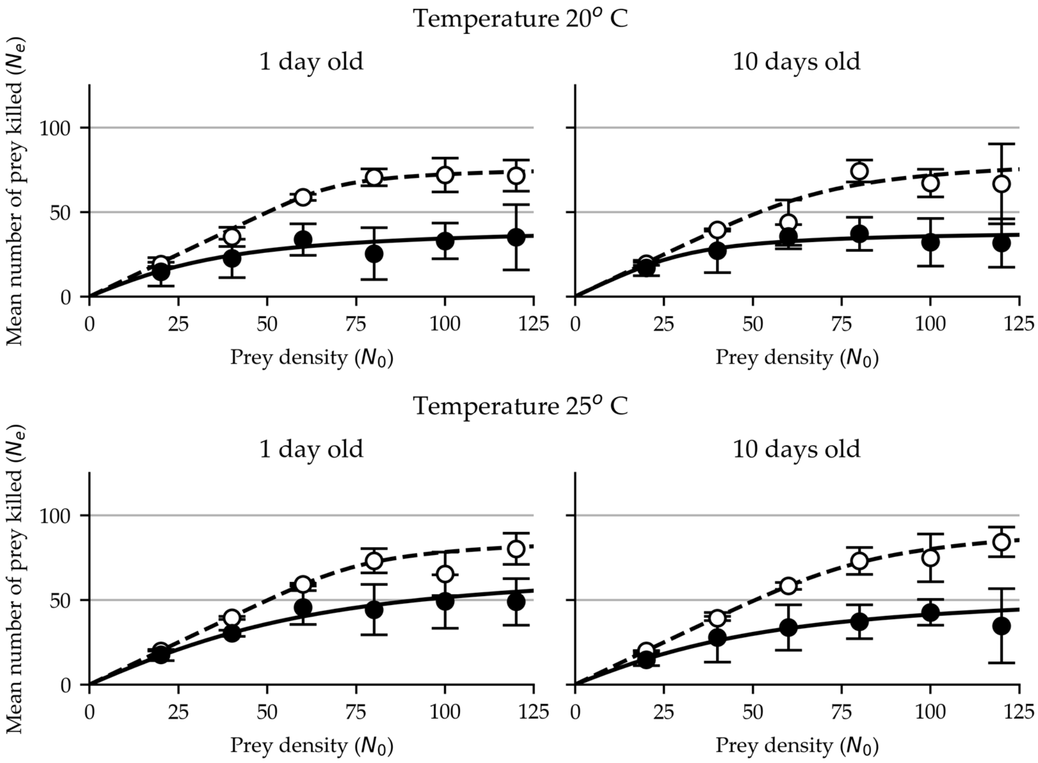

Figure 1. Functional response of 1 day and 10-day old females (open circles) and males (closed circles) of *M. pygmaeus* on eggs of *T. absoluta* at 20 and 25°C.

Table 1. Effect of sex, adult age and temperature on the attack rate (a) and handling time (***T_h***) of *M. pygmaeus* and *N. tenuis* adults feeding on eggs of *T. absoluta* on tomato leaflets.

Species	Sex*	Age (days)	Temperature (°C)	*a* (±SE) (hrs^{-1})	T_h (±SE) (hrs)	R^2
M. pygmaeus	f	1	20	0.70±0.26	0.31±0.02	0.99
	f	1	25	0.51±0.12	0.27±0.02	0.83
	f	10	20	0.33±0.09	0.28±0.03	0.92
	f	10	25	0.36±0.04	0.24±0.01	0.99
	m	1	20	0.09±0.04	0.57±0.08	0.81
	m	1	25	0.10±0.01	0.33±0.03	0.93
	m	10	20	0.19±0.08	0.61±0.05	0.71
	m	10	25	0.08±0.01	0.42±0.03	0.88
N. tenuis	f	1	20	0.22±0.02	0.18±0.02	0.99
	f	1	25	0.14±0.01	0.10±0.01	0.99
	f	10	20	0.18±0.07	0.25±0.06	0.87
	f	10	25	0.09±0.01	0.14±0.03	0.96
	m	1	20	0.20±0.07	0.24±0.03	0.98
	m	1	25	0.10±0.03	0.19±0.06	0.88
	m	10	20	0.09±0.01	0.37±0.05	0.93
	m	10	25	0.04±0.01	0.19±0.11	0.88

*f=female, m=male.

3.2. Predation of N. tenuis on Eggs of T. absoluta

As in *M. pygmaeus,* the functional response data of adult females and males of *N. tenuis* on eggs of *T. absoluta* was successfully fitted to the Royama (1971) and Rogers (1972) equation (Figure 2, Table 1). For *N. tenuis* the estimated values of the functional response parameters of the attack rate (a) and handling time (Th) are lower than the respective ones for *M. pygmaeus* (Table 1).

In 1-day old females of *N. tenuis* the estimated values of attack rate (a) were ~ 0.22 (compared to 0.70 for *M. pygmaeus (M.p.)*) and ~ 0.14 (0.51 for *M.p.*) and those of handling time ~0.18 (0.31 for *M.p.*) and ~0.10 (0.27 for *M.p.*) hours at 20° and 25°C respectively, whereas in males the respective estimated values of attack rate were ~0.20 (0.09 for *M.p.*) and ~0.10 (0.10 for *M.p.*) and those of the handling time ~0.24 (0.57 for *M.p.*) and ~ 0.19 (0.33 for *M.p.*) hours.

In 10 day old females the estimated values of attack rate were ~0.18 (compared to 0.33 for *M. pygmaeus(M.p.)*) and ~0.09 (0.36 for *M.p.*) and those of handling time were ~0.25 (0.28 for *M.p.*) and ~0.14 (0.24 for *M.p.*) hours respectively at 20° and 25°C, whereas in males the respective values of attack rate were ~0.09 (0.19 for *M.p.*) and ~ 0.04 (0.08 for *M.p.*) and those of handling time ~0.37 (0.61 for *M.p.*) and ~0.19 (0.42 for *M.p.*) hours.

Our results show that in *N. tenuis*, as in *M. pygmaeus*, females exhibited higher attack rates and shorter handling times, than their male counterparts at both tested temperatures and age groups. Based on these laboratory results we may assume that *M. pygmaeus* could be a more effective predator than *N. tenuis* for eggs of *T. absoluta* on tomato leaves.

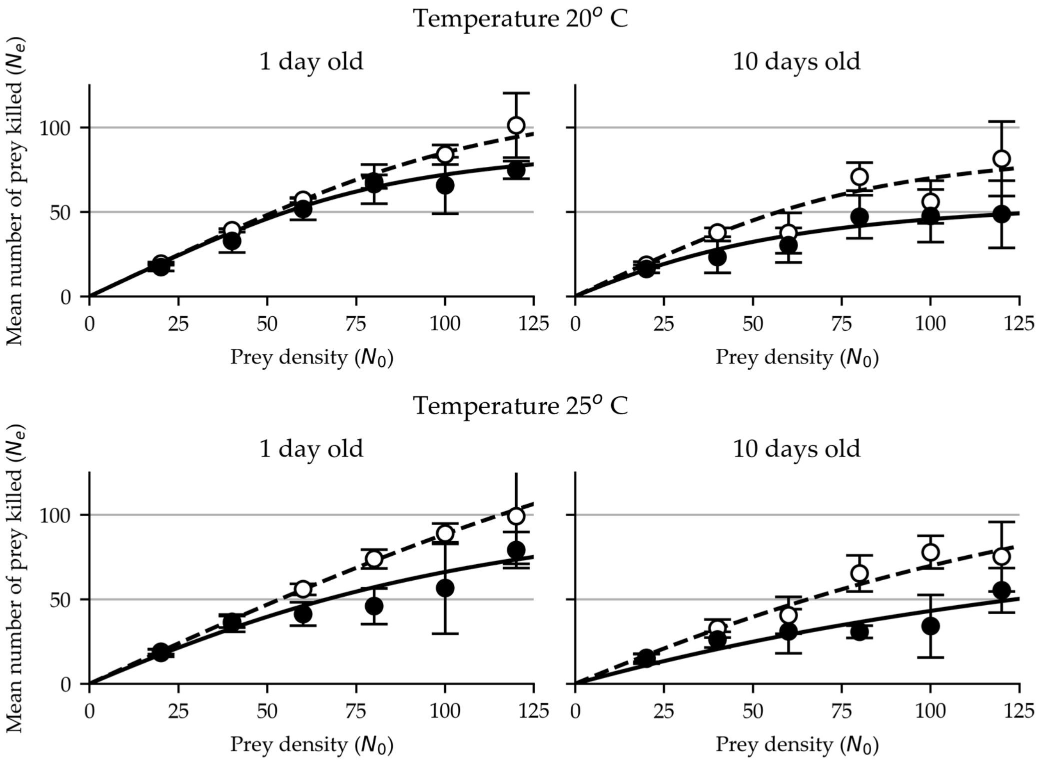

Figure 2. Functional response of 1 day and 10-day old females (open circles) and males (closed circles) of *N. tenuis* on eggs of *T. absoluta* at 20 and 25°C.

3.3. Comparison of Predation Efficacy of M. pygmaeus and N. tenuis

Three-way ANOVA indicated that the effects of predator species, sex, age at both experimental temperatures and the interactions of predator*sex and predator*age at 20°**C** on the consumption of *T. absoluta eggs* were significant.

Table 2. Three-way ANOVA (quasi-poisson distribution) on factors affecting consumption of eggs of *T. absoluta* by *M. pygmaeus* and *N. tenuis*, at 20 °C and 25 °C.

Source	**df**	**Deviance**
20 °C		
Predator	1	211.73[a]
Sex	1	591.80[a]
Age	1	104.83[b]
Predator*Sex	1	88.28[b]
Predator*Age	1	51.49[c]
Sex*Age	1	5.82
Predator*Sex*Age	1	11.89
25 °C		
Predator	1	52.80[c]
Sex	1	578.81[a]
Age	1	136.81[b]
Predator*Sex	1	24.74
Predator*Age	1	24.66
Sex*Age	1	23.53
Predator*Sex*Age	1	5.70

a: P<0.001, b: P<0.01, c: P<0.05.

4. Discussion

Functional response experiments illustrate the relationship between the initial prey density and the number of prey killed or parasitized and, based on this, the type of functional response with its parameters, the attack rate and handling time are estimated. From this determination, valuable practical conclusions can be derived of the suitability of a potential biological control agent against a pest of a crop. The dynamics of this predator-prey interactions are defined by a plethora of factors, such as predator age and sex, developmental instar and prey species [50]. In our experiments, we determined the functional responses, and estimated the values of attack rate and handling time of newly emerged (1 day old) and old (10 day old) *M. pygmaeus* and *N. tenuis* adult females and males feeding on different densities of *T. absoluta* eggs, laid on tomato leaves. We found that**,** in *M. pygmaeus*, the estimated values of attack rate were higher and handling time lower than the respective values for *N. tenuis.* The age and sex of both predator species affected the functional response parameters, i.e. handling time was lower and attack rate higher in female and young individuals than in males and old individuals.

The functional responses of *M. pygmaeus* and *N. tenuis* have been studied by other researchers following different experimental designs, prey densities and host plants [40–42,44–49]. Our findings are in accordance with the functional response type obtained in a study by Sharifian et al., 2015 [41], where 1-day old adults of unspecified sex of *M. pygmaeus* and *N. tenuis* fed on different densities of *T. absoluta* eggs, at 25°C. However, in this study, the values of attack rate and handling time did not differ between *M. pygmaeus* and *N. tenuis*, and were quite different to those obtained in our study. These observed differences between the obtained results of our and the aforementioned study, may be due to, among other factors, the different design of the experimental arenas, and the different sex of the experimental predators. It is known that the arena size and the varying spatial distribution of the prey affect functional response parameters, namely the attack rate [52,53]. The unique experimental design described in our experiments was adopted in an attempt to align the experimental conditions more closely with field conditions, where the predator consumes *T. absoluta* eggs laid by adult females freely on both sides of the leaf surface. By contrast, Sharifian et al., (2015) utilized an experimental arena consisting of three tomato leaflets in a petri dish, onto which the desired densities of eggs were added. These factors may provide possible explanations for the differential values of the functional response parameters.~~–~~

In our functional response experiments the predators *M. pygmaeus* and *N. tenuis* preyed on *T. absoluta* eggs and showed a type II response. Prey type and even prey developmental stage may modify the functional response of predators. *M. pygmaeus* adult females have been shown to exhibit a Type II functional response when feeding on 2nd – 4th instar nymphs of *Myzus persicae* Sulzer (Hemiptera: Aphididae) [44,48], pupae of *T. vaporariorum* [54], eggs of *E. kuenhiella* and eggs of *T. absoluta* [41], the latter in accordance with the results of our experiments and Type III when feeding on eggs of *T. vaporariorum* [55]. Adult females of *N. tenuis* have been shown to exhibit a Type II response when feeding on *T. vaporariorum* nymphs [56], *Tetranychus urticae* Koch (Tetranychidae) adults [57], and eggs of *T. absoluta* [41], the latter in accordance with the results of our experiments and Type III when consuming eggs and nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) [49].

In certain predator insect and mite species, the functional response is affected by adult age. The functional response of the predator mite *Amblyseius swirski* Athias-Henriot (Acari: Phytoseiidae) feeding on *T. urticae* was of Type II for a major part of its life, with the exception of 12-day old females that exhibited a Type III functional response. This was attributed to the greater energy demands, due to the higher reproductive output of females at that age [58]. A Type III response was also obtained with 1-day old, mated females of the parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), when feeding on *E. kuehniella* eggs and a Type II response, with 2-9 day old females which may be due to the effect of age progression on searching efficacy (varied effect) and handling time (increase) [59]. For the mirid bugs *Dicyphus bolivari* and *Dicyphus errans* Wolff

(Hemiptera: Miridae), both fifth instar nymphs and seven-day old females feeding on *T. absoluta* eggs exhibited a Type II response [60]. In the case of *M. pygmaeus*, the last nymphal instar and adult females, generally, have been shown to consume greater numbers of *M. persicae* Sulzer [61]. In our experiments, *M. pymaeus* and *N. tenuis* females and males maintained a Type II functional response as newly emerged (1 day old) and old (10 day old) adults.

We determined the functional responses of *M. pygmaeus* and *N. tenuis*, at two temperatures (20° and 25°C) and found a type II functional response with no substantial differences in the estimated values of attack rate and handling time between the two temperatures. It has been noted that changes in the ecological settings may be reflected in the functional response and associated parameters [52]. Investigations into the temperature thresholds of dicyphine species have determined that those vulnerable to low temperatures, are more resilient to high temperatures and vice versa. *N. tenuis* is considered the most thermophilous of the dicyphine species that have been investigated thus far, with optimum performance at temperatures between 20 and 30°C [62], and individuals reaching adulthood at a maximum temperature of 35°C [63]. Interestingly, in the context of the functional response, attack rate and handling time are expected to be optimized at intermediate temperatures [64,65]. As such, *N. tenuis* females have been indicated to exhibit a Type II response when consuming *B. tabaci* (Hemiptera: Aleyrodidae) at relatively low temperatures (15 and 25°C) and Type III response at a higher temperature of 35°C [66]. Conversely, *M. pygmaeus* maintained a Type II functional response over a range of temperatures [67].

We provide the first evidence of a *M. pygmaeus* population outperforming a population of *N. tenuis*. The previous history of an individual, such as previous host, prey that it was reared on, may influence its behaviour during the functional response experiment [43]. The two species we examined were reared in the lab on bean pods and *E. kuehniella* eggs. A preparatory period, where the predators were allowed to remain on tomato plants and feed on *T. absoluta* eggs, could have benefited and altered the outcome of the experiments.

In conclusion, our results provide evidence into the combined effect of sex and ageing on the functional response parameters of *M. pygmaeus* and *N. tenuis* at two temperatures and show that in both predator species, females have higher predation efficiency than males. In addition, newly emerged females have higher predation efficiency than 10-day old ones. Based on the estimated values of attack rate and handling time, we conclude that *M. pygmaeus* may have a better predation efficiency than *N. tenuis* for eggs of *T. absoluta* on tomato. However, further field experiments are required in order to verify our present laboratory results.

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