Temperature-dependent functional response of *Nesidiocoris tenuis* (Hemiptera: Miridae) to different densities of pupae of cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract. The effect of temperature on the functional response of female adults of *Nesidiocoris tenuis* Reuter to different densities of *Bemisia tabaci* (Gennadius) pupae was assessed. Three constant temperatures (15, 25, and 35°C) and six prey densities (5, 10, 20, 35, 50, and 70) were tested over a 24-h period. *Nesidiocoris tenuis* exhibited a type II functional response at 15 and 25°C, and a type III response at 35°C. The number of prey consumed by the predator increased with increase in the prey density at all temperatures. Temperature influenced attack rates and handling times. The highest attack rate occurred at 35°C at high densities (35, 50, and 70 prey) and the lowest handling time was recorded at 35°C. The maximum attack rates (T/Th) were 17.13, 42.12, and 45.07 whitefly pupae per day at 15, 25 and 35°C, respectively. As a result, the value of a/Th indicates that *N. tenuis* was relatively more efficient in attacking *B. tabaci* at 35°C than at lower temperatures. Results suggest that the ability of *N. tenuis* to detect and consume *B. tabaci* over a broad range of temperatures, especially high temperatures (25–35°C), makes this mirid a good candidate for the biological control of whiteflies in warm environments, such as greenhouses.

INTRODUCTION

*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous pest that causes damage to plants both through direct feeding and by transmitting plant viruses (Gerling et al., 2001). *B. tabaci* damages crops by sucking sap and also producing considerable amounts of honeydew that contaminates the leaves and results in the development of sooty mould (He et al., 2013). It is also an efficient vector of the tomato yellow leaf curl virus (TYLCV) and causes irregular ripening in tomato (Calvo et al., 2009; Stansly & Naranjo, 2010). In tomato cultivation, this pest is usually controlled by integrating several pest control tools, such as plant resistance (Fekri et al., 2016; Taggar & Gill, 2016), mass trapping (Böckmann et al., 2015) and biocontrol using parasitoids (Drobnjakovic et al., 2016) and predators (Calvo et al., 2009).

Including efficient biological control agents in pest management programs, not only provides more stable control (Lundgren et al., 2009; Aggarwal et al., 2016; Konecka et al., 2016), but compared to chemical control has benefits, including the pest not becoming resistant (Liang et al., 2012), no side effects on non-target organisms (Biondi et al., 2012, 2015; Abbess et al., 2015) and of toxic residues in the marketable products. The efficiency of a predator, or more broadly of a natural enemy, in suppressing pest populations in the field can be affected by several factors, such as intrinsic growth rates, numerical response, prey/host location, environmental complexities and dispersal ability (Desneux & O’Neill, 2008; Zappalà et al., 2012; Mollà et al., 2014; Naselli et al., 2017a).

*Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is a zoophytophagous mirid, naturally occurring in tomato crops in the Mediterranean basin, which has biological control potential (Biondi et al., 2013; Perdikis & Arvaniti, 2016). This predator is mass reared commercially for releases aimed at controlling small arthropod pests such as, whiteflies (Hemiptera: Aleyrodidae), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and mites (Zappalà et al., 2013; Sanchez et al., 2014; Campos et al., 2017), which are made routinely regardless of the damage caused to tomato plants under certain circumstances (Sanchez & Lacasa, 2008; Perdikis et al., 2009; Biondi et al., 2016, 2018). In addition,
*N. tenuis* is also known to activate indirect plant defenses, through its feeding activity (Pérez-Hedo et al., 2015; Naselli et al., 2016) and to contribute synergistically to multiple pest suppression together with parasitoids (Chailleux et al., 2013; Naselli et al., 2017b).

However, to provide a better picture of the efficiency of a given species of natural enemy in controlling targeted pests, several biological and behavioural traits need to be evaluated. One way of evaluating such features is to study its foraging behaviour, such as its functional response to prey density (Pakyar et al., 2009; Salehi et al., 2016). The functional response of predators is affected by several factors among which temperature is known to be an important abiotic driver. Indeed, temperature can influence the type of functional response of insect pest biocontrol agents, i.e., hymenopteran parasitoids and predatory stinkbugs and ladybirds (Mahdian et al., 2006; Zamani et al., 2006; Jalali et al., 2010).

In order to model predation by a long-lived, polyphagous predator, it is essential to know how the key parameters of the functional response (i.e. the attack coefficient and handling time) scale with temperature (Jalali et al., 2010). However, to the best of our knowledge, there is no study on the effect of temperature on the type and magnitude of the functional response of the mirid predator *N. tenuis*. Thus, the objective of the present study was to determine the functional response of *N. tenuis* to different densities of its common prey, *B. tabaci* pupae, at three constant temperatures.

**MATERIALS AND METHODS**

**Insects**

Adults of *N. tenuis* and the cotton whitefly, *B. tabaci* were originally collected from tomato fields and eggplant greenhouses, respectively, in Jiroft, Iran, in May, 2014. The mirid predator was reared on tomato plants and provided with frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) as prey in a chamber under controlled conditions (25 ± 1°C, 65 ± 10% RH, 16L : 8D photoperiod). The whiteflies were reared on tobacco plants in a heated greenhouse. Stocks were renewed periodically by adding individuals collected from the same area. Only the pupal stage of the whitefly was used in our experiments.

**Functional response**

The functional response of female adults of *N. tenuis* to different densities of *B. tabaci* pupae was assessed at three temperatures (15, 25 and 35°C) in the laboratory. These temperatures were chosen as typical of the minimum, average and maximum temperatures usually recorded during the typical tomato growing season (from September to May) in Mediterranean and Middle-East unheated greenhouses. Moreover, *N. tenuis* can complete its development over this temperature range and the optimum temperature for adults is between 20 and 30°C, and their minimum and maximum temperature thresholds for survival are substantially lower and higher than 15 and 35°C, respectively (Sanchez et al., 2009; Martinez-Garcia et al., 2016). Prior to each experiment, 24/48-h old mated females were fed the prey for 24 h and then starved for the following 24 h. Very young and mated females were chosen for this bioassay because females of most predatory Heteroptera need to mate and feed in order to start maturing eggs for reproduction (Legaspi & Legaspi Jr, 2008). In doing so, we ensured that all the insects tested were similar in terms of their physiological status and voracity.

The experimental arena consisted of a single tobacco leaf disk (6 cm in diameter) placed upside down on water saturated paper toweling in a plastic Petri dish 6 cm in diameter (Mollá et al., 2014). Six different densities of whitefly pupae (5, 10, 20, 35, 50 and 70 individuals per arena) were presented to each female, by transferring them to the leaf disks gently using a fine paint brush (Montserrat et al., 2000). The pupal stage of whiteflies was used as prey because it is a non-feeding immobile stage, thus easier to both manipulate and maintain at the desired densities. These densities were chosen based on preliminary observations in which the maximum number of prey consumed by young mated females provided with thousands of prey for 24 h was lower than 68, thus ensuring that the highest density was above the *ad libitum* level. The experiments were carried out in a growth chamber (65 ± 10% RH, 16L : 8D photoperiod). Twenty four hours after introducing the predator, the numbers of prey consumed were counted and recorded. At each prey density and temperature, a single replicate consisted of ten groups of prey and ten females. All the experiments were replicated three times.

**Data analysis**

For analysis of variance (ANOVA) of the results and of the functional responses the software SAS was used (SAS Institute Inc., 2003). Results were analyzed using a two-way ANOVA in order to determine the effects of prey density, temperature and their interaction on the predation rates. The Fisher’s least significant difference (LSD) post hoc analysis was used to compare the mean numbers of prey eaten by *N. tenuis* at different temperatures and prey densities. In order to compare the two functional response parameters (attack rate and handling time), binomial comparisons of the results obtained at the different temperatures were carried out using Mann-Whitney U-test (SAS Institute Inc., 2003).

The analysis of functional responses includes two steps. First, the shape of the curve or the functional response is determined by logistic regression of the proportion of prey eaten expressed as a function of the initial density. This is done by fitting a polynomial function:

\[
\frac{N_f}{N_0} = \frac{\exp\left[P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right]}{1 + \exp\left[P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right]} \quad (1)
\]

where \(N_f\) is the number of prey consumed, \(N_0\) is the initial number of prey, and \(P_0, P_1, P_2, P_3\) are the intercept, linear, quadratic and cubic coefficients, respectively, estimated using the method of maximum likelihood (Juliano, 2001). If \(P_0 < 0\), the proportion of prey eaten declines monotonically with the initial number of prey offered, which indicates a type II functional response (Juliano, 2001). If \(P_0 > 0\) and \(P_1 < 0\), the proportion of prey eaten is initially positively dependent on the density and consequently indicates a type III functional response.

In the second step, involved estimating the attack coefficients (\(a\)) and handling times (\(T_h\)), which was done by fitting the random predator equation to data using a nonlinear least squares regression (NLLIN) (Juliano, 2001). These equations take into account the depletion in prey density during the experiment (Rogers, 1972). For type II functional responses this equation can be written as follows:

\[
N_e = N_i \left[1 - \left(\exp[-a(T - T_a)N_i]\right)\right] \quad (2)
\]

where \(N_e\) is the number of prey eaten, \(N_i\) the initial prey density, \(T\) the total available time (24 h), \(a\) is the attack rate (h\(^{-1}\)), and \(T_a\) the handling time in hours.
For a type III response, the equation suggested by Rogers (1972) and Juliano (2001) was used:

\[ N_a = N_0 \left( 1 - \exp \left[ d - bN_0 \right] \right) \cdot \left[ 1 + cN_0 \right] \text{ (3)} \]

where \( b, c \) and \( d \) are constants from the function that relate \( a \) and \( N_0 \) in type III functional responses:

\[ a = \frac{d + bN_0}{1 + cN_0} \text{ (4)} \]

**RESULTS**

Functional response curves of female adults of *N. tenuis* to various densities of *B. tabaci* pupae at the three temperatures are illustrated in Fig. 1. As the prey density increased, the number of prey consumed also increased at all temperatures. At low (5) and high (70) prey densities, the consumption of prey by *N. tenuis* at 15, 25, and 35°C ranged from 3.9, 4.2, 4.5 to 16.9, 34.4, 42.4, respectively (Table 1). There were significant differences in the predation rates at the different temperatures (15, 25, and 35°C) and different prey densities (Table 2). The negative linear coefficient (\( P < 0 \)) indicates a type II functional response at 15 and 25°C. When the temperature was 15 and 25°C, the estimate of the linear coefficient in the original cubic model was not significantly different from 0 (\( P > 0.05 \)) and the subsequent elimination of the cubic terms was applied until all the remaining coefficients were significant (Juliano, 2001; Jalali et al., 2010). In the reduced models for 15°C (quadratic model: \( P1 = -0.1171, P = 0.0001 \) and 25°C (quadratic model: \( P1 = -0.0190, P = 0.0264 \)), the negative estimates of the linear coefficient (\( P1 \)) confirmed the functional response indicated by the original model. As indicated by the logistic regression, a type III functional response occurs at 35°C, with a positive linear coefficient and a negative estimate of the quadratic coefficient.

**Table 1.** Mean (± SE) number of prey (*Bemisia tabaci* pupae) consumed by adult females of *Nesidiocoris tenuis* at various prey densities (number of pupae offered per female during 24 h) and three different temperatures.

<table>
<thead>
<tr>
<th>Prey density</th>
<th>15°C</th>
<th>25°C</th>
<th>35°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.9 ± 0.27a</td>
<td>4.2 ± 0.22a</td>
<td>4.5 ± 0.24a</td>
</tr>
<tr>
<td>10</td>
<td>8.0 ± 0.55a</td>
<td>9.2 ± 0.24a</td>
<td>8.7 ± 0.51a</td>
</tr>
<tr>
<td>20</td>
<td>12.8 ± 0.95b</td>
<td>16.1 ± 0.85a</td>
<td>18.5 ± 0.71a</td>
</tr>
<tr>
<td>35</td>
<td>13.1 ± 1.24b</td>
<td>28.3 ± 1.12a</td>
<td>29.4 ± 1.58a</td>
</tr>
<tr>
<td>50</td>
<td>14.9 ± 0.93c</td>
<td>30.0 ± 2.46b</td>
<td>37.5 ± 1.89a</td>
</tr>
<tr>
<td>70</td>
<td>16.9 ± 1.19c</td>
<td>34.4 ± 1.74b</td>
<td>42.4 ± 2.04a</td>
</tr>
</tbody>
</table>

Values followed by different letters within rows are significantly different (LSD, \( P < 0.05 \)).

**Table 2.** Results of the logistic regression analysis of the proportion of *B. tabaci* pupae eaten by adult females of *N. tenuis* at three different temperatures over a period of 24 h.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Coefficient</th>
<th>Estimate</th>
<th>SE</th>
<th>Chi-squared value DoF (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Intercept ((P))</td>
<td>3.0700</td>
<td>0.3199</td>
<td>82.0710</td>
</tr>
<tr>
<td></td>
<td>Linear ((P))</td>
<td>-0.1398</td>
<td>0.0293</td>
<td>22.8544</td>
</tr>
<tr>
<td></td>
<td>Quadratic ((P))</td>
<td>0.00148</td>
<td>0.000779</td>
<td>3.6313</td>
</tr>
<tr>
<td></td>
<td>Cubic ((P))</td>
<td>-4.98E-6</td>
<td>6.135E-6</td>
<td>0.6601</td>
</tr>
<tr>
<td>25</td>
<td>Intercept ((P))</td>
<td>2.2938</td>
<td>0.3147</td>
<td>53.1414</td>
</tr>
<tr>
<td></td>
<td>Linear ((P))</td>
<td>-0.0212</td>
<td>0.0300</td>
<td>0.5001</td>
</tr>
<tr>
<td></td>
<td>Quadratic ((P))</td>
<td>-0.00014</td>
<td>0.000814</td>
<td>0.0283</td>
</tr>
<tr>
<td></td>
<td>Cubic ((P))</td>
<td>-5E-7</td>
<td>6.451E-6</td>
<td>0.0000</td>
</tr>
<tr>
<td>35</td>
<td>Intercept ((P))</td>
<td>2.6733</td>
<td>0.4767</td>
<td>31.4437</td>
</tr>
<tr>
<td></td>
<td>Linear ((P))</td>
<td>0.1011</td>
<td>0.0432</td>
<td>5.4786</td>
</tr>
<tr>
<td></td>
<td>Quadratic ((P))</td>
<td>-0.00458</td>
<td>0.00113</td>
<td>16.4566</td>
</tr>
<tr>
<td></td>
<td>Cubic ((P))</td>
<td>0.000039</td>
<td>8.71E-6</td>
<td>19.6264</td>
</tr>
</tbody>
</table>

Fig. 1. Functional response of *Nesidiocoris tenuis* feeding on various densities of *Bemisia tabaci* pupae at three different temperatures over a period of 24 h.
As is shown in Table 2, the functional responses of *N. tenuis* attacking *B. tabaci* at 15 and 25°C, over a 24-h period, were fitted by Rogers’ random predator equation (equation 2) and at 35°C by equation 3. Table 3 presents the relationships between temperature, attack rates and handling times. The non-parametric Mann-Whitney U-test revealed that the attack rates of *N. tenuis* were significantly different at 15 and 25°C (15°C vs. 25°C: \( Z = -2.763, P = 0.004 \)). In terms of handling times, there were significant differences between the results recorded at all temperatures (15°C vs. 25°C: \( Z = 3.977, P < 0.001 \); 15°C vs. 35°C: \( Z = -3.490, P < 0.001 \); 25°C vs. 35°C: \( Z = -2.862, P = 0.003 \)).

Based on the results of the non-linear regression the parameters \( c \) and \( d \) do not differ significantly from zero at 35°C. In the reduced type III model at 35°C, there was a linear relationship between attack rate (\( a \)) and initial prey density (\( N_0 \)) with a slope (\( b \)) of 0.00979 (Table 2). The estimated attack rates at densities of 5, 10, 20, 35, 50 and 70 at 35°C were 0.0489, 0.0979, 0.1958, 0.3425, 0.4895 and 0.6853 h⁻¹, respectively. As the temperature increased from 15 to 35°C, handling time decreased from 1.4005 to 0.5324. The values of \( a/T_\text{h} \) at 15 and 25°C were 0.1622 and 0.3443, respectively, and at 35°C 0.0919, 0.1838, 0.3677, 0.6435, 0.9194 and 1.2871, respectively for prey densities ranging from 5 to 70. The maximum attack rates (\( T/T_\text{a} \)) at 15°C, 25°C, and 35°C were 17.13, 42.12 and 45.07 whitefly pupae per day, respectively. The coefficients of determination (\( R^2 \)) revealed a greater variation in predation at 15°C than at 25 and 35°C (Table 3).

**DISCUSSION**

The findings of the present study revealed the form of the functional response of *N. tenuis* is temperature dependent. Temperature variation and deviation from the optimal temperature affect predation rates (Logan & Wolensky, 2007; Bonsignore, 2016). To the best of our knowledge, the present study is the first to provide insights into the effect of temperature on the functional response of *N. tenuis*. The results indicate that female adults of *N. tenuis* exhibited a type II functional response at 15 and 25°C, but a type III response at 35°C. This supports previous findings on how environmental conditions (namely temperature) can affect the type of functional response of a predator (Kfir, 1983; Wang & Ferro, 1999; Mohaghegh et al., 2001; Kalyebi et al., 2005).

For arthropod predators, the type II functional response of Holling (1959), obtained in this study, is commonly reported (Sivapragasam & Asma, 1985; Song & Heong, 1997). Several studies report a type II functional response for mirid bugs (Foglar et al., 1990; Song & Heong, 1997; Montserrat et al., 2000; Fantinou et al., 2008, 2009). Nevertheless, a type III functional response is also reported for mirid bugs by Abdel-Aziz (1994), Enkegaard et al. (2001) and Hamdan (2006), in which *Macrolophus cauginosus* Wagner foraged for *Trialeurodes vaporariorum* (Westwood). In the present study, the type III functional response was recorded only at the highest temperature used. This kind of response indicates a relatively poor prey consumption at low prey densities, but that the attack rate increases (the rate at which the predator encounters prey) with increase in prey density (Mahdian et al., 2006). Theoretically, predators with a type III functional response are more suitable for suppressing prey populations, because at low prey densities the predation rates increase exponentially, rather than linearly as in the type II functional response (Holling, 1965; Jalali et al., 2010). In our specific case, it is known that the prey species, *B. tabaci*, and other common prey such as *T. absoluta*, have an optimal thermal development below 35°C (Guo et al., 2012; Martins et al., 2016). Therefore, in greenhouses at high temperatures, this predator could forage and consume prey at higher rates, whereas the pest can barely develop. This would ultimately result in the pest population becoming locally extinct and in successful biological control.

Our findings indicate that both attack rate and handling time are temperature dependent. At high densities (35, 50 and 70 prey) the highest attack rates and shortest handling times were recorded at 35°C. The longest handling time was recorded at 15°C, which indicates this predator allocates a larger amount of time to non-searching activities (e.g., resting) at low temperatures, whereas at high temperatures it spends more time searching and feeding (Jalali et al., 2010; Moayeri et al., 2013). Consequently, the ratio \( a/T_\text{h} \) indicates that *N. tenuis* acted more efficiently against *B. tabaci* at 35°C than at 25°C. Overall, the effect of increase in temperature in poikilothermic organisms, such as insects, is that it increases their activity and metabolism. The consequent higher consumption of energy needs somehow to be fueled, for instance, by increased predation (Sentsi et al., 2012). Among all the species of Dicyphine recorded on vegetable crops in Mediterranean areas *N. tenuis* is the most thermophilous species (Sanchez et al., 2009). The maximum developmental rate of first instars nymphs and eggs of this predator is reported to be between 31.9 and 35.6°C (Martinez-Garcia et al., 2016), therefore, this predator is expected to be more efficient at 35°C than at the two lower temperatures used in this study.

**Table 3.** Parameters (mean ± SE) of the random predator equation, which indicate the type of functional response of females of *Nesiodorsus tenuis* to different densities of *Bemisia tabaci* measured at three different temperatures over a period of 24 h.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Type</th>
<th>Mean ± SE</th>
<th>B</th>
<th>Mean ± SE</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>II</td>
<td>0.2273 ± 0.0708</td>
<td>0.0875–0.3672</td>
<td>–</td>
<td>1.401 ± 0.0577</td>
</tr>
<tr>
<td>25</td>
<td>II</td>
<td>0.1962 ± 0.0440</td>
<td>0.1093–0.2831</td>
<td>–</td>
<td>0.5698 ± 0.0364</td>
</tr>
<tr>
<td>35</td>
<td>III</td>
<td>–*</td>
<td>–</td>
<td>–</td>
<td>0.0098 ± 0.0018</td>
</tr>
</tbody>
</table>

* in the best fit type III model, relationship between attack rate and initial number of prey is linear (\( a = bn_0 \)).
Due to its searching efficiency over a broad range of temperatures, especially high temperatures (25–35°C), *N. tenuis* is potentially a good biological control agent for use against whiteflies in warm environments such as greenhouses. Nonetheless, although the type of functional response is considered to be an important factor, it is not the only criterion determining the failure or success of a biological control agent. In addition, functional responses are measured in small laboratory arenas, which by their very simplicity are very unlike natural conditions and therefore the results should be interpreted carefully (Kareiva, 1990; Zamani et al., 2006). Further studies are thus needed to assess the effect of large searching areas, spatial complexity (Zamani et al., 2006), light intensity (Koski & Johnson, 2002), refuges measured in small laboratory arenas, which by their very simplicity are very unlikely natural conditions and therefore the results should be interpreted carefully (Kareiva, 1990; Zamani et al., 2006), different host plants (De Clercq et al., 2000), light intensity (Koski & Johnson, 2002), refuges for prey (Messina & Hanks, 1998) and of other common predators such as ants and spiders (Kareiva, 1990) on the functional response of *N. tenuis*.

**ACKNOWLEDGEMENTS.** Authors are grateful to the Department of Plant Protection at Vali-e Asr University of Rafsanjani (Rafsanjani, Iran), the Department of Plant Protection of Agricultural and Natural Resources Research Center in Southern Kerman (Jiroft, Iran), to the Italian Ministry of Education, Universities and Research (SIR project no. RBSI14H02A) for providing financial support for this research, Z. Tazerouni from the Tarbiat Modares University (Iran) for her helpful suggestions on the statistical analyses, S. AmiraZal from the Vali-e Asr University of Rafsanjani for technical assistance, three anonymous referees and the editors for their constructive comments, which greatly improved the quality of this manuscript.

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Received October 30, 2016; revised and accepted May 23, 2017
Published online July 13, 2017