

Bulletin of Entomological Research

Optimizing *Nesidiocoris tenuis* (Hemiptera: Miridae) as a biological control agent: mathematical models for predicting development as a function of temperature --Manuscript Draft--

Manuscript Number:	
Full Title:	Optimizing <i>Nesidiocoris tenuis</i> (Hemiptera: Miridae) as a biological control agent: mathematical models for predicting development as a function of temperature
Article Type:	Full research paper
Keywords:	<i>Nesidiocoris tenuis</i> , developmental rate, thermal thresholds, predatory mirids, pest management tools
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Abstract:	<p>In order to achieve an optimal use of <i>N. tenuis</i> as biological control agent, adequate field management and programmed mass rearing are essential. Mathematical models can establish the temperature-dependent developmental rate, and therefore represent extremely useful tools for optimization.</p> <p>This study was designed to determine the development rate of <i>N. tenuis</i> according to temperature, establish models, and validate them.</p> <p><i>N. tenuis</i> was able to achieve complete development from egg to adult at constant temperatures between 15 and 35 °C with a high survivorship (>80%) in the range 18-32 °C. Total developmental time decreased from a maximum at 15 °C (63.4 days) to a minimum at 33 °C (12.67 days) and after that, increased at 35 °C (13.98 days). Linear and nonlinear developmental models demonstrated a high level of accuracy ($R^2 > 0.86$). A field validation revealed that Logan type III and Lactin offered the best prediction (95.0% and 94.5%, respectively). While the maximum development was obtained between 31.9 °C and 35.6 °C for N1 in Loga type III and Brière model and for egg stage in Brière model. Optimal survival and highest developmental rate fall within the range of 27 to 30 °C.</p> <p>Mathematical models were established to predict the developmental time of <i>N. tenuis</i>. These models provide a valuable tool for using <i>N. tenuis</i> as a biological control agent. The data obtained on developmental time and mortality at different temperatures are useful for planning the mass rearing of this predator.</p>

1 **Optimizing *Nesidiocoris tenuis* (Hemiptera: Miridae) as a biological control agent:**
2 **mathematical models for predicting development as a function of temperature**

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11 **SHORT TITLE:** Mathematical models for predicting *Nesidiocoris tenuis* development.

12 **ABSTRACT**

13 In order to achieve an optimal use of *N. tenuis* as biological control agent, adequate field management and
14 programmed mass rearing are essential. Mathematical models can establish the temperature-dependent
15 developmental rate, and therefore represent extremely useful tools for optimization.

16 This study was designed to determine the development rate of *N. tenuis* according to temperature, establish models,
17 and validate them.

18 *N. tenuis* was able to achieve complete development from egg to adult at constant temperatures between 15 and 35
19 °C with a high survivorship (>80%) in the range 18-32 °C. Total developmental time decreased from a maximum at
20 15 °C (63.4 days) to a minimum at 33 °C (12.67 days) and after that, increased at 35 °C (13.98 days). Linear and
21 nonlinear developmental models demonstrated a high level of accuracy ($R_a^2 > 0.86$). A field validation revealed that
22 Logan type III and Lactin offered the best prediction (95.0% and 94.5%, respectively). While the maximum
23 development was obtained between 31.9 °C and 35.6 °C for N1 in Loga type III and Brière model and for egg stage
24 in Brière model. Optimal survival and highest developmental rate fall within the range of 27 to 30 °C.

25 Mathematical models were established to predict the developmental time of *N. tenuis*. These models provide a
26 valuable tool for using *N. tenuis* as a biological control agent. The data obtained on developmental time and mortality
27 at different temperatures are useful for planning the mass rearing of this predator.

1

2 **Key Words:** *Nesidiocoris tenuis*, developmental rate, thermal thresholds, predatory mirids, pest management tools.

3

4 1 INTRODUCTION

5 *Nesidiocoris tenuis* (Reuter 1985) (Hemiptera: Miridae) is a common predatory mirid bug widely distributed in the
6 Mediterranean region and in other places with a similar climate (Castañé et al., 2011). This mirid occurs
7 spontaneously and colonizes those horticultural crops (e.g. tomato), both in greenhouses and outdoor, which are not
8 heavily sprayed with insecticides (Arnó & Gabarra, 2011).

9 This predator makes significant contributions to the control of important horticultural and ornamental pests such as
10 the whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), the lepidopteran species *Tuta absoluta* (Meyrick)
11 (Lepidoptera: Gelechiidae), the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), the aphid
12 *Myzus persicae* (Sulzer) and the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and is utilized
13 commercially to control these pests (Castañé et al. 2011; Urbaneja, 2003; Calvo, 2009; Moreno-Ripoll, 2012; Pérez-
14 Hedo & Urbaneja, 2014). However, *N. tenuis* is also able to feed on certain host plants (e.g. tomato, beans, eggplant,
15 sweet pepper), causing necrotic rings in leaves and flower petioles, or flower abortion (Urbaneja et al., 2005; Arnó et
16 al., 2006; Sánchez, 2009). The extent of the damage varies depending on the availability of prey or the ratio
17 prey/control agent (Sánchez, 2009). This phytophagous character has long been a point of controversy and must be
18 taken into account when using this predator as a biological control agent (Arnó et al., 2010).

19 Many studies demonstrate that temperature plays a crucial role in arthropod development (Marco et al., 1998;
20 García-Ruiz et al., 2011; Jafari et al., 2012). Developmental rate, survival, reproduction, movement and dynamics of
21 insect pests and their natural enemies are all influenced by temperature (Sharpe & DeMichele, 1977; Roy et al.,
22 2002). Modeling the development of a predator as a function of temperature not only offers useful and precise
23 information that improves the effectiveness of biological control, but these models can also entail a significant cost
24 reduction for this kind of control, as fundamental tools for appropriately incorporating models into Integrated Pest
25 Management programs. Moreover, knowledge of the relationship between *N. tenuis* development and mortality as a
26 function of temperature offers valuable information for programming biofactory mass breeding for commercial
27 purposes as has been shown with other natural enemies like *Neoseiulus californicus* (McGregor 1954) (Acari:
28 Phytoseiidae) (Kim et al., 2013) or *Stethorus punctillum* (Coleoptera: Coccinellidae) (Roy et al. 2002).

1 Although several studies have reported practical application of this mirid (Sánchez, 2009; Calvo, 2012, Moreno-Ripoll
2 et al., 2012), no prior modeling study has been performed. A population model of natural enemies, as *N. tenuis*,
3 could be used to optimize the timing of release and enhance their potential in suppressing important pests. Better
4 knowledge of the population dynamics of both prey and predator would allow developing an optimal control strategy
5 (Kim et al., 2013). Previous studies have reported the effect of constant temperatures on the life cycle parameters of
6 *N. tenuis* (Sánchez et al., 2009). Nevertheless, these studies consider the complete nymph stage of the predator; a
7 separate analysis, however, has yet to be conducted for each one of the nymphal instars (N1 to N5) due to the fact
8 that the model of each would be different. Furthermore, in order to establish a valid developmental model, the
9 developmental rate under fluctuating temperatures and a field validation are also necessary. Mathematical models
10 can play an important role in predicting aspects of insect life cycles and events (Johnson et al., 2007). A variety of
11 linear and nonlinear temperature-dependent functions have been proposed to describe the relationship between
12 temperature and arthropod development (Stinner et al., 1974; Logan et al., 1976; Sharpe & DeMichele, 1977; Lactin
13 et al., 1995; Brière et al., 1999). The linear model is simple and allows for easy estimation of the lower temperature
14 thresholds and thermal constant, but it is not able to estimate at high temperatures. Nonlinear models more
15 accurately describe the rate of developmental over a broad temperature range (Gilbert & Raworth, 1996); and they
16 can estimate upper and lower temperature thresholds and optimal temperature for all life stages (Roy et al., 2002).

17 Under natural climatic conditions, arthropods are exposed to frequent temperature fluctuations (thermoperiods), and
18 such an influence on poikilotherm development may differ from that of exposure to constant temperatures (Beck,
19 1983). Some authors have already described a great number of species with different developmental rates in
20 constant and alternating regimes (Hagstrum & Milliken, 1991; Mironidis & Savopoulou-Soultani, 2008; García-Ruiz et
21 al., 2011).

22 This study investigates the temperature developmental rate and survival of all stages of *N. tenuis*, at different
23 constant and alternating temperatures in order to establish a complete and valid developmental model for this
24 important biological control agent. This information will improve the use of *N. tenuis* as a biological control agent
25 utilized in inoculative (especially in pre-plant releases) and conservation strategies for local populations. It will also
26 reduce the probability of crop damage caused by the mirid and improve programming in mass rearing biofactories.

27

28 **2 MATERIALS AND METHODS**

29 **2.1 Insects**

1 A stock colony of *N. tenuis* was established in 2012 in the laboratory of Crop Protection at the University of La Rioja
2 from a population provided by Biobest, BE (Wasterlo, Belgium). To rear the insects, prismatic methacrylate cages
3 (23.5 x 21.5 x 5.5 cm), which contain four 2.1 cm diameter holes in the cover, were utilized. The holes were closed
4 by a filter paper which permitted the exchange of gases, avoided excess humidity, and prevented the mirids from
5 escaping. A zig-zag filter paper was also deposited on the bottom of the cage to avoid humidity and provide shelter to
6 the insects. Commercial sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Nutrimac®) provided
7 by Biobest were supplied twice a week to feed *ad libitum* the insects. Then, *Phaseolus vulgaris* L. pods were also
8 provided because adult females laid their eggs inside the plant tissue and provide the necessary water source. The
9 plant choice was based on management simplicity, especially referring to insect isolation. It is important to note how
10 difficult it is to see clearly these eggs under the stereomicroscope.

11 The stock colony was maintained in a growth chamber (Sanyo MLR- 350H®, Sanyo, Japan) at 24 ± 1 °C, $60\pm 5\%$ RH
12 and a 16:8 L:D photoperiod.

13 In order to obtain the insects needed for the experiments, new bean pods were introduced in the methacrylate cages
14 of the stock colony. 24 hours later, the bean pods were deposited into a new empty cage of the same characteristics
15 as those used in mass rearing. Bean pods were observed under a stereomicroscope every 24 hours expecting the
16 non-visible eggs to hatch to obtain newly emerged *N. tenuis* nymphs to be used in the bioassays. The entire process
17 was performed at the corresponding temperature, $60\pm 5\%$ RH and 16:8 L:D photoperiod.

18

19 **2.2 Laboratory bioassays**

20 The development of *N. tenuis* nymphs was monitored at ten constant temperatures: 12, 15, 18, 21, 24, 27, 30, 33, 35
21 and 36 ± 1 °C. With the aim of establishing possible differences in mean developmental time between constant and
22 variable temperatures, one type-day was constructed simulating a typical summer day in Mediterranean climates.

23 The temperature fluctuated every hour with a minimum of 17 °C, a maximum of 31 °C and a mean of 24 °C (Fig. 1).

24 Between 50 and 60 newly emerged *N. tenuis* nymphs obtained as indicated above were removed with a fine brush
25 and introduced into individual cylindrical plastic cages (1.6 cm height x 3 cm diameter). Every two days, a small piece
26 of bean pod (0.5 x 0.5 cm) was incorporated as a host plant. *E. kuehniella* eggs were supplied to feed the insects *ad*
27 *libitum*. Each cage lid had a hole with a diameter of 0.8 cm covered with filter paper to prevent humidity and the
28 mirids from escaping. The cages were maintained in the growth chamber at the corresponding temperature and at
29 the same relative humidity and photoperiod conditions as those used in rearing.

1 Daily determinations of insect development and survival were made (by observing the presence of exuviae) until
 2 adults emerged. To estimate the sex ratio, the adults were sexed by observing abdominal differences between males
 3 and females. Due to the difficulty of observing most part of the eggs laid by the females inside the plant tissue, egg
 4 mortality were not quantified.

5

6 **2.3 Mathematical models**

7 The relationship between temperature (T) and developmental rate ($r = 1/d$, where d is the mean developmental time
 8 in days), under constant and alternating temperatures was determined by both linear and nonlinear regression
 9 models. The empirical nonlinear models were selected because these models are superior quantitatively at both high
 10 and low temperatures as compared to the traditional degree-day model; and unlike other nonlinear models, these
 11 models can predict the lower (LT) and upper (UT) temperature threshold for development (Logan et al., 1976; Lactin
 12 et al., 1995; Brière et al., 1999).

13 *Linear model.* The relationship between T and r was predicted by a regression analysis where $r = a + bT$; a and b
 14 were estimated by least-squares regression. *The lower developmental threshold (T_0)* and the degree-day
 15 requirements (DD) were estimated by the equations $T_0 = -a/b$ and $DD = 1/b$.

16 *Logan type III model.* The mathematical expression of this model is a combination of 2 functions (Hilbert & Logan,
 17 1983). The first function (representing the ascending rate of development with the increasing temperatures) is
 18 sigmoid. The second function, developed by Logan et al. (1976), represents the descending portion of developmental
 19 rate with increasing temperatures. The expression of the model is:

$$20 \quad r(T) = \psi \left(\frac{(T-T_b)^2}{(T-T_b)^2 + D^2} \right) - e^{-((T_m - (T-T_b))^{\Delta T})}$$

21 where T is the temperature; $r(T)$ is the developmental rate at T temperature; T_b is the base temperature (for
 22 temperatures below T_b , the rate of development presumably equals 0); T_m is the lethal maximum temperature
 23 threshold ($^{\circ}\text{C}$ above T_b); ΔT is the width of the high-temperature boundary area; and ψ and D are empirical constants
 24 (Logan et al., 1976).

25 *Lactin model.* Lactin et al. (1995) modified the nonlinear model proposed by Logan et al. (1976) to obtain another
 26 model capable of estimating the upper temperature threshold. To do so the ψ parameter is suppressed and a new
 27 one (λ) that permits this estimation is introduced:

$$28 \quad r(T) = e^{\rho T} - e^{\rho(T_{max} - (T_{max} - T)^{\lambda})} + \lambda$$

1 where T is the temperature; $r(T)$ is the developmental rate at T temperature; T_{max} is the supraoptimal temperature at
 2 which $r(T) = \lambda$; Δ is the range of temperature between T_{max} and the temperature at which $r(T)$ is maximum; ρ
 3 describes the acceleration of the function from the LT to optimal temperature and λ is a parameter that allows the
 4 curve to intersect the abscissa at suboptimal temperatures, making the estimation of the lower developmental
 5 threshold (LT) possible. This parameter represents the asymptote to which the function tends at low temperatures.

6 *Brière model.* With the objective of obtaining a more simplified developmental model, Brière et al. (1999) developed a
 7 model with only 3 parameters:

$$8 \quad r(T) = a T (T - T_0) (T_L - T)^{1/2} \text{ for } T_0 < T < T_L$$

9 where T is the temperature; $r(T)$ is the developmental rate at T temperature with a value of 0 for temperatures $T < T_0$
 10 and $T > T_L$; T_0 is the lower developmental threshold; T_L is the upper developmental threshold and a an empirical
 11 constant (Brière et al., 1999).

12

13 **2.4 Field validation**

14 To validate the models estimated at constant temperature, field measurements were recorded of embryonic
 15 development and of nymphal development and survival. In this case, cages identical to those used in the laboratory
 16 bioassays were placed inside a covered structure to protect them from rain and direct insolation. The ambient
 17 temperature was recorded at 30 minutes intervals, using a data-logger (Testostor 175). Insect development and
 18 mortality were recorded every 24 hours.

19 To compare the observed and the predicted developmental times, an integration of the increments in development at
 20 30 minute intervals within the lower and upper developmental temperature limits given by each model was used. The
 21 fit percentage of the models to the field development was calculated by dividing the developmental time predicted by
 22 each model by the mean field developmental time.

23

24 **2.5 Statistical analyses**

25 Developmental time of female and male *N. tenuis* and survival percentages and developmental times between
 26 temperature regimes were compared using the t-Student test. Nymphal developmental times were analyzed using a
 27 one-way analysis of variance (ANOVA). A significance level of 5% ($\alpha=0.05$) and SPSS (version 20.0; SPSS Inc.,
 28 Chicago, IL, USA) were utilized in all cases.

1 The fit of the linear and nonlinear models and the estimation of the different associated parameter values were
2 established with the help of the Tablecurve 2D program. The initial parameter estimation for the nonlinear Logan type
3 III and Lactin models was developed according to the suggestions of Logan (1998).

4

5 **3 RESULTS**

6 **3.1 Survivorship**

7 *N. tenuis* was able to achieve complete development from egg to adult at constant temperatures between 15 and 35
8 °C. Throughout this range, the percentage mortality for each nymphal instar fits three different patterns (Fig. 2): a
9 backward J-shape is characteristic for N1; a U-shaped valley for N2 to N5; and a U-shape for the total nymphal
10 development. The lowest cumulative survival rates of 56.41 and 43.40% were recorded at 15 °C and 35 °C,
11 respectively.

12 There were no significant differences among the mortality of *N. tenuis* nymphs developed under constant, alternating
13 and field temperatures: 16.0 ± 5.10 ; 18.0 ± 3.74 ; and 10.0 ± 3.16 , respectively ($F=1.04$; $P=0.3832$; $df=2$).

14

15 **3.2 Developmental time**

16 The developmental times of the immature stages of *N. tenuis* at each experimental temperature between 15 and 35
17 °C) are presented in Table 1. There was no egg survival at lower and upper tested temperatures (12 and 36 °C). The
18 mean total developmental time of immature *N. tenuis* progressively decreased from 63.40 ± 0.83 days at 15 °C, until
19 reaching the lowest value (12.67 ± 0.07 days) at 33 °C. Thereafter, an increase in temperature was associated with an
20 increase in developmental time (13.98 ± 0.12 days at 35 °C).

21 No significant differences were observed between the total developmental time of males and females at all constant,
22 alternating and field tested temperatures.

23

24 **3.3 Model description**

25 The estimated parameters as well as the adjusted coefficients of determination of the four tested models are
26 displayed in Table 2. The adjusted coefficients of determination for all nonlinear models were high ($R_a^2 > 0.96$), slightly
27 higher than those of the linear model ($R_a^2 > 0.86$).

1 The upper and lower thresholds and the optimal temperatures (T_{max} , T_0 and T_{opt} , respectively) for the different models
2 are presented in Table 3. T_{max} , and T_{opt} were not obviously estimated by the linear model. T_0 ranged between 5.9 and
3 12.5 °C, T_{opt} was estimated to be between 31.9 and 35.6 °C, and T_{max} varied between 35.1 °C and 43.4 °C. The
4 values of T_0 estimated by the Logan type III model were lower than those estimated by the other models due to the
5 fact that its equation approaches zero asymptotically for temperatures close to the T_0 (Fig. 3).

6

7 **3.4 Model evaluation**

8 Table 1 shows the developmental time resulting from the alternating temperature assay. These values were
9 significantly the same as those obtained at the constant temperature of 24 °C for N1 and N5. Although, for eggs, N2,
10 N3, N4, and total development times were significantly different it could be assumed that these differences are due to
11 the natural variability as outlined in the discussion.

12 The duration of life stages development obtained under field conditions and the values estimated by the different
13 models are detailed in Table 4. All the aforementioned models present an acceptable fit for each stage and for the
14 total immature stage, the most accurate being the nonlinear models Logan type III and Lactin. The adjusted
15 coefficients of determination were lower for egg stage with the linear model (129.51%) and the Brière model
16 (134.78%).

17

18 **4 DISCUSSION**

19 A U-shaped curve of mortality versus constant temperature is common for insects, with low and nearly constant
20 mortality at favorable temperatures and high mortality beyond certain critical constant temperatures (Wagner et al.,
21 1984; Fornasari, 1995; Lamana & Miller, 1995; Smith & Ward, 1995). However, the range within which minimum
22 mortality takes place is species-specific (Li, 1995).

23 Throughout the range of constant temperatures used in this study, the percentage mortality of *N. tenuis* fits a
24 backward J-shaped pattern is characteristic for the 1st instar. The U-shaped valley pattern describes the mortality of
25 the 2nd to the 5th instars and the entire nymphal stage (Fig. 1). The U-shaped curve is common for insects, with low
26 and nearly constant mortality at favorable temperatures and high mortality beyond certain critical constant
27 temperatures. This pattern has also been previously established for the mortality rate of this specie (Sánchez et al.,
28 2009).

1 Percentages of N1 mortality indicate that it is more sensitive only to the lower tested constant temperature (41.03%
2 of mortality at 15°C). And furthermore, the temperature-dependent mortality of N2-N5 were low (<20%) at all tested
3 temperatures. And what's more, mortality was also lower under alternating temperatures and field conditions
4 (<8.33% and <2.13%, respectively). *N. tenuis* is primarily used as a biological control agent in horticultural crops,
5 especially eggplant and tomato in greenhouse (Castañé et al., 2008). These results demonstrate optimal behavior in
6 these temperature conditions wherein these kinds of crops grow.

7 *N. tenuis* was able to complete its development between 15 and 35 °C. This result concurs with results previously
8 obtained by Hughes et al. (2009) and Sánchez et al. (2009). This temperature range is common in Mediterranean
9 Spring and Summer temperatures. *N. tenuis* eggs hatching at 12 °C and 36 °C failed.

10 The developmental times at constant temperatures obtained in our study were similar to those previously reported by
11 Hughes et al. (2009) and Sánchez et al. (2009), except for the lower assayed temperatures (63.4 days against 93.0
12 at 15.5 °C and 86.7 at 15 °C, respectively). Potential inter-population differences or host plant (beans vs. tobacco and
13 tomato, respectively) would explain the differences in developmental rates, especially those observed at the low
14 temperature where developmental time is considerably slower.

15 The embryonic developmental time was notably higher than those of the nymphal stages. Developmental time of N1
16 and N5 was longer than N2, N3 and N4.

17 The total developmental times of males and females were always the same. Urbaneja et al. (2005) obtained similar
18 results considering only nymphal development and using three different host plants at 25 °C.

19 In accordance with the obtained data for developmental rate at constant temperatures, linear and nonlinear models
20 were established. The adjusted coefficients were high for all cases (Table 2), and better for nonlinear models
21 ($R_a^2 > 0.96$) than for the linear model ($R_a^2 > 0.86$). This result may be considered expected, because the linear model
22 does not predict the developmental rate for the lower temperatures to approach zero asymptotically and it is no
23 possible to determine the T_0 and T_{max} (Wagner et al., 1984). However, a linear model may be interesting in certain
24 cases because of its simplicity and high prediction capacity in the lineal portion of the function temperature-
25 developmental rate (Fan, 1992).

26 Lower thermal thresholds obtained in our study ranged from 5.9 (Logan type III) to 12.5 (Lactin) for eggs and 6.2
27 (Logan type III) to 12.2 (Lactin) for nymphal stages (Table 3). Sánchez et al. (2009) reported values for tomato host
28 of 10.3 and 11.7 °C, for eggs and total nymphal stage respectively, while the developmental threshold on *Nicotina*

1 *tabacum* L. plants was estimated to be 12.9 °C for each immature stage (Hughes et al., 2009), both predicted by a
2 lineal regression analysis.

3 In the present study, T_{max} and T_0 were first observed in the different developmental stages of *N. tenuis*. Upper
4 thresholds obtained ranged from 35.1 °C (Logan type III) to 43.3 °C (Brière) at the egg stage, and 36.3 °C (Logan
5 type III) to 40.5 °C (Brière) at the nymphal stage. The high value obtained in the Brière model for the egg stage is,
6 unlike the other cases, due to the fact that the fall in the developmental rate beyond the T_0 was not as marked. A
7 similar trend was observed for the Lactin and Brière models setting for *A. bipunctata* L. (Coleoptera: Coccinellidae)
8 by Jalali et al. (2010). T_0 ranges from 31.9 °C (Logan type III and Brière for N1 in both cases) and 35.6 °C (Brière for
9 egg stage); these optimum temperatures only indicate the temperature at which the development rate is maximum,
10 but are also associated with high mortality rates. Therefore, the most suitable temperature range for *N. tenuis*,
11 considering developmental rates and mortality, was determined to be between 27 to 30 °C, approximately.

12 Campbell et al. (1974) stated that the developmental rate under variable temperatures should not significantly differ
13 from that observed with constant temperature regimes. However, some authors suggest that alternating
14 temperatures play an important role in the development of several insect species (Hagstrum & Milliken, 1991;
15 Fornasari, 1995; García-Ruiz et al., 2011). Developmental time results were significantly different at 24 °C constant
16 temperature and alternating (mean 24 °C) for egg, N2, N3, N4 and total stages. Nevertheless, by observing the
17 results one can appreciate that the differences between means are very low (less than the elapsed time between
18 observations in all cases). In addition, standard errors reach particularly low values due to the low intrinsic variability
19 of developmental time; this reduced variability within each treatment makes very similar means result appear
20 different in statistical analysis. Considering both of these reasons, one can assume that these differences are due to
21 natural variability between individuals from the same population rather than to the effect of intraday temperature
22 variations. In fact, the fit percentages between developmental times for all immature stages under constant and
23 alternating regimes ranged from 88.1% to 97.7%, which is approximately similar to the best adjustment percentages
24 provided by the models for developmental time obtained under field conditions (82.2-98.5% for type III Logan and
25 81.4-100.0% for Lactin).

26 The field validation established that the nonlinear models with the best prediction of *N. tenuis* developmental time
27 and with high percentages of fit were Logan type III and Lactin.

28 These models are favored in the case of *N. tenuis*, given that they present the possibility of establishing an accurate
29 biofix associated with an early pre-plant release in the nursery, usually implemented in the control of *T. absoluta* and

1 *B. tabaci* in greenhouse tomato crops. A different biofix should be used if a subsequent release of *N. tenuis* adults in
2 the greenhouse is necessary because of a sufficiently high population density of one of the target pests (Calvo et al.,
3 2012).

4 The success of *N. tenuis* as a biological control agent using an inundative strategy depends heavily on effectively
5 planning its mass rearing, as well as on optimizing the timing of release. The present results can serve as a useful
6 tool for achieving both objectives. Therefore, these results will contribute to improving the use of *N. tenuis* as a
7 biological control agent of important agricultural pests.

8

9 **ACKNOWLEDGEMENTS**

10 The authors would like to express their gratitude to Dr. Eduardo Sáenz de Cabezón (Universidad de La Rioja) for
11 supporting the mathematical and statistical analysis.

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- 4

1 Table 1. Mean developmental time (days \pm SE) of immature stages of *N. tenuis* at constant and alternating (mean of
2 24 °C) temperatures.

Temperature (°C)	Life stage						
	Egg	N1	N2	N3	N4	N5	Total
15	26.65 \pm 0.12 (39)	11.43 \pm 0.26 (22)	8.48 \pm 0.23 (21)	8.08 \pm 0.10 (17)	8.76 \pm 0.18 (17)	13.34 \pm 0.21 (16)	63.4 \pm 0.83 (16)
18	17.66 \pm 0.10 (48)	7.64 \pm 0.20 (42)	5.28 \pm 0.15 (41)	4.93 \pm 0.12 (41)	5.63 \pm 0.08 (40)	8.79 \pm 0.10 (39)	49.41 \pm 0.55 (39)
21	11.12 \pm 0.10 (42)	4.41 \pm 0.10 (36)	2.96 \pm 0.09 (33)	2.72 \pm 0.09 (33)	3.14 \pm 0.07 (33)	5.14 \pm 0.12 (30)	29.31 \pm 0.17 (30)
24*	8.14 \pm 0.05 a (47)	3.42 \pm 0.11 a (40)	2.51 \pm 0.08 a (39)	2.21 \pm 0.08 a (39)	2.58 \pm 0.06 a (39)	4.26 \pm 0.09 a (39)	23.16 \pm 0.14 a (39)
27	5.83 \pm 0.00 (50)	2.36 \pm 0.07 (49)	1.78 \pm 0.08 (48)	1.65 \pm 0.06 (48)	1.88 \pm 0.07 (47)	2.80 \pm 0.09 (43)	16.29 \pm 0.08 (43)
30	4.98 \pm 0.06 (49)	2.18 \pm 0.05 (48)	1.56 \pm 0.07 (47)	1.34 \pm 0.07 (43)	1.57 \pm 0.12 (41)	2.37 \pm 0.09 (37)	14.00 \pm 0.10 (37)
33	4.00 \pm 0.00 (55)	2.16 \pm 0.05 (45)	1.50 \pm 0.07 (43)	1.30 \pm 0.07 (43)	1.43 \pm 0.09 (42)	2.32 \pm 0.09 (41)	12.67 \pm 0.07 (41)
35	4.41 \pm 0.06 (53)	2.33 \pm 0.06 (43)	1.56 \pm 0.09 (38)	1.31 \pm 0.08 (35)	1.74 \pm 0.08 (29)	2.49 \pm 0.09 (22)	13.98 \pm 0.12 (22)
Alternating* temperatures	7.90 \pm 0.04 b (48)	3.16 \pm 0.07 a (44)	2.21 \pm 0.07 b (43)	2.02 \pm 0.05 b (43)	2.85 \pm 0.11 b (41)	4.16 \pm 0.09 a (39)	22.20 \pm 0.18 b (39)

3 (n), number of individuals used to obtain the developmental time at each temperature treatment.

4 *Means followed by the same letter show significant differences: Egg ($t=3.84$; $P<0.01$); N1 ($t=1.99$; $P=0.05$); N2
5 ($t=2.75$; $P<0.01$); N3 ($t=2.10$; $P=0.04$); N4 ($t=-2.16$; $P=0.03$); N5 ($t=0.79$; $P=0.43$); Total ($t=4.28$; $P<0.01$).

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1 Table 2. Estimated parameters and coefficients of determination for linear and nonlinear models describing the
 2 relationships between temperature ($^{\circ}\text{C}$) and developmental rate (r) for immature stages of *N. tenuis*.

Model	Parameter	Life stage					
		Eggs	N1	N2	N3	N4	N5
Linear	a	-0.1339	-0.1966	-0.2988	-0.3913	-0.2959	-0.2100
	b	0.0110	0.0212	0.02927	0.03529	0.02862	0.01926
	T_0	12.1	9.8	10.2	11.1	10.3	10.9
	R_a^2	0,9585	0.8580	0.9212	0.9479	0.8657	0.9012
Logan Type III	ψ	1.2359	8.8748	6.2417	6.859	2.3433	27.9382
	D	-54.1430	80.3407	60.2880	60.0015	37.8873	180.0851
	ΔT	0.1032	5.4012	5.0744	4.6708	1.4161	3.3744
	T_m	29.5208	43.1807	41.3055	40.5921	31.4446	44.1504
	T_b	5.8069	6.935	6.4530	6.7938	6.6215	6.1158
Lactin	R_a^2	0.9921	0.9678	0.9766	0.9838	0.9861	0.9683
	ρ	0.0096	0.01932	0.0235	0.0254	0.0220	0.0167
	T_{max}	35.3715	42.0198	42.2016	41.9215	38.0831	40.2181
	Δ	0.1068	3.3413	3.4036	3.1464	1.5089	2.1308
	λ	-1.1271	-1.2660	-1.3161	-1.3522	-1.2882	-1.2259
Brière	R_a^2	0.9849	0.9686	0.9822	0.9873	0.9898	0.9671
	a	0.0001	0.0003	0.0004	0.0004	0.0004	0.0002
	T_0	11.5566	11.8110	11.1840	11.5308	12.1622	12.0881
	T_L	43.4273	38.4030	39.6210	40.4480	38.5920	39.3817
	R_a^2	0.9687	0.9774	0.9856	0.9888	0.9620	0.9664

3 R_a^2 is the adjusted coefficient of determination.

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1 Table 3. Upper and lower thresholds and optimal temperatures (°C) estimated by linear and nonlinear models for the
 2 development of *N. tenuis* immature stages.

Life stage	Linear model			Nonlinear models								
				Logan type III			Lactin			Brière		
	T ₀	T _{opt}	T _{max}	T ₀	T _{opt}	T _{max}	T ₀	T _{opt}	T _{max}	T ₀	T _{opt}	T _{max}
Egg	12.1	-	-	5.9	35.0	35.1	12.5	34.6	35.2	11.6	35.6	43.4
N1	9.8	-	-	7.2	31.9	39.5	12.2	32.2	38.8	11.8	31.9	38.4
N2	10.2	-	-	6.8	33.1	40.3	11.7	32.4	39.9	11.2	32.3	39.6
N3	11.1	-	-	7.1	33.0	40.4	11.8	33.0	39.5	11.5	33.0	40.5
N4	10.3	-	-	6.7	32.8	36.3	11.5	32.7	36.8	12.2	32.3	38.6
N5	10.9	-	-	6.2	32.3	38.6	12.2	32.6	37.9	12.1	32.6	39.4

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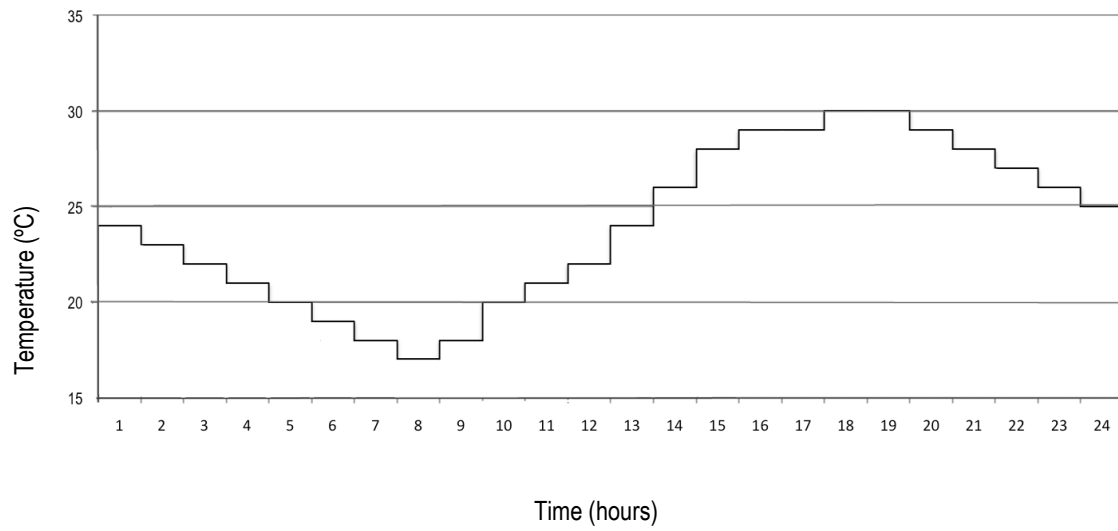
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- 1 Table 4. Days necessary for the immature stages development of *N. tenuis* under field conditions and the models
 2 estimates for constant temperatures.

		Life stage						Total
		Egg	N1	N2	N3	N4	N5	
Field*		8.00±0.00	3.68±0.07	2.47±0.67	2.48±0.08	3.24±0.09	4.45±0.10	24.33±0.08
		(47)	(43)	(43)	(42)	(42)	(42)	(42)
Linear		6.12	4.04	2.71	2.02	2.69	4.38	21.98
		(129.5)	(110.3)	(109.6)	(81.4)	(83.0)	(98.4)	(90.4)
Logan type III		8,12	3.50	2.40	2.04	2.94	4.06	23.10
		(98.5)	(95.0)	(97.0)	(81.4)	(90.7)	(91.4)	(95.0)
Lactin		8.00	3.46	2.50	2.02	2.92	4.04	22.98
		(100.0)	(94.1)	(101.2)	(81.2)	(90.1)	(90.9)	(94.5)
Brière		5.94	3.10	2.40	2.02	2.46	4.08	20.91
		(134.8)	(84.1)	(97.0)	(81.2)	(76.0)	(91.8)	(86.0)

- 3 *(n), number of individuals used to obtain the developmental time in field conditions.
 4 **(%), percentage of fit of developmental times predicted by each model in relation to field developmental time.

- 1 Figure 1. Type-day constructed for the immature stages temperature-dependent development monitoring at variable
- 2 temperatures.



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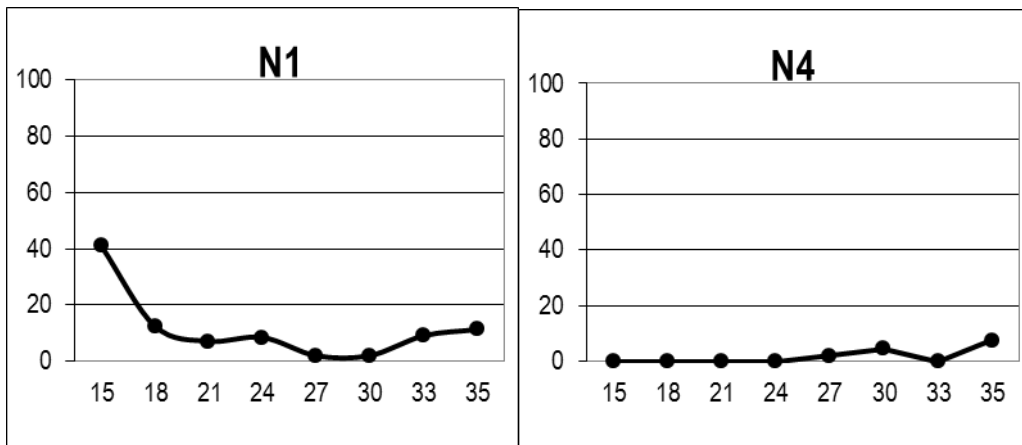
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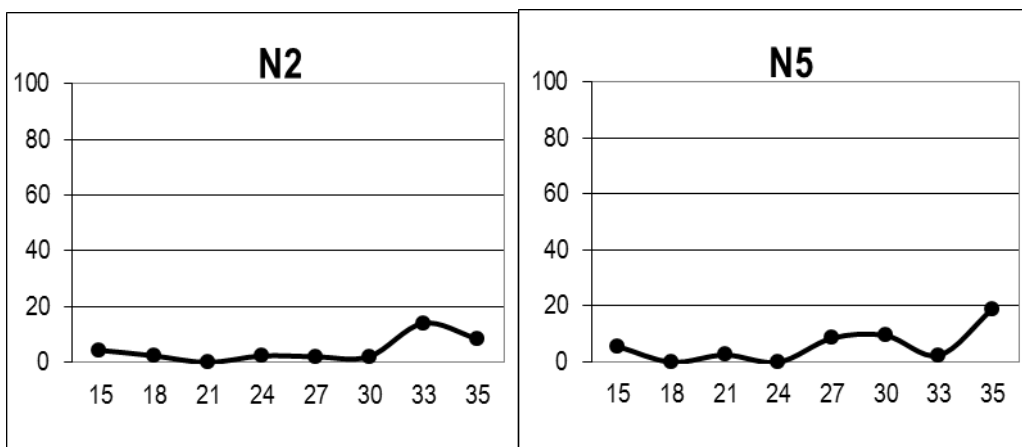
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1 Figure 2. Percentage mortality of *N. tenuis* nymphal instars at constant temperatures (°C).

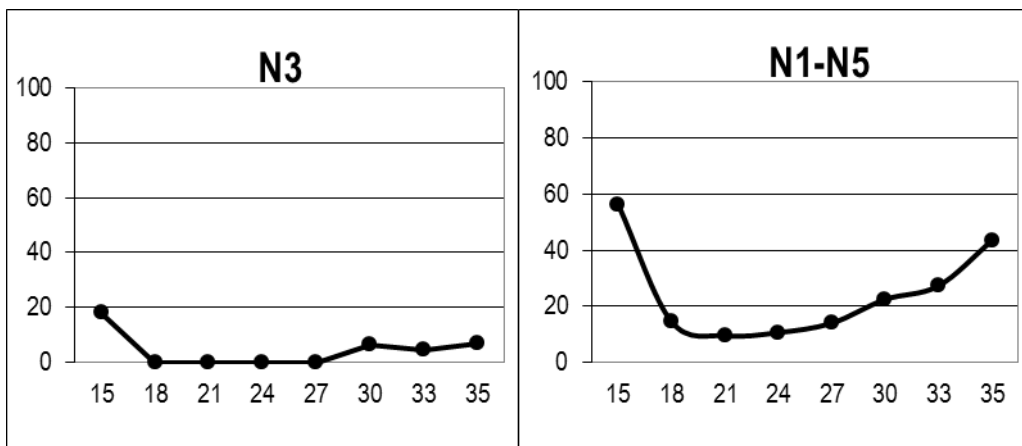
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Figure 3. Predicted rate of development of *N. tenuis* as a function of temperature (°C). (•): Observed values.