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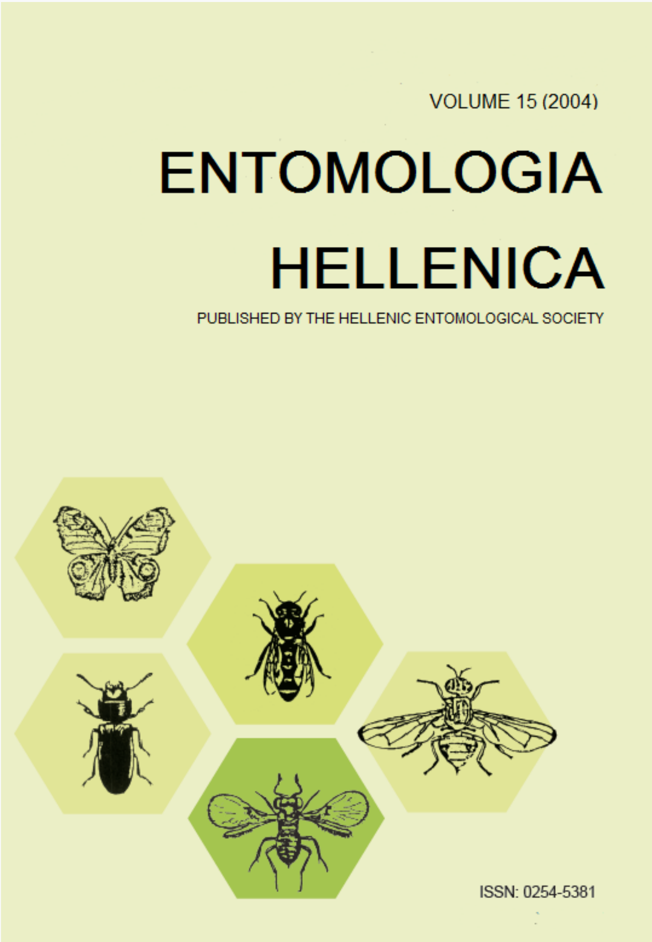

The effect of temperature on the development of Nephus includens (Kirsch) and Nephus bisignatus (Boheman) (Coleoptera: Coccinellidae), predators of Planococcus citri Risso (Hemiptera: Pseudococcidae)

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The effect of temperature on the development of *Nephus includens* and *Nephus bisignatus* (Coleoptera: Coccinellidae) predators of *Planococcus citri* (Hemiptera: Pseudococcidae)

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ABSTRACT

The effect of temperature on the development of the predators *Nephus includens* (Kirsch) and *N. bisignatus* (Boheman) (Coleoptera: Coccinellidae), was studied. The development time of immature stages and the pre-oviposition period of adult females for the two predators was recorded at eight constant temperatures (10, 15, 20, 25, 30, 32.5, 35 and 37.5°C). The beetles were reared on eggs, nymphs and female adults of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) that had developed on squash (*Cucurbita pepo*) and on sour orange leaves (*Citrus aurantium*). Using the linear model for the biological cycle of *N. includens* on squash and on sour orange leaves, the developmental zeros (lower temperature thresholds) were estimated to be 10.9 and 11.0°C respectively and the thermal constants, 490.5 and 472.8 day-degrees respectively. Using the Lactin model the lower thresholds were estimated to be 11.1 and 11.2°C respectively and the upper thresholds 36.1 and 36.0°C respectively. For the biological cycle of *N. bisignatus*, using the linear model, the lower thresholds were estimated to be 9.4°C on squash and 9.3°C on sour orange leaves and the thermal constants were 614.3 and 647.9 day-degrees respectively. Using the Lactin model the lower thresholds were estimated to be 9.9 and 10.0°C respectively and the upper thresholds, 34.7 and 35.0°C respectively. The survival rate of *N. includens* instars at 10, 15, 20, 25, 30, 32.5, 35 and 37.5°C on squash and on sour orange leaves was respectively 0, 34.9, 63.2, 70.6, 63.3, 54.5, 19.8, 0, and 0, 32.2, 61.0, 68.0, 68.3, 56.6. 17.6, 0%. The survival rate of *N. bisignatus* instars at 10, 15, 20, 25, 30, 32.5 and 35°C on squash and on sour orange leaves was respectively, 0, 39.9, 61.1, 60.7, 47.2, 26.4, 0 and 0, 35.7, 65.7, 68.0, 44.2, 29.1, 0%. The results show that *N. includens* has a shorter biological cycle than *N. bisignatus*, whereas the latter species has lower temperature thresholds.

Introduction

Temperature is the main abiotic factor influencing the biology, ecology and population dynamics of pests and their natural enemies. In biological control, details concerning such responses are useful to select natural enemies that are best adapted to conditions favoring target pests (Jervis and Copland 1996, Obrycki and Kring 1998). Biological control, whether using introduction, conservation or augmentation approaches, is facilitated when the climatic responses of biocontrol agents, especially to temperature, are known.

Nephus bisignatus (Boheman) and *N. includens* (Kirsch) (Coleoptera: Coccinellidae) are species of the palearctic region and important indigenous predators of mealybugs (Homoptera: Pseudococcidae) in Greece (Argyriou et al. 1976, Kontodimas 1997). Both species are among the less studied coccinellids with limited knowledge about their biology, and no experimental data concerning the influence of temperature on their development are available.

Nephus bisignatus is distributed throughout Northern Europe (South Norway, Finland, Sweden, Denmark, Netherlands and Germany) (Pope 1973), but it has also been reported in Morocco, South France, Italy and Portugal (Pope 1973, Francardi and Covassi 1992, Magro and Hemptinne 1999, Magro et al. 1999). It has been recently reported in Greece on *Thuja orientalis* L. (Cupressaceae) and *Pistacia lentiscus* L. (Anacardiaceae) infested by *Planococcus citri* (Kontodimas 1997). There is no data concerning any biological features of *N. bisignatus*. *Nephus includens* has been reported in Greece, Turkey, Italy, Spain, and Portugal (Bodenheimer 1951, Viggiani 1974, Argyriou et al. 1976, Longo and Benfatto 1987, Suzer et al. 1992, Katsoyannos 1996, Magro and Hemptinne 1999, Magro et al. 1999). Tranfaglia and Viggiani (1972) found that the female laid 150.6 eggs and lived 74 days at 25-27°C preying on *P. citri*. Kontodimas (2003, 2004) studied the effect of temperature on many biological features of *N. includens*. The average total fecundity was 49.2, 97.8, 162.8, 108.5, 87.4 and 31.1 eggs / female at 15, 20, 25, 30, 32.5 and 35°C, respectively, while females lived 99.5, 84.7, 69.5, 61.1, 49.6 and 30.1 days, respectively, at the above mentioned temperatures. The predator completes five generations in Greece, whereas *N. bisignatus* four. They both overwinter as adults and reach population peak during August and September (Kontodimas 2004).

The effect of temperature on the duration of immature stages and the preoviposition period of *N. bisignatus* and *N. includens* were studied here. The thermal thresholds were estimated using the linear and the Lactin model.

Materials and Methods

Nephus bisignatus and *N. includens* were originally collected in 1997 from *Thuja orientalis* L. (Cupressaceae) in Attiki (Central Greece), and *Citrus sp.* (Rutaceae) in Preveza (Northwestern Greece), respectively, infested by *P. citri*. The same mealybug was used as prey for predator rearing in the laboratory. Citrus mealybug was reared with two ways:

i) on pumpkins (*Cucurbita pepo*)

ii) on *Citrus aurantium* leaves,

at 25°C, L:D 16:8h photoperiod and 65% relative humidity, in large plastic boxes (30x40x15cm) tightly covered in the top with mesh (hole: 0.3x0.4cm). Both predators were reared in large cylindrical plexiglass cages (50cm height x 30cm diameter) containing excess of prey under controlled conditions of temperature (10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1, 30 ± 1, 32.5 ± 1, 35 ± 1 and 37.5 ± 1°C), relative humidity (65 ± 2 %) and photoperiod (L:D 16:8h). Additionally, male-female pairs of each coccinellid were kept separately in

Plastic petri dishes (9 cm diameter x 1.6 cm height) with abundance of prey in same conditions as above. The eggs for development measurements were collected from these pairs. All experiments and rearings were conducted in SANYO incubators, models MLR-3500T, 3500HT.

In order to study the development and survivorship of immatures of the two predators, newly-laid eggs were placed individually in plastic Petri dishes in the above mentioned temperatures in the incubators. Upon hatching coccinellid larvae were constantly supplied with excess of *P. citri* of various stages. Progress in development and survival was assessed every 12 hours. In case of immature mortality the dead individual was removed and replaced by another of the same age, taken from laboratory rearing. Thereby, twenty-five individuals of each species completed their development until adult emergence. The pre-ovipositional period (time interval required for ovary maturation and initiation of mature egg production) was measured for newly emerged females (N=25) of both species. Each female was isolated with a male in plastic petri dishes with excess of prey. Observations for initiation of oviposition were made every 12 hours. The total time for completion of the biological cycle (time elapsed from egg stage until adult oviposition) was estimated by adding the duration of immature stages with the respective pre-ovipositional period. Data were submitted to analysis of variance at a=0.05. Means were separated by using the Tukey – Kramer HSD Test (Sokal and Rohlf 1995). Data were also submitted to 3-way ANOVA at a=0.05 for the significance of the main effects and interactions. The main effects were: a) species b) temperature and c) host of prey. Statistical analysis was performed by using the statistical package with JMP v. 4.02 (SAS 1989).

TABLE 1. Mathematical models that were used to describe the effect of temperature on the development of *N. bisignatus* and *N. includens*.

	Equation		Model	Reference
1	$D = K/(temp - t_{min})$	(1)	Linear or thermal summation	Uvarov 1931, Wigglesworth 1953, Campbell et al. 1974, Campbell and Mackauer 1975, Johnson et al. 1979, Obrycki and Tauber 1982, Logan 1988, De Clerq and Degheele 1992, Lamb, 1992, Fornasari, 1995, Lactin and Johnson 1995, Stathas 2000, Muniz and Nombela 2001.
	$K = D \cdot (temp - t_{min})$	(2)		
	$\frac{1}{D} = \frac{1}{K} \cdot temp - \frac{t_{min}}{K}$	(3)		
	$r = b \cdot temp + a$	(4)		
2	$\frac{1}{D} = e^{\rho \cdot temp} - e^{\left(\rho \cdot t_m - \frac{t_m - temp}{\Delta}\right)} + \lambda$	(5)	Lactin	Lactin et al. 1995, Lactin and Johnson 1995, Briere and Pracros 1998, Royer et al. 1999, Muniz and Nombela 2001, Tobin et al. 2001, Roy et al. 2002

In addition, the Linear model and the Lactin model (Table 1) was used and the following standard thermal indices were calculated:

The lower developmental threshold (t_{min}). The minimum temperature at which the rate of development is zero or there is no measurable development. The SE of t_{min}, when calculated from linear models, is:

$$SEt_{\min} = \frac{\bar{r}}{b}\sqrt{\frac{s^2}{N \cdot \bar{r}^2} + \left[\frac{SE_b}{b}\right]^2}$$

where s^2 is the residual mean square of r, $\bar{r}$ is the sample mean, and N is the sample size (Campbell et al. 1974).

The upper developmental threshold (t_{max}). The maximum temperature at which the rate of development is zero or life cannot be maintained for a prolonged period.

The optimum temperature for development (t_{opt}). The temperature at which the rate of development is maximum. It is estimated as the parameter value for which their first derivatives equals to zero.

The thermal constant (K). The amount of thermal energy (day-degrees) needed to complete development. The thermal constant K can be estimated only by the linear equation as the reciprocal of the slope b, $K=1/b$. The SE of K is:

$$SE_K = \frac{SE_b}{b^2} \quad \text{(Campbell et al. 1974)}$$

The survival of the two predators under the experimental temperatures were described by the equation

$$Survival = a \cdot temp^2 + b \cdot temp + c \quad (6)$$

(Izhevsky and Orlinsky 1988), that could be also be written

$$Survival = a \cdot (temp - t_{min})^2 \cdot (t_{max} - temp) \quad (7).$$

Two statistics were used to evaluate goodness of fit. The coefficient of determination (for linear model) or the coefficient of non-linear regression (for non-linear models) (R^2) and the residual sum of squares (RSS). The higher the values of R^2 and lower of RSS, the better the fit is.

In the linear regression, the last data value, which deviated from the straight line, was omitted. The omission was necessary for the correct calculation of the parameters t_{min} and K (De Clerq and Degheele 1992). Furthermore, equations (1) and (3)(Table 1) were considered as equivalent, and parameters K and t_{min} were estimated from the linear regression. In other studies, these equations have been considered as different models and the parameters K and t_{min} have been estimated from the nonlinear regression of equation (1) (Johnson et al. 1979, Fornasari 1995, Muniz and Nombela 2001).

The non-linear regression was analyzed with the Marquardt algorithm (Marquardt 1963) using the statistical programs JMP v.4.0 (SAS 1989) and SPSS v.9.0. (SPSS 1999)

Results and Discussion

The development time, pre-oviposition period and duration of biological cycle of both predators at eight constant temperatures are presented in Tables 2 and 3. None of the species succeeded in completing development at 10 and 37.5 °C, while *N. bisignatus* did not complete it at 35 °C, as well. The rate of development was positively correlated with temperature until the upper limit of 32.5°C and 30°C for *N. includens* and *N. bisignatus*, respectively. As far as pre-ovipositional period is concerned, the ovaries of *N. includens* showed a higher maturation rate as they start ovipositing about 1-2 days earlier than *N. bisignatus.*

Differences in the total time of the biological cycle of the two predators were only marginally significant, biologically meaningless, at temperatures ≤ 20°C (Table 4). At higher temperatures *N. includens* completed development faster than *N. bisignatus*. The t_{min},

t_{max} and t_{opt} for the biological cycle of the two predators showed that *N. bisignatus* had generally lower temperature thresholds than *N. includens*.

TABLE 2. Duration (Mean±S.D.) of development *Nephus includens* at various constant temperatures (n, in parentheses).

Host of prey A: *Planococcus citri* on *Cucurbita pepo* pumpkins

Tempe-rature (°C)	Egg	1st instar larva	2nd instar larva	3rd instar larva	4th instar larva	Prepupa	Pupa	Pre-oviposition Period (adult - egg)	Total im-mature (egg-adult)	Biological cycle (egg-egg)
10±1	-	-	-	-	-	-	-	-	-	-
15±1	26.84±1.28 (33)	8.18±0.24 (30)	6.36±0.34 (31)	7.26±0.36 (29)	15.12±0.79 (28)	6.08±0.66 (26)	24.14±1.13 (27)	20.34±0.59	93.98±1.12	114.32±1.61
20±1	13.18±1.07 (29)	4.82±0.24 (26)	3.78±0.25 (27)	4.04±0.20 (26)	5.84±0.31 (29)	2.96±0.35 (26)	11.72±1.08 (27)	9.48±0.37	46.34±0.85	55.82±1.10
25±1	8.28±0.36 (27)	2.56±0.22 (27)	2.08±0.19 (27)	2.34±.024 (26)	4.10±0.33 (25)	1.64±0.45 (26)	8.12±0.30 (26)	5.78±0.38	29.12±0.65	34.90±0.46
30±1	5.38±0.30 (28)	2.04±0.14 (28)	1.58±0.19 (26)	1.88±0.22 (26)	3.46±0.29 (26)	1.32±0.35 (26)	5.28±0.38 (28)	4.56±0.17	20.94±0.53	25.50±0.66
32.5±1	4.98±0.39 (29)	1.84±0.24 (28)	1.52±0.34 (28)	1.68±0.35 (27)	3.04±0.32 (26)	1.18±0.24 (26)	4.44±0.58 (27)	4.12±0.46	18.68±1.14	22.80±1.53
35±1	5.68±0.89 (36)	2.42±0.66 (32)	1.68±0.56 (31)	1.74±0.29 (31)	3.32±0.48 (30)	1.52±0.51 (29)	5.06±0.55 (32)	4.42±0.31	21.42±1.44	25.84±1.57
37.5±1	-	-	-	-	-	-	-	-	-	-

Host of prey B: *Planococcus citri* on *Citrus aurantium* leaves

Tempe-rature (°C)	Egg	1st instar larva	2nd instar larva	3rd instar larva	4th instar larva	Prepupa	Pupa	Pre-oviposition Period (adult - egg)	Total im-mature (egg-adult)	Biological cycle (egg-egg)
10±1	-	-	-	-	-	-	-	-	-	-
15±1	27.04±2.73 (32)	7.58±1.03 (30)	5.62±0.73 (29)	7.32±0.64 (30)	13.92±1.39 (27)	5.78±0.75 (27)	22.56±1.56 (27)	20.90±1.70	89.82±3.79	110.72±3.87
20±1	12.74±1.41 (28)	4.52±0.47 (27)	3.58±0.34 (27)	3.88±0.42 (25)	5.68±0.48 (25)	2.84±0.40 (28)	11.26±1.19 (28)	8.96±0.73	44.50±2.17	53.46±2.70
25±1	7.84±1.05 (28)	2.70±0.58 (27)	2.00±0.25 (26)	2.22±0.25 (26)	3.96±0.43 (26)	1.56±0.42 (26)	7.98±0.59 (26)	5.62±0.46	28.26±1.66	33.88±1.49
30±1	5.16±0.49 (28)	2.08±0.24 (27)	1.54±0.35 (25)	1.88±0.36 (25)	3.42±0.45 (26)	1.34±0.35 (26)	5.10±0.50 (28)	4.62±0.36	20.52±1.11	25.14±1.30
32.5±1	4.48±0.53 (30)	1.80±0.32 (27)	1.42±0.37 (27)	1.60±0.32 (27)	2.90±0.35 (26)	1.14±0.23 (26)	4.18±0.50 (26)	3.90±0.54	17.52±1.18	21.42±1.59
35±1	5.44±0.98 (37)	2.10±0.71 (31)	1.62±0.55 (33)	1.80±0.29 (33)	3.54±0.78 (32)	1.36±0.57 (30)	4.96±0.71 (29)	4.54±0.38	20.82±2.04	25.36±2.20
37.5±1	-	-	-	-	-	-	-	-	-	-

The fitting of linear and Lactin equations on data of Tables 2 and 3 is presented in Figure 1. The survival of the two predators under the experimental temperatures is presented in Figures 2–5. Using the equations (3) and (5) (Table 1) the lower thresholds for *N. includens* and *N. bisignatus* were estimated to be 9.0–11.1°C and 7.0–10.6°C, whereas using the equation (7) they were estimated to be 9.4–12.2°C and 9.5–11.3 °C respectively. For *N. bisignatus* using Lactin equation the upper thresholds for *N. includens* and *N. bisignatus* were estimated to be 36.0–38.8°C and 34.3–36.4°C respectively, whereas using the equation (7) were estimated to be 37.5–39.4°C and 35.1–36.5°C respectively. Furthermore, a 3-way ANOVA for duration of development with species, temperature and host of prey as factors is presented in Table 5.

Table 3. Duration (Mean±S.D.) of development *Nephus bisignatus* at various constant temperatures (n, in parentheses).

Host of prey A: *Planococcus citri* on *Cucurbita pepo* pumpkins

Temperature (°C)	Egg	1st instar larva	2nd instar larva	3rd instar larva	4th instar larva	Prepupa	Pupa	Pre-oviposition Period (adult - egg)	Total immature (egg-adult)	Biological cycle (egg-egg)
10±1	-	-	-	-	-	-	-	-	-	-
15±1	27.02±1.08 (32)	8.52±0.57 (30)	6.58±0.43 (29)	7.90±0.60 (29)	13.28±0.78 (27)	5.16±0.51 (27)	22.76±1.20 (26)	21.76±0.90	91.22±2.26	112.98±2.51
20±1	14.02±0.99 (30)	5.08±0.37 (27)	3.92±0.37 (26)	4.94±0.58 (26)	6.38±0.65 (26)	2.88±0.44 (26)	10.82±0.89 (27)	10.02±0.59	48.04±2.35	58.06±2.90
25±1	9.10±0.48 (28)	3.36±0.40 (27)	2.48±0.42 (27)	3.34±0.55 (27)	4.26±0.44 (26)	1.76±0.25 (26)	7.28±0.66 (27)	6.48±0.76	31.58±1.59	38.06±2.25
30±1	7.10±0.58 (30)	2.92±0.47 (28)	2.10±0.41 (27)	2.68±0.66 (27)	3.56±0.46 (28)	1.50±0.25 (27)	5.22±0.60 (29)	5.24±0.46	25.08±2.11	30.32±2.14
32.5±1	8.04±0.75 (34)	3.08±0.43 (30)	2.32±0.35 (31)	3.14±0.70 (30)	4.06±0.71 (29)	1.78±0.33 (28)	5.62±0.92 (30)	6.26±0.63	28.04±1.46	34.30±1.84
35±1	-	-	-	-	-	-	-	-	-	-

Host of prey B: *Planococcus citri* on *Citrus aurantium* leaves

Temperature (°C)	Egg	1st instar larva	2nd instar larva	3rd instar larva	4th instar larva	Prepupa	Pupa	Pre-oviposition Period (adult - egg)	Total immature (egg-adult)	Biological cycle (egg-egg)
10±1	-	-	-	-	-	-	-	-	-	-
15±1	27.80±2.30 (31)	8.76±0.91 (31)	7.02±0.77 (29)	8.40±0.60 (30)	13.96±1.07 (27)	5.76±0.74 (27)	23.38±1.83 (28)	22.22±1.38	95.08±4.05	117.30±3.84
20±1	14.88±1.25 (29)	5.50±0.56 (26)	4.14±0.55 (27)	4.70±0.71 (25)	7.02±0.68 (26)	2.76±0.46 (27)	11.28±0.71	10.52±1.03	50.28±2.35	60.80±2.87
25±1	9.22±0.66 (27)	3.56±0.63 (27)	2.66±0.51 (26)	3.52±0.55 (27)	4.48±0.39 (25)	1.80±0.25 (26)	7.42±0.76 (27)	6.64±1.07	32.66±1.41	39.30±2.30
30±1	7.56±0.73 (31)	3.04±0.48 (29)	2.26±0.44 (27)	2.72±0.72 (26)	3.74±0.58 (27)	1.56±0.30 (27)	5.58±0.79 (30)	5.54±0.59	26.46±2.19	32.00±2.43
32.5±1	8.48±0.78 (33)	3.20±0.50 (29)	2.58±0.55 (29)	3.18±0.79 (31)	4.36±0.77 (29)	1.94±0.30 (29)	5.98±0.87 (29)	6.64±0.77	29.72±1.34	36.36±1.74
35±1	-	-	-	-	-	-	-	-	-	-

TABLE 4. Comparison of host plants and predators by using the Tukey – Kramer HSD test.

Tempe-rature (°C)	Egg: *N. includens* on *C.pepo*	*N. includens* on *C.aurantium*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*	1st instar larva: *N. includens* on *C.aurantium*	*N. includens* on *C.pepo*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*
15±1	26.84 a	27.04 a	27.02 a	27.80 a	8.18 a	7.58 a	8.52 a	8.76 a
20±1	13.18 a	12.74 a	14.02 a	14.88 a	4.82 a	4.52 a	5.08 a	5.50 a
25±1	8.28 a	7.84 a	9.10 b	9.22 b	2.56 a	2.70 a	3.36 b	3.56 b
30±1	5.38 a	5.16 a	7.10 b	7.56 b	2.04 a	2.08 a	2.92 b	3.04 b
32.5±1	4.98 a	4.48 a	8.04 b	8.48 b	1.84 a	1.80 a	3.08 b	3.20 b
35±1	5.68 a	5.44 a			2.42 a	2.10 a		

Tempe-rature (°C)	2nd instar larva: *N. includens* on *C.pepo*	*N. includens* on *C.aurantium*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*	3rd instar larva: *N. includens* on *C.aurantium*	*N. includens* on *C.pepo*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*
15±1	6.36 a	5.62 a	6.58 a	7.02 a	7.26 a	7.32 a	7.90 a	8.40 a
20±1	3.78 a	3.58 a	3.92 a	4.14 a	4.04 a*	3.88 a*	4.94 b*	4.70 b*
25±1	2.08 a*	2.00 a*	2.48 b*	2.66 b*	2.34 a	2.22 a	3.34 b	3.52 b
30±1	1.58 a	1.54 a	2.10 b	2.26 b	1.88 a	1.88 a	2.68 b	2.72 b
32.5±1	1.52 a	1.42 a	2.32 b	2.58 b	1.68 a	1.60 a	3.14 b	3.18 b
35±1	1.68 a	1.62 a			1.74 a	1.80 a		

Tempe-rature (°C)	4th instar larva: *N. includens* on *C.pepo*	*N. includens* on *C.aurantium*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*	Prepupa: *N. includens* on *C.aurantium*	*N. includens* on *C.pepo*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*
15±1	15.12 a	13.92 a	13.28 a	13.96 a	6.08 a	5.78 a	5.16 a	5.76 a
20±1	5.84 a*	5.68 a*	6.38 b*	7.02 b*	2.96 a	2.84 a	2.88 a	2.76 a
25±1	4.10 a	3.96 a	4.26 ab	4.48b	1.64 a	1.56 a	1.76 a	1.80 a
30±1	3.46 a	3.42 a	3.56 a	3.74 a	1.32 a	1.34 a	1.50 a	1.56 a
32.5±1	3.04 a	2.90 a	4.06 b	4.36 b	1.18	1.14	1.78 b	1.94 b
35±1	3.32 a	3.54 a			1.52 a	1.36 a		

Tempe-rature (°C)	Pupa: *N. includens* on *C.pepo*	*N. includens* on *C.aurantium*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*	Pre-oviposition period (adult - egg): *N. includens* on *C.aurantium*	*N. includens* on *C.pepo*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*
15±1	24.14 a	22.56 a	22.76 a	23.38 a	20.34 a*	20.90 ab*	21.76 b*	22.22 b*
20±1	11.72 a	11.26 a	10.82 a	11.28 a	9.48 ab*	8.96 a*	10.02 bc*	10.52c*
25±1	8.12 a*	7.98 a*	7.28 b*	7.42 b*	5.78 a	5.62 a	6.48 b	6.64 b
30±1	5.28 a	5.10 a	5.22 a	5.58 a	4.56 a	4.62 a	5.24 b	5.54 b
32.5±1	4.44 a	4.18 a	5.62 b	5.98 b	4.12 a	3.90 a	6.26 b	6.64 b
35±1	5.06 a	4.96 a			4.42 a	4.54 a		

Tempe-rature (°C)	Total immature (egg-adult): *N. includens* on *C.pepo*	*N. includens* on *C.aurantium*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*	Biological cycle (egg-egg): *N. includens* on *C.aurantium*	*N. includens* on *C.pepo*	*N. bisignatus* on *C.pepo*	*N. bisignatus* on *C.aurantium*
15±1	93.98 a*	89.82 b*	91.22 b*	95.08 a*	114.32 a*	110.72 b*	112.98 b*	117.30 c*
20±1	46.34 a *	44.50 b*	48.04 c*	50.28 d*	55.82 a*	53.46 b*	58.06 c*	60.80 d*
25±1	29.12 a	28.26 a	31.58 b	32.66 b	34.90 a	33.88 a	38.06 b	39.30 b
30±1	20.94 a	20.52 a	25.08 b	26.46 b	25.50 a	25.14 a	30.32 b	32.00 b
32.5±1	18.68 a	17.52 a	28.04 b	29.72 b	22.80 a	21.42 a	34.30 b	36.36 b
35±1	21.42 a	20.82 a			25.84 a	25.36 a		

* differences marginally significant

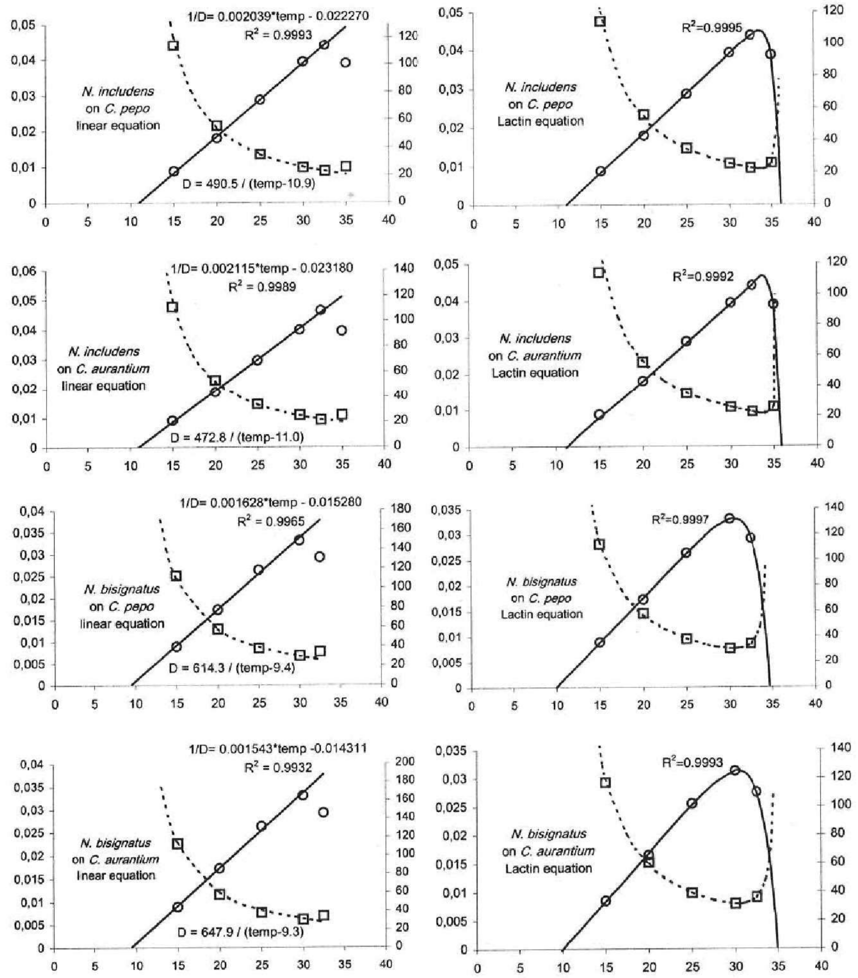

FIG. 1. Fitting of linear and Lactin equations on data of the development of *Nephus includens* and *N. bisignatus* at constant temperatures. Prey: *Planococcus citri* reared on Cucurbita pepo pumpkins or *Citrus aurantium* leaves. In all charts the left ordinate is the rate of development (1/D, in days^{-1}), the right ordinate is the development (D, in days) and the abscissa is the temperature (in °C). The solid line is the 1/D equation whereas the drop line is the D equation. In the linear regression last data values have been omitted, due to deviation from the straight line.

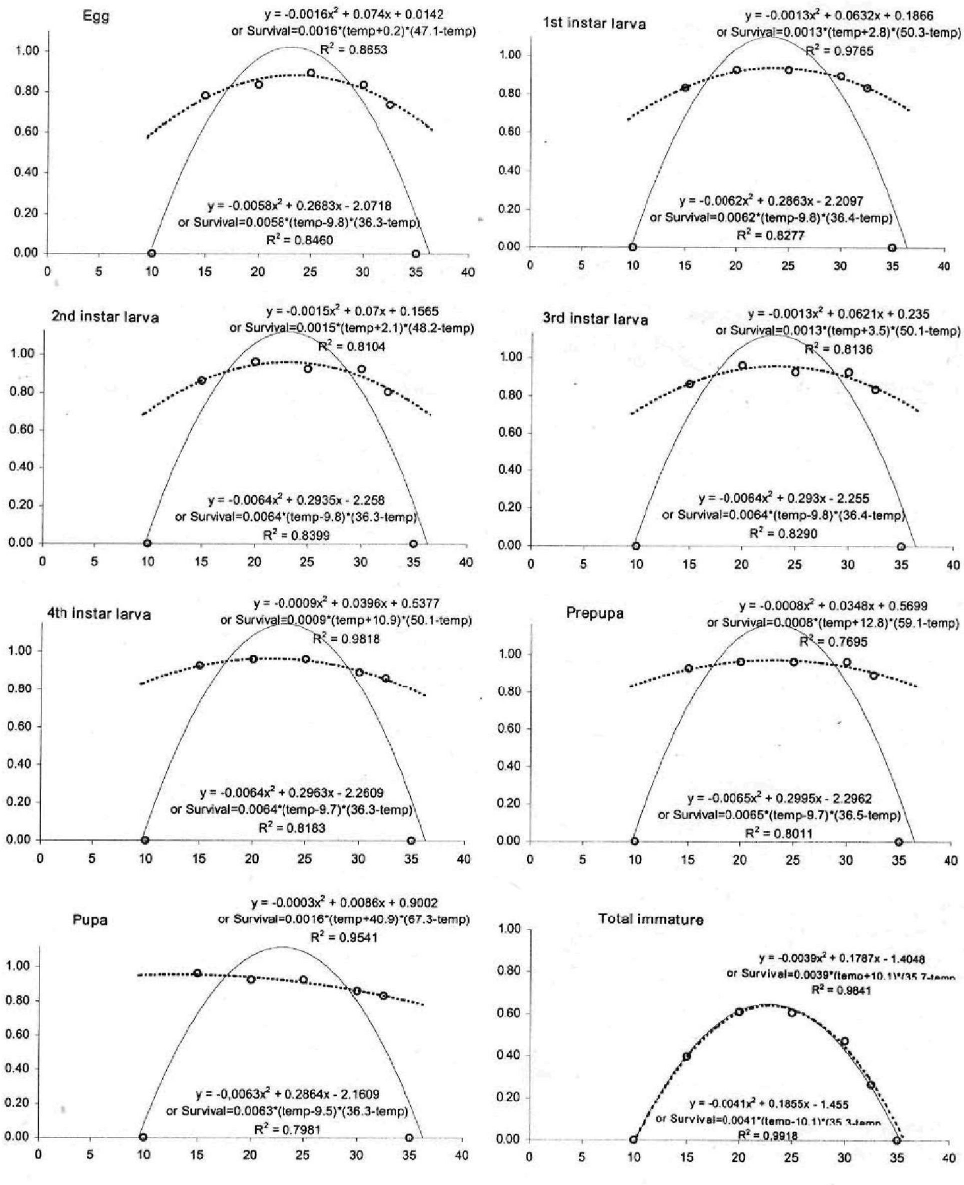

FIG. 2. Survival of immatures of *Nephus bisignatus* at constant temperatures. Prey: *Planococcus citri* reared on *Cucurbita pepo* pumpkins. The solid line include the zero data. In all charts the ordinate is the survival (%) and the abscissa is the temperature (in °C).

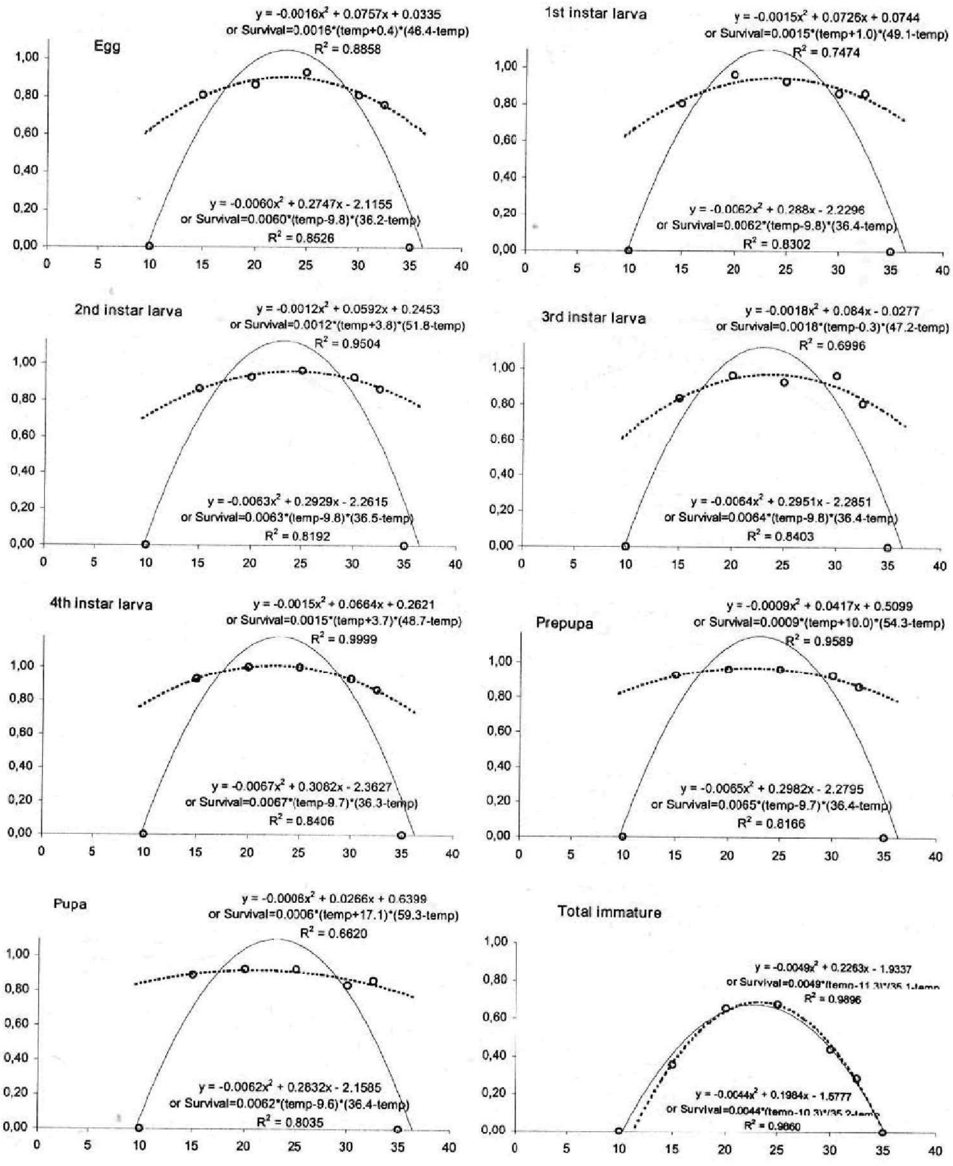

FIG. 3. Survival of immatures of *Nephus bisignatus* at constant temperatures. Prey: *Planococcus citri* reared on *Citrus aurantium* leaves. The solid line include the zero data. In all charts the ordinate is the survival (%) and the abscissa is the temperature (in °C).

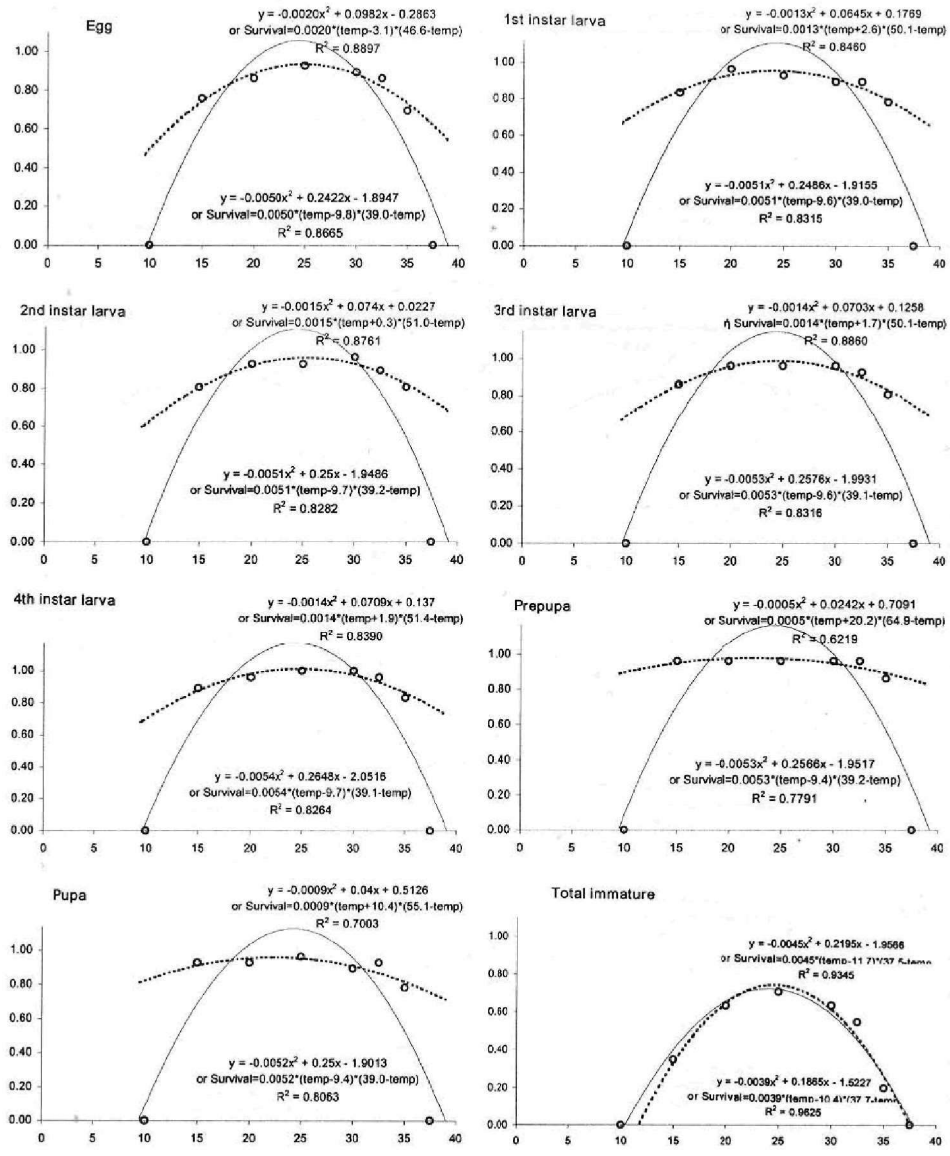

FIG. 4. Survival of immatures of *Nephus includens* at constant temperatures. Prey: *Planococcus citri* reared on *Cucurbita pepo* pumpkins. The solid line include the zero data. In all charts the ordinate is the survival (%) and the abscissa is the temperature (in °C).

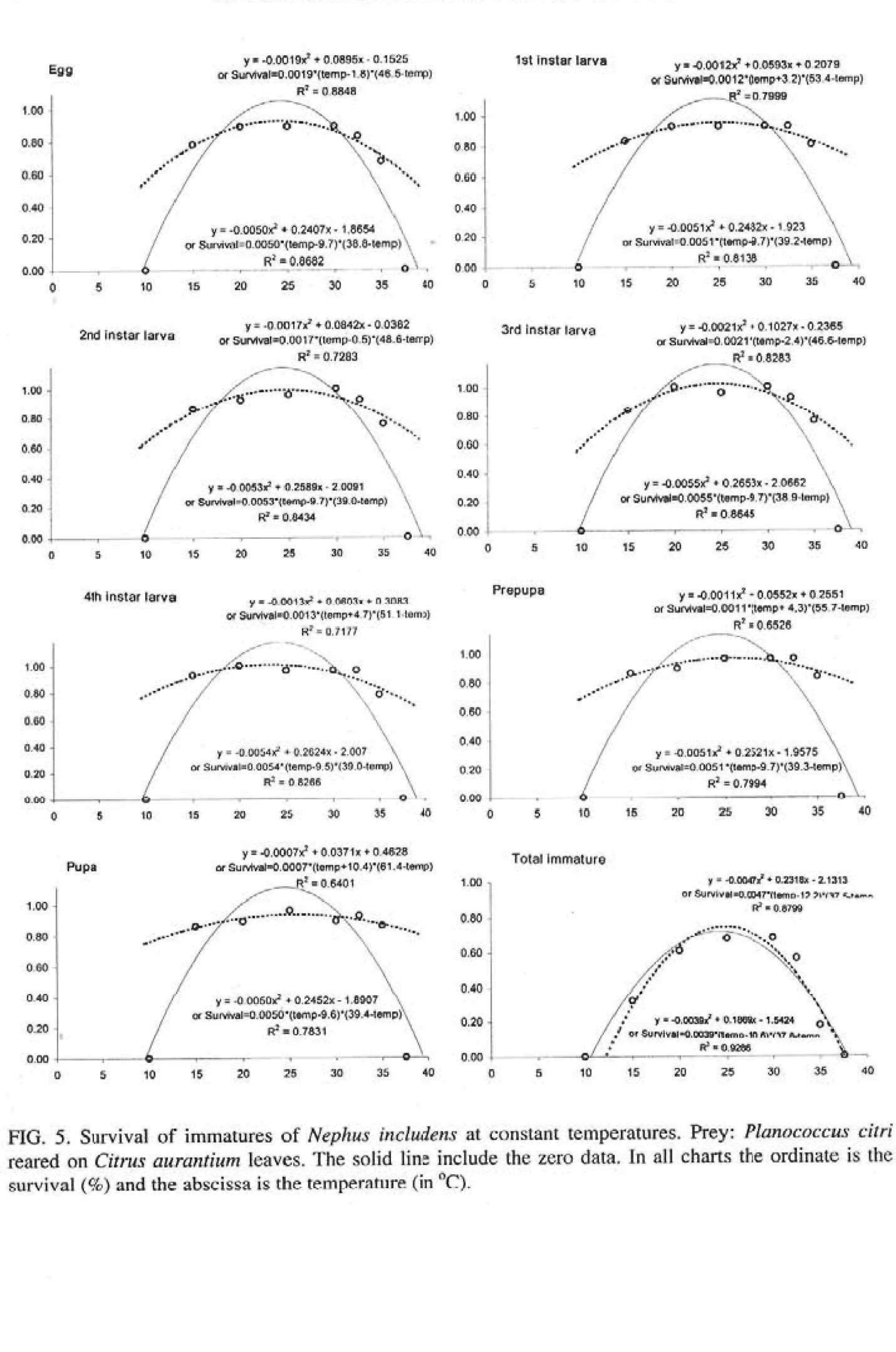

FIG. 5. Survival of immatures of *Nephus includens* at constant temperatures. Prey: *Planococcus citri* reared on *Citrus aurantium* leaves. The solid line include the zero data. In all charts the ordinate is the survival (%) and the abscissa is the temperature (in °C).

TABLE 5. Three-way ANOVA at a=0.05 for the significance of the main effects and interactions of species, temperature and host of prey.

Developmental stages	Statistical parameter	Temperature	Species	Host of *P.citri*	Temperature x Host	Temperature x Species	Species x Host	Temperature x Species x Host
Egg	F	6667.1	34.5	0.9397	1.2839	215.4	16.7	0.7174
	P	<0.0001	<0.0001	0.3328	0.2692	<0.0001	<0.0001	0.6105
1st instar larva	F	2590.4	80.0	0.0017	1.9721	171.2	19.8	2.8532
	P	<0.0001	<0.0001	0.9674	0.0810	<0.0001	<0.0001	0.0148
2nd instar larva	F	2160.0	61.4	0.0091	0.9837	128.1	35.1	5.4
	P	<0.0001	<0.0001	0.9239	0.4269	<0.0001	<0.0001	<0.0001
3rd instar larva	F	2409.1	208.5	0.3453	2.4891	146.8	2.5441	1.1276
	P	<0.0001	<0.0001	0.5570	0.0304	<0.0001	0.1113	0.3444
4th instar larva	F	5109.0	28.0	0.8564	1.7975	193.1	33.1	8.4
	P	<0.0001	<0.0001	0.3551	0.1114	<0.0001	<0.0001	<0.0001
Prepupa	F	1805.2	19.4	0.0210	1.3676	76.5	11.8	3.9
	P	<0.0001	<0.0001	0.8847	0.2347	<0.0001	0.0006	0.0018
Pupa	F	7391.7	123.0	0.8363	1.4394	161.0	29.9	4.7
	P	<0.0001	<0.0001	0.3608	0.2082	<0.0001	<0.0001	0.0003
Pre-oviposition Period	F	8921.5	33.2	5.3631	1.8236	284.2	7.7	2.3
	P	<0.0001	<0.0001	0.2090	0.1063	<0.0001	0.0058	0.0472
Total immature	F	24289.1	11.5	0.4238	0.5688	866.6	109.4	12.2
	P	<0.0001	0.0008	0.5153	0.7240	<0.0001	<0.0001	<0.0001
Biological cycle	F	29127.0	24.8	1.8743	0.5025	1012.8	104.8	9.7
	P	<0.0001	<0.0001	0.1715	0.7744	<0.0001	<0.0001	<0.0001
	df	5	1	1	5	5	1	5
	error df	599						

Nephus includens has a shorter biological cycle than *N. bisignatus*. However, the latter species has t_{min} about 2-3°C lower than the former. This corresponds to the known distribution of *N. bisignatus* in Northern Europe (Norway, Finland, Sweden, Denmark, Netherlands and Germany) (Pope 1973, Francardi and Covassi 1992), and *N. includens* exclusively in countries with warm climate (Turkey, Spain, Italy, Portugal) (Bodenheimer 1951, Tranfaglia and Viggiani 1972, Viggiani 1974, Longo and Benfatto 1987, Suzer et al. 1992, Magro et al. 1999, Magro and Hemptinne 1999). The comparison of the thermal constants leads us to conclude that *N. includens* can complete more generations per year than *N. bisignatus* in temperate climatic conditions. In fact in Greece, they complete five and four generations per year, respectively (Kontodimas 2004).

The geographical distribution of the two predators in composition with our results also support the hypothesis that *N. bisignatus* could be more tolerant to cold than its conspecific. Both predators have lower temperature thresholds than *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), a cosmopolitan predator of *P. citri*, given that its lower developmental threshold for total development is 13.7°C (Babu and Azam 1988). In contrast, the respective threshold of another pseudococcid predator *Nephus reunioni* is 10.9°C (Izhevsky and Orlinsky 1988) almost identical to *N. includens* lower developmental threshold. No other experimental data about critical temperatures of citrus mealybug predators are available in the literature.

The comparison of the two hosts of *Planococcus citri* did not show any biologically statistical differences although the mean total development times for *N. includens* were getting shorter in *C. aurantium* leaves in contrary to *N. bisignatus*.

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