Effects of Temperature on Development and Reproduction of a Predatory Beetle, *Nephus includens* Kirsch (Coleoptera: Coccinellidae)¹

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**ABSTRACT**

The effect of different temperatures on some biological properties of *Nephus includens* Kirsch (Coleoptera: Coccinellidae) was investigated. This species is one of the most important predators of *Planococcus citri* Risso (Homoptera: Pseudococcidae). The development time, mortality and fecundity were determined at constant temperatures of 15, 20, 25, 30, and 35°C and at the variable temperatures, 25–35°C (12 hours 25°C, 12 hours 35°C). Life tables were also constructed for 25, 30, 35, and 25–35°C. The mortality was lower and the mean generation time was shorter at 30°C than at all other temperatures except 35°C. The intrinsic rate of increase was the highest at 30°C (0.081), followed by 0.076 at 25–35°C. The net reproductive rate was higher at 25–35°C than at 30°C. From biological data and population growth parameters calculated from the life tables, 30°C and 25–35°C were determined to be the most suitable temperatures for mass rearing of *Nephus includens*. However, mass rearing at a temperature as high as 35°C could cause deterioration of sprouted potatoes on which the citrus mealybug is reared. Therefore, 30°C would be better than 25–35°C.

**KEY WORDS** *Nephus includens*, citrus mealybug, mass-rearing, biological control

The citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae) is one of the most important citrus pests in Turkey. It causes significant damage by feeding on all parts of citrus except the roots. It sometimes causes 30–60% fruit drop, which ranks it as a key pest in orchards where the natural balance of the pest predator complex has been damaged by overspraying of pesticides (Ozkan et al. 1991).

Biological control against the citrus mealybug has been carried out since 1972, with releases of the ladybeetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera; Coccinellidae) and the parasitoid, *Leptomastix dactilopii* Howard (Hymenoptera: Encyrtidae). These beneficial insects have been mass reared and sold to farmers. However, these nonindigenous species are not capable of overwintering in Tur-

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Prod. #18205
key, probably due to inability to survive low temperature and/or lack of alternative prey (Bodenheimer 1951, Ebeling 1959, Kececioglu 1975, Uygun 1981, Soylu et al. 1983). Therefore, they must be mass-reared and released every year. On the other hand, about 27 natural enemies of citrus mealybug have been reported in the Mediterranean region and they suppress this pest in orchards where broad-spectrum pesticides are not used (Soylu & Urel 1977, Kansu & Uygun 1980, Uygun et al. 1991, Uygun et al. 1992). One of the most promising of these is the ladybeetle, *Nephus includens* Kirsch (Soylu & Urel 1977, Kansu & Uygun 1980, Uygun 1981, and Soylu et al. 1983).

The objective of this study was to assess some biological properties of *N. includens* at different temperatures to serve as a basis for the use of this predatory coccinellid in a biological control program. Development time and mortality of different immature stages, longevity and fecundity were determined. Life tables were constructed using these data.

**Materials and Methods**

**Maintenance of citrus mealybug colony.** Immature stages were collected from Washington navel citrus trees in the orchard of Adana Plant Protection Research Institute, Adana, Turkey in 1993 and were used to infest sprouted potatoes in crispers (26 by 35 by 6 cm). This colony was maintained throughout the study.

**Maintenance of Nephus includens.** Sprouted potatoes infested with citrus mealybugs in two crispers were put into rearing cages (45 by 60 by 60 cm) and predators were released into cages at the rate of 30 individuals/crisper, 60 individuals/cage. When needed, additional food was added and new cages were established by the same method when potatoes became too old to support the mealybugs.

**Development times and mortality rates of Nephus includens.** This test was conducted at constant temperatures of 15, 20, 25, 30, 35°C and at fluctuating temperatures of 25–35°C (12 hours 25°C, 12 hours 35°C). 20–25 adults of *N. includens* were placed for 24 hours in plexiglas containers (25 cm diameter × 30 cm height) containing sprouted potatoes infested with citrus mealybug. Eggs were collected from the containers and put in petri dishes. The larvae that hatched were placed singly on navel citrus fruits infested with citrus mealybugs, using a thin brush. A plastic ring (3-cm diam. by 2-cm height) which had mesh cloth on the top was placed over the larvae on each fruit. Eggs and larvae were checked twice daily and development time and mortality of eggs, larvae, and pupae were determined. Trials were conducted using a completely randomized design, with 50 replicates of the egg stage and 30 replicates of the larval and pupal stages.

**Preoviposition, oviposition, postoviposition, and progeny of Nephus includens.** Adults of *N. includens* that had newly emerged were put onto navel citrus fruit infested with citrus mealybugs. The fruit was placed in plexiglas cells 4 cm in diameter, and 3 cm in height. The predators were supplied with more citrus mealybugs daily than they could consume and they were kept in these cells until they died. They were checked daily for preoviposition, oviposition, postoviposition and progeny. There were 5 replicates of the treatments in a completely randomized design. Data were analyzed using the MSTAT-C computing package.
Table 1. Development times for immature stages of *Nephus includens* reared at different temperatures in the laboratory.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>n</th>
<th>Mean development time (days) and range for each stage&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total</th>
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<tr>
<td></td>
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<td>Egg 1&lt;sup&gt;st&lt;/sup&gt; ins. 2&lt;sup&gt;nd&lt;/sup&gt; ins. 3&lt;sup&gt;rd&lt;/sup&gt; ins. 4&lt;sup&gt;th&lt;/sup&gt; ins. Pupa</td>
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<tr>
<td>15</td>
<td>30</td>
<td>26.1a (23–29.3) No larval development</td>
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<tr>
<td>20</td>
<td>30</td>
<td>9.7b (8.9–11.3) 6.2a (4.2–7.8) 3.9a (2.8–4.8) 4.5a (3.5–5) 6.9a (4.2–8.7)</td>
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<td>25</td>
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<td>7.3c (5.6–8.6) 3.3b (3.0–4.1) 2.3b (1.9–3.0) 2.6b (1.9–3.4) 3.6b (3.0–4.9)</td>
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<td>30</td>
<td>30</td>
<td>5.4e (4.4–6.1) 3.0cd (2.4–3.3) 2.0cd (1.4–2.6) 2.2c (1.9–2.6) 3.1c (2.1–4.0)</td>
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<td>35</td>
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<td>4.6f (3.4–5.3) 2.8d (2.0–3.3) 1.9d (1.1–2.4) 2.1cd (1.3–3) 2.5d (2.0–3.4)</td>
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<td>25–35</td>
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<td>6.1d (5.3–6.4) 3.2bc (3.0–3.9) 2.2c (1.8–3.0) 2.0d (1.8–3.1) 3.1c (2.3–3.9)</td>
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<sup>a</sup>Means followed by same letter within a column are not significantly different, at α = 0.05.
The method of Southwood (1978) was used to construct the life tables. The formula is
\[ S_{lx}m_xe^{-rm_x} \]
where: 
- \( l_x \) = percentage survival at age \( x \),
- \( m_x \) = expected number of daughters per female at age \( x \) (female/female/day),
- \( e \) = natural log base,
- \( r_m \) = intrinsic rate of increase,
- \( x \) = age of females in days.

\( R_0 \) is a parameter that defines the number of daughters that replace an average female during her life. The method of estimating \( R_0 \) from information in the life table is the sum of the reproduction expectation (\( \Sigma l_xm_x \)) for each age group.

The mean period elapsing from birth of parents to birth of offspring (\( T \)) was estimated by the formula of Laing (1968): 
\[ T = \log_e R_0/r_m \]

### Table 2. Mortality rates of *Nephus includens* for immature stages reared at different temperatures in the laboratory.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>%Mortality for egg stage</th>
<th>%Mortality for larval and pupal stages</th>
<th>Total mortality (%)</th>
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<td>25–35</td>
<td>50</td>
<td>28</td>
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**Life tables of *Nephus includens* reared at different temperatures.** The method of Southwood (1978) was used to construct the life tables. The formula is 
\[ \Sigma l_xm_xe^{-r_m}x = 1 \]
where: 
- \( l_x \) = percentage survival at age \( x \),
- \( m_x \) = expected number of daughters per female at age \( x \) (female/female/day),
- \( e \) = natural log base,
- \( r_m \) = intrinsic rate of increase,
- \( x \) = age of females in days.

\( R_0 \) is a parameter that defines the number of daughters that replace an average female during her life. The method of estimating \( R_0 \) from information in the life table is the sum of the reproduction expectation (\( \Sigma l_xm_x \)) for each age group. The mean period elapsing from birth of parents to birth of offspring (\( T \)) was estimated by the formula of Laing (1968): 
\[ T = \log_e R_0/r_m \]

### Development times and mortality rates of *N. includens.* The incubation period of eggs decreased significantly as temperature increased. The period was 26.1 days at 15°C and 4.6 days at 35°C (Table 1). Although 5–6 eggs hatched at 15°C, larval development was not observed. Larval and pupal development times at other temperatures decreased as temperature increased. Development time for immature stages was the longest at 20°C (48.8 days) and shortest at 35°C (22 days) (Table 1), and differences between temperatures were statistically significant.

Tranfaglia and Viggiani (1973) reported that the development time from hatching to adult of *N. includens* was 25.9 days at 25–27°C. This period is between the 31.4 days at 25°C and 24.6 days at 30°C reported in this test. Soylu et al. (1983) found development times to be 32.6 and 23.3 days at 25 and 28°C, respectively which are similar to the results that we obtained.

The mortality rate of eggs was higher than that of any other stage except at 30°C. Total mortality decreased as temperature increased up to 30°C. Mortality was highest at 35°C with 71.6% (Table 2). Temperatures higher than 30°C are not suitable for development of *N. includens.* Tranfaglia and Viggiani (1973) found that the mortality rate of eggs was 29% at 25–27°C, which is similar to the 24% at 25°C found in this test. Yigit (1989) stated that the mortality rate of eggs and 1st instar larvae of the coccinellid *Stethorus punctillum* Weise decreased as temperature increased to 30°C; these results are similar to ours.
Table 3. Preoviposition, oviposition, and postoviposition periods and longevity of *Nephus includens* adults reared at different temperatures in the laboratory.

| Temp (°C) | Mean longevity of females and range for each period (days)
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| Mean longevity and range (days)
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<td>18</td>
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</table>

*Means within a column followed by the same letter (lower case) are not significantly different at α = 0.05.*

*Means within a line followed by the same letter (upper case) are not significantly different at α = 0.05 (t test).*
Preoviposition, oviposition, postoviposition, and progeny of *Nephus includens*. Increasing temperature decreased longevity of adults (Table 3). The highest female longevity was 105 days at 15°C and the lowest was 57 days at 35°C. Longevity for females at 15°C was significantly different from longevity of females reared at other temperatures except 20°C. Male longevity was longest at 15°C (85 days) and decreased as temperature increased. Differences in male longevity between temperatures were not significant except between 15 and 35°C.

Tranfaglia and Viggiani (1973) reported that the female longevity of *N. includens* was 74 days and the male longevity 62 days at 25–27°C and 50–70% relative humidity. While the 74 days for female longevity is similar to our result (70 days) at 25°C, male longevity was quite different in the two studies (62 versus 78).

The preoviposition, oviposition and postoviposition periods decreased as temperature increased (Table 3). The 10.8 days of preoviposition at 25-27°C determined by Tranfaglia and Viggiani (1973) is different from the 5.7 days at 25°C in this study.

The total number of eggs laid by *N. includens* at 25, 30, and 35 also decreased with increasing temperature (Table 4). Egg numbers were highest (133.5) at 25°C and lowest (50.8) at 35°C. However, temperature did not influence total egg numbers except at 35°C, which resulted in significantly lower numbers (50.8). Tranfaglia and Viggiani (1973) stated that *N. includens* laid 150.9 eggs at 25-27°C. This amount differs from 133.5 at 25°C and 123 at 30°C in this study.

**Life tables of *Nephus includens* reared at different temperatures.** Life graphs of *N. includens* for different temperatures were constructed using life tables (Fig. 1). One of the important factors that affects population growth is starting time for egg deposition. Females at 35, 30, 25–35 and 25°C started to lay eggs on the 26th, 29th, 31st and 38th day, respectively (Fig. 1). Early egg deposition is another important parameter for population growth. Females at 25, 30, 35, and 25–35°C laid about 50 % of their total eggs by the 58th, 52nd, 32nd and 55th day, respectively.

Total mortality rates were higher at 35 and 25–35 °C than at 25 and 30°C, and mortality was seen earlier. Mortality rates were 36.7, 34.4, 71.6 and 46.7 at 25, 30, 35 and 25–35°C, respectively (Table 2). The net reproductive rate ($R_n$), the mean length of a generation ($T$) and the intrinsic rate of increase ($r_m$) were calculated from life tables (Table 5). $R_n$, the
number of daughters that replace an average female during her life, was highest at 25°C, with 54.4. \( R_0 \) was 38.7, 7.7 and 46.1 at 30, 35 and 25–35°C, respectively. Mean generation time (\( T \)) was longest (59.6 days) at 25°C, and was 44.9, 38.4 and 50.4 days at 30, 35 and 25–35°C, respectively. The intrinsic rate of increase (\( r_m \)) was highest at 30°C (0.081), followed by 0.076 at 25–35°C, 0.067 at 25°C and 0.053 at 35°C.

**Conclusions**

As one of the most effective indigenous predators of the citrus mealybug, \( N. \) includens could be a valuable component of a biological control program if suitable mass-rearing procedures can be developed for augmentative releases. In this study we demonstrated that the most suitable rearing temperatures for \( N. \) includens were 30 and 25–35°C because the intrinsic rate of increase (\( r_m \)) was
highest at 30°C and very close to the highest at 25–35°C. In addition, mortality rates for immature stages were less and mean generation time (T) was shorter at 30°C than for other temperatures. However, mass rearing at temperatures as high as 35°C could cause deterioration of sprouted potatoes on which the citrus mealybug grows. Therefore 30°C would be better than 25–35°C for mass rearing of *N. includens*.

**Acknowledgment**

We thank Abdurrahman Yigit for useful comments at all stages of this work; Ismail Karaca for his technical assistance in constructing life tables; and our colleagues at Adana Plant Protection Research Institute and Plant Protection Department of Cukurova University for their assistance in laboratory studies. This research project was supported by Adana Plant Protection Research Institute of the Ministry of Agriculture of Turkey.

**References Cited**


