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тепловой шок замедляет развитие личинок и созревание самок, но увеличивает вес выходящих имаго!
Exposing eggs to high temperatures affects the development, survival and reproduction of *Harmonia axyridis*

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Abstract

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is a well-known biological control agent for aphids and soft-bodied insects. We investigated the developmental, survival and reproductive traits of *H. axyridis* when its eggs were exposed to 25 (control), 37, 39 and 41\degree C for 1 h, and then transferred to ambient condition (25\degree C). The effects of heat stress on the hatching success greatly differed among temperature treatments. No *H. axyridis* larvae hatched out at 41\degree C. The development, survival, weight, reproduction and longevity of *H.*
Harmonia axyridis exhibited significant differences with temperature treatment and gender. The survival rate of immatures declined, while the adult fresh weight of both sexes markedly increased with the increase of temperature. Heat exposure of the eggs caused a subsequent reduction in longevity, oviposition period and reproduction, while the pre-oviposition period became longer as the temperature increased. These may imply that the reproductive investment increased in higher level stressful environments, and the response of adult individuals could be linked to the experiences from early stages of the life history. Our findings provide useful information for predicting population dynamics and understanding the potential for H. axyridis as a biological control agent under variable environments.

Highlights

- Heat stress on Harmonia axyridis eggs reduced hatching rates.
- The subsequent effects of heat stress on eggs result in longer larval development time and lower survival rate.
- Heat stress on eggs also increased weight, lowered reproduction, and shortened longevity of H. axyridis adults.

Keywords

Harmonia axyridis; Heat stress; Survival; Longevity; Reproduction
1. Introduction

Temperature is one of the most important ecological factors affecting growth, reproduction, distribution, abundance and phonology, and even small changes potentially have a significant impact on fitness and life history characteristics of insects (Angilletta et al., 2002; Bale et al., 2002; Parmesan, 2006; Wang et al., 2009b). The rate of global warming in the future is expected to increase much faster than that in the last century, and global mean surface temperature is predicted to increase by 1.5 - 6.0°C by the end of this century (IPCC, 2007). There is no doubt that global warming will increase the exposure of insects and other ectothermic species to high temperatures exceeding their upper physiological limits (Deutsch et al., 2008). Temperatures above the normal optimum are perceived as heat stress by all living organisms. Heat stress may result in water loss (Yoder et al., 2009), disruption of structure of membranes (Hochachka and Somero, 2002), protein denaturation (Chown and Nicholson, 2004), and damage in neurons (Robertson, 2004; Chown and Terblanche, 2006). These injuries can lead to severe fitness consequences such as decreased growth and development and increased mortality (Krebs and Loeschcke, 1994; Hoffmann et al., 2003; Mironidis and Savopoulou-Soultani, 2010). When threatened by extreme temperatures, most insect species employ behavioral, physiological or genetic adaptation mechanisms to adjust their body temperature, or the extremes they can withstand (McMillan et al., 2005; Overgaard and Sorenson, 2008; Kalosaka et al., 2009; Nyamukondiwa and Terblanche, 2009; Karl et al., 2011). The eggs are the initial stage of insects’ life cycles, and they have an upper and a lower temperature limits that they can
tolerate. The temperatures outside of the limits would retard or completely inhibit the insect’s development or kill the insects (Heming, 2003). When embryonic development of insect eggs is stressed by environmental factors, especially temperature, subsequent development and reproduction could be affected. However, how the thermal environment experienced in early ontogeny affects biological characteristics of both sexes and thermal tolerance capacities in later development stages is not well-studied (Bowler and Terblanche, 2008).

The predaceous coccinellids are important natural enemies of numerous small phytophagous insects and acarines, and are therefore considered as potentially good biological control agents (Obrycki and Kring, 1998). Aphidophagous coccinellids occur in most cropping systems and their impact on aphid populations is known to be important (Hodek and Honek, 1996; Obrycki et al., 2009). Predaceous coccinellids represent a third trophic level, which must cope with their own thermal stress. To our knowledge, however, little is known about the impact of heat stress on the life history traits of aphidophagous coccinellids.

*Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is indigenous to many regions of Asia, and is a generalist predator that feeds primarily on aphids and soft-bodied insects as well as pollen and nectar (Koch, 2003; Pervez and Omkar, 2006; Pell et al., 2008; Lundgren, 2009). *H. axyridis* has several characteristics that make it useful for biological control of aphids, such as high voracity, dispersal capacity, multivoltine pattern of seasonal development and low host specificity (Koch, 2003; Brown et al., 2011). It has been used successfully in greenhouses, orchards, gardens, and outdoor crops for aphid
management (Obrycki et al., 2009). The aphid *Megoura japonica* (Matsumura), a severe pest on a variety of fabaceous plants in the genus *Vicia* and *Lathyrus*, such as broad bean (*Vicia faba* L.), garden pea (*Pisum sativum* L.), soy bean (*Glycine max* (L.) Merr.), as well as grass pea (*Lathyrus sativus* L.), is one of the major prey species of *H. axyridis*.

In northern China, temperature is normally above 35°C during summer months, and the highest temperature during the day could well exceed 40°C (Climate Databases, Chinese Academy of Forestry). An interesting question concerning the effects of the temperature extremes is whether they affect the development and reproduction of *H. axyridis*. The present paper describes laboratory experiments designed to study the effect of brief exposure of *H. axyridis* eggs to high temperatures in an attempt to discover how the subsequent developmental and reproductive activity of *H. axyridis* are affected.

### 2. Materials and methods

#### 2.1. Insect species

*Harmonia axyridis* colony was initiated from approximately thirty pairs of adults, collected from a garden pea (*Pisum sativum* L.) field and a nearby corn field in the Experimental Farm of the Northwest A&F University (34°17'37.01" N, 108°01'03.34" E). The lady beetles were reared in mesh covered cages (40×40×40 cm). Four potted broad bean seedlings with aphids were placed in each cage, and the seedlings were replaced with fresh ones with aphids when necessary. The lady beetles were reared at 23 ± 1°C, 50 ± 10% RH and 16L: 8D for at least 2 generations, and were then used in all experiments. *Megoura japonica* culture was initiated in the laboratory using aphids collected on garden
pea in the same location as the lady beetles. The culture was developed on potted broad bean plants in mesh covered cages (60×60×60 cm).

2.2. Effects of heat shock on *H. axyridis* preimaginal development

Newly laid eggs (less than 12 h old) of *H. axyridis* were randomly divided among four groups: one group were placed in Petri dishes (12.0 cm in diameter and 2.0 cm in height) at a constant temperature of 25°C (control), while other three groups were separately heat-shocked for one hour in Petri dishes in a climate chamber at 37, 39 and 41°C, and were then transferred to 25°C under an ambient conditions at L16 : D8 h and RH 60 ± 10% in the laboratory. A piece of filter paper was placed at the bottom of the Petri dish, and a few drops of water were added as needed to maintain sufficient humidity. For each treatment about 90-120 *H. axyridis* eggs (each bean leaf with one egg mass, total three to five egg masses) were used. The eggs were inspected every 12 h and numbers of hatched larvae were recorded. The first instar larvae were individually transferred from Petri dishes to an experimental arena using a soft camel-hair brush. The arena consisted of a 100 mL centrifugal tube, and each tube contained a broad bean shoot with its end inserted into a 5 mL Eppendorf tube filled with water. The tube was covered with a fine muslin cloth, secured with a rubber band. Newly hatched first instars were individually placed in close vicinity to the prey. At least thirty newly hatched larvae (i.e., each larva as one replicate) were randomly selected for each experimental group. The aphid along with host plant shoot was supplied during the entire larval life span. Aphids were replaced every 12 h to avoid microbial contamination, and the broad bean shoot was replaced with a fresh one when necessary. The development duration for each immature stage of the predators
was recorded, and newly-emerged adults were sexed and weighed individually using an electronic balance (Mettler-Toledo XS64, Switzerland) with a precision of 0.1 mg to record their initial body mass.

2.3. Effects of egg heat shock on longevity and reproduction of *H. axyridis* adults

To evaluate longevity and reproductive performance of the adults, individual newly-emerged adults from stressed eggs were sexed and paired. Each pair in each temperature treatment was placed in a transparent hard plastic truncated cylindrical cup (500 mL) covered with a fine muslin cloth. Along with the aphids, each cup contained a broad bean shoot with its end inserted into a 50 mL conical flask filled with water. The pair was transferred to a new cup twice a day until the female died, and the number of eggs laid each day was counted. Twenty pairs (i.e., each pair as one replicate) were selected for each experimental group. Pre-oviposition period, oviposition period, fecundity, and longevity were simultaneously determined. Females that did not lay any eggs were excluded from the analysis. The adults maintained at 25°C under a L16 : D8 h and RH 60 ± 10% were used as controls.

2.4. Statistical analysis

The effects of temperature on developmental time of immature stages were subjected to one-way analysis of variance (ANOVA). The effects of temperature and sex on developmental duration and adult weight were subjected to two-way ANOVA. The hatching rate and survival rate were arcsine-transformed and then analyzed using χ²-test. All data were analyzed using the statistical package software SPSS 11.5 (2002).
3. Results

3.1. Development and survival of immature H. axyridis

The effects of heat shock on the eggs hatching greatly differed among temperature treatments. No H. axyridis larvae were hatched at 41°C, so the hatching rates were compared with those eggs from 39, 37 or 25°C. The eggs hatching rates were highest at 25°C (92.6%), following at 37°C (47.1%) and 39°C (43.3%) ($\chi^2 = 24.407$, df = 2, $P < 0.001$). The subsequently preimaginal developmental duration of H. axyridis exhibited significant differences with temperature treatment and sex (Table 1). The mean developmental durations of the first instars ($F = 0.472$; df = 2, 39; $P = 0.627$), the second instars ($F = 2.070$; df = 2, 39; $P = 0.141$), the third instars ($F = 1.816$; df = 2, 39; $P = 0.177$), the pupae ($F = 1.095$; df = 2, 39; $P = 0.345$), and the preimaginal development ($F = 1.276$; df = 2, 39; $P = 0.291$) were not significantly different among the female larvae at different temperature treatments. However, the fourth instars ($F = 4.261$; df = 2, 39; $P = 0.022$) showed significant difference. Similarly, the development durations of the first instars ($F = 1.314$; df = 2, 38; $P = 0.281$), the second instars ($F = 1.570$; df = 2, 38; $P = 0.222$), the third instars ($F = 1.966$; df = 2, 38; $P = 0.155$) did not differ among male larvae at different temperature, while the fourth instars ($F = 3.609$; df = 2, 38; $P = 0.037$), the pupae ($F = 3.763$; df = 2, 38; $P = 0.033$), the total immature ($F = 4.166$; df = 2, 38; $P = 0.024$) exhibited significant differences. There were no interactions of temperature and sex on preimaginal development durations of H. axyridis, except pupal developmental durations ($F = 3.583$; df = 2, 73; $P = 0.033$).
Survival rates from the first instars larva to adult stage were significantly higher at 25°C (73.3%) or 37°C (67.5%) than 39°C (55%) ($\chi^2 = 12.658$, df = 2, $P = 0.002$, Fig. 1). The first instars from stressed eggs showed higher mortality (35% at 39°C and 27.5% at 37°C, respectively) than that of control (10%).

3.2. Weight, reproduction and longevity of *H. axyridis*

The female adult fresh weight ($F = 14.380$; df = 2, 39; $P < 0.001$) and male adult fresh weight ($F = 31.278$; df = 2, 38; $P < 0.001$) exhibited significant differences (Fig. 2). The weights of fresh female *H. axyridis* from stressed eggs at 37°C and 39°C increased by 34.95% and 27.18% as compared with that of control, respectively. Similarly, the fresh weights of the male ladybird from stressed eggs at 37°C and 39°C increased by 36.67% and 25.56% as compared with the control. The sex and temperature significantly affected adult weight, with the adult females being heavier than males, but not the interaction between them ($F = 0.186$; df = 2, 73; $P = 0.830$). In addition, the adult weight between female and male showed significant difference under each temperature treatment (25°C: $t = 4.751$, df = 24, $P < 0.001$; 37°C: $t = 2.643$, df = 25, $P = 0.014$; 39°C: $t = 2.351$, df = 24, $P = 0.027$).

The longevity, pre-oviposition, oviposition periods and reproductive parameters of *H. axyridis* were presented in Table 2. The mean adult longevity (females and males) decreased significantly with the increase of heat shock temperatures and ranged from 62.1 to 41.5 days for females and from 51.6 to 29.3 days for males (female: $F = 13.089$; df = 2, 48; $P < 0.001$; male: $F = 57.434$; df = 2, 48; $P < 0.001$). The average lifespan of the females was longer than that of the males, and the lifespans of both sexes differed
significantly among the stressed temperature treatments \( t = 2.121, \text{df} = 28, P = 0.043 \) at 37°C; \( t = 3.802, \text{df} = 34, P = 0.001 \) at 39°C, respectively). The average preoviposition period increased significantly with the increase of heat-shocked temperatures from 8.6 days at 25°C to 12.3 days at 39°C \( (F = 12.141; \text{df} = 2, 48; P < 0.001) \). The oviposition period and fecundity remarkably declined from 50.5 days and 540.6 eggs (control) to 22.3 days and 336.3 eggs (39°C) (oviposition period: \( F = 29.784; \text{df} = 2, 48; P < 0.001 \); fecundity: \( F = 44.930; \text{df} = 2, 48; P < 0.001 \)). There was no significant difference in the fecundity between 37 and 39°C. In addition, the oviposition intervals of adults were different, i.e., 1-2 days and more than 2 days intervals from stressed eggs accounted for 62.9% and 37.1% at control, 78.6% and 21.4% at 37°C, 81.8% and 18.2% at 39°C, respectively.

4. Discussion

Temperature plays a critical role in survival and reproductive success of insects. Our results show that high temperature during the egg stages had deleterious effects on the subsequent development, survival, reproduction and longevity of the male and female H. axyridis.

4.1. Impact of heat exposure on the fitness of subsequently preimaginal development

The eggs of H. axyridis could not hatch at 41°C, which suggests that the temperature is detrimental to embryonic development of the lady beetle eggs because such a high temperature may exceed physiological limits of the eggs. This may be one reason that the ladybird must aestivate in summer in northern China (Wang et al., 2009a). Bergh and
Arking (1984) reported that the embryo development of *Drosophila* was reduced by heat stress due to the presence of high concentrations of heat shock proteins that are produced following stress exposure.

The accumulated survival rates from the first instar larvae to adults of *H. axyridis* were significantly different among the temperature treatments. However, the survival rate did not differ between 37°C and 25°C (Fig. 1). Moreover, we found that differences exist in subsequent preimaginal developmental time between male and female larvae (Table 1). Cong et al. (2010) found that the survival rates of *Bemisia tabaci* from egg to adult after exposure at 41°C for 1 h at egg stage were significant lower. Arbogast (1981) reported that the exposure of the pupal stage of *Ephestia cautella* and *Plodia interpunctella* at 50°C for 2 h caused a zero survival rate. Krebs and Loescheke (1995) documented that the pupae of *Drosophila buzzatii* were most resistant to high temperature stress, followed by eggs. Mahroof et al. (2003) observed the mortality of different life stage of *Tribolium castaneum* under elevated temperature, and found that young larvae were the most heat tolerant. The effects of heat shock could accumulate slowly and be displayed at later stages of development when late third instars of *Bactrocera dorsalis* were stressed by high temperatures (Xie et al., 2008). Cui et al. (2008) found that the survival rates of *B. tabaci* and *Trialeurodes vaporariorum* were significantly affected when adults were exposed for 1 h under different high temperature, while in both whitefly species females were more tolerant to high temperatures than males. In contrast, Mironidis and Savopoulou-Soultani (2010) found that the survival of *Helicoverpa amigera* adults did not vary between the sexes with the heat-shock treatments. Most recently, Li et al. (2011) reported that
temperature, exposure time and gender all significantly affected the survival of *Frankliniella occidentalis* adults, and the females were more tolerant to extreme temperature than males. The results obtained above indicate that the survival of insects suffer when exposed to high temperature depended on life stage, sex, and interaction of exposure temperature and duration. Also, differences in heat tolerance between sexes can vary among species.

4.2. Impact of heat exposure on reproduction and survival of subsequent adult stage

Reproductive success and survival are important for insect population development. In the present study, we found that the pre-oviposition period of *H. axyridis* from stressed eggs was 34.9% (37°C) and 43.0% (39°C) slower (i.e., prolonged 3.0 and 3.7 d compared with controls, respectively) than that at 25°C, while the oviposition period, fecundity and longevity were markedly reduced compared with controls (Table 2). Rinehart et al. (2000) observed that heat-shock in adults of both sexes of *Sarcophaga crassipalpis* at 45°C for 1 h severely affected fecundity. Lale and Vidal (2003) reported that females of *Callosobruchus maculatus* and *C. subinnotatus* when exposed at high temperature (40 to 50°C) significantly reduced oviposition biomass. Cui et al. (2008) found that high temperatures significantly reduced the number of eggs oviposited by *T. vaporariorum*. Mironidis and Savopoulou-Soultani (2010) found that *Helicoverpa armigera* adult longevity declined significantly with the increase in exposure time in heat-shock treatments (40 to 46.5°C) and fecundity was inversely related to the exposure time of the adults to high temperatures. The parasitoid *Aphidius avenae* adults’ exposure to 36°C for 1 h resulted in high mortality and affected the fitness of survivors by drastically reducing
reproductive output (Roux et al., 2010). In addition, Saxena et al. (1992) reported that the exposure of three stored-product insects in pupal stage at 45°C for 48 or 72 h also significantly decreased oviposition. Most recently, Janowitz and Fischer (2011) found that heat shock (40°C for 1 h) of Bicyclus anynana females caused a subsequent reduction in longevity, fecundity and egg size.

High temperatures can disturb the normal functioning of the reproductive physiology of insect species (Arbogast, 1981; Saxena et al., 1992; Cui et al., 2008; Janowitz and Fischer, 2011). It has been reported that heat shock can cause injury to oocytes and ovarian development in females that could lead to the decrease in egg production (Krebs and Loeschcke, 1994; Rinehart et al., 2000). Furthermore, heat shock also can cut down on male fertility due to direct injury to the testes and sperm (Chihrane and Lauge, 1994, 1997; Scott et al., 1997; Nguyen et al., 2013). Our data also showed the reproduction of subsequent adults significantly decrease when H. axyridis eggs suffered from high temperature. Interestingly, the fecundity displayed increase from 37°C (287 eggs) to 39°C (336 eggs), though there was no significant difference (Table 2). Moreover, the latter oviposition period was shorter than that of the former. We speculate that the results might be caused by a trade-off in subsequent reproductive and survival investment when H. axyridis egg stage suffered from high temperature stress. When H. axyridis eggs were exposed to higher level stress, the later adult females could allocate more resources to reproduction at the cost of a reduction in longevity so as to maintain population development. Further investigations are needed to elucidate the intrinsically physiological mechanisms that underlie the changes produced by heat stress on the subsequent fitness
and life history characteristics of *H. axyridis*.

Ectothermic species live in changeable environments, which have to face substantial challenges to survival and reproduction. Under natural conditions in northern China, the maximum temperature often exceeds 35°C and even 40°C during the summer. *H. axyridis* is considered as one important biological control agent in China (Wang et al., 2009a), so it may adopt different strategies (for example, adaptation, dispersion or extinction) to cope with environmental heat stress. Due to the increasing effects of global warming and climate change on natural systems, understanding mechanisms by which natural enemies (third trophic level) respond to environmental stress is very important because it determines the success or failure of biological control of pest insects (Thomson et al., 2010). This study investigated the effects of heat stress at egg stage on subsequent survival and reproduction of *H. axyridis*, and contributes to understanding the potential for *H. axyridis* to evolve in response to environmental changes. The results should be used for predicting population dynamics, distribution and dispersal of this insect.

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Rinehart, J.P., Yocum, G.D., Denlinger, D.L., 2000. Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the


Table 1 Developmental time (Mean days ± SE) of different life stages of *Harmonia axyridis* after different levels of heat stress at egg stage.

Table 2 Longevity, pre-oviposition and oviposition periods, reproductive parameters of *Harmonia axyridis* [mean ± SE (range)] after different levels of heat stress at egg stage.

Figure Captions

Fig. 1. Survival rates of *Harmonia axyridis* pre-imaginal stages after different levels of heat stress at egg stage.

Fig. 2. Fresh weight (mean mg ± SE) of *Harmonia axyridis* adults after different levels of heat stress at egg stage. Different letters at the top of bars indicate significant difference at *P* < 0.05.
The effects of heat stress on the eggs hatching of *Harmonia axyridis* greatly differed among temperature treatments.

The subsequent development, survival, weight, reproduction and longevity of *H. axyridis* exhibited significant differences with temperature treatment and gender.

The reproductive investment increased in higher level stressful environments, and the response of adult individuals could be linked to the experiences from early stages of the life history.
Table 1 Developmental time (Mean days ± SE) of different life stages of *Harmonia axyridis* after different levels of heat stress at egg stage

<table>
<thead>
<tr>
<th></th>
<th>First instar</th>
<th>Second instar</th>
<th>Third instar</th>
<th>Fourth instar</th>
<th>Pupa instar</th>
<th>Total immature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females 25°C</strong></td>
<td>2.0±0.1a</td>
<td>1.6±0.2a</td>
<td>2.2±0.1a</td>
<td>5.5±0.4a</td>
<td>4.1±0.2a</td>
<td>15.5±0.3a</td>
</tr>
<tr>
<td><strong>Females 37°C</strong></td>
<td>2.0±0.3a</td>
<td>1.9±0.1a</td>
<td>2.6±0.1a</td>
<td>4.4±0.2b</td>
<td>4.3±0.1a</td>
<td>15.1±0.4a</td>
</tr>
<tr>
<td><strong>Females 39°C</strong></td>
<td>1.8±0.2a</td>
<td>2.0±0.2a</td>
<td>2.2±0.3a</td>
<td>5.7±0.4a</td>
<td>4.3±0.1a</td>
<td>16.0±0.2a</td>
</tr>
<tr>
<td><strong>Males 25°C</strong></td>
<td>2.2±0.2a</td>
<td>1.9±0.1a</td>
<td>1.9±0.2a</td>
<td>5.6±0.5a</td>
<td>4.6±0.2a</td>
<td>16.4±0.5a</td>
</tr>
<tr>
<td><strong>Males 37°C</strong></td>
<td>1.7±0.2a</td>
<td>2.3±0.3a</td>
<td>2.5±0.2a</td>
<td>4.5±0.2b</td>
<td>4.1±0.1b</td>
<td>15.0±0.3b</td>
</tr>
<tr>
<td><strong>Males 39°C</strong></td>
<td>1.9±0.3a</td>
<td>2.1±0.2a</td>
<td>2.3±0.1a</td>
<td>5.1±0.2ab</td>
<td>4.4±0.2ab</td>
<td>15.7±0.3ab</td>
</tr>
</tbody>
</table>

*Means followed by the same letters in the same row among the same sex are not significantly different at 0.05 level.*
Table 2 Longevity, pre-oviposition and oviposition periods, reproductive parameters of *Harmonia axyridis* [mean ± SE (range)] after different levels of heat stress at egg stage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Female adults longevity</td>
<td>62.1±3.5a</td>
</tr>
<tr>
<td></td>
<td>(37-81)</td>
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<tr>
<td>Male adults longevity</td>
<td>51.6±1.2a</td>
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<tr>
<td></td>
<td>(34-60)</td>
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<tr>
<td>Pre-oviposition period</td>
<td>8.6±0.3a</td>
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<tr>
<td></td>
<td>(7-10)</td>
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<tr>
<td>Oviposition period</td>
<td>50.5±3.5a</td>
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<tr>
<td></td>
<td>(32-67)</td>
</tr>
<tr>
<td>Fecundity (eggs)</td>
<td>540.6±18.7a</td>
</tr>
<tr>
<td></td>
<td>(412-680)</td>
</tr>
</tbody>
</table>

*Means followed by the same letters within a row were not significantly different at 0.05 level.

Note: first and second values in brackets showed the numbers of minimum and maximum, respectively.