

## RESEARCH

# Influence of Constant Temperature on Reproductive Parameters of *Holotrichia oblita* (Coleoptera: Scarabaeidae)

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**ABSTRACT.** *Holotrichia oblita* Faldermann (Coleoptera: Scarabaeidae) is a major pest both in field crops and forests because the larvae could eat the roots of most crops in the field, and the adults damage the leaves of trees and field crops. In this study, we focused on the effects of temperature on *H. oblita* reproductive parameters. The results indicated *H. oblita* female adults at 25°C could lay more eggs (84.0 eggs per female) and have the shortest preoviposition period (19.1 d), the greatest oviposition rate (2.8 eggs per female per 3 d), and largest percentage of life span spent in oviposition (59.5%). The longevity and the time to 50% egg laying decreased with increasing temperature, and female longevity was always longer than male longevity. The preoviposition and postoviposition period decreased with increasing temperature from 15 to 25°C and then increased when the temperature increased from 25 to 30°C. These results show that 25°C is the optimal temperature for reproduction of *H. oblita*.

**Key Words:** *Holotrichia oblita*, temperature, egg laying, longevity

The scarab beetle *Holotrichia oblita* Faldermann (Coleoptera: Scarabaeidae) is the main underground field pest in northern China, and it causes conspicuous root damage to field crops, forests, nurseries, and lawns. Adults eat the leaves of plants such as Siberian elm (*Ulmus pumila* L.), Chinese white poplar (*Populus tomentosa* Carrière), pin oak (*Quercus palustris* Muenchh), Chinese lacquer tree (*Toxicodendron vernicifluum* Stokes), Chinese mulberry (*Morus australis* Poir), Chinese chestnut (*Castanea mollissima* Blume), and Chinese walnut (*Juglans regia* L.), and they particularly like the leaves of peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.). They usually congregate to eat and mate in bushes and weeds (Luo and Cui 1995). Larvae usually feed on the roots of peanut, soybean, potato (*Solanum tuberosum* L.), sweet potato (*Dioscorea esculenta* Burkill), sugar beet (*Beta vulgaris* L.), hemp (*Cannabis sativa* L.), sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and cotton (*Gossypium* spp.) (Wei et al. 1989). It is difficult to prevent infestation by and to control *H. oblita* due to their ability to conceal themselves and because of their broad host range (Wang et al. 2013), so they cause great economic loss in China each year (Deng et al. 2012).

The generation cycle for *H. oblita* is as long as 2 yr, and the last instar larvae overwinter in soil (Wang et al. 1989). The development of *H. oblita* is influenced by food, temperature, water content of soil, and even by the microorganisms in the soil. Adult feeding on different plants such as perennial ryegrass (*Lolium perenne* L.), Japanese hops (*Humulus japonicus* Siebold and Zucc), soybean, peanut, oilseed rape (*Brassica napus* L.), corn, elm, Chinese white poplar, and black locust (*Robinia pseudoacacia* L.) has been well studied, and Siberian elm leaves are considered as the best food for adult survival, oviposition, and egg hatch (Liu et al. 2008, Zhou et al. 2009). Dang et al. (2009) reported that the optimal soil moisture content for survival and development was 10–20% for eggs, 10–15% for larvae, and 15–20% for pupae. The adult sex ratio was close to 1:1 when the soil moisture content was 10–15%, and the survival rate was highest when the soil moisture content was 15%. Luo and Cui (1995) reported that soil type influenced the occurrence of *H. oblita*. Clay and clay loam were suitable for development of *H. oblita* because of their ability to retain water and organic material, but sandy soil and sandy loam were less suitable due to their poor water retention and lack of organic material.

Although some factors impacting development of *H. oblita* have been investigated, the impact of temperature on adults has not been determined. Therefore, the objective of this study was to assess survival, development, reproduction, and population growth of *H. oblita* at four temperatures ranging from 15 to 30°C. The results will help to improve rearing methods for *H. oblita* and aid in understanding their biology and improving control.

## Materials and Methods

**Insects.** *H. oblita* adults were collected from Cishan village, Feixi County, Hefei, Anhui Province, China (31.63 N, 117.11 E) on 15–21 April 2012. This is the first emergence period for *H. oblita* in this area, so we assumed that adults collected were newly emerged and unmated. The *H. oblita* were collected between 19:30 and 22:00 in night on *U. pumila* L. (Lu 2008). Adults were placed in plastic boxes (64 cm by 44 cm by 37 cm), which have a square hole (46 cm by 20 cm) in the lid that was covered with nylon screen to ensure that air could circulate but the insects could not escape. Boxes were 2/3 filled with clay loam, and fresh branches and leaves of *U. pumila* L., which is one of the favorite foods for *H. oblita* adults, were added onto the soil surface (Liu et al. 2008).

**Effects of Temperature on Reproductive Parameters.** Experiments were conducted in rearing boxes (25.4 cm by 16.5 cm by 7.6 cm) with a square hole (20 cm by 12 cm) in the lid that was covered by nylon screen. The rearing box filled to a depth of 5 cm with clay loam, and the clay loam included one quarter of sand and was sieved through a no. 10 wire mesh screen before use. The sand insured that the clay loam could pass the no. 10 mesh screen when the water content of the clay loam was as high as 20%. Fresh branches and leaves of *U. pumila* were placed onto the surface of the soil to provide food for adults and a place for them to move in the night.

One pair of mated *H. oblita* adults was introduced onto the *U. pumila* branches in a rearing box. Sixty such rearing boxes were randomly assigned to one of four incubators (SPX-250C, Shanghai Boxun Industry and Commerce Co., Ltd, Shanghai, China) set at temperatures of 15, 20, 25, and 30°C. We were unable to collect 60 pairs of adults on a single night, so we ensured that a similar number of pairs of adults were allocated to each of the four temperatures each day until each incubator received 15 pairs.

Each rearing box was checked every 3 d until both the male and female died. Eggs were counted and removed at each check, and the *U. pumila* leaves were replaced and the soil moisture was maintaining between 15 and 20% (Hu and Xu 1986, Zheng et al. 1996, Liu et al. 2008, Zhou et al. 2009). Soil moisture content was measured by using a soil moisture meter (YZS-1, Top Instrument, Hangzhou, Zhejiang Province, China), and water was added as necessary. *U. pumila* leaves used in winter were cut at the end of October and stored at  $-20^{\circ}\text{C}$ . Eggs were removed by sieving the soil over a no. 10 wire mesh screen. Eggs were retained on the mesh screen, and the soil passed through the screen. Preoviposition period, oviposition period, postoviposition period, longevity, and fecundity were recorded, and time to 50% egg laying and oviposition rate (eggs laid per female every 3 d during the oviposition period) were calculated.

**Table 1. Effects of temperature on adult longevity ( $\pm$  SE) of *H. oblita***

| Temp ( $^{\circ}\text{C}$ ) | Female (d)     | Male (d)       | Both sexes (d) |
|-----------------------------|----------------|----------------|----------------|
| 15                          | 313.4 (35.7)bB | 206.0 (25.8)bA | 259.7 (23.8)b  |
| 20                          | 194.0 (17.1)aB | 144.3 (11.8)aA | 171.0 (11.6)a  |
| 25                          | 155.5 (15.8)aB | 109.8 (5.9)aA  | 132.6 (9.4)a   |
| 30                          | 119.3 (5.9)aB  | 86.3 (5.6)aA   | 103.6 (5.4)a   |

The numbers in parentheses are standard errors. Means within a column followed by the same lowercase letter or those within a row followed by the same uppercase letter are not significantly different ( $P > 0.05$ , analysis of variance with Tukey's-b).

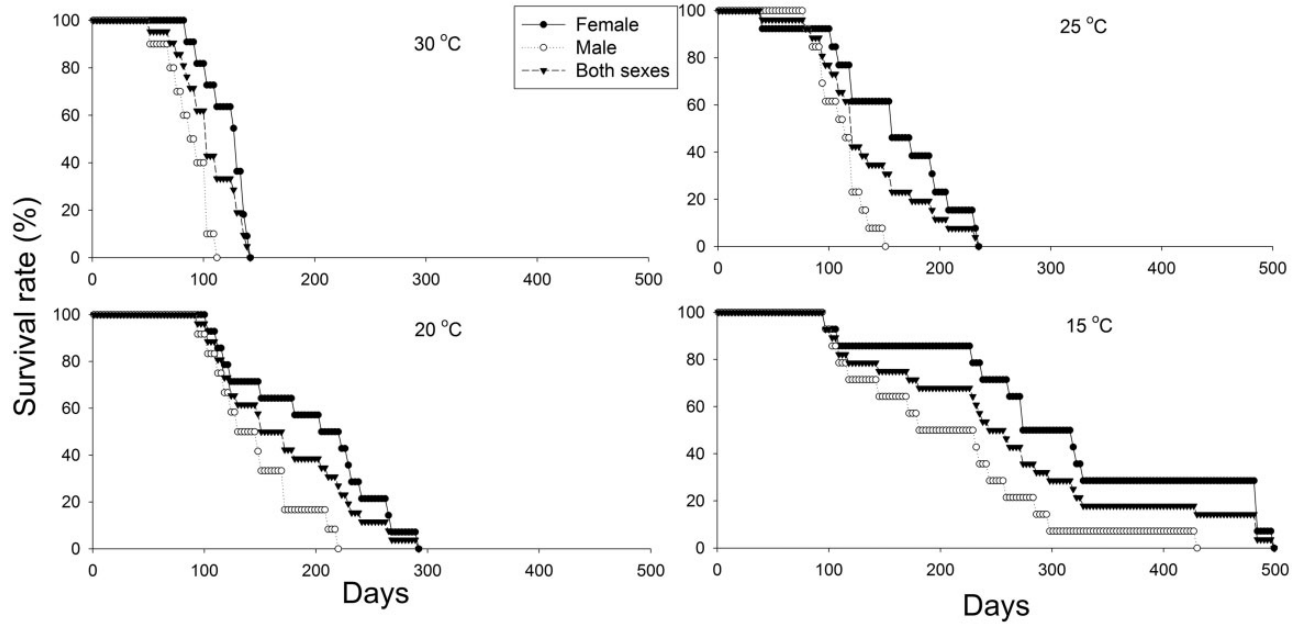
**Statistical Analysis.** The experimental design was a completely randomized block, and data were analyzed using SPSS 16.0 (SPSS Inc. 2007). Preoviposition period, oviposition period, postoviposition period, longevity, fecundity, percentage life span spent in oviposition, and time to 50% egg laying were compared across temperatures by using one-way analysis of variance and Tukey's-b test ( $P = 0.05$ ) (SPSS Inc. 2007).

## Results

**Longevity of Adults.** The longevity of females, males, and both sexes combined decreased with increasing temperature (female:  $F = 13.6$ ;  $df = 3, 48$ ;  $P < 0.001$ ; male:  $F = 10.7$ ;  $df = 3, 45$ ;  $P < 0.001$ ; sexes combined:  $F = 20.7$ ;  $df = 3, 97$ ;  $P < 0.001$ ) (Table 1). Longevity of females was longer than that of males at each temperature ( $15^{\circ}\text{C}$ :  $F = 6.0$ ;  $df = 1, 26$ ;  $P = 0.021$ ;  $20^{\circ}\text{C}$ :  $F = 5.3$ ;  $df = 1, 24$ ;  $P = 0.030$ ;  $25^{\circ}\text{C}$ :  $F = 7.3$ ;  $df = 1, 24$ ;  $P = 0.012$ ;  $30^{\circ}\text{C}$ :  $F = 15.6$ ;  $df = 1, 19$ ;  $P = 0.001$ ). The longest longevity of 498 d was recorded at  $15^{\circ}\text{C}$ .

Survival rate decreased more rapidly with increasing temperature (Fig. 1). The survival rate of females was greater than for males at all temperatures. Complete mortality occurred at 498, 291, 234, and 141 d at  $15, 20, 25,$  and  $30^{\circ}\text{C}$ , respectively.

**Preoviposition, Oviposition, and Postoviposition Periods.** The preoviposition period decreased with temperature from 15 to  $25^{\circ}\text{C}$ , and the mean values varied from 63 to 19 d. However, the preoviposition period was prolonged to 24 d at  $30^{\circ}\text{C}$  (Table 2). The longest preoviposition period of 87 d was recorded at  $15^{\circ}\text{C}$ , and the shortest was recorded as 3 d at  $25^{\circ}\text{C}$ . The temperature at which the shortest preoviposition



**Fig. 1.** Effect of temperatures on survival rate of *H. oblita*.

**Table 2. Effects of constant temperatures on preoviposition period ( $\pm$  SE), oviposition period ( $\pm$  SE), postoviposition period ( $\pm$  SE), the percentage of adult life span spent in oviposition ( $\pm$  SE), and fecundity ( $\pm$  SE) of *H. oblita***

| Temp ( $^{\circ}\text{C}$ ) | Preoviposition period (d) | Oviposition period (d) | Postoviposition period (d) | Fecundity (eggs per ♀) |
|-----------------------------|---------------------------|------------------------|----------------------------|------------------------|
| 15                          | 63.3 (4.1)c               | 71.3 (14.9)ab          | 171.8 (34.8)b              | 16.6 (3.7)a            |
| 20                          | 35.9 (3.6)b               | 98.1 (13.1)b           | 60.0 (13.7)a               | 72.3 (8.5)b            |
| 25                          | 19.1 (2.3)a               | 90.9 (2.4)b            | 45.5 (11.7)a               | 84.0 (17.3)b           |
| 30                          | 24.4 (4.5)ab              | 33.3 (11.2)a           | 61.6 (8.6)a                | 23.3 (10.0)a           |

Analysis of variance results were  $F = 28.4$ ;  $df = 3, 46$ ;  $P < 0.001$  for the preoviposition period;  $F = 5.4$ ;  $df = 3, 46$ ;  $P = 0.003$  for oviposition period;  $F = 8.6$ ;  $df = 3, 46$ ;  $P < 0.001$  for the postoviposition period; and  $F = 9.9$ ;  $df = 3, 46$ ;  $P < 0.001$  for fecundity. The numbers in parentheses are standard errors. Means within columns followed by the same lowercase letter are not significantly different ( $P > 0.05$ , analysis of variance with Tukey's-b).

period occurred was estimated by a quadratic model at 26.6°C (Table 3, Fig. 2A).

The oviposition period did not differ among 15, 20, and 25°C (Table 2), but oviposition periods at 20 and 25°C were longer than at

30°C. The temperature at which the longest oviposition period occurred was estimated by the quadratic model as 21.1°C (Table 3, Fig. 2B).

The postoviposition period did not differ among 20, 25, and 30°C (Table 2), but postoviposition period was shorter at 15°C. The longest postoviposition period was recorded as 396 d at 15°C. The temperature at which the shortest postoviposition period occurred was estimated by the quadratic equation as 25.2°C (Table 3, Fig. 2C).

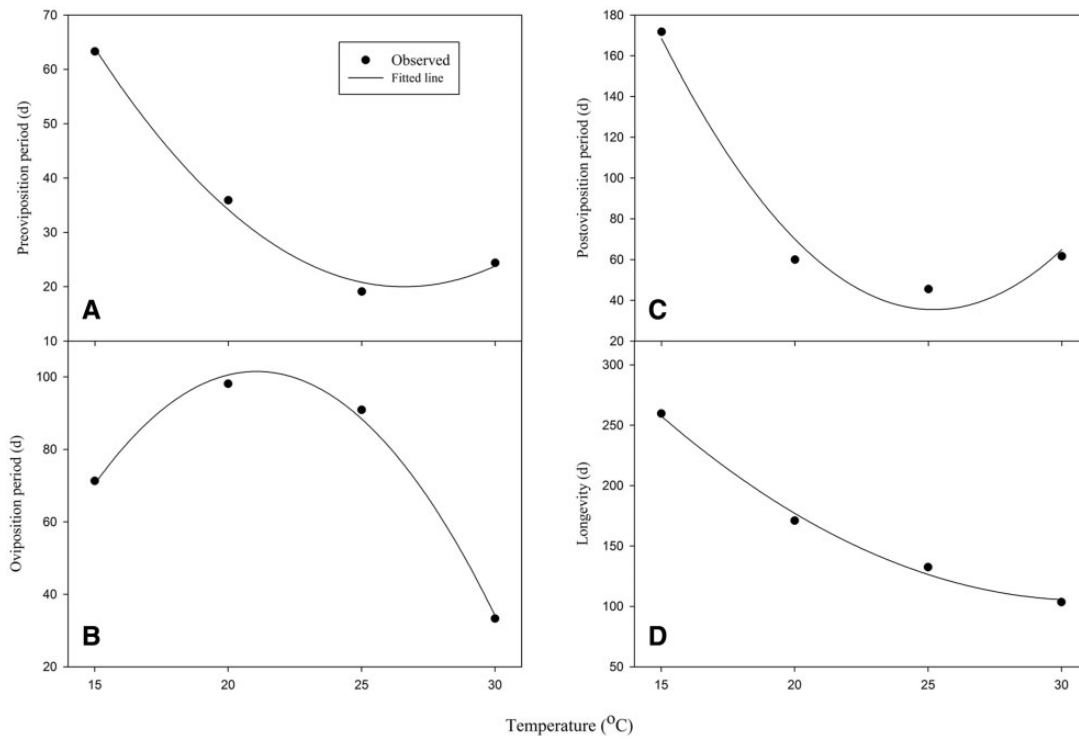
The percentage of life span spent in oviposition increased from 24.4% at 15°C to 59.5% at 25°C, and then declined to 27.4% at 30°C and the highest percentage of life span spent in oviposition was recorded as 89.1% at 25°C.

**Table 3. Parameters of a quadratic equation describing the effects of constant temperatures on *H. obliqua* preoviposition period, oviposition period, postoviposition period, oviposition rate, and longevity**

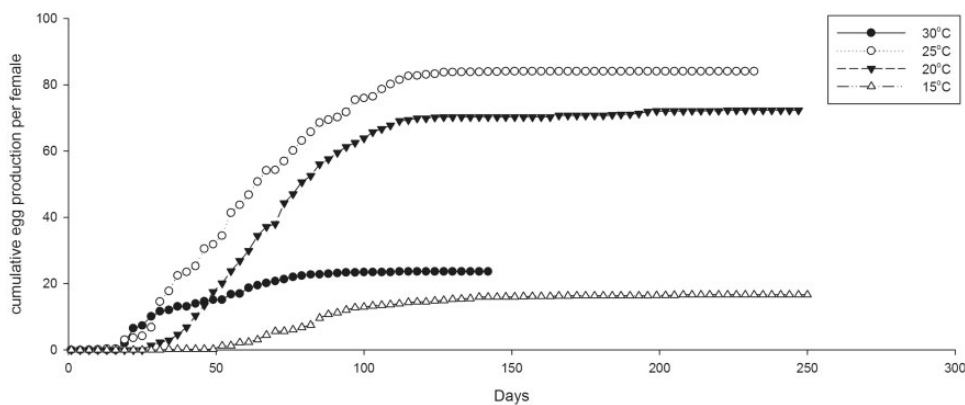
| Subject                    | R <sup>2</sup> | F      | p    | a       | b      | c     |
|----------------------------|----------------|--------|------|---------|--------|-------|
| Preoviposition period (d)  | 0.98           | 87.59  | 0.08 | 251.08  | -17.39 | 0.33  |
| Oviposition period (d)     | 0.98           | 93.52  | 0.07 | -272.96 | 35.56  | -0.84 |
| Postoviposition period (d) | 0.94           | 22.58  | 0.15 | 847.55  | -64.46 | 1.28  |
| Longevity (d)              | 0.98           | 82.07  | 0.08 | 678.32  | -37.00 | 0.60  |
| Oviposition rate           | 0.99           | 356.33 | 0.04 | -10.46  | 1.07   | -0.02 |

Equation is of the type  $y = a + bx + cx^2$  with an adjusted R<sup>2</sup> value, in all cases, df = 2, 21.

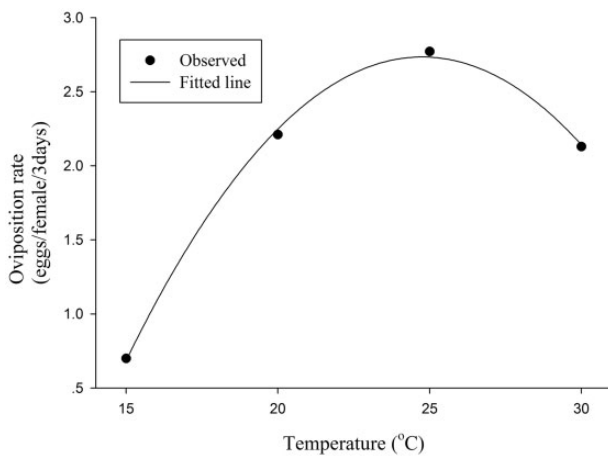
**Fecundity.** The number of eggs deposited per female increased from 16.6 at 15°C to 84.0 at 25°C but decreased to 23.3 eggs per female at 30°C (Table 2). The greatest number of eggs deposited by one female was 168 at 25°C. The cumulative egg production at 20°C and 25°C were similar, but the cumulative egg production at 25°C was always more than at 20°C. At 20°C and 25°C, females almost lay no egg after 120 d (Fig. 3).



**Fig. 2.** Preoviposition period, postoviposition period, oviposition period, and longevity of *H. obliqua* at constant temperatures. (A) Preoviposition period. (B) Postoviposition period. (C) Oviposition period. (D) Longevity. Parameters for the quadratic equation are listed in Table 3.



**Fig. 3.** Cumulative egg production per female of *H. obliqua* by the females age increased.



**Fig. 4.** Oviposition rate of *H. oblita* at constant temperatures. Parameters for non-linear model are in Table 3.

The effect of temperature was similar on eggs laid per female and the daily rate of oviposition. The 3-d rate of oviposition increased from 0.7 eggs per female at 15°C to 2.8 eggs per female at 25°C, and then decreased to 2.1 eggs per female at 30°C. The temperature at which a female would produce the maximum number of eggs per day was 24.8°C, estimated using the quadratic equation (Table 3, Fig. 4).

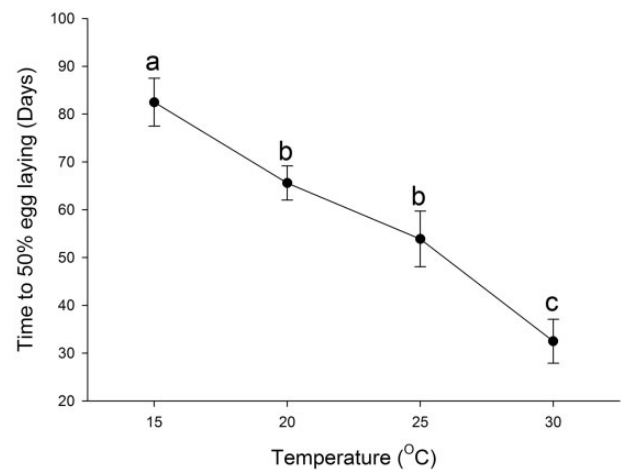
The time to 50% egg laying decreased with increasing temperature (Fig. 5), varying from 82.5 d at 15°C to 32.5 d at 30°C. The longest time to 50% egg laying was recorded as 129 d at 15°C, and the shortest time to 50% egg laying was recorded as 21 d at 30°C.

## Discussion

All reproductive parameters of *H. oblita* were affected by temperature. Our results indicated that the performance of *H. oblita* was optimal at moderate temperatures (20°C and 25°C), and 25°C is considered as the optimal temperature for reproduction with insects exhibiting the shortest preoviposition period, 60% of the adult life was spent in oviposition, and most importantly, the most eggs were laid at this temperature. No other studies examined the effects of temperature on reproductive parameters of *H. oblita*, but some studies determined reproductive parameters using *U. pumila* as food at 25°C (Dang et al. 2007, Liu et al. 2008, Zhou et al. 2009).

The longevity of *H. oblita* in our study decreased with increasing temperatures, as also happened in other species of Coleoptera such as *Agasicles hygrophila* Selman and Vogt, *Hylobius transversovittatus* Goeze, and *Otiorhynchus sulcatus* F. adults when in the temperature range of 15–30°C, 12.5–30°C, and 18–36°C, respectively (McAvoy and Kok 1999, Stewart et al. 1999, Son and Lewis 2005). The longevity of *H. oblita* was 102 d when *U. pumila* was used as food at 25°C (Liu et al. 2008), and longevity in our study was a little longer at 132.6 d. One possible reason for differences in longevity between studies may be that different geographic populations were used. Liu et al. (2008) collected their insects in Cangzhou, Hebei Province (38.29 N, 116.82 E), and we collected our population in Hefei, Anhui Province (31.63 N, 117.11 E). Another reason for differences may be the rearing container used in the studies. Liu et al. (2008) used a glass jar (8 cm in height by 18 cm in diameter), while we used a plastic box (25.4 cm by 16.5 cm by 7.6 cm), which had 1.5 times the volume of the glass jar.

In the study by Liu et al. (2008), the preoviposition period of *H. oblita* was 18.3 d at 25°C when using *U. pumila* as food, and this result was similar to our result of 19.1 d. Son and Lewis (2005) investigated the effects of temperature on reproduction of black vine weevil, *O. sulcatus*, between 11 and 36°C, and they found the shortest preoviposition period at 24°C which is similar to our result of 25°C. The oviposition period in the Dang et al. (2007) study was 90 d when using *U. pumila*



**Fig. 5.** Time to 50% egg laying of *H. oblita* at different temperatures. Analysis of variance results were  $F = 17.8$ ;  $df = 3, 46$ ;  $P < 0.001$ . Means with the same lowercase letter are not significantly different ( $P > 0.05$ , Tukey's-b).

leaves as food at 25°C, which is similar to our result of 90.9 d. The optimal reproductive temperatures for the Coleoptera species *A. hygrophila*, *H. transversovittatus*, and *Anoplophora glabripennis* Motschulsky were between 23 and 25°C (McAvoy and Kok 1999, Stewart et al. 1999, Keena 2006), and this is similar to results from our study of 25°C for *H. oblita*.

Fecundity of *H. oblita* was 145.5 eggs per female in the Liu et al. (2008) study and 107.1 eggs per female in the Zhou et al. (2009) study when using *U. pumila* as food at 25°C, but fecundity was lower at 84 eggs per female when using *U. pumila* as food at 25°C in our study. However, our results are similar to those of the Dang et al. (2007) study with fecundity of 82.4 when using fresh leaves of *U. pumila* at 24–26°C. The differences with the Liu et al. (2008) study may be because of the reasons previously mentioned (different geographic populations and rearing containers). The differences between the Zhou et al. (2009) study and our study may be because of using different sizes of rearing containers and the density of *H. oblita*. The size of the rearing box that they used was 26.8 cm by 26.8 cm by 45.5 cm and they introduced 15 pairs of *H. oblita* in each rearing box, but our rearing box was 25.4 cm by 16.5 cm by 7.6 cm and we introduced only one pair of *H. oblita* into each box. Additionally, the population in the Zhou et al. (2009) study was collected in Qingdao, Shandong Province (36.33 N, 120.40 E). The fecundity of *H. oblita* was recorded as 38.7 eggs per female in natural conditions (Hu and Xu 1986), which is much less than when using the favorite food (*U. pumila*) and optimal temperature (25°C). The peak of egg laying in the Zhou et al. (2009) study was at 20 d when using *U. pumila* as food at 25°C, and then the egg laying decreased to zero at 40 d (Zhou et al. 2009). However, in our study, the peak of egg laying for *H. oblita* at 25°C was after 30 d, and egg laying continued until about 120 d.

The optimal reproductive temperature for *H. oblita* was 25°C, which may indicate the adverse effects of high temperatures and why it is underground during the daytime and active at night because the temperatures in Hefei, Anhui Province, after *H. oblita* emerge are usually around 30°C in daytime and 20–25°C at night. The adults of *H. oblita* occur in the field at Hefei from the middle of April to the beginning of July (Hu and Xu 1986), so adult longevity in the field was around 90 d, which was similar to adult longevity in our study at 30°C (Table 1). The fecundity in the field of *H. oblita* (38.7 eggs per female) was more than in this study at 30°C (23.3 eggs per female). However, in our study, both longevity and fecundity at 25°C were greater than in the field study.

There was less information on how to mass rear *H. oblita*, because the development time in field was as long as 2 yr in most parts of China

(Hu and Xu 1986, Wang et al. 1989, Liu et al. 2008). *H. oblita* could be reared in the greenhouse to shorten the development time, but it is only shortened by several months. Determining how to optimize fecundity would be useful for mass rearing. The information provide in this study is useful for developing *H. oblita* management programs in the field and for optimizing mass rearing.

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