

Demography and Mass Rearing of the Medicinal Blister Beetle *Epicauta impressicornis* (Pic) (Coleoptera: Meloidae) at Different Temperatures

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Abstract

The population dynamics of the blister beetle, *Epicauta impressicornis* (Pic) (Coleoptera: Meloidae) had been investigated from 2014 to 2016 in field. The results showed that *E. impressicornis* is univoltine and overwintered in the fifth instar in the Luodian area of Guizhou, China. The biological and ecological characteristic of the beetle were analyzed at six temperatures (i.e., 21, 24, 27, 30, 33, and 36°C) in the laboratory using the age-stage, two-sex life table. The developmental duration, longevity, and total preoviposition period were significantly shortened with increases in temperatures. The adult preoviposition periods at 24, 27, and 30°C were significantly longer than at other temperatures, while the fecundities at 27 and 30°C were significantly higher than at other temperatures. Life expectancy (e_{xj}) decreased with age and the reproductive value (v_{xj}) increased with age. The intrinsic rate of increase ($r = 0.0921 \text{ d}^{-1}$) and finite rate ($\lambda = 1.0965 \text{ d}^{-1}$) were the highest at 33°C, followed by 30°C ($r = 0.0758 \text{ d}^{-1}$, $\lambda = 1.0788 \text{ d}^{-1}$). Although the net reproductive rate ($R_0 = 17.63$ offspring) was the lowest at 36°C, the values of r (0.0724 d^{-1}) and λ (1.0751 d^{-1}) were higher at 36°C than those at 21, 24, and 27°C. Our analysis for a mass-rearing system showed the most efficient and economical strategy would be to rear *E. impressicornis* at 30–33°C.

Key words: *Epicauta impressicornis*, age-stage two-sex life table, temperature, mass rearing

The blister beetle *Epicauta impressicornis* (Pic) (Coleoptera: Meloidae) is well known for secreting the vesicant, cantharidin, an insect toxin containing sesquiterpenoids that are known to have pharmaceutical effects (National Pharmacopoeia Editorial Board 2010, Seabrooks and Hu 2017). Blister beetles have been used in traditional Chinese medicine for more than 2000 years (Wu et al. 2010). In modern medicine, cantharidin has been widely used in the treatment of several human diseases, e.g., dropsy, warts and molluscum, furuncles and piles, cancers (stomach, lung, liver, and esophageal), and got good results in the past decades (Carrel et al. 1993, Moed et al. 2001, Zou et al. 2002, Gao et al. 2004, Li et al. 2009, Wu et al. 2010, Hu 2011, Liu 2013, Prasad and Verma 2013, Verma and Prasad 2013).

Because the main supply of meloidae beetles needed for medicinal cantharidin relies on field collection and the mass rearing of these beetles is difficult due to the beetle's unique life cycle (hypermetamorphic), Meloidae beetles have been classified as one of the animal species of medicinal importance that is in chronic short supply

(Keele and Armstrong 1964, Dauben et al. 1980, Wang et al. 2000, Schmidt 2002, Nikbakhtzadeh et al. 2007, Wang et al. 2010, Yin and Jin 2010, Li et al. 2013). Mu and Chen (2014 and 2015) reported on the development, breeding habits, and larval feeding of *E. impressicornis*. Since blister beetles are ectothermic like many other insects, it is critical to understand their development, survival and fecundity under different temperature regimes prior to planning an efficient mass-rearing system. The large-scale production of cantharidin, however, relies on the mass-rearing and harvesting theory (Chi and Getz 1988). Life tables are essential tools that enable researchers to understand the development, survival and reproduction of an insect population as thoroughly as possible. Because traditional female based age-specific life tables (Lotka 1907, Lewis 1942, Leslie 1945, Birch 1948, Carey 1993) ignore the male population and are incapable of correctly describing the stage differentiation, their practical application is limited in studies involving insect ecology, pest management, biological control, and mass rearing (Huang and Chi 2012, Huang et al. 2018). In contrast to the female-only life tables, the

age-stage, two-sex life table (Chi and Liu 1985, Chi 1988) takes both sexes into consideration, precisely describes the stage differentiation, and has been widely adopted in research related to insect population ecology, pest management, predator-prey relationships, and practical aspects of biological control.

To devise an efficient and economical mass rearing system for *E. impressicornis*, it is necessary to comprehensively understand the demographic characteristics. Therefore, we conducted a preliminary 2-yr field survey first. After that, we collected the life history data (development, survival, fecundity, sex composition) at different temperatures (21, 24, 27, 30, 33, and 36°C) in the laboratory, and analyzed the demographic data using the age-stage, two-sex life table. Then, we constructed the mass-rearing system according to Chi and Getz (1988). This information will not only provide basic knowledge on the life history of *E. impressicornis*, but will also serve as a theoretical basis to enable the large-scale mass rearing of these insects for cantharidin production.

Materials and Methods

E. impressicornis

Adult males and females of *E. impressicornis* were originally collected from Luokun village (106.58°E, 25.42°N), Luodian, Guizhou Province, China, in August 2015. Subsequently, insects have been cultured on fresh leaves of *Vicia faba* L. and *Solanum nigrum* L. and fresh fruit of *Cucurbita moschata* Duchesne in a climate chamber (Jiangnan Instrument RXZ-380A, Ningbo, China) set at 28 ± 1°C, 75 ± 5% RH, and a photoperiod of 14: 10 h (L: D). Egg masses laid within 24 h time periods were transferred to a glass petri dish (9 cm in diameter) with a dampened filter paper on the bottom. Larvae were reared in plastic cups (8.5 cm in height, 7 cm in diameter) half-filled with sterilized soil (moisture content approximately 30%). An egg pod of the Chinese rice grasshopper, *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae), was supplied as food for the larvae. The hatched larvae were transferred into the cup, covered with damp water-absorbent cotton, and then sealed with plastic wrap. Several needle holes were punctured in the plastic wrap for ventilation. The emerged adults were transferred to a glass beaker (1,000 ml) containing 0.5 kg sterilized soil (30% moisture content). Adults were supplied with fresh leaves of *V. faba* and *S. nigrum*, and pieces of young *C. moschata* fruit (fruit size 10–20 cm in diameter) (Mu and Chen 2014, 2015). Insects were reared in the laboratory for more than three generations before beginning the life table study.

Field population survey

Field investigations of *E. impressicornis* were conducted from 1 March 2014 to 1 March 2016 at 10-d intervals, in Luokun Town (106.58°E, 25.42°N), Luodian County, Guizhou Province, China. Five sampling squares were randomly selected in a rice paddy field. At each sampling area, a soil sample (30 cm in length, 30 cm in width, and 10 cm in depth) was dug and then carefully inspected. The number and stage of insects were recorded. No samples were taken from November to February of the following year because of the low temperatures and the insects were overwintering and inactive.

Life table construction

Life table data were collected in growth chambers set at 21, 24, 27, 30, 33, and 36°C, 75 ± 5% RH, and a photoperiod of 14: 10 h (L: D). An initial number of 50 eggs laid within a 24 h period were used for each treatment. The survival rate and time of each egg to hatch

instar and the development and survival of larvae were observed twice daily at 8:00 and 20:00. Because the second to fourth instar larvae fed inside the egg pod, we grouped them as a single stage (L2–4) to avoid damaging the insects. The larval stages were, therefore, grouped as L1, L2–4, L5 (pseudopupa), and L6 instar. Being supplied with a mixture of *V. faba* leaves (4 g), *S. nigrum* leaves (5 g), and a section (4 g) of young *C. moschata* fruit (10–20 cm in diameter). The survival and adult fecundity were recorded daily (8:00) until the death of all individuals.

Statistical Analysis

The developmental rates at different temperatures were fitted to the linear equation to estimate the thermal summation (K) and development threshold temperature (T_0) according to Campbell et al. (1974).

The life table raw data of *E. impressicornis* were analyzed according to the age-stage, two-sex life table theory (Chi and Liu 1985, Chi 1988) by using the computer program TWSEX-MSChart (Chi, 2018a). The age-stage specific survival rate (s_{xj}), which is the probability that an individual can survive from the egg to age x and develop to stage j , and the age-specific fecundity (f_{xj}), which is the daily number of eggs laid by a female adult at age x and stage j , were calculated according to Chi and Liu (1985). The age-specific survival rate (l_x), i.e., the survival rate from age 0 to age x , was calculated as:

$$l_x = \sum_{j=1}^{\beta} s_{xj} \quad (1)$$

where β is the number of stages. The age-specific fecundity (m_x), i.e., the average number of eggs produced by an individual at age x , was calculated as:

$$m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}} \quad (2)$$

The product of l_x and m_x is the age-specific net maternity ($l_x m_x$). The sum of $l_x m_x$ over all ages gives the net reproductive rate (R_0), the number of offspring produced by an individual during its lifetime, was calculated as:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (3)$$

The life expectancy (e_{xj}), which represents the length of time that an individual of age x and stage j is expected to survive, was calculated according to Chi and Su (2006) as:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{\beta} s'_{iy} \quad (4)$$

where s'_{iy} is the probability that an individual of age x and stage y will survive to age i and stage y by assuming $s'_{xj} = 1$. The intrinsic rate of increase (r) was estimated by using the iterative bisection method from the Euler-Lotka formula with age indexed from 0 (Goodman 1982) as:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (5)$$

The finite rate (λ) was then calculated as:

$$\lambda = e^r \quad (6)$$

The mean generation time (T) is the length of time that a population needs to increase to R_0 -fold when the stable increase rate r and λ are reached, that means, $e^{rT} = R_0$ or $\lambda^T = R_0$, and was calculated as:

$$T = \frac{\ln R_0}{r} \quad (7)$$

The reproductive value (v_{xj}) represents the contribution an individual of age x and stage j makes to the future population, it was calculated as:

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{\beta} s_{iy}' f_{iy} \quad (8)$$

The variances and standard errors of the life table statistics were estimated using the bootstrap technique (Efron and Tibshirani 1993). The differences among treatments were compared using the paired bootstrap test. These procedures were embedded in the TWOSEX-MSChart program (Chi 2018a).

The intrinsic rate of increase at different temperatures was also fitted to the model of Stinner et al. (1974) as:

$$r_{\tau} = \frac{C}{1 + e^{k_1 + k_2 \tau}} \quad (9)$$

where r_{τ} is the intrinsic rate of increase at temperature τ , C is the asymptote. It was calculated as:

$$C = r_{\max} \times (1 + e^{k_1 + k_2 t_{opt}}) \quad (10)$$

t_{opt} is the temperature at which the maximum intrinsic rate of increase (r_{\max}) occurs, k_1, k_2 are empirical constants, $\tau' = \tau$, for $\tau \leq t_{opt}$ and $\tau' = 2 \times t_{opt} - \tau$, for $\tau > t_{opt}$.

Population projection

The TIMING-MSChart program (Chi 2018b) was used to project the population dynamics of *E. impressicornis* at different temperatures.

Mass-rearing system

Since pupae could be readily collected and kept for subsequent harvesting of adults, we used the age-stage survival rate (s_{xj}) and fecundity (f_{xj}) to calculate the sustainable harvest rate h that satisfied the net reproductive rate after harvesting 1,000 new pupae, i.e., $R_0(h) = 1$, according to Chi and Getz (1988) as:

$$R_0(h) = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} s_{xj} f_{xj} (1-h) = 1 \quad (11)$$

We then used the harvest rate h to calculate the number of daily new recruits to each stage (N_j) and the total number of individuals in each stage (T_j) necessary for a stable mass-rearing system according to Chi and Getz (1988). Because adults of the blister beetle contain the highest amount of cantharidin, the adults that can be harvested from 1,000 harvested pupae can be calculated as:

$$1000 \times S_A = H_A \quad (12)$$

where S_A is the survival rate from pupa to female and male adults, and H_A is the expected daily harvest number of adults. Because eggs and pupae do not feed, their rearing costs was zero; since each larva requires a single grasshopper egg pod (costing 0.45 Renminbi [RMB], 1 RMB = 0.16 USD) which lasts through the entire larval stage, we set the one-time rearing cost for the first instar as 0.45 RMB and the remaining larval instars as zero. The daily cost for rearing each adult using the previously discussed mixture was 0.03 RMB, while the cost of labor was 0.15 RMB. The fixed expenses includes petri dishes for egg masses (4.5 RMB each), plastic cups for larvae (1 RMB each), and beakers for adults (12 RMB each).

Results

Field Population Survey

E. impressicornis is a univoltine species in Luodian town (Guizhou Province, China). The fifth instars (also called pseudopupae) overwinter in the soil and ridges of rice paddy fields at a depth of 5–8 cm when the temperature drops below 20°C. The fifth instar molts to the sixth instar in March of the following year and pupation begins in early April. Adults emerge in late May and mate in mid-June. Oviposition begins in early July. Under field conditions, the egg stage lasts from 45 to 55 d. Larvae emerge in late August and feed on grasshopper eggs. The fifth instars begin to overwinter in early October, and continue as dormant fifth instar larvae for 5–6 mo under field conditions (Fig. 1).

Life Table

The developmental duration, longevity, preadult survival rate, adult preoviposition period (APOP), and total preoviposition period (TPOP) of *E. impressicornis* under different temperatures are listed in Table 1. In general, the developmental stages of *E. impressicornis* were significantly shortened with increases in temperature. The durations of the fifth instar were significantly extended at 21°C (96.7 d), 24°C (59.4 d), and 27°C (21.3 d), and were significantly longer than the durations at 30, 33, and 36°C. However, the durations of the fifth instar were shorter in all temperature treatments of lab reared larvae than they were in larvae developing at comparable temperatures in the field. The longest total preadult duration (189.3 d) at 21°C, was significantly longer than those at other temperatures, while the shortest preadult duration was 36.1 d at 36°C. The lifespans of both males and females were also significantly shortened with increasing temperature. The longest (232.8 d for females and 219.2 d for males) and shortest (43.7 d for females and 46.6 d for males) were at 21°C and at 36°C, respectively. The developmental rate was a close fit to the linear model, with a thermal summation (K) of 622.36 degree-days and a developmental threshold (T_0) of 18.41°C (Fig. 2A).

The overall preadult survival rates (s_a) between 24 and 33°C were all $\geq 70\%$ and were significantly higher than those at the lowest and highest temperatures (21 and 36°C). The TPOP differed significantly among temperatures, progressively decreasing with increases in temperature, i.e., the longest 191.3 d at 21°C and the shortest 38.7 d at 36°C. The fecundities at 27°C (198.4 eggs) and 30°C (188.1 eggs) were significantly higher than those at other temperatures (Table 2).

The age-stage specific survival rate (s_{xj}) of *E. impressicornis* was presented in Fig. 3. Due to the variation in developmental rate among individuals, the s_{xj} curves showed obvious overlap. Significant extension of the fifth instar could be observed at 21 and 24°C. The higher s_{x7} curves (females) showed that there were more female adults than males (s_{x8} curves).

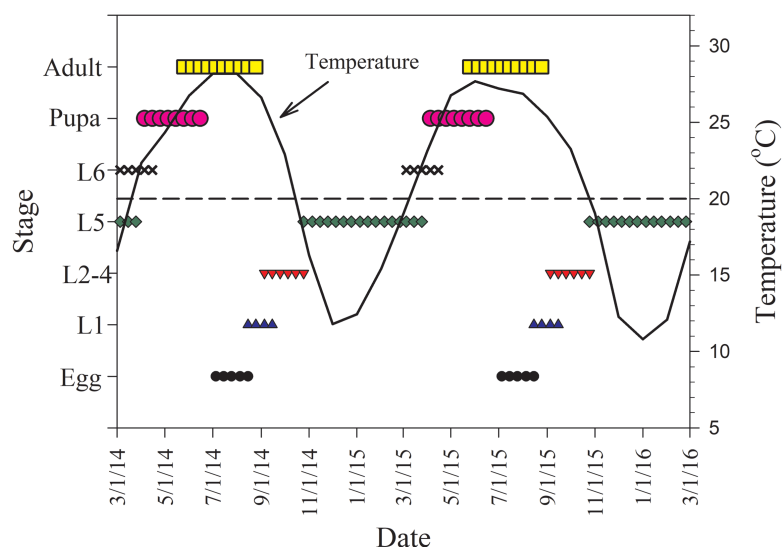


Fig. 1. Occurrence of different stages of *E. impressicornis* during the field survey from 2014 to 2016.

Table 1. Mean (\pm SE) of the developmental period, total preadult duration, preadult survival rate (s_p), longevity, adult preoviposition period (APOP), total preoviposition period (TPOP), and fecundity (F) of *Epicauta impressicornis* at different temperatures

Statistics	N	21°C	n	24°C	n	27°C	n	30°C	n	33°C	n	36°C
Egg (d)	50	32.4 \pm 0.3 ^a	50	27.2 \pm 0.3 ^b	50	21.8 \pm 0.6 ^c	50	16.8 \pm 0.4 ^d	50	13.1 \pm 0.1 ^e	50	10.0 \pm 0.3 ^f
L1: first instar larva (d)	44	7.6 \pm 0.1 ^a	48	6.2 \pm 0.1 ^b	44	5.4 \pm 0.1 ^c	46	4.7 \pm 0.1 ^d	46	4.0 \pm 0.2 ^e	47	3.0 \pm 0.1 ^f
L2-4: second-fourth instar larva (d)	37	18.1 \pm 0.3 ^a	42	13.6 \pm 0.2 ^b	41	11.4 \pm 0.2 ^c	42	9.1 \pm 0.1 ^d	43	6.2 \pm 0.1 ^e	33	5.9 \pm 0.1 ^e
L5: fifth instar larva (d)	35	96.7 \pm 0.4 ^a	41	59.4 \pm 0.5 ^b	40	21.3 \pm 0.5 ^c	40	6.5 \pm 0.2 ^d	41	6.2 \pm 0.1 ^d	30	6.2 \pm 0.2 ^d
L6: sixth instar larva (d)	30	15.9 \pm 0.6 ^a	39	10.0 \pm 0.3 ^b	37	8.9 \pm 0.3 ^c	37	5.2 \pm 0.3 ^d	40	4.2 \pm 0.2 ^e	29	3.7 \pm 0.2 ^f
Pupa (d)	29	19.1 \pm 0.4 ^a	37	12.8 \pm 0.3 ^{b,c}	35	11.7 \pm 0.6 ^c	37	8.3 \pm 0.3 ^d	39	8.0 \pm 0.3 ^{d,e}	29	7.6 \pm 0.2 ^e
Total preadult (d)	29	189.3 \pm 1.0 ^a	37	129.4 \pm 0.9 ^b	35	80.3 \pm 1.1 ^c	37	50.1 \pm 0.7 ^d	39	41.4 \pm 0.5 ^e	29	36.1 \pm 0.4 ^f
Preadult survival rate (s_p)	50	0.580 \pm 0.070 ^b	50	0.740 \pm 0.062 ^a	50	0.700 \pm 0.065 ^a	50	0.740 \pm 0.062 ^a	50	0.780 \pm 0.058 ^a	50	0.580 \pm 0.070 ^b
Adult longevity (d)	29	38.4 \pm 1.5 ^a	37	29.4 \pm 0.4 ^b	35	23.7 \pm 0.9 ^c	37	20.0 \pm 0.6 ^d	39	12.8 \pm 0.5 ^e	29	8.8 \pm 0.3 ^f
Total lifespan Female (d)	18	232.8 \pm 2.2 ^a	24	158.1 \pm 1.5 ^b	20	101.7 \pm 2.5 ^c	22	69.0 \pm 1.3 ^d	25	54.5 \pm 0.7 ^e	17	43.7 \pm 0.4 ^f
Male (d)	11	219.2 \pm 2.1 ^a	13	159.9 \pm 1.6 ^b	15	107.0 \pm 1.8 ^c	15	71.5 \pm 1.3 ^d	14	53.7 \pm 1.3 ^e	12	46.6 \pm 0.9 ^f
APOP (d)	18	2.1 \pm 0.3 ^d	24	4.4 \pm 0.6 ^{a,b}	20	4.4 \pm 0.3 ^a	22	4.0 \pm 0.3 ^{a,b}	25	2.8 \pm 0.2 ^c	17	3.3 \pm 0.4 ^b
TPOP (d)	18	191.3 \pm 1.4 ^a	24	133.6 \pm 1.2 ^b	20	84.0 \pm 1.9 ^c	22	53.4 \pm 0.8 ^d	25	44.0 \pm 0.7 ^e	17	38.7 \pm 0.5 ^f

Means within the same row followed by the same letter are not significantly different according to the paired bootstrap test at 5% level.

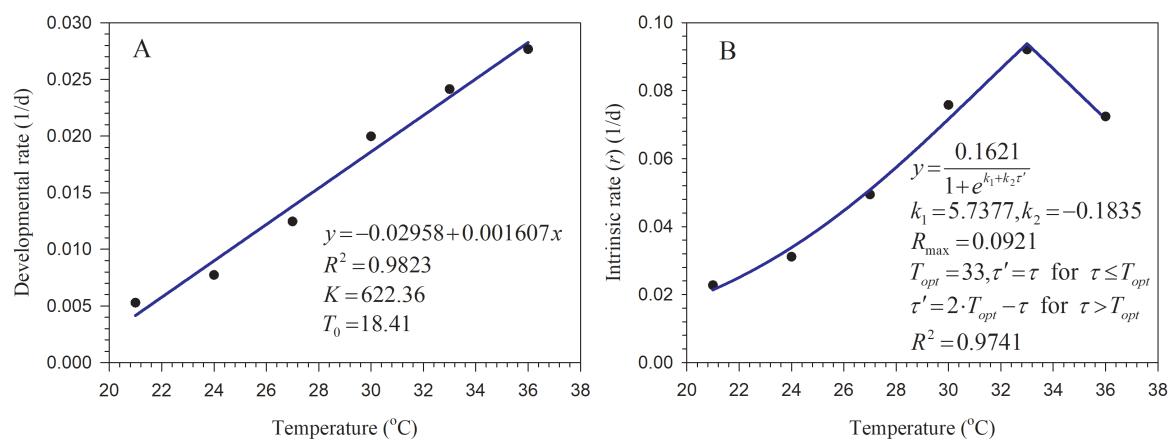
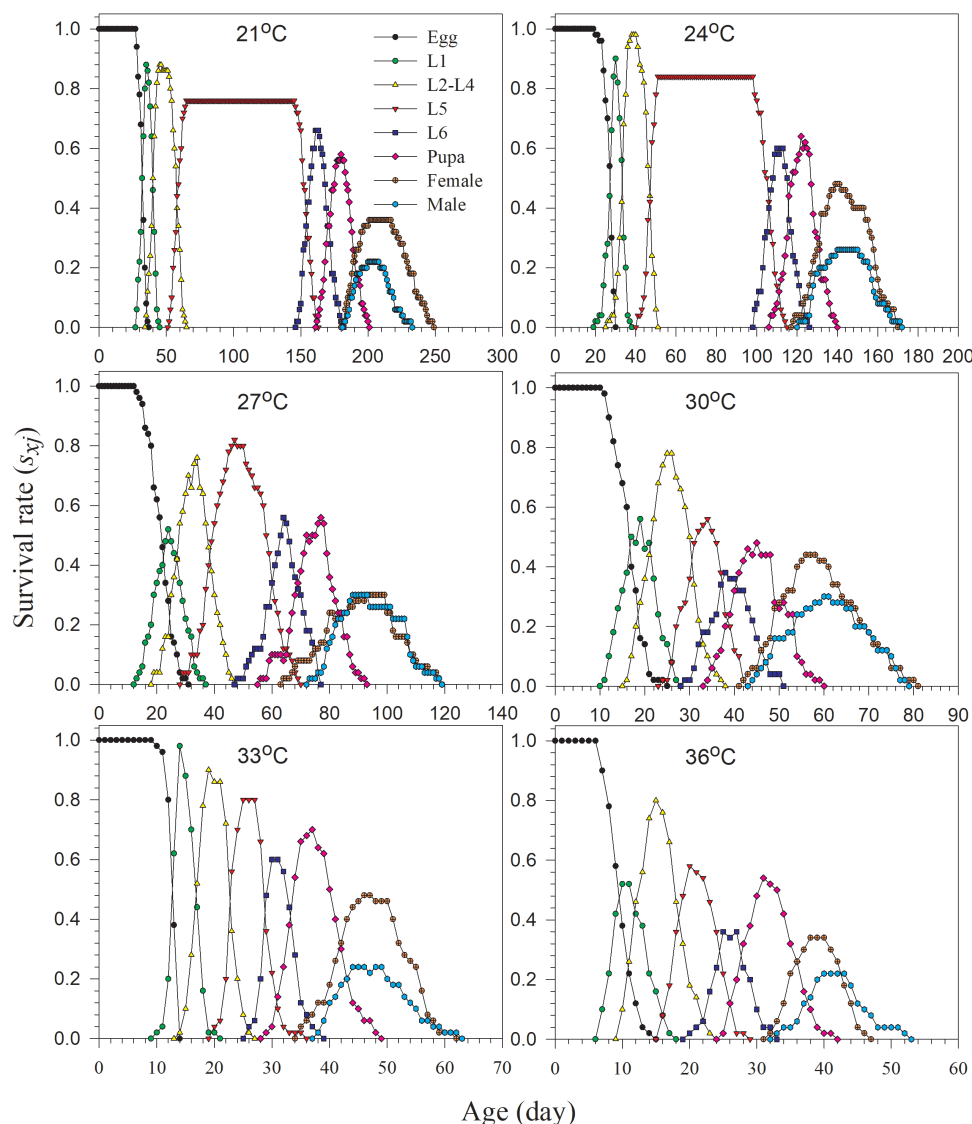


Fig. 2. (A) The developmental rate at different temperatures fitted to the linear model of Campbell et al. (1974). (B) The intrinsic rate of increase at different temperatures fitted to the model of Stinner et al. (1974).

Table 2. The population parameters (mean \pm SE) of *Epicauta impressicorins* at different temperatures

Population parameters	21°C	24°C	27°C	30°C	33°C	36°C
Proportion of females in cohort (N_f/N)	18/50	24/50	20/50	22/50	25/50	17/50
Oviposition days (O_d) (d)	3.2 \pm 0.1 ^a	2.6 \pm 0.1 ^b	2.6 \pm 0.1 ^b	2.3 \pm 0.1 ^b	1.7 \pm 0.1 ^c	1.0 \pm 0.0 ^d
Fecundity (F) (all females)	159.4 \pm 7.4 ^c	161.6 \pm 8.3 ^{b,c}	198.4 \pm 8.7 ^a	188.1 \pm 9.5 ^a	146.2 \pm 7.6 ^c	51.8 \pm 10.1 ^d
Intrinsic rate (r) (d ⁻¹)	0.0227 \pm 0.0011 ^f	0.0311 \pm 0.0012 ^e	0.0494 \pm 0.0024 ^d	0.0758 \pm 0.0032 ^{b,c}	0.0921 \pm 0.0036 ^a	0.0724 \pm 0.0075 ^c
Finite rate (λ) (d ⁻¹)	1.0209 \pm 0.0011 ^c	1.0316 \pm 0.0012 ^f	1.0507 \pm 0.0025 ^d	1.0788 \pm 0.0034 ^{b,c}	1.0965 \pm 0.0039 ^a	1.0751 \pm 0.0080 ^c
Net reproductive rate (R_0) (offspring)	57.38 \pm 11.14 ^a	77.58 \pm 12.06 ^a	79.39 \pm 14.20 ^a	82.74 \pm 13.85 ^a	73.08 \pm 10.99 ^a	17.62 \pm 4.81 ^b
Mean generation time (T) (d)	195.5 \pm 1.6 ^a	139.8 \pm 1.2 ^b	88.5 \pm 2.2 ^c	58.2 \pm 1.1 ^d	46.6 \pm 0.7 ^e	39.6 \pm 0.51 ^f

Means within the same row followed by the same letter are not significantly different according to the paired bootstrap test at 5% level.

**Fig. 3.** Age-stage-specific survival rate (s_{xj}) of *E. impressicorins* at different temperatures.

The age-specific survival rates (l_x), female age-specific fecundity rates (f_{xj}), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) curves are shown in Fig. 4. Because l_x is the pooled survival rate of all stages, it is the simplified version of s_{xj} .

Life expectancy (e_{xj}) is the length of time that an individual of age x and stage j is expected to survive (Fig. 5). In general, e_{xj} decreased

with increases in age and temperature. The reproductive value (v_{xj}) is the contribution an individual of age x and stage j will make to the future population of *E. impressicorins* (Fig. 6). The reproductive value gradually increased with increases in age and stage, while the reproductive value of the female stage dramatically increased when they began to oviposit. The maximum reproductive value noted in

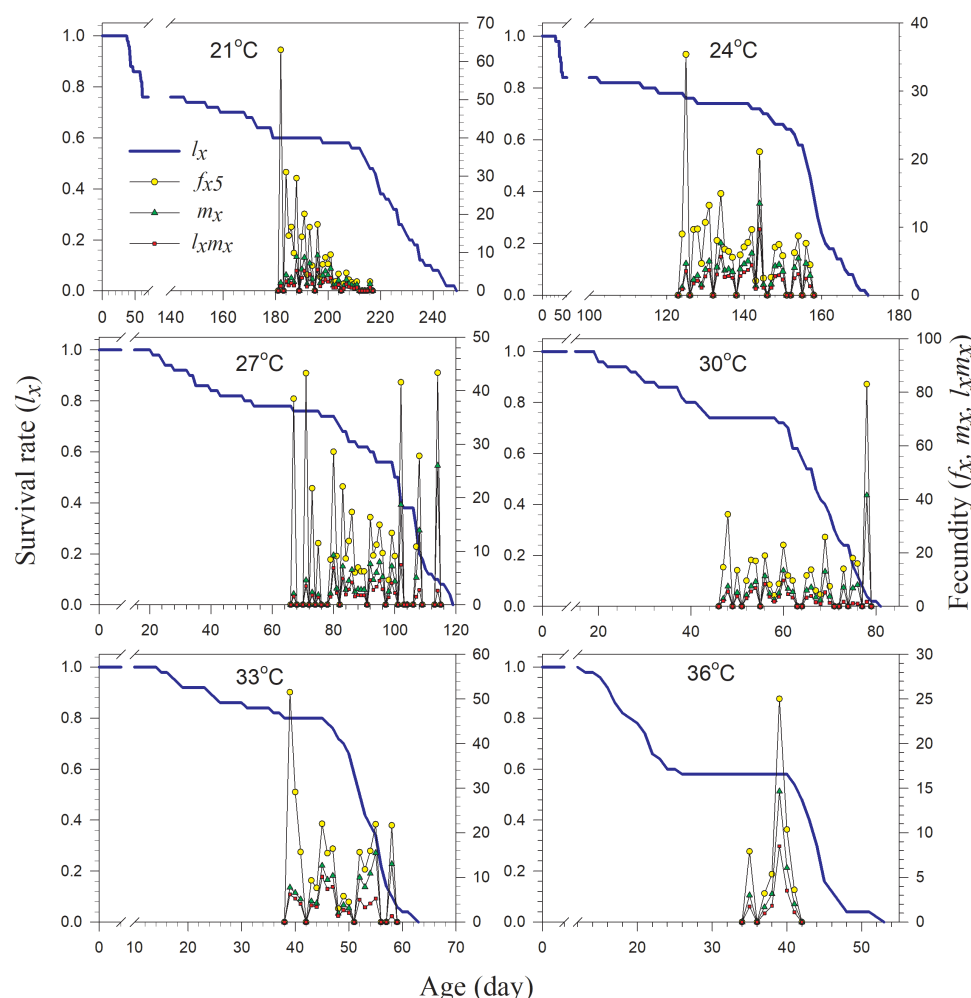


Fig. 4. Age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity of total population (m_x), and age-specific maternity ($l_x m_x$) of *E. impressicornis* at different temperatures.

the six temperature regimes occurred at age 182 d (260.91 d⁻¹), 124 d (161.69 d⁻¹), 67 d (167.41 d⁻¹), 47 d (126.39 d⁻¹), 39 d (145.56 d⁻¹), and 35 d (42.97 d⁻¹).

The population parameters are listed in Table 2. The intrinsic rate of increase (r) and finite rate of increase (λ) of *E. impressicornis* increased from 21°C ($r = 0.0227$ d⁻¹, $\lambda = 1.0209$ d⁻¹) to 33°C ($r = 0.0921$ d⁻¹, $\lambda = 1.0965$ d⁻¹), but decreased at 36°C ($r = 0.0724$ d⁻¹, $\lambda = 1.0751$ d⁻¹). The net reproductive rate (R_0) was the lowest (17.62 offspring) at 36°C, and the greatest at 30°C (82.74 offspring). The intrinsic rates at different temperatures fit Stinner's model (1974) well ($R^2 = 0.9741$).

Population Projection

The projected population growth of *E. impressicornis* for a 60-d period is shown in Fig. 7 for the six temperature regimes. At 36°C, the second generation emerged after 35 d and had developed to L2-4, L5, and L6 instars by 60 d. At 33°C, the eggs of the second generation hatched after 40 d and were only able to develop to L1, L2-4, and L5. At temperatures below 27 and 24°C, the population had not begun to reproduce by 60 d. Significant stage overlaps could be observed. When reared at 33 and 36°C, the population would be capable of producing over five generations per year.

Mass-rearing System

Using the life table data, the stage structure information, and the calculated number of daily recruits needed for each stage, the cost,

and revenue of the mass-rearing systems needed to achieve a daily harvest rate of 1,000 pupae are shown in Table 3. The highest daily harvest rate of adults ($H_A = 1,000$ d⁻¹) were obtained at 30 and 36°C. However, the minimum fixed capital per year (47,906 RMB) and the minimum of daily cost (785 RMB) occurred at 33°C. The minimum cost for each adult (0.940 RMB) was obtained at 33°C in a mass-rearing system (25 workers, each with a monthly salary of 1,800 RMB) with daily harvest rate of 975 adults. Because the price of individual adult (female or male) is normally around 3–5 RMB, the mass-rearing system at 30 or 33°C can potentially generate a profit of 60,000–120,000 RMB/month ($\approx 37,500$ USD).

Discussion

E. impressicornis is not only an important medicinal insect, but also an important enemy insect (the larvae feed on grasshopper egg) and a pest insect (it causes damage to crops of Leguminosae, Solanaceae, and Amaranthaceae) (Carrel et al. 1993, Gao et al. 2004, Li et al. 2009, Wang et al. 2010, Liu et al. 2016). However, due to its unusual life history and reproduction, there are still many unknown features that need to be understood when attempting to rear the species. Similar to the leafhopper, *Kolla paulula* (Walker) (Hemiptera: Cicadellidae) (Tuan et al. 2017), *E. impressicornis* showed good fitness when reared on a mixture of leaves from *V. faba* leaf and *S. nigrum* along with fruit from *C. moschata* including beans and

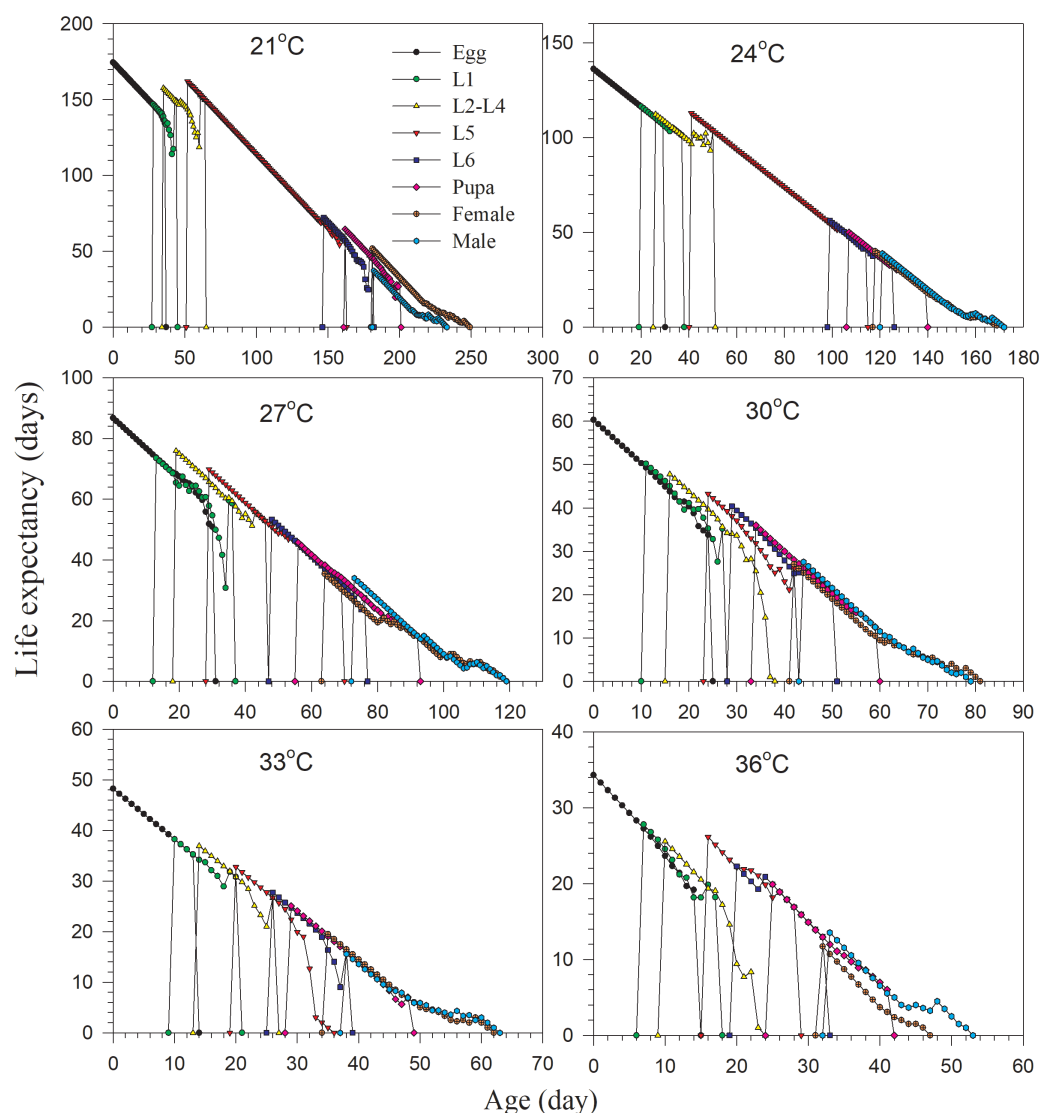


Fig. 5. Life expectancy of each age-stage group of *E. impressicornis* at different temperatures.

melons. However, the physiological mechanism involved in feeding mixed host plants needs further study.

Our field survey showed that *E. impressicornis* had only one generation per year in the Luodian area, Guizhou Province, China and overwintered as fifth instar larvae in the soil when temperatures dropped below 20°C. The extended developmental period of the fifth instar at 21 and 24°C in our life table study was consistent with the field survey. However, no obvious overwinter was observed in this laboratory study. Because *E. impressicornis* overwinters as fifth instar larvae in soil for an extended period of time, tillage accompanied by releasing chickens (and other fowl) and watering can be an effective strategy for the control of *E. impressicornis* in areas where it is considered a pest.

Many factors, including temperature, humidity, light, natural enemies, food, etc. can affect the population dynamics of insects. Each of these factors can result in changes in the developmental rate, survival rate, fecundity, reproductive age, etc. (Hoffmann et al. 2003, Broufas et al. 2009). Because life tables are capable of providing the most comprehensive and detailed description on the effect of these factors on various biological characteristics, they play a crucial role

in studies on insect population ecology and pest management (Chi 1988, Chi 1990).

As an ectothermic organism, the developmental rate of *E. impressicornis* increased at higher temperatures (Fig. 2A). Because the developmental rate is only one aspect of an insect's responses to temperature change, it is not justifiable to draw conclusions based on the developmental rate alone. On the other hand, according to Equation 5, the intrinsic rate of increase is calculated by including the survival rate (s_{xj} and l_x), the fecundity (f_{x5} and m_x), and the reproductive age (x), which gives a more accurate description of the effect of temperature on the population increase rate. Therefore, fitting the intrinsic rate at different temperatures can reveal the overall effect of temperature on the population fitness. As shown in Fig. 2B, the intrinsic rate decreased at 36°C. Zhou et al. (2010) showed a similar effect that temperature had on the development, reproduction and survival of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae); the fecundity of *O. communa* increased from 22 to 28°C, then dropped at 30 and 32°C. In our study, the fecundity of *E. impressicornis* significantly decreased at 33 and 36°C (Table 2). A similar effect due to temperature changes was observed in the

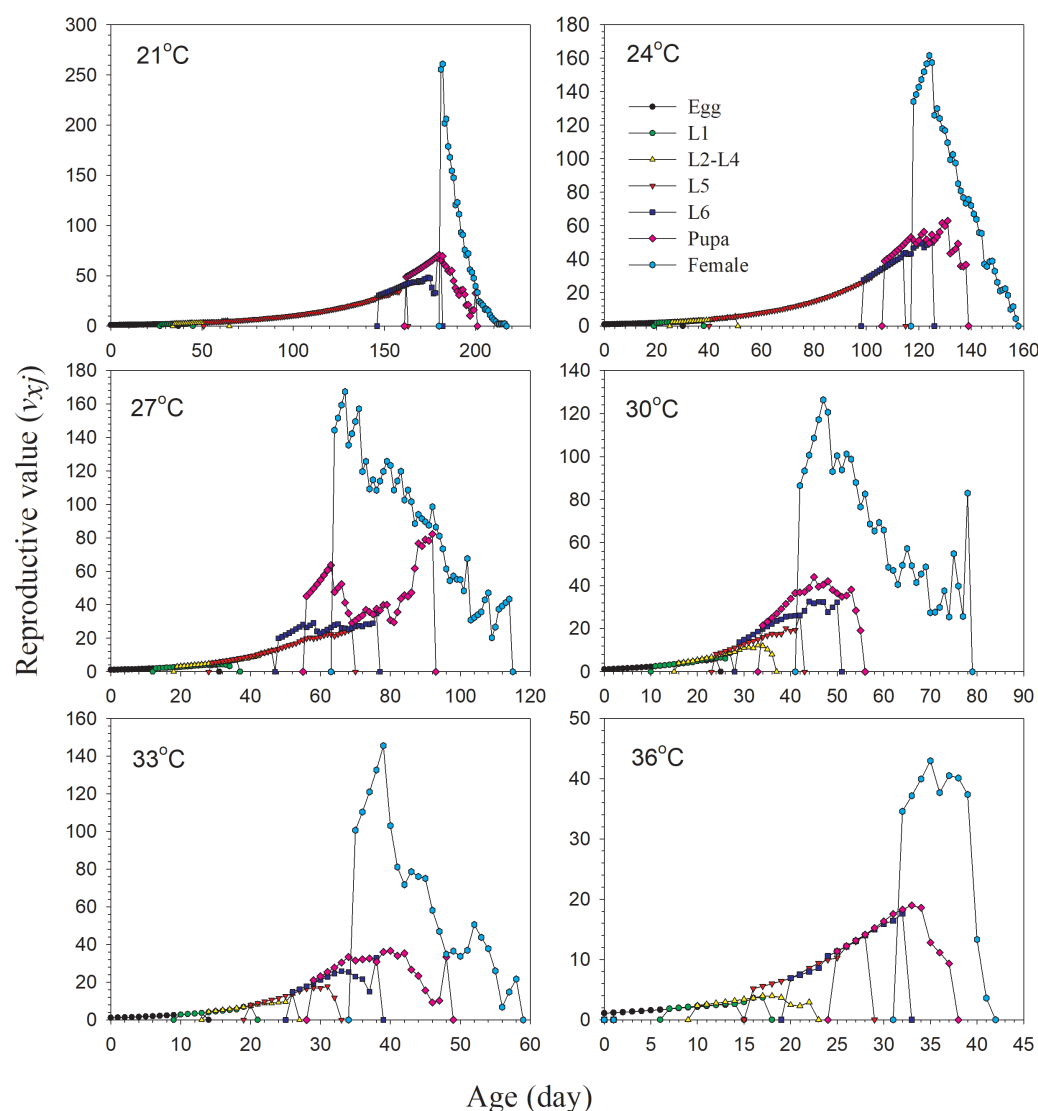


Fig. 6. Reproductive value (v_{xj}) of each age-stage group of *E. impressicornis* at different temperatures.

development of *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae) (Xu et al. 2012).

Because the two-sex life table is capable of describing the variable developmental rate among individuals, overlaps were observed at all temperatures (Fig. 3). Chi (1988) proved the relationship between the mean fecundity (F) and the net reproductive rate (R_0) as $R_0 = F \times (N_f/N)$. All our values for F and R_0 were consistent with the proof given by Chi (1988).

There were no significant differences in the length of the APOP at 24, 27, and 30°C. Because the APOP excludes the preadult period, it cannot be used to determine the effect of reproductive age on the population increase rate. As Gabre et al. (2005) pointed out, the peak value of the reproductive value (v_{xj}) is close to the TPOP. In this study, the female adults emerged at age 181, 118, 64, 42, 35, and 32 d (Fig. 3), while the first reproductive age was 182, 124, 67, 47, 39, and 35 d at 21, 24, 27, 30, 33, and 36°C (Fig. 4), respectively. It is notable that the peak values of v_{xj} appeared at the same age as the first reproductive age (i.e., 182, 124, 67, 47, 39, and 35 d) (Figs. 4 and 6). It is atypical when the peak value of v_{xj} occurs at the same age of the first reproduction and it is true when insects reproduce by laying egg masses. In general, the reproductive value will

jump to a high value when female adults emerge. Therefore, the peak reproductive value occurs after the first reproductive age and is close to the TPOP. These results were consistent with the observation of Lewontin (1965). The importance of survival rate and reproductive age can be seen in Equation 8, which explains why TPOP is a more appropriate statistic than the commonly used APOP.

Based on the developmental time, survival rate, fecundity, and population parameters, *E. impressicornis* demonstrated a greater fitness in the temperature range 27–33°C. This is similar to the findings described by Mu et al. (2014). When the fixed capital, daily rearing cost, and the number of daily harvested adults is considered, the most suitable temperature for the most efficient and economic mass rearing of *E. impressicornis* would be between 30 and 33°C. Our study and analysis will be useful in implementing a system for designing a facility to economically mass produce medicinal or edible *E. impressicornis*.

Temperature has a prominently effect on many physiological processes of insects, such as body size (Atkinson 1994), immune function (Karl et al. 2011), feeding ability and feeding rate (Kingsolver and Woods 1997, Karl and Fischer 2008), mating behavior (Geister and Fischer 2007), etc. In addition, the development and reproduction of

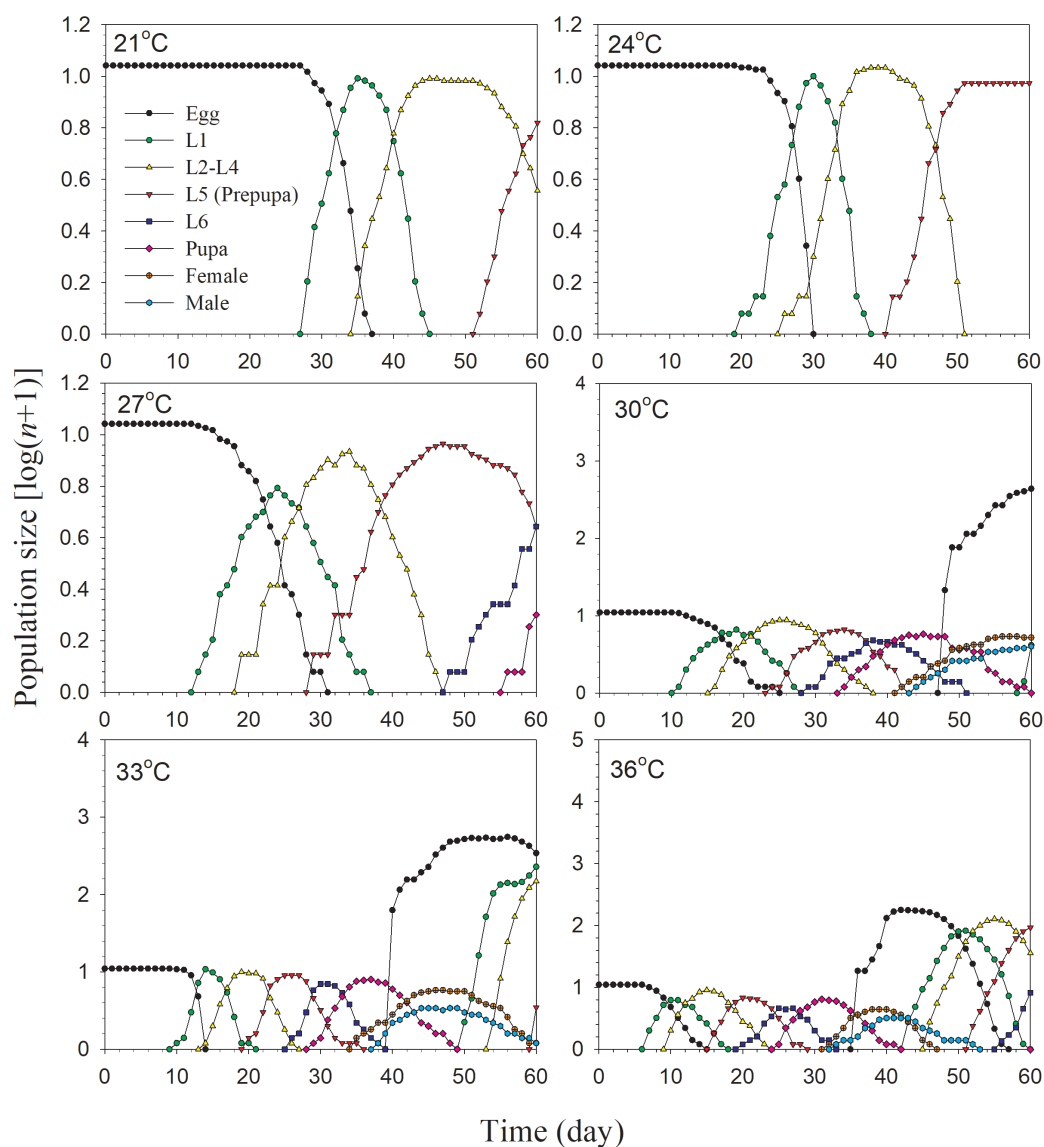


Fig. 7. Population projections over a 60 d period for *E. impressicornis* at different temperatures.

Table 3. Stage structure (T_i), daily recruits to each stage (N_i), survival rate from pupae to adults (S_A), daily harvested pupae (H_p), and adults (H_A) and cost of mass-rearing system for a daily harvest rate of 1,000 pupae (H_p) of *Epicauta impressicornis* at different temperatures

Temperature		Egg	L1	L2-4	L5	L6	Pupa	Female	Male	H_p	S_A	H_A	Fixed capital per year	Daily cost	Cost per adult (RMB)
21°C	T_i	54962	13028	27176	124546	19271	336	463	194	1000	0.9667	967	360,142	1127	2.186
	N_i	1696	1696	1493	1289	1187	18	11	6						
24°C	T_i	35276	8131	17014	64681	10702	164	230	131	1000	0.9487	949	196,938	838	1.451
	N_i	1299	1299	1273	1091	1065	13	8	4						
27°C	T_i	29811	7802	13660	23487	9417	150	152	133	1000	0.9459	946	107,802	866	1.227
	N_i	1369	1369	1204	1122	1068	13	7	5						
30°C	T_i	23035	6484	11381	7359	5608	101	141	101	1000	1.0000	1000	62,345	857	1.028
	N_i	1368	1368	1258	1149	1067	12	7	5						
33°C	T_i	16628	5171	7224	6844	4461	111	115	57	1000	0.9750	975	47,906	785	0.940
	N_i	1267	1267	1166	1090	1039	14	9	5						
36°C	T_i	18353	5594	10090	7275	4022	458	303	224	1000	1.0000	1000	56,552	1183	1.337
	N_i	1828	1828	1718	1206	1097	60	35	25						

insect populations is also influenced by a multitude of factors such as food, photoperiod, larval diapause and cannibalism (Zhu et al. 2006; Shintani et al. 2011; Terao et al. 2012, 2015; Shintani et al. 2017). Because the age-stage, two-sex life table (both individual-reared and group-reared) is able to take into account all of the above factors (survival rate, developmental rate, fecundity, sex, etc.), offers the most comprehensive understanding of these parameters, and is the basis of constructing a mass-rearing system, we strongly encourage the use of the age-stage, two-sex life table in the construction of mass-rearing systems for medicinal, or other economically important insects.

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Conflict of Interest

The authors declare that they have no conflicts of interest to this work.

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