J. Entomol. Res. Soc., 26(3): 451-463, 2024 *Research Article*
Doi: 10.51963/jers.v26i3.2705 *Online ISSN:2651-3579*

The Morphology, Effects of Temperature and Photoperiod on Pseudopupal Diapause and Low Temperature Storage of *Epicauta impressicornis* (Coleoptera: Meloidae)

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ABSTRACT

The gross morphology of each phase of the hypermetamorphic development of *Epicauta impressicornis* Pic (Coleoptera: Meloidae) was observed. The effects of temperature and photoperiod on pseudopupal (5th instar larva) diapause and low temperature storage on eggs and pseudopupae were determined. Low temperatures had a dominant effect on diapause incidence, regardless of the photoperiod. Pseudopupae stored at temperatures lower than 18 °C entered diapause, but completed their development without entering diapause at 21, 24, and 27 °C. The critical temperature for inducing diapause was between 16 °C and 16.5 °C, regardless of the photoperiod. The viability of low temperature storage of eggs and pseudopupae decreased with prolongation of storage time. The optimal storage condition for both eggs and pseudopupae was 4 °C for 30 days. Our results will provide a theoretical basis for additional studies on the biological characteristics of *E. impressicornis* and will also function as a prerequisite basic reference for the artificial large-scale rearing of *E. impressicornis*.

Keywords: Medicinal insect, *Epicauta impressicornis*, diapause induction, life cycle, low temperature storage.

Liu, Y., Zhou, Z., & Chen, X. (2024). The Morphology, Effects of Temperature and photoperiod on pseudopupal diapause and low temperature storage of *Epicauta impressicornis* (Coleoptera: Meloidae). *Journal of the Entomological Research Society*, 26(3), 451-463.

Received: August 20, 2024 *Accepted: October 16, 2024*

INTRODUCTION

The blister beetle *Epicauta impressicornis* Pic, 1913 (Coleoptera: Meloidae), as all species of this family, produces the terpenoid named cantharidin, which, in addition to its purported historical use as an aphrodisiac, is an important medicinal compound (Selander, 1981; National Pharmacopoeia Editorial Board, 2010). With the increasing discoveries of clinical therapeutic effects of cantharidin on cancer and other diseases, *e.g.*, dropsy, molluscum and warts, furuncles and piles, cancers (stomach, liver, lung, and esophageal), the demand for cantharidin has increased dramatically (Carrel et al., 1993; Moed, Shwayder, & Chang, 2001; Zou, Zhang, & Feng, 2002; Gao, Wang, Wang, & Wang, 2004; Li, Hou, & Chen, 2009; Wang, Pan, & Ren, 2010; Hu, 2011; Liu, 2013; Prasad & Verma, 2013; Verma & Prasad, 2013; Ren & Kinghorn, 2021; Schwarz, Hofmann, & Neumann, 2024). Within China, large numbers of native blister beetles are ground into powder for medicinal purposes, which is a crude drug in Chinese medicine (National Pharmacopoeia Editorial Board, 2010). The major source of medicinal cantharidin and its derivatives has continued to be from field-collected blister beetles. However, obtaining natural products from the meloid beetles is difficult and unreliable due to their peculiar hypermetamorphic life history. To date, artificial large-scale rearing of meloid beetles has not been successful. Meloid beetles have been classified as an animal species of medicinal importance that is in chronic short supply (Dauben, Kessel, & Takemura, 1980; Wang, Wu, Hsieh, Yen, & Yang, 2000; Nikbakhtzadeh, Dettner, Boland, & Dötterl, 2007; Wang, Pan, & Ren, 2010; Yin & Jin, 2010; Li et al., 2013; Liu, Li, Yang, Chi, & Chen, 2018)

Epicauta impressicornis, as almost all Meloidae (except the basal subfamily Eleticinae), has a hypermetamorphic pre-imaginal with a life cycle that is composed of egg, larvae (L1-L6), pupa, and adult, a typical metamorphosis in the meloid life cycle (e.g. Paoli, 1937; Cros, 1940; MacSwain, 1956; Selander & Mathieu, 1964; Selander & Weddle, 1969, 1972; Selander, 1981; Selander & Agafitei, 1982; Bologna & Pinto, 2001; Shintani, Hirose, & Terao, 2011; Pan et al., 2014). The 1st through 4th larval of *E. impressicornis* are semi-parasitic predators feeding on the egg pod of the Chinese rice grasshopper, *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). Adults of this species are phytophagous on Fabaceae and leaves of other plants (Bologna, 1991; Nikbakhtzadeh, Dettner, Boland, & Dötterl, 2007; Shintani, Terao & Tanaka, 2017). Pre-imaginal morphology and biological characteristics of only a few species within three genera have been described in detail (*i.e.*, *Mylabris* Fabricius, 1775, *Pyrota* Dejean, 1834, and *Epicauta* Dejean, 1834) (Paoli, 1937; Selander & Mathieu, 1964; Selander & Weddle, 1969, 1972). A distinctive phase of the blister beetle life cycle is that of the named pseudo pupa (5th instar larva). However, the hypermetamorphic habit of Meloidae has been useful in the classification and definition of phylogenetic relationships of meloid genera (Bologna & Pinto, 2001). Diapause, a period in which many insects enter an inactive physiological state in response to adverse environmental factors, has a critical role in regulating seasonal life cycles, and may be activated during one developmental stage or another depending on species (Koštál, 2006). Little is known about diapause induction in meloids (Selander & Weddle, 1972; Zhu, Xue, & Lei, 2006; Shintani, Hirose, & Terao, 2011; Terao, Hirose, & Shintani, 2012). Additionally, research on cold storage in meloids has not been reported. Scientific research on *E. impressicornis* has mainly focused on its morphology (Liu, Pan & Chen, 2016; Liu, Li, Yang, & Chen, 2019), ethology (Mu & Chen, 2014, 2015), life tables under different temperatures (Liu, Li, Yang, Chi, & Chen, 2018), and cantharidin synthesis regulates in fat body (Zhou et al., 2024). To date, there are few basic studies on hypermetamorphosis within *E. impressicornis*, and no reports available on diapause induction and cold storage of that species.

A portion of this study is aimed at providing a morphological characterization of the growth stages of *E. impressicornis.* Additionally, the study sought to identify the effects that the variation of in temperature and photoperiod, separately and in combination, have on diapause induction, and determine the tolerance to various cold storage temperatures relative to the length of storage time. The purpose of these tests was to determine how to effectively avoid diapause, and prolong the shelf life of the eggs and pseudopupae (5th instar larvae). Acquiring this information will provide technical data and serve as a theoretical basis for biological characteristics of the beetles. This study is intended to benefit efforts to initiate the large-scale artificial rearing of *E. impressicornis* needed for use in traditional Chinese medicine.

MATERIALS AND METHODS

Rearing and Collection

Initial number of 306 adults of *E. impressicornis* were collected from Luokun Village (106.58°E, 25.42°N), Luodian, Guizhou Province, China, during August 2018. They were reared 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 30 ± 1 °C, 75 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Egg masses laid within 24-h time periods were transferred to a glass Petri dish (9 cm diam.) with dampened filter paper on the bottom. After hatching, each first instar larva was removed and placed in a covered plastic Petri dish (9 cm diam.) which was subdivided into four compartments with dampened filter paper on the bottom, which was moistened daily with a sprayer. An egg pod of *O. chinensis* was divided into strips (2 cm in length) and supplied as food for the 1st to 4th instar larvae. Egg pods had been stored at 4 °C prior to use. Each pseudopupae (5th instar larva) was placed in a plastic 2-ml centrifuge tube (3.5 cm ht., 1 cm diam.) that was half filled with sterilized soil (moisture content *ca.* 30%) until their emergence as adults. The emerged adults were transferred to a 1000-ml glass beaker containing 0.5 kg of sterilized soil (moisture content *ca.* 30%). Adults were supplied with fresh leaves of *V. faba* and *S. nigrum*, and pieces of young *C. moschata* fruit (fruit size 10-20 cm diam.). Insects were reared in the laboratory for more than three generations before beginning the diapause induction and low temperature storage studies (Mu & Chen, 2014, 2015; Liu, Li, Yang, Chi, & Chen, 2018).

Field Population Survey

The occurrence of each phase of *E. impressicornis* was recorded from 1 January 2018 to 31 December 2018 at 10-day intervals, in Luokun Village. Five sampling squares were randomly selected in a rice paddy field. Each sampling area (30 cm in length, 30 cm in width, and 10 cm in depth) was dug and examined in detail. Local temperatures during 2018 were obtained from the meteorological observation system of Luokun Village (106.58°E, 25.42°N) (Luodian, Guizhou Province, China). We made use of the daily maximum and minimum temperatures record.

Effects of Temperature and Photoperiod on Diapause

The combinations of photoperiod and temperature used to study diapause induction in pseudopupae were set at 12, 15, 18, 21, 24, and 27 °C, and photoperiods of 8:16, 10:14, 12:12, 14:10, or 16:8 (L:D) h. The pseudopupae were reared in plastic 2-ml centrifuge tubes (3.5 cm ht., 1 cm diam.), half filled with sterilized soil (moisture content ca. 30%) and placed in artificial climate chambers set for each of the different photoperiod and temperature treatments. An initial number of 50 individuals were used for each treatment.

Effect of Low Temperature Storage on Eclosion Rate

Groups of 50 eggs and 50 pseudopupae were each placed in glass Petri dishes (9 cm diam.) with dampened filter paper on the bottom, covered with matching lid, and stored at either 2, 4, 6, 8, or 10 °C for 30, 60, or 90 days. The eggs and pseudopupae were moved to a climate chamber (Jiangnan Instrument RXZ-380A, Ningbo, China) set at 30 °C, 75 ± 5% RH, and a photoperiod of 14:10 (L:D) h and allowed to complete their development. There were two control groups, one consisting of 50 eggs and another of 50 pseudopupae, that were placed directly in a chamber set at 30 °C, 75 ± 5% RH, and a photoperiod of 14:10 (L:D) h and allowed to develop. The eclosion rate of all *E. impressicornis* eggs/pseudopupae was recorded daily.

Critical Temperature for Diapause Induction

This consisted of five photoperiod treatments of 8:16, 10:14, 12:12, 14:10, and 16:8 (L:D) h, and seven temperatures of 15, 15.5, 16, 16.5, 17, 17.5, and 18 °C. Pseudopupae were reared in 2-ml plastic centrifuge tubes (3.5 cm ht., 1 cm diam.) that were half filled with sterilized soil (moisture content ca. 30%) and placed in an artificial climate chamber (75 ± 5% RH) set at one of the above temperatures. Pseudopupae were observed for the onset of diapause under each variable. The incidence of diapause using the equation: (diapause incidence [%] = number of diapausing pseudopupae / total number of pseudopupae × 100) was used to determine the critical temperature for initiating diapause.

Diapause Identification

The pseudopupal stage is creamy-yellow, pliable and quiescent, characterized by limited roaming and creeping before entering the soil, and lasts until diapause occurs.

In diapausing individuals, the body surface is converted into a dark yellowish-brown color, forming a hard shell, and the individual is inactive, whereas non-diapausing individuals continue to develop into the next stage.

Data collection and statistical Analysis

Each developmental stage of *E. impressicornis* was photographed using a SMZ125 Nikon camera. The incidence rates of diapause, the duration of the pseudopupal stage, the eclosion rates after storage at low temperatures, and the duration of the egg and pseudopupal stages in the other treatments were compared using analysis of variance (ANOVA) and Tukey's HSD method for significance. Critical temperatures causing 50% of the individuals to enter diapause were estimated by using logistic regression analysis. All experimental statistical data were analyzed using IBM SPSS software version 22.0 (Landau & Everitt, 2004). Data for eggs and pseudopupae that died in each experiment were excluded from analysis, since the death rates for both were negligible.

RESULTS

Morphological Characters

The life history of *E. impressicornis* involves a complex system of hypermetamorphic development (Fig. 1). Bodies of both male and female adults of *E. impressicornis* are black with red heads (Figs. 1i-1j). The antennae of *E. impressicornis* adults are sexually dimorphic with the antennomeres being slightly serrated in males, and filiform in females. Males also have a pair of shiny black, rounded, "tumor-like" protrusions at the base of their antennae, which are absent in the females. Body length and head width are 13.80 mm and 2.04 mm, respectively, in the males, and 15.10 mm and 2.92 mm in the females. Eggs (Fig. 1a) are milky-white in color, smooth, and rice-grain-shaped with a length of approximately 2.5 mm. First instar larvae (Fig. 1b), also called triungulins, are active, campodeiform in shape, well-sclerotized in appearance, and black and shiny on the pronotum and abdominal segments II-IV and VIII-X. The head is wider than the pronotum. Larvae in the 2nd, 3rd, and 4th instars are first-grub-like and increasingly scarabaeiform, their bodies become softer, heads become larger, and thoracic legs shorten as they age (Figs. 1c, d). The 5th instars (Fig. 1f), also called pseudopupae (coarctate), are mainly quiescent and are the overwintering stage. They have a thick, dark brown and highly sclerotized cuticle, mastoid shape of thoracic legs, and aborted appendages, which are adaptations for diapause. Sixth instar larvae (Fig. 1g) are second-grub-like and are a non-feeding instar that precedes pupation. The pupa (Fig. 1h) is yellow, exarate, and not enclosed in a cocoon.

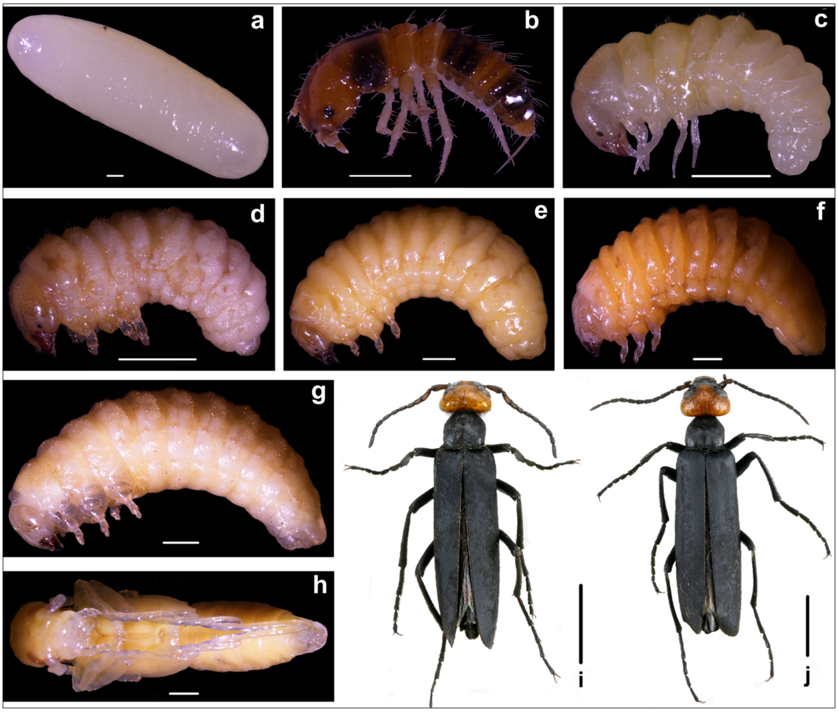


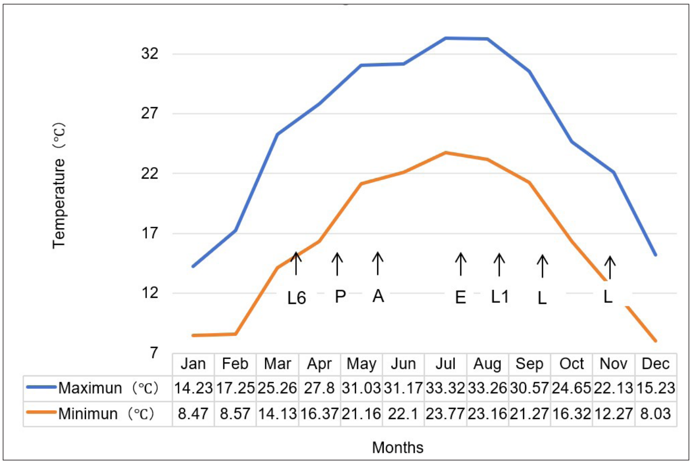
Figure 1. Developmental stages of *E. impressicornis.* a) Egg, b) L1 (1st instar larva), c) L2 (2nd instar larva), d) L3 (3rd instar larva), e) L4 (4th instar larva), f) L5 (5th instar larva or pseudopupa), g) L6 (6th instar larva), h) Pupa, i) Male adult, j) Female adult. Scale bars: a: 100 μm, b: 500 μm, c-h: 1 mm, i-j: 0.5 mm.

Field Population Survey

Our field survey in the Luodian Village (Guizhou Province, China) showed that when the highest temperature did not exceed 30 °C and the lowest temperature was above 10 °C at the end of October, *E. impressicornis* had only one generation per year and the 5th instars (pseudopupae) were overwintering and inactive from November to February (Fig. 2). We collected adults (approximately 350) of *E. impressicornis*, while other stages were only observed at the time of emergence.

Effects of Temperature and Photoperiod on Diapause

Temperature, especially low temperatures, were found to have a significant effect on the diapause incidence of pseudopupae, while photoperiod did not significantly influence the incidence or duration of diapause at 12, 15, and 18 °C. At 18 °C, only a portion of the pseudopupae entered diapause (Tables 1). With decreasing temperatures, the diapause rate gradually increased, with the diapause rate reaching 100% at 12 °C. However, 100% of pseudopupae completed development without diapausing at temperatures above 18 °C (21, 24, and 27 °C). Temperature was the primary factor in causing diapause regardless of the photoperiod (Tables 1-2). With decreases in temperature, the duration of diapause was prolonged. The developmental duration of non-diapausing pseudopupae was less than 121 days. The duration of diapause did not differ significantly at 12, 15, and 18 °C under different photoperiods. However, the duration of the non-diapausing stage during the 14:10 (L:D) h photoperiod at the elevated temperatures (21, 24, and 27 °C) was not only significantly different from the duration of other photoperiods at 21, 24, and 27 °C, but the duration of the pseudopupal stage was also the shortest at these temperatures (Tables 1-2).



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Maximun （℃）	14.23	17.25	25.26	27.8	31.03	31.17	33.32	33.26	30.57	24.65	22.13	15.23
Minimun （℃）	8.47	8.57	14.13	16.37	21.16	22.1	23.77	23.16	21.27	16.32	12.27	8.03

Figure 2. The life cycle of *E. impressicornis* and the daily minimum (blue line) and maximum (orange line) temperatures in Luokun Village during 2018. Different developmental stages of *E. impressicornis* are indicated by arrows. E = Egg, A = Adult, L1 = 1st instar larva, L2 = 2nd instar larva, L5 = 5th instar larva (overwintering stage), L6 = 6th instar larva, P = Pupa.

Temperature (°C)	Photoperiod				
	8L:16D	10L:14D	12L:12D	14L:10D	16L:8D
12	100%	100%	100%	100%	100%
15	79.3%	79%	79%	79.3%	79%
18	27.3%	27%	27.3%	27.3%	27%
21	0	0	0	0	0
24	0	0	0	0	0
27	0	0	0	0	0

Table 1. Diapause incidence of pseudopupae of *E. impressicornis* at different temperatures and photoperiods. The sample size (n =50) is the same under each treatments.

Temperature (°C)	Duration of the pseudopupal stage (days)				
	8L:16D	10L:14D	12L:12D	14L:10D	16L:8D
12	125.92 ± 10.99Aa	127.58 ± 9.15Aa	124.50 ± 14.31Aa	128.70 ± 11.87Aa	127.34 ± 11.11Aa
15	124.06 ± 14.73Aa	124.62 ± 11.98Aab	125.28 ± 14.18Aa	124.58 ± 14.69Aab	127.20 ± 6.85Aa
18	124.28 ± 11.92Aa	121.60± 14.56Ab	124.96 ± 11.29Aa	123.86 ± 13.83Ab	123.88 ± 10.64Ab
21(ND)	97.18 ± 3.72ABb	95.76 ± 4.26Bc	97.06 ± 8.86ABb	96.90 ± 7.86ABc	98.70 ± 5.50Ac
24(ND)	64.02 ± 9.70Ac	62.88 ± 8.00Ad	62.84 ± 10.34Ac	63.32 ± 11.39Ad	60.54 ± 7.30Ad
27(ND)	20.58 ±4.10ABd	19.76 ± 3.70Be	20.28 ± 3.12ABd	19.76 ± 4.24Be	21.80 ± 6.04Ae

Table 2. The effects of temperature and photoperiod on the duration of the pseudopupal stage of *E. impressicornis*. Means (n = 50) of the same temperature in a row followed by different capital letters indicate significant difference in different photoperiods ($P < 0.05$). Means (n = 50) in a column with the same lowercase letters are not significantly different ($P < 0.05$). ND: Non-diapausing pseudopupae.

Effect of Low Temperature Storage of Eggs and Pseudopupae on Eclosion Rate

Adult eclosion rates were affected when eggs and pseudopupae were stored at various low temperatures and different lengths of time (Figs. 3-4). The pseudopupae that were stored under these various conditions, however, had relatively higher adult eclosion rates than the corresponding egg hatch rates in almost all comparable treatments. Maximum eclosion rates were achieved for both eggs and pseudopupae that were stored at 4 °C for 30 days, where 78.0% of the eggs successfully hatched and 86.0% of the adults emerged. The second-best storage treatment was at 4 °C for 60 days where the egg hatch and adult emergence rates were 70.0% and 78.6%, respectively. In most instances the lowest emergence rates for eggs and adults occurred at 2 °C and 10 °C.

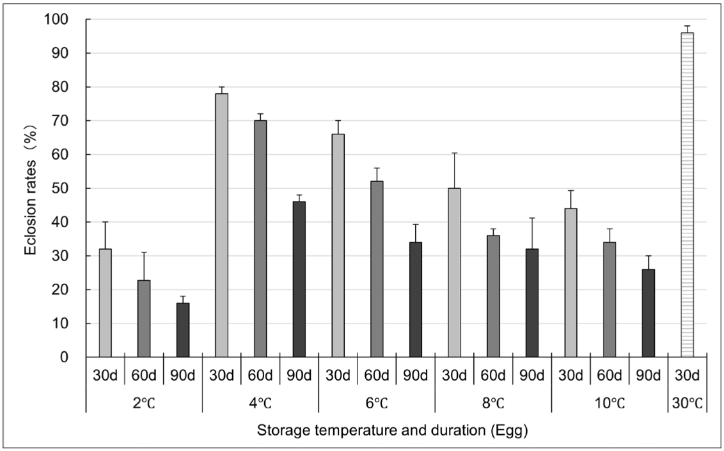


Figure 3. Eclosion rates of *E. impressicornis* eggs at different temperatures and storage durations.

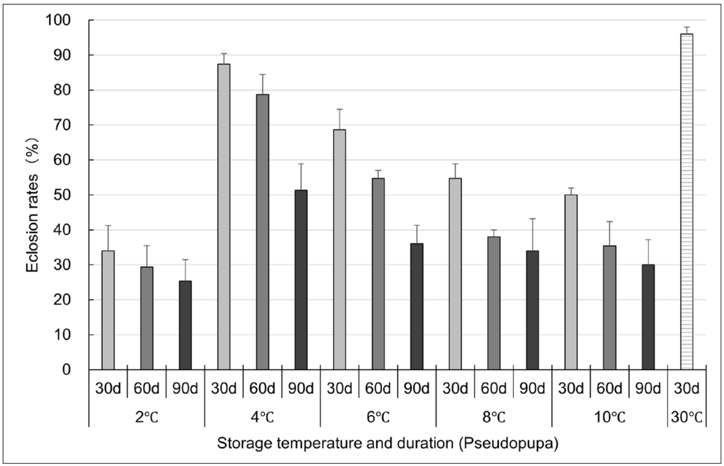


Figure 4. Eclosion rates of *E. impressicornis* pseudopupae at different temperatures and storage durations.

Critical Temperature for Diapause Induction

When temperature equaled or exceeded 21 °C, the pseudopupa of *E. impressicornis* could develop normally without entering diapause (Tables 1-2). In contrast, diapause rates in the pseudopupa increased with decreasing temperatures (18, 15, and 12 °C). The critical temperature triggering diapause was found to be between 16 °C and 16.5 °C (Table 1, Fig. 5). Within this narrow temperature range, at 16 °C and with a 10:14 (L:D) h photoperiod, the diapause incidence was 51.67% higher than at other treatment conditions, while at 16.5 °C and with a 16:8 (L:D) h photoperiod, the diapause incidence was 49.33% lower than in other treatment conditions.

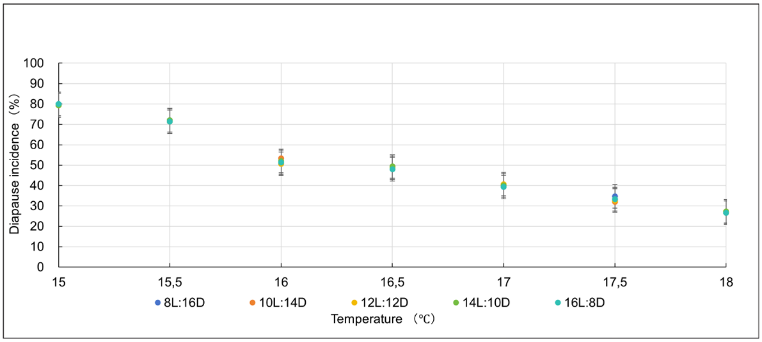


Figure 5. Critical temperatures for inducing diapause in *E. impressicornis* pseudopupae at different photoperiod regimes (8:16, 10:14, 12:12, 14:10 and 16:8 [L:D] h).

DISCUSSION

One result of the present study is that, when the temperature exceeded 20 °C at the beginning of May, adults of *E. impressicornis* began to emerge (Fig. 2), entering a gregarious aggregating phase, and caused considerable damage to crops (e.g., Leguminosae, Solanaceae, and Amaranthaceae). The adults at this time were active primarily during the morning and evening hours. The 1st to 4th instar larvae feed on eggs of *O. chinensis*. Thus, *E. impressicornis* is not only an important medicinal insect, but also an important insect predator, as well as a crop pest (Bologna, Oliverio, Pitzalis, & Mariottini, 2008; Wang, Pan, & Ren, 2010; Liu, Pan & Chen, 2016; Liu, Li, Yang, Chi, & Chen, 2018).

Since diapause is a genetically determined pattern of response to environmental stimuli, the expression of diapause is subject to both environmental and genetic factors (Xue, Spieth, Li, & Hua, 2002). Many investigators in China and abroad have studied insect diapause in response to exogenous regulators, i.e., temperature, photoperiod, food, population density, etc. (Xue, Spieth, Li, & Hua, 2002; King & MacRae, 2015; Lehmann et al., 2014). This research has greatly increased our knowledge regarding the diapause phenomenon. Both *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Shintani, Tatsuki, & Ishikawa,1996; Shintani & Ishikawa, 1997) and *Kytorhinus sharpianus* Bridwell (Coleoptera: Chrysomelidae: Bruchinae) (Ishihara & Shimada 1995) were induced to enter diapause based on temperature. In general, incidence of diapause increased significantly with decreasing temperature.

Until now, there have been no studies involving diapause in *E. impressicornis*, although diapause has been studied in some other species of Meloidae. Zhu, Xue, & Lei (2006) analyzed diapause induction in *Hycleus phaleratus* (Pallas, 1781) (Pan et al., 2014, for the taxonomic definition), using temperature, soil humidity, and photoperiod. They found that temperature played a crucial role in inducing diapause in *H. phaleratus* larvae, and that soil humidity and photoperiod had no effect on diapause. In their study of diapause induction in larvae of the blister beetle *Epicauta gorhami* (Marseul, 1873), Shintani, Hirose, & Terao (2011) found that diapause pseudopupa in *E. gorham*i was controlled by temperature and photoperiod, although photoperiod had an effect only within a narrow temperature range. They found that higher temperatures prevented larvae from entering diapause, whereas lower temperatures induced diapause.

Within our study, regardless of photoperiod conditions, when temperatures reached or exceeded 21 °C, pseudopupae did not enter diapause, whereas when temperatures were as low as 18 °C these beetles were able to diapause. Under identical photoperiod conditions, as temperature decreased, the diapause incidence in the pseudopupa increased. When the temperature was lowered to 12 °C, the diapause rate reached 100% (Table 1). However, at temperatures below 12 °C, the epidermis of the pseudopupa progressively blackened and their body became turgid, causing reductions in the emergence rate. We speculated that 12 °C was the optimum temperature for initiating diapause in pseudopupae. Varying the photoperiod conditions did not significantly affect the developmental period of diapause at the same or at different temperatures (≤ 18 °C). There was no significant difference in diapause rates at different photoperiod conditions at the same temperature. Our result similar to that reported by Zhu, Xue, & Lei (2006) for in *H. phalerataus*, in which they showed that diapause induction was dependent only on temperature. We found that when temperature remained constant and the beetles were exposed to different photoperiod conditions, the duration of the non-diapausing pseudopupal stage was the shortest in the 14:10 (L:D) h photoperiod regime. This was significantly different from the developmental duration of other photoperiods. Why this occurs is unclear and will require further study to determine the cause.

Temperature plays a crucial role in the life history and in many physiological processes of insects, including body size (Atkinson, 1994), mating behavior (Geister & Fischer, 2007), immune function (Karl, Stoks, Block, Janowitz, & Fischer, 2011), development, reproduction, and diapause induction (Zhu, Xue, & Lei, 2006; Shintani, Hirose, & Terao, 2011; Terao, Hirose, & Shintani, 2012, 2015; Shintani, Terao, & Tanaka, 2017; Liu, Li, Yang, Chi, & Chen, 2018). In the present study, it was determined that the critical temperature for the starting of diapause in pseudopupa of *E. impressicornis* was between 16 °C and 16.5 °C (Fig. 5, Table 1). Knowledge of this temperature range is vital for successful artificial mass-rearing of *E. impressicornis* in order to prevent untimely or unexpected, detrimental diapause “outbreaks” in the culture.

On the other hand, successful storage of cultured medicinal insects is often an important aspect of artificial rearing. By determining the optimal conditions for effective long-term storage of these insects in artificial mass-rearing programs, including identifying the appropriate large-scale and precise feeding requirements, we can successfully prolong their shelf-life and ensure availability of the culture as needed. In the present study, it was found that the effectiveness of low-temperature storage of eggs and pseudopupae decreased with prolonged storage time. The optimal storage temperature for both eggs and pseudopupae was at 4 °C for 30 days, and the more effective stage for low-temperature storage was the pseudopupa. It is anticipated that the data will provide a reference basis for further studies on the biology and behavioral mechanisms of *E. impressicornis.*

ACKNOWLEDGMENTS

We thank Dr. Cecil Smith (University of Georgia, USA) very much for language editing of this manuscript. This research was funded by the National Natural Science Foundation of China (grant number 81460576), the Program of Science and Technology Innovation Talents Team, Guizhou Province, China (grant number 20144001), the Scientific Research Project of Higher Education Institutions of Guizhou Provincial Education Department (Youth Project) [Qianjiaoji (2022) 299], the Program of Excellent Innovation Talents, Guizhou Province, China (grant number 20154021), the Guizhou Province Science and Technology Basic Project [QKHJC-ZK (2023) 018], and the Qiandongnan Prefecture Science and Technology Support Plan Project, Qiandongnan Science and Technology Support [(2023) No.06]. The authors declare that they have no conflicts of interest related to this work.

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