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Study on the Hatching Characteristics and Diet of the Stick Insect, *Baculum elongatum* (Phasmida:Phasmatidae) for Artificial Mass Rearing

Jin Gu Lee[a]*, Hee Dong Kim[a], Chang Sung Kang[a], Ae Gyeong Seo[a], Sang Hyun Lee[c], Young Kyu Park[d], and Young Bo Lee[b]

[a]*Gyeonggi-do Agricultural Research and Extension Services, Hwaseong 445-972, Korea*
[b]*National Academy of Agricultural Science, Suwon 441-100, Korea*
[c]*Sunyou. Co. Ltd., Namyangju 472-851, Korea*
[b]*Korea Beneficial Insects Lab. Co. Ltd. Anseong 456-913, Korea*

Abstract

Baculum elongatum has several peculiarities such as parthenogenesis, unique external features, changing body color, and dropping oviposition, which makes it a potential economically useful insect. Oviposition was performed by females without fertilization by the sperm of males. The oviposition period was 42.2 ± 22.7 days and the number of eggs per female was 109.5 ± 70.5 eggs. The hatch rate was 73.3% at 25°C and 66.7% after low temperature treatment (8°C for 60 days). In nature, B. elongatum overwinters as an egg, but it can be assumed from the results that cold temperatures were not required for hatching. The hatch rate was 98.2% in the treatment using floral foam, fermented sawdust, and leaves. The developmental period was 100.9 ± 4.2 days for eggs, 55.3 ± 4.6 days for nymphs, and 49.7 ± 16.0 days for adults. The length of the eggs was 0.33 ± 0.0 cm and the lengths of the nymphs were clearly distinguishable according to the instar stage. Clover(Trifolium repens) was an excellent diet as it was similar to the host plant and could be used as an alternative diet. The rate of reaching adulthood for the insects was 66.7% on an artificial diet containing 25% acacia leaves. For sustainable mass rearing of nymphs or adults of B. elongatum, a natural diet could be used such as acacia (Robinia pseudoacacia), white oak (Quercus aliena), chestnut (Castanea crenata var. dulcis), and bush clover (Lespedeza bicolor), or an alternative diet such as clover or artificial diet.

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Introduction

Insects can provide economic resources as pets, pollen vectors, natural enemies, food, or medical uses. Allomyrina dichotoma and Dorcus titanus are gorgeous beetles and popular pet insects in Korea,

***Correspondence :**
Jin Gu Lee
Gyeonggi-do Agricultural Research and Extension Services, Hwaseong 445-972, Korea
Tel: +82-31-229-6192 / FAX: +82-31-834-3107
E-mail: seamt@gg.go.kr
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but industrial insect farmers require new, diverse pet insects as a source of income.

Stick insects are terrestrial phytophagous insects found in nearly all temperate and tropical ecosystems. Over 3000 species exist in the world, including 5 species in Korea. To avoid predators, they either play dead or try to scare the predator with a startle display such as leg kicking or spastic motion. Some species can purposely lose some of their legs to aid their escape from a predator's grasp and can regenerate the lost limbs during successive molts; further, these species are popular as pets and as displays at zoological gardens (John, 2008). Their body color can change depending on the environmental conditions; in Carausius morosus, the body pigmentation can be affected by visual stimulation of its compound eyes (Detlef, 1977). Much research has been carried out on their oviposition behavior, maturation divisions of parthenogenesis, preying by spiders, and leg movements (Carlberg, 1984; Eva and Ulrich, 1985; Pijnacker, 1966; Wolfgang, 1990).

Baculum elongatum has a long, thin shape that resembles a twig, propagates by parthenogenesis, changes body color, and drops eggs, all of which suggests its potential in the commercial market, especially as an educational pet insect.

Thus, this study was carried out to determine the best hatching environment and an alternative or artificial diet for rearing *B. elongatum* to be developed as a commercial and educational pet insect.

Materials and Methods

Baculum elongatum were collected from Mt. Mubong in Hwaseong, Gyeonggi Province, in 2010, and successively reared to the 4th generation in a rearing room. The diets used for rearing included fresh leaves from acacia (*Robinia pseudoacacia*), white oak (*Quercus aliena*), chestnut (*Castanea crenata var. dulcis*), bush clover (*Lespedeza bicolor*), and clover (*Trifolium repens*).

Hatching characteristics

To investigate the characteristics of oviposition of *B. elongatum*, nymphs were reared individually in rearing cage (20 × 15 × 15 cm) set in a rearing room maintained at 25 ± 1^{o}C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h. Observations were made every day to record the day of molting for adults, the day of first and last oviposition, and the number of oviposited eggs.

To investigate the hatch rate of *B. elongatum* after a period of exposure to cold temperatures, 16 treatments were conducted. The rearing cages (30 × 20 × 20 cm) were half-filled with soil, and the eggs were spread on the soil and covered with fallen leaves. The hatching of eggs of *B. elongatum* was very difficult; hence, in this study, we considered the environmental conditions observed in the natural habitat of *B. elongatum*. Five cages were placed in each of 3 growth chambers set to a temperature of 0, 4, or 8^{o}C and after 30, 60, 90, 120 or 150 days 1 cage from each chamber was placed in the rearing room at 25^{o}C. One cage was retained in the rearing room at 25^{o}C. The eggs were checked every day to see if they had hatched.

To investigate the hatch rate of *B. elongatum* under different hatching conditions, 4 treatments were conducted. In the 1st treatment, rearing cage (30 × 20 × 20 cm) were half-filled using fermented sawdust, which is generally used to rear *Allomyrina dichotoma*, and the eggs were spread on the sawdust. The 2nd treatment was similar to 1st treatment, except that the eggs were covered using fallen leaves. The 3rd treatment was similar to the 1st except that the floral foam, which is commonly used for flower arrangement, was embedded in the sawdust. The 4th treatment was similar to the 3rd except that the eggs covered using fallen leaves. The cages were placed in the rearing room at 25 ± 1^{o}C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h. The eggs were checked every day to see if they had hatched.

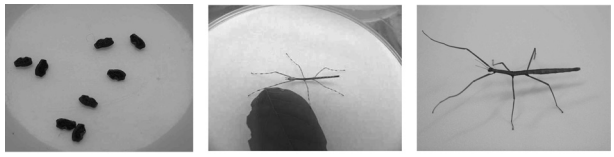

Fig. 1. Developmental stages of Baculum elongatum.

To investigate the hatch rate of *B. elongatum* under different temperatures, the eggs treated using the methods from the 3rd treatment of previous experiment were put on a weighing dish (3 × 3 cm). One cage each was placed in rearing rooms set at 20, 22.5, 25, 27.5, or 30°C and 65 ± 5 % relative humidity under a photoperiod of light-dark (LD) 16:8 h. The eggs were checked every day to see if they had hatched.

To investigate the developmental period and body length of insects of each stage, individual nymphs and adults were reared in rearing cages (20 × 15 × 15 cm) at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h. Insects were checked every day for hatching molting and mortality. The length of eggs and nymphs were measured under a stereomicroscope and using a ruler, respectively.

Alternative and artificial diet

To select an alternative diet, leaves of several plants were tested such as acacia, white oak, chestnut, bush clover, clover, grape, pepper, gyeol-myeong-ja, rice, corn, adlay, and bean. A nymph was reared on a diet of each test plant in the rearing room for 3 days. If the nymph ate the plant, then the test was continued. This was to determine whether the insects that consumed the test plant leaves developed to the next stage.

To develop an artificial diet, acacia leaf powder was used as the main host plant source and agar was used as the coagulating agent. Fresh acacia leaves were dried using a drier at 50°C for 48 h and were ground into powder by using electric grinder. After boiling in 100 mL distilled water, 1, 2, of 3 g of agar corresponding to 1, 2, or 3% treatments, respectively were added and boiled for further 10 min. Leaf powder in the amount of 5, 10, 15, 20, of 25 g corresponding to 5, 10, 15, 20, or 25% treatments, respectively, were added and mixed along with several additives, including malt (14 g), yeast (7.5 g), vitamins (1.9 g), sorbic acid (0.3 g), salt mixture (1 g), ascorbic acid (1.9 g) and MPH (0.6 g).

Results and Discussion

Hatching characteristics

The pre-oviposition and post-oviposition periods were 14.5 ± 2.2 and 1.4 ± 2.0 days, respectively, and their deviations were not high. The oviposition period and the number of eggs per female were 42.2 ± 22.7 days and 109.5 ± 70.5, respectively, with high deviations(Table 1). These results differed from that shown in a study where the oviposition period and number of eggs per female were 54.23 days and 60, respectively (Park et al., 2003).

The hatch rates of *B. elongatum* after 30 and 60 days at 8°C was 70.0 and 67.7%, respectively, and 73.3% at 25°C. In nature, *B. elongatum* overwinters as an egg, but cold temperatures were not required for hatching (Table 2). The hatch rate of *B.*

Table 1. Characteristics of oviposition of *Baculum elongatum*

Pre-oviposition period (days ± SD)	Oviposition period (days ± SD)	Post-oviposition period (days ± SD)	Number of eggs (eggs/female ±SD)
14.5 ± 2.2	42.2 ± 22.7	1.4 ± 2.0	109.5 ± 70.5

* Nymphs (n = 30) were reared in a rearing room at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h.

Table 2. Hatch rate(%) of *Baculum elongatum* after exposure to different durations of cold temperature

Period (days) / Temperature (°C)	0	30	60	90	120	150
0	73.3	63.3	60.0	30.0	26.7	0
4		50.0	46.7	53.3	13.3	10.0
8		70.0	66.7	10.0	6.7	0

* For each treatment, 30 × 20 × 20 cm cage with soil, eggs (n = 30), and fallen leaves was used. After the cold temperature treatment, each cage was placed in a rearing room maintained at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h.

Table 3. Hatch rate of *Baculum elongatum* under different hatching conditions

Treatments	n	Hatch rate(%)
Fermented sawdust and Eggs	50	70.0
Fermented sawdust, Eggs, and Leaves	50	92.0
Floral foam, Fermented sawdust, and Eggs	50	86.0
Floral foam, Fermented sawdust, Eggs, and Leaves	55	98.2

* For each treatment, 30 × 20 × 20 cm cage was placed in a rearing room maintained at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h.

Table 4. Hatch rate of *Baculum elongatum* under different temperatures

Temperature (°C)	n	20	22.5	25	27.5	30
Hatch rate (%)	50-60	85.0	83.3	80.0	18.3	0

* For each treatment, 30 × 20 × 20 cm cage with floral foam, fermented sawdust, egg dish, and eggs was placed in a rearing room maintained at 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h.

elongatum was 98.2% in the treatment comprising floral foam, fermented sawdust, and leaves and 92.0% in the treatment comprising the fermented sawdust, leaves (Table 3). The optimal temperature for hatching was approximately 20-25°C. In the 27.5 and 30°C treatments, the hatch rate was very low (Table 4).

The developmental period of *B. elongatum* was 100.9 ± 4.2 days as an egg, 55.3 ± 4.6 days as a nymph and 49.7 ± 16.0 days as an adult. Further, a previous study showed that *B. elongatum* has 5 nymph stages(Park et al., 2003), but the nymphs in this study showed 5 (62.9%) or 6 (37.1%) stages. Nymph lengths were clearly separable according to the instar and the deviation in length was very low (Table 5). For the hatching of *B. elongatum* eggs, humidity is very important, and floral foam can help maintain the moisture. Kim et al. (2011) reported that when hatching the Emma field cricket, *Teleogryllus emma*, floral foam was moistened frequently with water so that the hatching of the eggs would not be affected by moisture loss.

Table 5. Developmental period and body length of each stage of *Baculum elongatum*

Stage	Egg	Nymph						Total	Adult
		1st instar	2nd instar	3rd instar	4th instar	5th instar♩	6th instar♪		
Developmental period (days ± SD)	100.9 ± 4.2	9.9 ± 1.6	7.3 ± 1.0	7.7 ± 1.3	10.3 ± 1.3	14.7 ± 3.2	13.5 ± 1.8	55.3 ± 4.6	49.7 ± 16.0
Body length (cm ± SD)	0.33 ± 0.0	1.6 ± 0.2	2.4 ± 0.1	3.4 ± 0.1	4.5 ± 0.1	5.8 ± 0.4	6.6 ± 0.3	-	7.5 ± 0.4

* Baculum elongatum (n = 35) were reared in the rearing room maintained at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h. ♩5th instar: 62.9%; ♪6th instar: 37.1%

Table 6. Potential of alternative diet plants for *Baculum elongatum*

Potential♩	Plants (Leaves)
5	Acacia, White oak, Chestnut, Bush clover, Clover
3	Grape, Pepper, Gyeol-myeong-ja
1	Rice, Corn, Adlay, Bean

♩5: Very high (as host plant); 3: Consumed but no normal growth; 1: Not consumed

Alternative and artificial diet

B. elongatum did not feed on rice, corn, adlay, or bean leaves. While the insects ate grape, pepper, and gyeol-myeong-ja leaves, they did not develop to the next stage. Clover was an excellent diet, as was acacia, white oak, chestnut, and bush clover (Table 6). It is known that acacia, white oak, chestnut, and bush clover are the host plants of *B. elongatum*. The host plants are deciduous woody plants, so it is difficult to harvest them for the diet during the winter in Korea. Clover, however, is an herbaceous plant and easy to manage in a green-house; hence, it could be promising as an alternative diet. Peanuts and beans have been shown as potential alternative diets for rearing *Poecilocoris lewisi* (Kim and Seol, 2003) and it is important that the alternative diet should be obtained cheaper and easier than the natural host plant.

The rate of reaching adulthood for *B. elongatum* was 66.7% by using an artificial diet of 25% acacia leaf powder and 1% agar and was 0% by using an artificial diet of approximately 5-10% acacia leaf powder (Table 7). Much research has been carried

Table 7. Rate of *Baculum elongatum* reaching adulthood when fed artificial diets of different media and leaf powder densities

Media \ Host plant		Leaf powder of acacia				
		5 %	10 %	15 %	20 %	25 %
Agar	1%	0	0	6.7	40.0	66.7
	2%	0	0	20.0	40.0	33.3
	3%	0	0	20.0	6.7	46.7

* Baculum elongatum (n = 30) were reared on an artificial diet in the rearing room at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of light-dark (LD) 16:8 h. The % was measured as g/100 mL of distilled water

out to develope an artificial diet for the harmful insect or industrial insect, for example, *Matsumuraeses phaseoli*, *Teleogryllus emma*(Jung *et al.*, 2007; Kim et al., 2007), and artificial diets can be used for sustainable rearing during seasons when it is difficult to obtain the host plant or other food.

The best results for hatching the eggs of *B. elongatum*, was by using a rearing cage (30 × 20 × 20cm) set with fermented sawdust, and leaves or floral foam, fermented sawdust, and leaves and placed in a rearing room maintained at approximately 20-25°C and 65 ± 5% relative humidity. To rear nymphs and adults of *B. elongatum*, natural diet such as acacia, white oak, chestnut, or bush clover leaves, or an alternative diet such as clover or an artificial diet can be used.

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