Research Article / Artículo de Investigación

Biological cycle of the greater wax moth *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae: Galleriinae) in Panama

Ciclo biológico de la polilla mayor de la cera *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae: Galleriinae) en Panamá

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Abstract. The greater wax moth *Galleria mellonella* (Linnaeus, 1758), is one of the principal pests in the honeycomb of the European bee *Apis mellifera* Linnaeus, 1758, around the world. In this experiment, the complete biological cycle of *G. mellonella* was determined for the first time in Panama, achieving an incubation period of 4 to 10 days with an average of 7.16 ± 1.42. The larvae went through eight larval instars with a total larval duration of ±38.63 days. The pupa period was 9.22 ± 1.40 days. The average daily oviposition varied from a maximum of 182 to a minimum of 1.8 eggs, and the maximum life span of the adult was eight days. The number of eggs hatched was 1,503 in 10 evaluation days; on day 7, the most hatching occurred with 410 newly emerged larvae. The temperature and relative humidity averages were 28 °C and 76%, respectively, and varied from 25-33 °C to 58%-86% during the evaluation period. The life cycles of females and males were similar, of 39.00 and 39.53 days, respectively. The artificial diet used in this study guaranteed the successful completion of the larval stage in the life cycle of this wax moth.

Key words: Artificial diet; honeybee; larval instars; pest moth.

Resumen. La polilla mayor de la cera *Galleria mellonella* (Linnaeus, 1758) es una de las principales plagas presentes en panales de la abeja europea *Apis mellifera* Linnaeus, 1758 alrededor del mundo. En este experimento por primera vez se determino el ciclo de vida de *G. mellonella* en Panamá, logrando un período de incubación de 4 a 10 días con un promedio de 7,16 ± 1,42 días. Las larvas atravesaron por ocho estadios larvarios, alcanzando una duración larvaria de ±38,63 días. La fase de pupa fue de 9,22 ± 1,40 días. La oviposición diaria promedio varió de un máximo de 182 a un mínimo de 1,8 huevos, y la vida máxima del adulto fue de ocho días en promedio. El número de huevos eclosionados fue de 1.503 en 10 días de evaluación; el día 7, se produjo la mayor cantidad de eclosiones con 410 larvas emergidas. Los promedios de temperatura y humedad relativa fueron de 28 °C y 76%, respectivamente, y variaron de 25-33 °C a 58-86% durante el período de evaluación. Los ciclos de vida de hembras y machos fueron similares, de 39,00 y 39,53 días, respectivamente. La dieta artificial utilizada en este estudio garantizó la finalización exitosa de la etapa larvaria en el ciclo vital de esta polilla de la cera.

Palabras clave: Abeja melífera; dieta artificial; estadios larvales; polilla plaga.

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Introduction

Galleria mellonella (Linnaeus, 1758), colloquially known as a "greater wax moth," is part of the Pyralidae family, and they are amply distributed in almost all parts of the world, except for locations with excessively cold weather and the highest points of altitude (Llorente 2003).

This Lepidoptera is considered the world's main pest of mellifera bee colonies (Hanumanthan & Samy 2008; Kwadha *et al.* 2017). The main damage is caused by the larvae stage because of its nutritional habits which include wax, honey, propolis, and even stored pollen in active colonies. They make tunnels within the colonies to damage hive bees (Jackman & Dress 1998; Chandel *et al.* 2003). These reasons make *G. mellonella* one of the most important pests in apiaries due to the economic losses and the damage caused to the bee colonies.

Despite the significant damage they can cause, *G. mellonella* can be used as a model to assess the pathogenicity of various entomopathogenic microorganisms such as bacteria, fungi, and nematodes (Jander *et al*. 2000; Joyce & Gahan 2010; Harding *et al*. 2013). In addition, it is utilized as a type of trap for catching nematodes and entomopathogenic fungi (Vilcinskas 2010; Razia *et al.* 2011; Obando *et al.* 2013). It can also be used successfully as bait for fish and amphibian feeding; it is also being used as a complementary diet for poultry nutrition because of its higher content of proteins (Lloret 2005; Ortega 2020). Moreover, this insect is considered an alternative host to produce natural enemies and parasitoids, which are used against harmful pests in important agroecosystems (Ranjbar-Aghdam *et al*. 2015).

The literature indicates that *G. mellonella* is a common holometabolous insect, which means that they have complete metamorphosis (egg, larva, pupa, and adult) (Manthey *et al.* 2023). However, all of these stages are directly influenced by different factors such as temperature, humidity, and diet (Abdel-Naby *et al.* 1983; Gulati & Kaushik 2004; Restrepo-Garcia *et al.* 2019), for this reason, previously mentioned Central America climatological conditions are acceptable for their reproduction and utilization on biological control as a model organism, so this insect can get more than three generations in a year (Neira 2006; Ellis *et al.* 2013).

Hence, this insect is well-known and considered a model organism for developing diverse lines of research that range from host-pathogen interaction to its use as a lure to catch predatory insects (Ramarao *et al.* 2012; Atencio *et al.* 2020). Its value as a model organism is due to its immunological system which is tolerant to external pathogens such as bacteria, fungus, and nematodes (Tsai *et al.* 2016; Wojda 2016; Singkum *et al.* 2019). In addition, the biological cycle of this moth is relatively shorter if we compare it to other insects used as model organisms for studying microorganisms. Its diet is cheap, and its ingredients are easy to find in groceries or supermarkets, so its reproduction is not a problem (Asai *et al.* 2019).

The selection of the larvae stage is an important parameter to consider in establishing an experiment, especially in entomopathogenic studies, because it provides an accurate prediction of larvae stages, which is very important in evaluating the efficacy of the interactions between host and microorganism. Some authors have determined that *G. mellonella* has from six to eight larvae instars, but in all of the research, evaluation was done under controlled conditions, which include temperature, humidity, and day cycle (Realpe-Aranda *et al*. 2007; Desai *et al*. 2019). However, in Panama *G. mellonella* cycle has not been evaluated so far. Therefore, the objective of this study is to comprehensively document the biological cycle of *G. mellonella* under natural environmental conditions in Panama.

Methods and Materials

Galleria mellonella breeding was established using larvae found invading hives maintained by the faculty of Agriculture Sciences at the University of Panama (8°23'41.79" N - 82°19'47.60" W). The larvae were transported to the entomology laboratory located at the Institute of Agricultural Innovation of Panama in David-Chiriquí. The insect breeding stock was put on an artificial diet consisting of the following ingredients: 400 g of wheat bran, 120 g of wheat flour, 160 g of powdered calf's milk, and 250 ml of natural bee honey per 680 g of prepared diet. Environmental values were recorded for 62 days, which is the maximum lifespan *G. mellonella.* The average temperature was 28 °C with a maximum of 33 °C and a minimum of 25 °C, and the average relative humidity was 76% with a maximum value of 86% and a minimum of 58%. During the evaluation of the biological cycle, the number of stages under environmental conditions was determined, establishing eight larvae instars for *G. mellonella* using capsule cephalic exuviae (Florez-Perez *et al.* 2005).

Biological parameters used for evaluating *G. mellonella*

The biological cycle of *G. mellonella* was determined by evaluating the following stages: egg, larva, pupa, and adult (Fig. 1).

Eggs: For 10 days, daily samples of eggs were taken from each evaluated moth. In total, 15 female moths of *G. mellonella* were evaluated with their respective males and placed inside a glass jar with wax paper to facilitate egg collection. The eggs collected fluctuated from 2 to 40, for a total of 1,585 eggs. The embryonic development was determined by a daily collection of sub-samples of eggs per female. Sub-samples were stored separately on Petri plates for each female moth under environmental conditions (temperature and humidity). Additionally, the eclosion of eggs was recorded every 24 hours. The number of larvae emerged was recorded for 10 days.

Larvae: Newly hatched larvae were taken from the eggs and placed individually in a Petri with 0.1 g of artificial diet. Each Petri was covered with adhesive paper so that larvae could not escape from the dish. The experiment started with an experimental unit of 40 larvae and every 24 hours were evaluated for instar change throughout the cephalic capsule exuviae. The number of larvae that died during the experiment was also recorded.

Each cephalic exuviae was collected and placed in small vials numbered with a specific code for each larva. The cephalic capsule was measured according to the methodology previously proposed (Dyar 1890; Flores-Perez *et al*. 2005), where the distance between the genas in millimeters (mm) is considered the main parameter. A stereoscopic microscope, the Leica MZ 125, which incorporated the Leica Application Suite software was used.

Pupae: To evaluate the pupa stage, the same larvae from the previous step were used when they reached the pupal stage. These pupae were labeled and put on another Petri dish to be evaluated, and each pupae's number of days at this stage was recorded. Pupae were evaluated every 24 hours until the last moth emerged from the pupa, and the sex of each of them was determined using the scientific key (Smith 1965) by observing their genitals located on the eight abdominal segments of the pupa (Figs. 2A-2B).

Adults: The adults of *G. mellonella* were separated by sex (male-female), employing a morphological guide (Smith 1965). Then each female adult was introduced inside a glass container with its respective male and was fed a honey solution (10% of the honey in 90 ml of distiller water). Finally, square portions of wax paper were placed in the jar and collected to evaluate daily oviposition. Eggs collected were counted every 24 hours to know the total number of eggs oviposited by each female until they died.

The biological cycle diagram of *G. mellonella* was created using BioRender software.

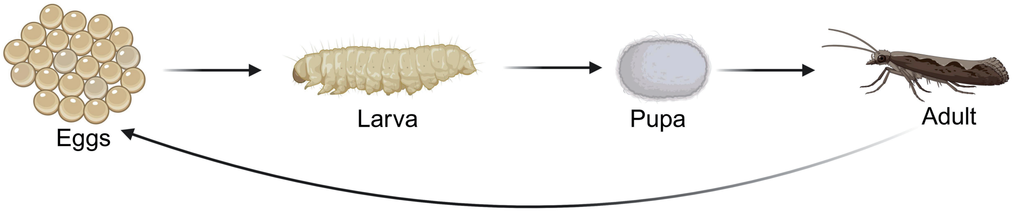

Figure 1. Biological cycle diagram of *G. mellonella.* / **Figura 1.** Diagrama del ciclo biológico de *G. mellonella*.

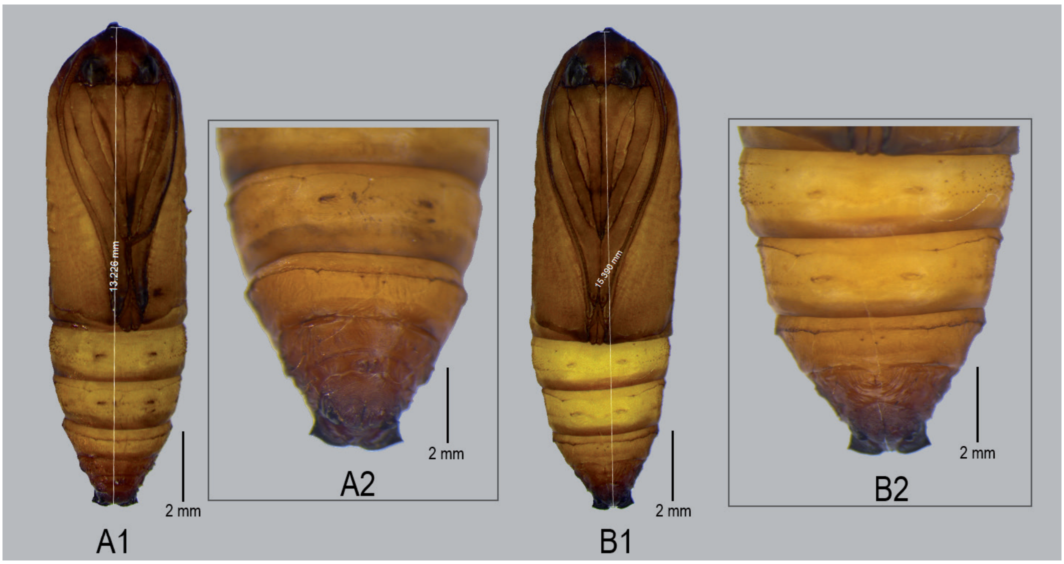

Figure 2. Sexual dimorphism in pupae of *Galleria mellonella*. **A1-A2.** Male. **B1-B2.** Female. / **Figura 2.** Dimorfismo sexual en pupas de *Galleria mellonella*. **A1-A2.** Macho. **B1-B2.** Hembra.

Results

According to our findings, the biological cycle of *G. mellonella* has the following stages: egg, eight larva instars, pupa, and adult (Fig. 1). The duration for each stage is 7.1, 38.6, 9.22, and 6.7 days, respectively. In Tab. 1 we can observe that some larva instars differed in days, with the lowest being instars II and III, with an average of 2.83 days, and the longest being instar VIII, with 11.37 days. Notably, larvae I and IV had the highest mortality percentage, which may indicate that the younger instars are more sensitive to mechanical damage.

During the evaluation of the biological cycle, eight larvae instars were established for *G. mellonella,* taking as a reference the width between the genas, where the average of the smallest was 0.18 mm for instar I and 2.49 mm for instar VIII (Tab. 2).

The average daily oviposition rate for 15 females was evaluated; the highest oviposition rate was recorded on the third day and subsequently decreased until day 8 when death and the lowest oviposition rate were recorded with 1.8 eggs (Fig. 3). Moreover, the daily rate of emerged larvae was estimated after observing 1,503 eggs and being evaluated for 10 days for assessing the eclosion percentage; it was observed that day 4 had the lowest number of emerged larvae (Fig. 4), which might be due to the temperature and humidity fluctuation during the day (Jorjão *et al.* 2018).

Table 1. Duration in days of the different stages of the biological cycle of *G. mellonella* under local environmental conditions. / **Tabla 1.** Duración en días de los diferentes estados del ciclo biológico de *G. mellonella* bajo condiciones ambientales locales.

Complete biological cycle					Female				Male			
Stage	**N**	**X Days ± S.D**	**Min Days**	**Max Days**	**N**	**X Days**	**Min Days**	**Max Days**	**N**	**X Days**	**Min Days**	**Max Days**
Egg	1585	7.16 ± 1.42	4	10	–				–			
Larva I	29	5.72 ± 1.39	4	10	–	5.73±1.74	4	10	–	6.13±1.97	4	9
Larva II	24	2.83 ± 0.64	2	4	–	2.91±0.54	2	4	–	3.13±0.64	2	4
Larva III	24	2.83 ± 1.05	2	6	–	2.91±1.30	2	6	–	3.13±0.83	2	4
Larva IV	19	3.47 ± 1.12	2	7	–	3.36±0.67	3	5	–	3.63±1.60	2	7
Larva V	19	3.63 ± 0.76	3	6	–	3.64±0.92	3	6	–	3.63±0.52	3	4
Larva VI	19	3.47 ± 0.84	2	5	–	3.45±0.82	2	5	–	3.50±0.93	2	5
Larva VII	19	5.31 ± 1.37	3	7	–	5.55±1.44	3	7	–	5.00±1.31	7	5
Larva VIII	19	11.37 ± 4.10	6	18	–	11.45±4.25	7	18	–	11.38±4.27	6	17
Pupa	19	9.22 ± 1.40	6	12	10	9.10±1.37	7	12	9	8.63±1.77	6	12
Adult	19	6.72 ± 1.84	5	11	10	6.80±2.98	4	10	9	6.63±1.85	4	10

Table 2. Average of the cephalic capsule width (in mm) for eight larval instars of *G. mellonella* under local environmental conditions. / **Tabla 2.** Promedio del diámetro de las cápsulas cefálicas (en mm) de ocho instares larvales de *G. mellonella* bajo condiciones ambientales locales.

Instars	N	X (mm) ± S.D	Min	Max
I	29	0.18±0.02	0.16	0.23
II	24	0.25±0.04	0.21	0.35
III	24	0.35±0.06	0.25	0.48
IV	19	0.49±0.08	0.35	0.69
V	19	0.7±0.14	0.47	1.08
VI	19	1.06±0.21	0.75	1.44
VII	19	1.53±0.16	1.09	1.69
VIII	19	2.49±0.38	1.57	2.94

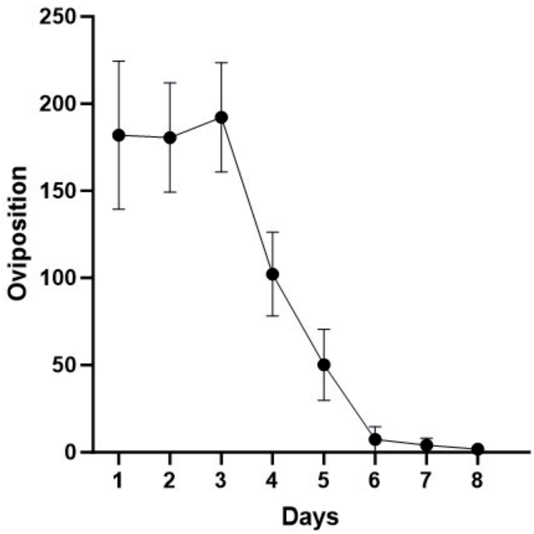

Figure 3. The trend of the mean values of oviposition per day of 15 females of *G. mellonella*. Black dots represent the mean and the bars are the standard error. / **Figura 3.** Tasa promedio de oviposición por día de 15 hembras de *G. mellonella*. Los círculos negros representan la media y las barras representan el error estándar.

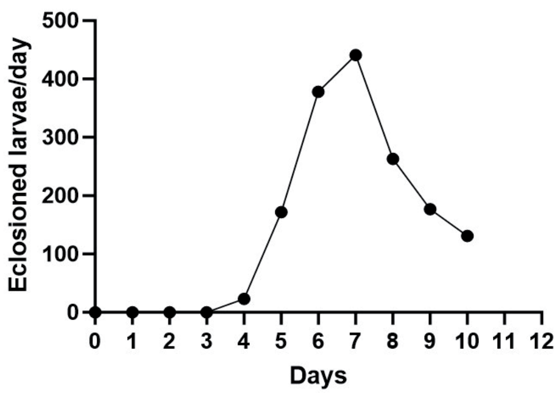

Figure 4. Mean values of larvae of *G. mellonella* emerged per day in 10 days. / **Figura 4.** Promedio de larva de *G. mellonella* emergida por día durante 10 días.

Discussion

According to the data, the biological cycle of *G. mellonella* has the following stages: egg, larva (VIII), pupa, and adult.

Even though this research was conducted under uncontrolled environmental conditions, these records coincide with some reports (Williams 1990; Marquina & Carbajal 2017), which mention that the ranges of hatching time for *G. mellonella* can vary from 5 to 8 days with temperatures that vary from 24-28 °C. Eight larvae stages (L) were recorded in this study where the longest stage was LVIII with an average of 11.37 days, and the shortest were instars LII and LIII with an average of 2.83 days. These data differ from those obtained by Desai *et al*. (2019) and Restrepo-Garcia *et al*. (2019), who reported seven larvae instars for *G. mellonella* at temperatures below 25 °C. However, they are similar to those obtained by Realpe-Aranda *et al*. (2007), who reported that *G. mellonella* larvae went through eight larvae instars at temperatures between 25 and 30 °C, but under 20 °C, *G. mellonella* obtained only VII larvae instars.

It is important to mention that the instar determination is made by measuring the diameter of the cephalic capsule. This parameter provides a consistent reference to be sure about the instar larvae stage, as established by Realpe-Aranda *et al*. (2007), to address the fact that some experiments require a very accurate determination of the larval instar because it is considered a critical parameter in both biological and microbiological experiments (Serrano *et al*. 2023).

According to our results, the duration in days for each stage was very different for some instars (Tab. 1). The instar coincides with Cardoso's (2007) findings, in which he mentions that the duration of the larval stages is inversely related to temperature and relative humidity. Because the test was carried out under uncontrolled environmental conditions; the average temperature and relative humidity were 28 °C and 76% (25-33 °C; 58-86%).

The instars with the highest mortality percentage were LI, LII, and LIV (27.5, 17.2, and 20.8%, respectively). This is possible because they are very small larvae and are sensitive to environmental variations in temperature and relative humidity as well as handling, so they may present a greater probability of being affected by mechanical damage when evaluated.

For the pupal stage, the duration was 9.22 days on average, very similar to those obtained by Realpe-Aranda *et al.* (2007) and Neira & Manquian (2008), who mention that for this phase the duration is approximately 9 days in a temperature range from 25-33 °C, with 0.22 more days than that obtained in this study.

In the adult phase, an average life of 6.72 days was recorded, in which the female obtained an average of 6.80 and the male 6.6 days. These findings are similar to those found by Williams (1990) and Realpe-Aranda *et al*. (2007), who mention that the adult stage of *G. mellonella* can survive for 1-3 weeks (7-21 days) in temperatures ranging from 28-35 °C depending on the availability of food. Under the environmental conditions previously mentioned, the total duration of the biological cycle was 62.7 days, and mortality rates of 12.4, 52.5, and 5.3% were recorded for the egg, larva, and pupal stages respectively; results were very similar to those obtained by Realpe-Aranda *et al*. (2007), in which they registered a total duration of 62.4 days at a constant temperature of 30 °C, and those mentioned by Neira (2006), which indicate that, according to the temperature conditions, the cycle can be from 30 to 62 days with optimum temperatures ranging from 26-38 °C, or five months with lower temperatures. Nevertheless, the duration of the biological cycle may be influenced by the physical and biological factors of the environment to which the insect is exposed (Kopacek *et al*. 1995).

Conclusions

The methodology and diet implemented in this study provide a consistent and reliable source of information for maintaining populations of *G. mellonella* for future research as a model organism in biological control studies.

For the first time in Panama, the biological cycle of *G. mellonella* was established under non-controlled conditions where it was possible to consistently determine the number of larval instars with LVIII for all the larvae evaluated.

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Author Contributions

DMS: Methodology, investigation, data curation writing original - draft. **ARM:** Conceptualization, methodology, data curation. **GGD:** Conceptualization, methodology, resources, data curation.

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