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# Biological parameter of cocoa pod borer *Conopomorpha cramerella* (Lepidoptera: Gracillariidae) under laboratory conditions

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**Abstract.** Cocoa pod borer (CPB), *Conopomorpha cramerella*, causes a 50% reduction in cocoa pod productivity. Effective pest management requires an understanding of the biological characteristics of CPB. This study aims to provide information of CPB's infestation symptoms, pupal duration, adult body length, adult lifespan, adult flight ability, and female fecundity. Field observations of CPB larval infestation were made at all pod development stages of cocoa clone M01. Infested cocoa pods were collected in the field and brought to the laboratory to obtain the pupal phase. The pods were placed on a layer of dried cocoa leaves in a bug dorm for pupation. The duration of pupal to adult emergence was recorded. The fecundity of females was measured after copulation. Flight abilities were conducted by measuring the distance and time travel from the adult take-off's starting point to landing. Young cocoa pods showed premature yellowing of the husk due to CPB's larval feeds on the placenta around the beans. Pupal duration ranges from 6 to 10 days. The body length of male adults is 5.4 - 6.4 cm and female is 4.7 - 6.7 cm. The average flight distance and travel time of females are  $392.5 \pm 124.4$  cm and  $30.6 \pm 16.6$  seconds, males  $379.7 \pm 118.7$  cm and  $39.1 \pm 26.5$  seconds. The number of eggs laid by females averaged  $114.21 \pm 91.32$  eggs (n=14). Female adults have longer body length and flight distances than males, but the duration of males travel longer than females.

**Keywords:** fecundity, flight capability, life cycle, symptoms of attack.

## 1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the economically important agricultural commodities that plays an important role as a source of income for farmers, especially in developing countries. Indonesia, Côte d'Ivoire, Ghana, and Nigeria are the world's leading producers and exporters of cocoa beans [1-3]. Cocoa beans are widely used in daily life as main ingredients of food, beverages, medicine, and cosmetics. The cocoa was produced by smallholder farms, large private estates, and large state-owned estates. In Indonesia, South Sulawesi is the third-largest cocoa-producing province. However, cocoa production in Indonesia has a constant decline yield. In 2018, cocoa bean production reached 767,280



tons, but by 2022, it had decreased to 650,612 tons [4]. One of the causes of the decline in cocoa productivity is the cocoa pod borer, *Conopomorpha cramerella* (Lepidoptera: Gracillariidae).

The cocoa pod borer (CPB) is one of the most serious pests in Indonesia and worldwide. The larvae attack young cocoa pods and disrupt the development of cocoa beans. CPB larvae can cause up to a 50% loss in yield [5]. In Indonesia, states CPB can cause cocoa yield losses ranging from 20% to 70% [6]. To date, there have been many reports on CPB pest control strategies. The implementation of biological control has been demonstrated by using the parasitoid egg of *Trichogrammatoidea bactrae fumata* [7]; mechanical control by using sleeving or wrapping pods with plastic on young cocoa pods (< 8cm length); and chemical control by spraying in adult resting sites [8-9]. In addition, a monitoring system for adult CPB has also been conducted using sex pheromones combined with sticky liner traps [10]. An effective pest management program requires a comprehensive understanding of the biological and developmental characteristics of cocoa pod borer pests. This study aims to observe cocoa pod borer attacked symptoms and to determine pupal duration, fitness, lifespan, flight capability, and fecundity of cocoa pod borer. Therefore, it is important to know the biological characteristics to help CPB pest control strategies and minimise crop damage.

## 2. Materials and Methods

### 2.1. The symptoms of cocoa pod borer's attack observation

Symptoms of cocoa pod borer infestation were observed at each phase of cocoa pods, starting from small size or around 10-15 cm in length. Observations were made by identifying abnormal signs on the pods, such as early yellowing of the husk surface and insect entry holes. In addition, cocoa pods are also cut open to see the symptoms of CPB feeding damage inside the pod. Ten healthy cacao pods of clone M01 were also observed as a comparison.

### 2.2. Sample collection and pupal duration observation

Pupae (100 samples) were collected from infected pods in the field. The infected pods were covered with dry leaves inside a large bug dorm (60 cm × 60 cm). The leaves served as a place for the larvae to pupate. The collection of larvae into pupae was conducted in the outside laboratory to adapt the environmental conditions. Average daily temperature ranged from 25.8 - 28.7 °C, and average daily humidity ranged from 80.3% - 93.5%. The pupae were then kept in a medium-sized bug dorm (30 cm × 30 cm) in the laboratory with an average daily temperature ranging from 21.4 - 29.5°C. The pupae emerged within a week. The number of adult emergences was recorded, and the pupal duration was also measured.

### 2.3. Adult fitness and lifespan observation

Newly emerged adults were placed in petri dishes (diameter 60 mm). The dorsal side of the adult, from the head to the tip of the abdomen, was measured (in mm) under a microscope using Leica Application Suite Version 3.4.0. The body weight of the adults was also measured using a 4-decimal analytical balance (in mg). The length and weight of the CPB were measured as fitness parameters, and potential malformations were recorded. After the measurements, the insects were placed in a bug dorm with a 10% honey solution, and their lifespan was observed. During the observation period, the temperature was recorded using a HOBO data logger and recorded that the average daily temperature ranged from 21.4°C - 29.5°C.

### 2.4. Fecundity observation of cocoa pod borer

Newly emerged adults were placed in a bug dorm (15 × 15 × 15 cm) consisting of two males and one female. An immature cocoa pod from clone M01 was placed inside of the bug dorm to serve as an egg-laying site for the female insect, while a 10% honey solution was provided as a food source for the adults. The number of eggs laid was checked and counted daily. The eggs laid by the adult insects were observed, and their hatchability was determined by assessing the success of larvae entering the fruit.

During the observation period, the temperature was recorded using a HOBO data logger and recorded that the average daily temperature ranged from 21.4°C - 29.5°C.

### 2.5. *Flight capability test*

The flight ability of adult insects was conducted in a laboratory room (10.1 m x 3.2 m) by determining flight time and observed of flight direction for the adult insects. The adult insects were released, and the flight direction of the cocoa pod borer (CPB) from the take-off point to the landing point was observed. The flight ability of the CPB was measured by the length, height, and diagonal as the hypotenuse of the length and height, as well as the time taken from take-off to landing.

### 2.6. *Data Analysis*

The collected data were analysed using Microsoft Excel software and subjected to a t-test to determine differences in pupal duration, body size, lifespan, and flight ability between males and females.

## 3. Results and Discussion

### 3.1. *The Symptoms of Cocoa Pod Borer's Attack*

3.1.1. *Healthy cocoa pod appearance.* An immature pod has a green pod shell and the colour of the cocoa pod clone M01 turns yellow when it enters the ripe phase (Figure 1.a). The skin of the M01 clone is distinctive, characterized by its thickness and rough texture with a wavy or textured surface. Cocoa pods are arranged in five rows with a white pulp enveloping the seeds (Figure 1.b). A healthy pod has pulp surrounding the beans, which appears white [11]. As additional information on clone M01, one technique to determine that cocoa pods are ripe and healthy is to hit the pods by hand. During that activity, the pods will make a different sound compared to unripe pods. This distinct sound indicates that the cocoa beans have separated from the pods.

3.1.2. *Cocoa pod damage.* Field observations of cocoa pod borer infestation on the M01 clone pod were conducted throughout all pod stages. CPB can attack starting from the small pod stage, which is about 10–15 cm in size (Figure 1.d), by laying eggs on the pod's surface. The mature eggs hatch into first-instar larvae, which immediately dig into the pod. However, the entry holes are difficult to observe with unaided vision due to their microscopic size (Figure 1.c). The entire larval instar period is spent inside the pod for 14-18 days, creating tunneling damage throughout the pod layers and completing 4-6 instars [12]. The intensity of a CPB attack can be either high or low at different pod stages. Symptoms of infestation can be identified from abnormal signs on the cocoa pod, such as premature ripening with colour changes to yellow or orange before reaching the ripe stage, indicating a CPB attack on the pod. Premature ripening can be caused by CPB infestation on young pod beans, preventing further pod development. The infestation of young pods caused bean malformation.

Black tunnelling paths revealed on the infested pods as the impact of feeding activity of larvae (Figure 1.i). CPB larvae are light green and have an eruciform larval type. A high level of infestation also caused pod decay (Figure 1.g). The pod damage and decay due to the secreted enzymes (hexokinase, malate dehydrogenase, fluorescein esterase, and polymorphic malate) from CPB larvae [14]. Furthermore, when larvae have completed their feeding activity inside the pod and enter the final instar stage, they will seek a way to approach the skin and create an exit hole for pupation. Unlike the entry holes, the exit holes are more extensive and can be observed unaided (Figure 1.h). Additionally, infested pods did not produce a loud sound when tapped, unlike healthy, ripe pods, caused by the clumping of beans and pulp from CPB infestation.

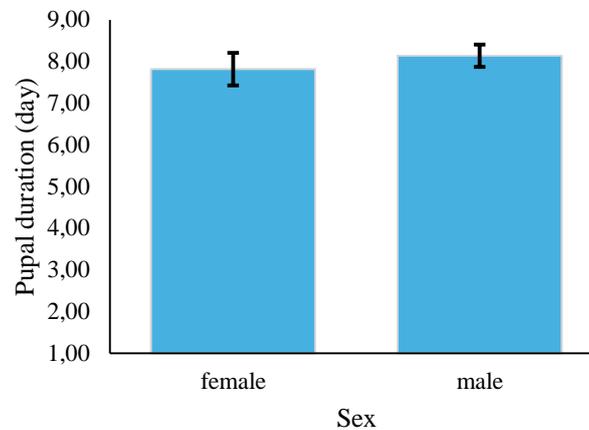


**Figure 1.** Symptoms of cocoa pod borer on the pod: (a) healthy ripe pod; (b) healthy cocoa bean; (c) neonate larval entrance hole under microscope (magnification 40x); (d) infested small immature pods; (e) infested large immature pods; (f) infested mature pods; (g) infested ripe pods; (h) last instar larvae on cocoa pod; (i) last instar larvae exit hole

### 3.2. Pupal Duration

The final instar larvae created a cocoon before becoming pupae. During observations in the laboratory, cocoons were formed on the provided dry leaves. The cocoon is transparent white, while the pupa is yellowish-green and darkens as it gets closer to emergence. In the field, pupae can be found on the pod surface, on cacao leaves still attached to the plant branches, or on leaf litter and dried leaves [15, 16]. In this observation, the formation of the pupa begins with the development of a transparent cocoon. After the cocoon is formed, the larvae inside then transform into pupae. During the early pupa stage, the pupa is yellow and turns black as it approaches the emergence time. At emergence, the adult insect comes out and leaves its shell in the cocoon. The pupal duration shows there is not a significant difference between males and females ( $t = -1.59$ ,  $df = 58$ ,  $p > 0.05$ ) (Figure 2). This stage lasts 6 to 10 days before emergence. Female pupae show a shorter duration  $7.8 \pm 0.7$  days ( $n = 40$ ) compared to

males  $8.1 \pm 1.0$  days ( $n = 35$ ). Aligns with Wassel-Riemens [17], who reported that the pupal stage lasts 6 to 8 days before emergence



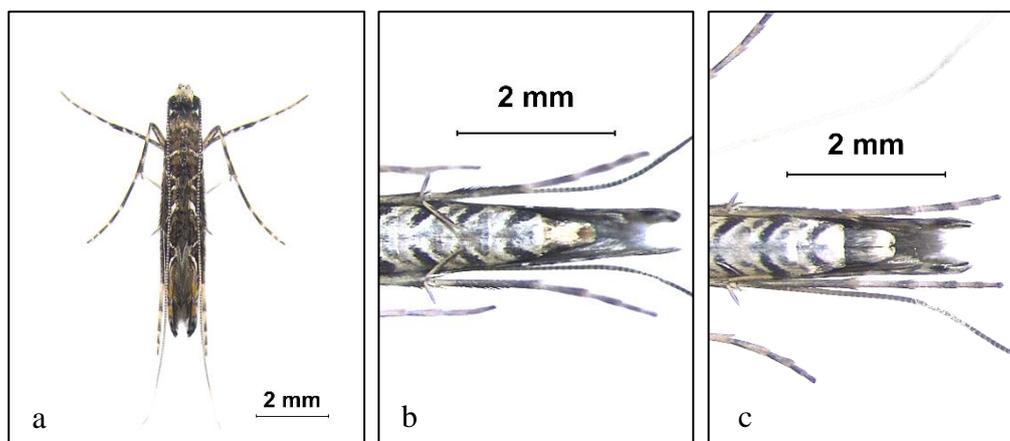
**Figure 2.** Pupal duration

Using the pupal stage information for integrated pest management can aid in determining the optimal timing for CPB control. Another study reported that *Beauveria bassiana* was able to infect *C. cramerella* pupae in the soil within seven days [18]. Furthermore, cultural practices such as sanitation of infected pods, breaking them into smaller pieces, and burying infested pods help prevent the successful emergence of adults that could infest healthy cocoa pods. These measures effectively disrupt the CPB life cycle, particularly targeting the larval and pupal stages [19].

### 3.3. Adult Morphology, Fitness, and Lifespan

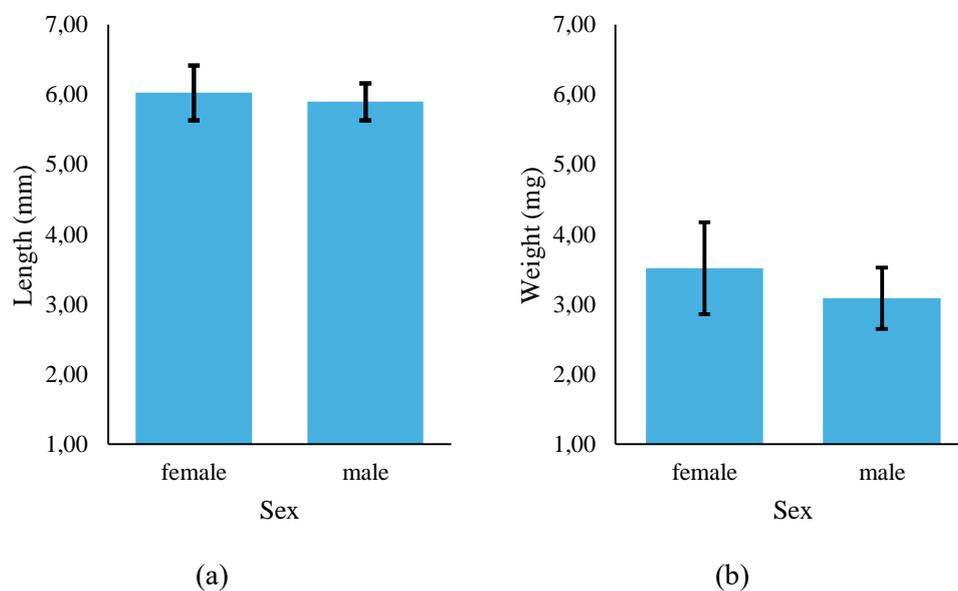
Adult insects have brown wings and yellow patterns at the tips of the forewing. Adults have a symmetrical forewing design. Females and males are characterised by the shape of the last abdominal segment. In females, the abdomen tip is white and slender

(Figure 3.b), while in males, the abdomen tip is black and broader (Figure 3.c). In general, the female adults have a hairy anal papillae of the ovipositor characterizing the female genitalia, and a darker and wider caudal segment and the presence of a hair pencil characterizing male's genitalia [20].



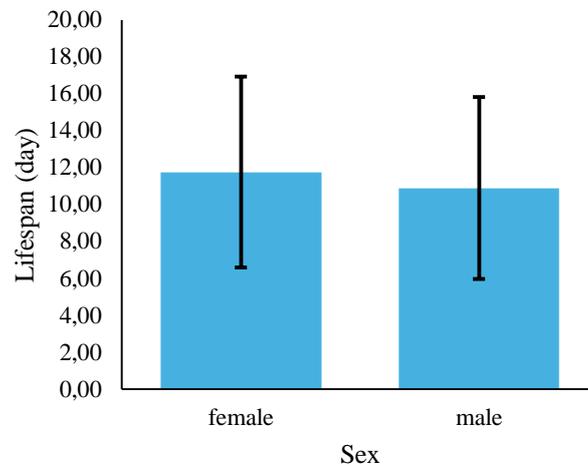
**Figure 3.** Adult morphology: (a) adult in resting position; (b) female abdomen; (c) male abdomen

**3.3.1. Adult fitness.** The female body more prolonged  $6.0 \pm 0.4$  mm ( $n = 50$ ) compared to the male  $5.9 \pm 0.3$  mm ( $n = 50$ ). The body length of males and females does not differ significantly ( $t = 1.92$ ,  $df = 85$ ,  $p > 0.05$ ) (Figure 4.a). Adult body length ranges from 5 to 7 mm. However, there is a significant difference in weight between males and females ( $t = 3.83$ ,  $df = 85$ ,  $p < 0.05$ ) (Figure 4.b). Females have an average weight of  $3.5 \pm 0.7$  mg, while males have an average weight of  $3.1 \pm 0.4$  mg. Although the weight range of adult insects is between 3 and 5 mg, the difference between the sexes indicates that females tend to be heavier. This difference relates to reproductive functions, where females produce eggs, which can affect body weight. Insect body size had association with insect fecundity, influenced by natural selection [21]. Moreover, most of insect orders tend to exhibit greater sexual size dimorphism in females due to fecundity selection [22]. Adult females with large size can carry more eggs and affects their reproduction.



**Figure 4.** Fitness of cocoa pod borer adult, (a) body length; (b) body weight

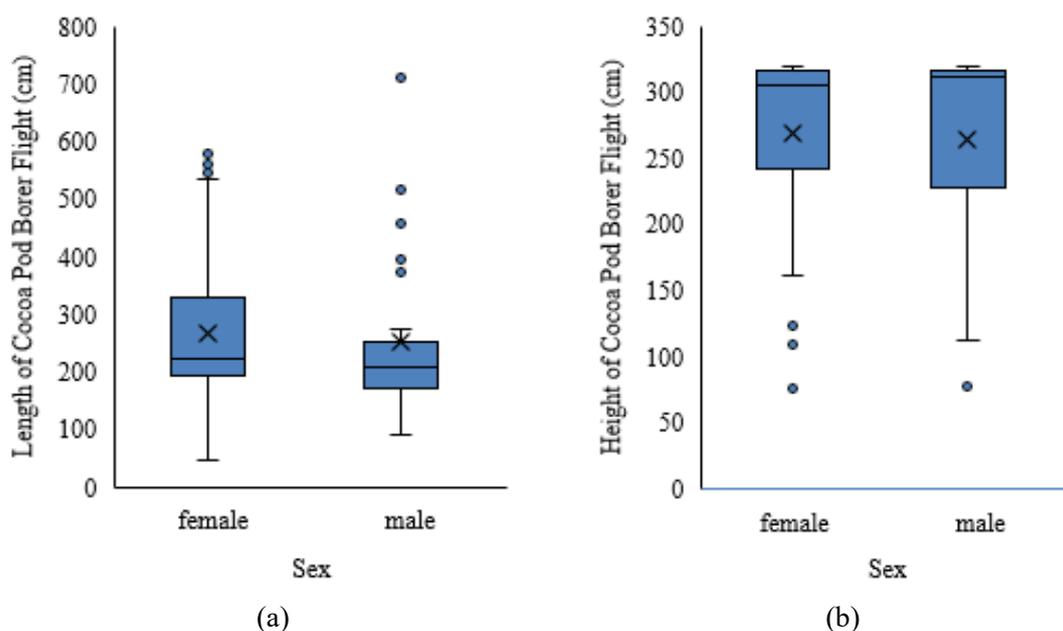
**3.3.2. Adult lifespan.** Adults were kept in a room with temperatures ranging from 25 to 30 °C. This temperature range is considered optimal for insect development. CPB adult lifespan up to two weeks after adult emergence. Longevity of female insects is more extended than males. The average lifespan of females was  $11.8 \pm 5.2$  days ( $n = 50$ ), while males have an average lifespan of  $10.9 \pm 4.9$  days ( $n = 50$ ), but this study shows that there is no significant difference in the lifespan of adult females and males ( $t = 0.85$ ,  $df = 97$ ,  $p > 0.05$ ). However, the effect of temperature caused *Conopomorpha sinensis* a longer lifespan for males than females, i.e., female adults living at 25-30°C had a lifespan of 5-11 days and 6-13 days for males [23]. In addition, CPB development is also influenced by humidity. High humidity (80%-90%) promotes CPB population growth [24]. Therefore, pruning to reduce humidity can help prevent CPB infestations.

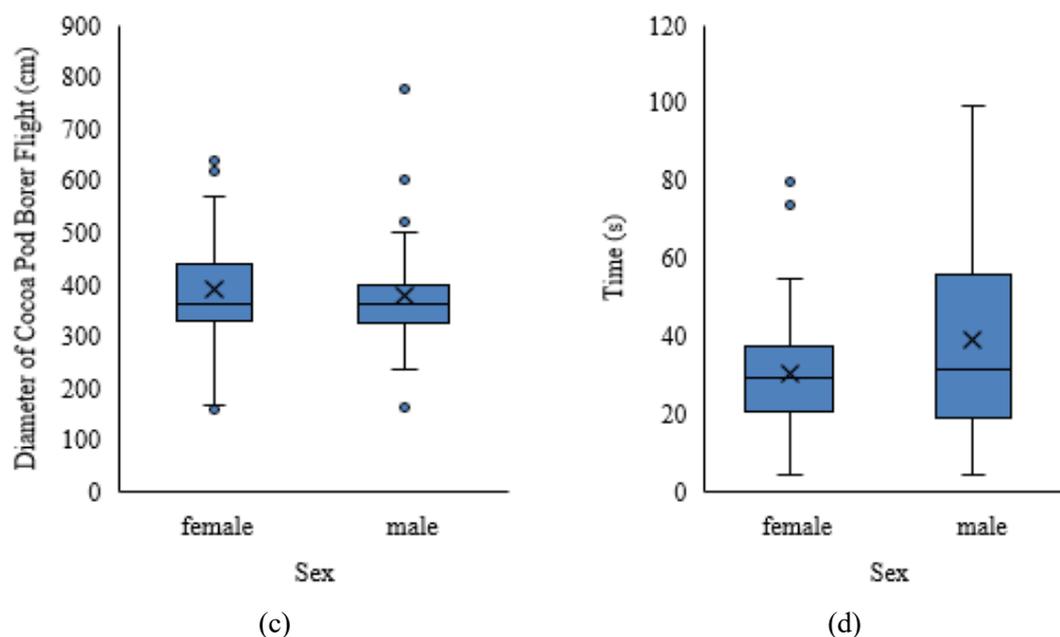


**Figure 5.** Adult lifespan

**3.4. Flight Capability**

This flight capability test was conducted in a laboratory with size 10.1 m × 3.2 m. The flight capability of the insects was measured using four variables: length (the distance flown by the adult), the height reached by the adult, diagonal (the hypotenuse of length and height calculated using the pythagorean theorem), and time (s) (the duration of the flight from take-off to the first landing). The diagonal plot (Figure 6) shows that female insects fly farther (length)  $268.6 \pm 144.2$  cm compared to males  $253.6 \pm 138.2$  cm, with  $(t = 0.407, df = 57, p > 0.05)$ . Females also achieve a greater height  $268.5 \pm 69.9$  cm compared to males  $263.8 \pm 75.2$  cm, with  $(t = 0.249, df = 57, p > 0.05)$ . Additionally, the hypotenuse or diagonal value of length and height also indicates that females fly farther  $392.5 \pm 124.4$  cm compared to males  $379.7 \pm 118.7$  cm, with  $(t = 0.405, df = 57, p > 0.05)$ . Female insects flight duration was 4.5 to 99.0 seconds with an average flight time is  $30.6 \pm 16.6$  seconds and 4.7 to 79.8 seconds with the average flight time for female insects is  $30.6 \pm 16.6$  seconds, while for males, it is  $39.1 \pm 26.5$  seconds. Males fly longer than females  $(t = -1.483, df = 57, p > 0.05)$ .





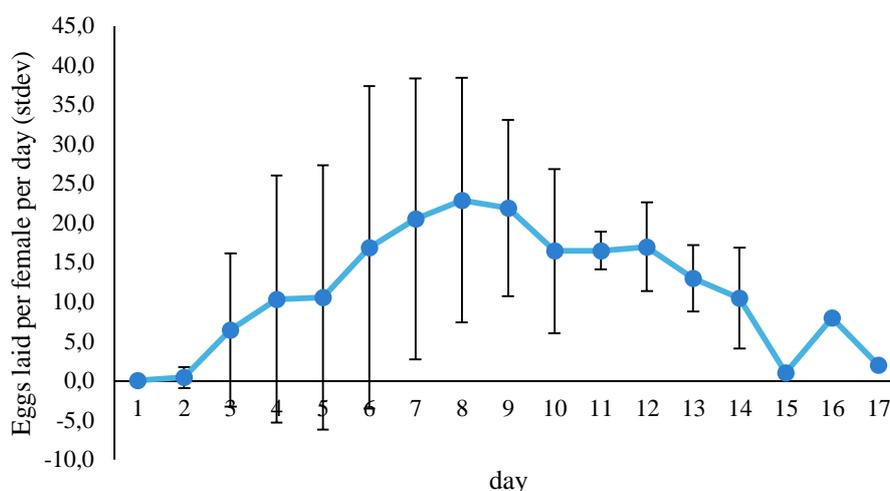
**Figure 6.** Flight capability of cocoa pod borer: (a) length of adult flight; (b) height of adult flight; (c) diameter as hypotenuse of length and height adult flight capability; (d) adult flight time.

When the female was released, they landed just after take-off. In contrast, males tend to circle the room before finally landing. Thus, although males fly more actively, they have a shorter flight distance when measuring the distance from take-off to landing. This resulted in a longer flight duration for males than females. This may be due to the larger body size of females compared to males. Additionally, female insects can start producing eggs as early as the first day after copulation (Figure 7). Male and female samples used were from the same bug dorm during pupal rearing until emergence. This may have led to the possibility of copulation between males and females before the flight capability test. Another study of the flight ability of *Lymantria xyliina* (Lepidoptera: Erebididae) showed that the energy available after mating for female flight becomes limited, leading to a decrease in flight muscle strength [25]. The limited space in the laboratory resulted in shorter flight distances in this study compared to previous field studies. In the field, females fly and rest around plant canopies and disperse pheromones that attract males and fly up to 5 metres higher than males, which can fly up to 2.5 metres [26]. The attraction of male insects to pheromones released by females influences the flight of males in the field. In addition, the field is an open space that does not restrict the adults from flying higher. The difference between outdoor and indoor conditions affects the flight distance of moths. This has been utilized in several studies to monitor *C. cramerella* adults using pheromones. For example, the implementation of sex pheromone monitoring and CPB adult trapping with sticky liners could be effective tools for Integrated Pest Management (IPM) as they have high specificity to their target and are environmentally friendly [27].

### 3.5. Egg Measurement and Fecundity of Cocoa Pod Borer

Females prefer to lay eggs in hard-to-reach places, such as in the concave grooves of pods, to avoid disturbances like washing by rain [12, 28]. CPB eggs are oval-shaped. Females laid eggs singly on the pod surface. Newly laid eggs are light yellow. The size of the eggs ranges from 0.4 mm to 0.5 mm. Most eggs are laid between days 7 and 9, and females can continue to produce eggs until day 17. Egg-laying observations were stopped when the female died, indicating that the female's lifespan plays a crucial role in determining total egg production. The varying lifespan of females causes the average number of eggs laid daily to fluctuate (Figure 7).

The number of eggs laid can reach up to 60 per day, and the total number of eggs a female can lay during her lifetime can exceed 300. The eggs start to hatch on the third day. On the fifth day of checking, it was found that the average number of eggs that had hatched was 64% from the average number of eggs laid, which was  $114.2 \pm 91.3$  eggs/female ( $n = 14$  replicates). During the observation, holes were observed on the part of the egg surface that was in contact with the pod. The presence of holes at the bottom of the egg and on the pod surface indicates that the eggs have hatched and the first-instar larvae have successfully entered the pod. Female fecundity can be influenced by temperature. According to a study by Syarkawi *et al.* [24], locations with temperatures ranging from 28-30°C are suitable for copulation and egg laying, and they also affect the lifespan of the adults, allowing them to produce more eggs.



**Figure 7.** Average of eggs laid per day

The oviposition by *C. cramerella* (CPB) adults can be prevented using pod sleeves as a mechanical control method to disrupt the insect's life cycle in the field [8]. In addition, the use of the natural enemy *Trichogrammatoidea bactrae fumata* as an egg parasitoid can help reduce yield losses caused by CPB infestations in the field [7]. Although these two control methods cannot be applied simultaneously, their development has the potential to reduce pesticide use in the field. On the other hand, pod sleeves can synergize with *Heterorhabditis* sp. which has been shown to effectively reduce CPB infestations and the level of cocoa bean damage [29].

#### 4. Conclusion

CPB causing the pod to early ripening. The pupal female stage having duration  $7.8 \pm 0.7$  days and males  $8.14 \pm 1.00$  days. Adult CPB had a body length for females were  $6.0 \pm 0.4$  mm and males  $5.9 \pm 0.3$  mm. The average weight of adult CPB were  $3.5 \pm 0.7$  mg for females and  $3.0 \pm 0.4$  mg for males. Adult CPB live for more than two weeks. Females fly higher than males, but females have shorter flight time than males. It was found that the average number of eggs that had hatched was 64%, from the average number of eggs laid of  $114.2 \pm 91.3$  eggs/females.

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