

Photoperiod effects on the growth stages of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions

Pengaruh fotoperiodisme pada tahapan perkembangan ulat grayak *Spodoptera frugiperda* (Lepidoptera: Noctuidae) di kondisi laboratorium

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ABSTRACT

Spodoptera frugiperda, commonly known as the fall armyworm, is a pest that attacks maize plants in Indonesia. Light is one of the crucial factors in the development of *S. frugiperda*. This study aims to observe and analyze the effects of different light exposure (photoperiod) on the duration of larval instars, pupal stage, and imago, as well as the body length and weight of *S. frugiperda*. The expected benefit of this research is to provide insights into how light influences the developmental stages of *S. frugiperda* under laboratory conditions. The test insects, *S. frugiperda*, were obtained from Agricultural Standardization Testing Center - Sweetener and Fiber Crops (BSIP-TAS) and were reared from the first instar larval stage, with a total of 56 individuals fed with young maize cobs. *S. frugiperda* undergoes complete metamorphosis, passing through egg, larval, pupal, and imago stages. The treatment with no light and 24 hours of darkness (0L:24D) resulted in the shortest duration for the larval, pupal, and imago stages, averaging 27.42 days, compared to other treatments. On the other hand, the 24L:0D treatment significantly affected body length and weight, showing distinct differences from other treatments (L: light; D: dark). The effect of light, using 238.89 lux illumination, was found to influence the development of *S. frugiperda* at each developmental stage.

ABSTRAK

Spodoptera frugiperda, yang dikenal sebagai ulat grayak, merupakan hama yang menyerang tanaman jagung di Indonesia. Cahaya adalah salah satu faktor krusial dalam perkembangan *S. frugiperda*. Penelitian ini bertujuan untuk mengamati dan menganalisis pengaruh paparan cahaya yang berbeda (fotoperiode) terhadap lama instar larva, fase pupa, dan imago, serta panjang dan berat tubuh *S. frugiperda*. Manfaat yang diharapkan dari penelitian ini adalah memberikan wawasan tentang bagaimana cahaya memengaruhi tahapan perkembangan *S. frugiperda* dalam kondisi laboratorium. Serangga uji, *S. frugiperda*, diperoleh dari Balai Standardisasi Instrumen Pertanian - Tanaman Pemanis dan Serat (BSIP-TAS) dan dipelihara sejak fase larva instar pertama, dengan total 56 individu yang diberi makan tongkol jagung muda. *S. frugiperda* mengalami metamorfosis sempurna, melewati fase telur, larva, pupa, dan imago. Perlakuan tanpa cahaya dan 24 jam dalam kondisi gelap (0L:24D) menghasilkan durasi terpendek untuk fase larva, pupa, dan imago, dengan rata-rata 27.42 hari dibandingkan dengan perlakuan lainnya. Di sisi lain, perlakuan 24L:0D secara signifikan memengaruhi panjang dan berat tubuh, menunjukkan perbedaan yang mencolok dibandingkan dengan perlakuan lainnya (L: cahaya; D: gelap). Pengaruh cahaya dengan pencahayaan 238.89 lux ditemukan mempengaruhi perkembangannya.

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INTRODUCTION

Maize (*Zea mays*) is a staple crop originating from the Americas and has become a key agricultural commodity worldwide (Bhato, 2016). In Indonesia, maize is the second most important food crop after rice. According to the Central Statistics Agency (BPS), maize production reached 19.56 million metric tons in 2023, a significant decrease from the 21.35 million tons produced during the rice, maize, and soybean (*padi, jagung, dan kedelai* – PAJALE) program in 2016 (Syachbudy, 2023). Maize plays a crucial role as a source of carbohydrates and is widely used in food and feed industries (Wahyudin et al., 2016). However, maize production in Indonesia faces several challenges, including pest infestations.

Spodoptera frugiperda, commonly known as the fall armyworm (FAW), is an invasive pest that has spread from the United States to Argentina. This species of *Lepidoptera* is highly destructive to maize, sugarcane, and rice in South America (Hannalene et al., 2018). In Indonesia, several studies have reported severe damage caused by *S. frugiperda* in maize crops, with damage rates ranging from 45% to 71% in Lamongan Regency (Damayanti et al., 2023) and 58% to 100% in Tuban Regency (Megasari & Khoiri, 2021). This pest is known for its high adaptability to a wide range of host plants, feeding on 353 plant species from 76 families (Montezano et al., 2018). The larval stage of *S. frugiperda* is the most destructive, while the imago (adult) stage has high dispersal capabilities (Azwana, 2021). Unlike other migratory pests, *S. frugiperda* does not enter diapause under extreme temperature conditions (Nonci et al., 2019), which allows it to thrive in a wide range of environments. Currently, control methods for *S. frugiperda* have predominantly relied on conventional chemical pesticides, which are effective but pose risks to the environment and non-target species (Purnama et al., 2024a). However, recent efforts have focused on developing and utilizing botanical pesticides as a more sustainable and eco-friendly alternative (Shai et al., 2023; Purnama et al., 2024b).

Throughout the maize growth cycle, from vegetative to reproductive stages, maize plants are vulnerable to *S. frugiperda* attacks. The larval stage is particularly destructive, feeding on the apical meristem, thereby inhibiting the plant's growth. The feeding behavior of *S. frugiperda* larvae has been associated with high efficiency, as noted by Maharani et al. (2019). Indications of infestation include the appearance of wood-like fragments on the leaf surface and damage to the young shoots, which can ultimately lead to plant death. Several studies, including Nonci et al. (2019), Trisyono et al. (2019), and Schmidt-Duran et al. (2015) have focused on the feeding habits and damage caused by *S. frugiperda* larvae, but there is limited information on the factors influencing their development under controlled conditions.

While extensive research has been conducted on the biology, feeding habits, and damage caused by *S. frugiperda*, there is a significant gap in understanding how environmental factors, particularly light exposure (photoperiod), affect its developmental stages. Light is a critical factor in insect development, influencing behavior, growth rates, and reproduction (Borges, 2022). Although previous studies have explored photoperiodism in various insect species (Hori et al., 2014; Yuan et al., 2023), there is limited research specifically addressing the effects of different light exposures on the growth stages of *S. frugiperda*. Understanding how light influences the developmental phases of this invasive pest could offer valuable insights for more effective pest management strategies. Therefore, this study aims to fill this research gap by examining the effect of different light exposure treatments (photoperiod) on the duration of the larval, pupal, and imago stages, as well as body length and weight, of *S. frugiperda* under laboratory conditions.

MATERIALS & METHODS

This study was conducted at the Plant Health Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The instruments used in this study included a digital microscope (endoscope camera magnifier 500 × 8 LED, China), a digital hygrometer (HTC-2, China), a lux meter (type AS803, China), and a digital scale (TN-series, accuracy 100.00g/0.001g, China). The materials used in this study included test insects (*S. frugiperda*), young maize cobs (baby corn), and 100% natural honey. LED lights were used as the light source in the rearing process of *S. frugiperda* (Kim et al., 2019).

Insect preparation and maintenance

The test insects were obtained from the Agricultural Standardization Testing Center for Sweetener and Fiber Crops (BSIP-TAS) at the first instar larval stage, totaling 56 individuals. Each larva was placed individually in a 25.00 ml plastic container. The containers were perforated at the top to ensure proper air circulation (Fadel & Anshary, 2023).

Feeding provision

S. frugiperda was fed young maize cobs that were cleaned before use. The maize used was organic, purchased from a local supermarket in Surabaya, Indonesia. The food was replaced daily to maintain freshness. Larvae were fed according to their developmental stage: 1 g for the first instar, 2 g for the second instar, and 3 g for instars 3-6. Upon entering the pre-pupal stage, the larvae stopped feeding and underwent metamorphosis into pupae. The daily food for the imago (adult) of *S. frugiperda* was a 10% honey solution provided on cotton (Fadel & Anshary, 2023).

Treatments on S. frugiperda

First instar larvae of *S. frugiperda* were placed individually in 25 mL plastic containers, with a total of 56 individuals divided into 14 replications. Upon pupation, the larvae were moved and paired (male and female pupae) in 5-liter plastic containers to mate after reaching the imago stage. Tissues were spread on the sides of the containers for egg-laying by the imagoes. Four photoperiod treatments (light ratios) were applied: 6L:18D, 12L:12D, 0L:24D, and 24L:0D (L: light; D: dark). The treatments were designed to examine the relative importance of light and darkness in the developmental stages. The "L" indicates the length of the light period, while "D" indicates the length of the dark period (Saunders, 2022). Daily observations were made to record the number of eggs, duration of each larval instar, pupal stage, imago stage, body length of each instar, and weight of the sixth instar, pre-pupa, and pupa. During the light period, the insects were exposed to a 25-watt lamp, while during the dark period, they were kept in a completely dark room, without exposure to sunlight or artificial light (Yuan et al., 2023).

Observation parameters

The number of eggs was observed from the start of egg-laying by the female imago until the process was completed. The duration of larval stages was monitored from the first to the sixth instar. Changes in the larval stages could be identified by color and body size (Fadel & Anshary, 2023). The duration of the pupal stage was observed from the time the larva entered pupation until the pupa became an imago. The length of the imago stage was recorded until death. Body length measurements for each larval instar, pupa, and imago were taken after complete pupation and the death of the imago, using a ruler and a millimeter block. The body weight of the sixth instar, pre-pupa, and pupa was measured using a digital scale (Wijaya, 2023).

RESULTS & DISCUSSION

Life cycle of S. frugiperda

Observations of the life cycle of *S. frugiperda* show that this species undergoes complete metamorphosis, beginning from the egg phase to the larval, pupal, and imago stages. The life cycle of *S. frugiperda* can vary depending on the season. The insect may experience a faster life cycle under normal temperature conditions, but the life cycle lengthens when exposed to extreme temperatures, either too hot or too cold (Fadel & Anshary, 2023). The eggs of *S. frugiperda* are whitish-green and later turn grayish-black as they near hatching. The head capsule of the first instar larva can be seen as a dark shadow (Figure 1). The eggs of *S. frugiperda* have an average diameter of about 0.51 mm and are dome-shaped (Sumaryati et al., 2023). Eggs are laid in clusters on tissue paper spread in a 5-liter plastic container. The eggs are arranged in one or two layers and covered with scales from the female imago.

The data show that the larval stage of *S. frugiperda* passes through six instars. Newly hatched first instar larvae are transparent gray and gather before dispersing. The first instar larvae, after feeding on young maize cobs, turn whitish-yellow. The second instar larvae have brown heads with a faint "Y" marking beginning to appear, and the body becomes

white-greenish with visible spots on each segment. The abdomen is wider than the head. By the third instar, the body turns green, and the "Y" marking on the head becomes more pronounced. The lateral lines on the abdomen are reddish-brown. In the fourth instar, the abdomen is brown, and the head becomes translucent brown, with the "Y" marking clearly visible. By the fifth instar, the larvae are mostly dark brown, with a brown head and a more prominent "Y" shape on the abdomen. The sixth instar larvae show significant changes, becoming larger and more compact, with four distinct spots on the back and a clearer "Y" marking (Figure 2).

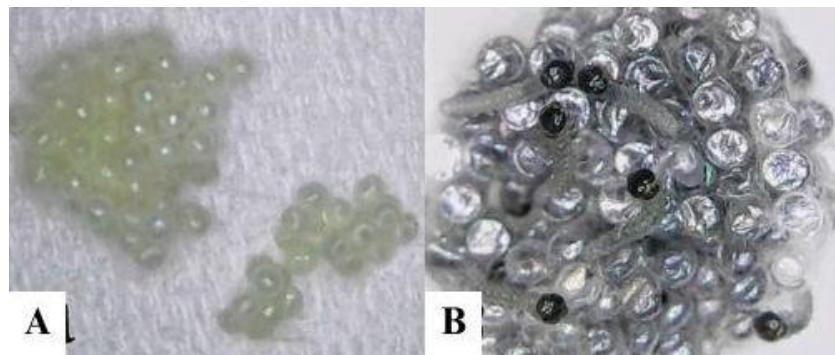


Figure 1. Eggs of *S. frugiperda*. A. Newly laid eggs; B. Eggs about to hatch.

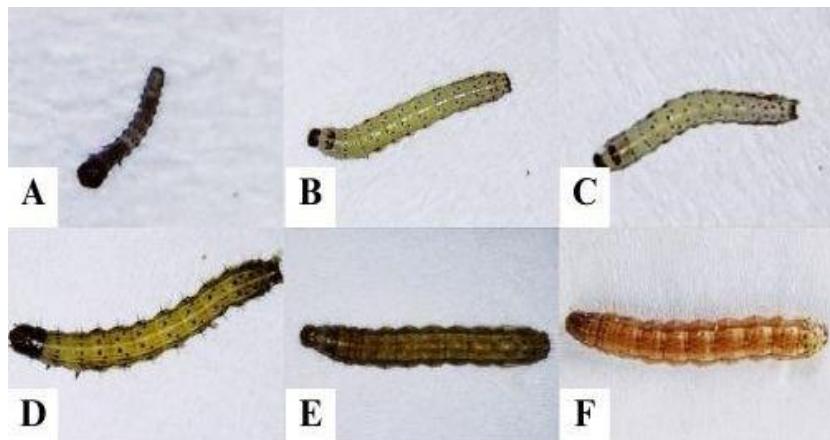


Figure 2. Larval instars of *Spodoptera frugiperda*. A. Instar 1; B. Instar 2; C. Instar 3; D. Instar 4; E. Instar 5; F. Instar 6.

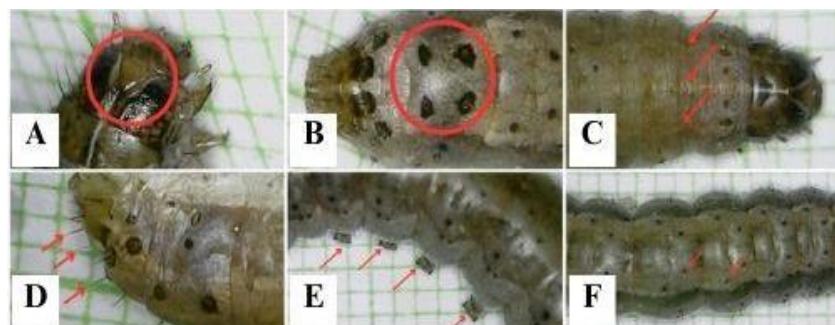


Figure 3. Distinct characteristics of *S. frugiperda*. A. Inverted Y on the head; B. Four black spots forming a square on the 8th segment and trapezoid on the 9th; C. Three yellow lines along the body; D. Hairs on each pinacula; E. Four pairs of prolegs; F. Black pinacula.

Distinct characteristics of *S. frugiperda*

The characteristic features of *S. frugiperda* start to become evident in the third instar larva stage and become more pronounced by the sixth instar. There are six distinguishing features of *S. frugiperda*: (1) The front of the skull is black,

displaying a faint inverted Y-shaped pattern; (2) The eighth abdominal segment has a trapezoidal pattern formed by four large spots called pinacula; (3) The dorsal side of the body is marked by three pale yellow or whitish-yellow lines, one running along the back and one on each side just below the back; (4) The dorsal pinacula are covered with single stiff black hairs (setae); (5) The abdomen is equipped with four pairs of false legs called prolegs, and the dorsal area contains black pinacula (Maharani et al., 2019) (Figure 3).

Following the larval phase, the pre-pupa ceases feeding. The larva protects its body by forming a cavity in the middle of the young maize cob, entering it, and sealing it with silk threads. The body shrinks, and its weight decreases compared to the sixth instar larva. Feeding the larvae with young maize cobs has the advantage of maintaining moisture compared to maize leaves. The nutritional content of maize cobs consists of 90.00% dry matter, 2.80% crude protein, 0.70% crude fat, 1.50% ash, 32.70% crude fiber, 80.00% cell wall content, 6.00% lignin, and 32% ADF (Astuti et al., 2020). *S. frugiperda* feeds on the sheath and the young kernels of the maize cob (Hardke et al., 2015). Before the pre-pupal phase, the larva seeks food and hides in the middle of the young maize cob for protection (Sumaryati et al., 2023).

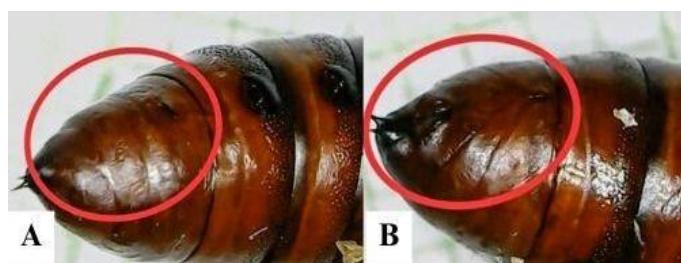


Figure 4. Pupa of *Spodoptera frugiperda*. A. Male; B. Female

Initially, the pupa is white and light green. During development, the pupa hardens, and its color changes to reddish-brown and then black. The presence of genitalia and the anal slit allows for sex determination. The distance between the anus and the genital opening is greater in females than in males (Figure 4). Sharanabasappa et al. (2018) noted that the pre-pupa that transforms into a pupa is initially green but later turns brown. In terms of sex differentiation, it is important to note that females have a greater distance between the anus and the genitalia.



Figure 5. Imago of *Spodoptera frugiperda*. A. Male; B. Female.

The wings of *S. frugiperda* can be used to differentiate males from females based on size and pattern. Male imagos are significantly larger than females. Male *S. frugiperda* has a pair of forewings and another pair of hindwings. The male's forewings are white on the ventral side with numerous scattered white spots, while the dorsal side of the wings is grayish-brown. The female imago has silvery-white hindwings and grayish-brown forewings adorned with small brown and gray spots (Figure 5). Hutagalung et al. (2021) explained that the shape of *S. frugiperda* imagos is distinguished by white

hindwings and two pairs of wings with a brown base color. When at rest, the wings fold over the back, covering the dorsal surface.

*The effect of photoperiod on *S. frugiperda* development*

The observation of the duration of larval, pupal, and imago stages of *S. frugiperda* was conducted in a laboratory setting with an average temperature of 27.63 °C, humidity of 73.41%, and light intensity of 238.89 lux. According to Liu et al. (2021), *S. frugiperda* under 650.00 lux illumination exhibited positive phototaxis toward the light source, similar to other nocturnal insects. The observations were conducted with a total of 14 replications to measure the duration of the life cycle phases of larvae, pupae, and imagos (Table 1). The larval phase includes instars 1 through 6, the pupal phase includes pre-pupa and pupa stages, and the imago phase refers to the adult stage.

Table 1. The duration of each life stage (larval, pupal, and imago) of *S. frugiperda*

Phase	Mean ± SD (day)			
	6L:18D	12L:12D	0L:24D	24L:0D
Larval Instar 1	3.00 ± 0.00 a	3.00 ± 0.00 a	3.00 ± 0.00 a	3.00 ± 0.00 a
Larval Instar 2	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a
Larval Instar 3	1.14 ± 0.36 a	1.14 ± 0.36 a	1.50 ± 0.52 a	1.43 ± 0.51 a
Larval Instar 4	1.21 ± 0.43 ab	1.86 ± 0.36 c	1.50 ± 0.52 b	1.14 ± 0.36 a
Larval Instar 5	1.64 ± 0.50 b	1.21 ± 0.43 a	1.21 ± 0.43 a	2.00 ± 0.00 a
Larval Instar 6	1.93 ± 0.27 b	1.79 ± 0.43 ab	1.86 ± 0.36 b	1.50 ± 0.52 a
Pre-pupa	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a
Pupa	7.57 ± 0.65 a	7.29 ± 0.99 a	6.93 ± 0.92 a	8.64 ± 0.63 b
Imago	9.30 ± 2.66 a	9.10 ± 1.85 a	8.42 ± 2.97 a	10.25 ± 4.03 a

Note: Means followed by the same letters indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

The results show that the 6L:18D treatment had a significant effect on the duration of the third instar larvae. The 12L:12D treatment significantly affected the duration of the third and fifth instar larvae. The 0L:24D treatment significantly shortened the duration of the fifth instar larvae, pupae, and imago. The 24L:0D treatment significantly affected the duration of the fourth and sixth instar larvae. The 0L:24D treatment resulted in shorter larval, pupal, and imago phases, averaging 27.42 days, compared to the other treatments. This is likely because the absence of light in the 0L:24D treatment accelerated the metamorphosis of *S. frugiperda*. Sumaryati et al. (2023) found that the life cycle of *S. frugiperda* reared in the laboratory on young maize cobs lasts between 33 to 42 days. In a study by Tiwari & Deole (2021), *S. frugiperda* had a life cycle of 34.50 ± 0.72 days in the laboratory.

Other studies have also shown that photoperiodism affects insect development. According to Yuan et al. (2023), the effects of photoperiodism on insect development are not always consistent and depend on the biological characteristics of the species. The egg-to-imago phase of *S. frugiperda* is extended under the 24L:0D treatment compared to 0L:24D. This is supported by He et al. (2021), who found that the imago of *S. frugiperda* lived the longest under full light conditions (24L:0D). *Telenomus remus*, for example, had a shorter life cycle under 24L:0D conditions (Chen et al., 2021), while *Sclerodermus pupariae* developed faster in short-day conditions (Hu et al., 2019). Additionally, nighttime artificial lighting (during the dark period) positively influenced the development of *Mythimna separata* (Kim et al., 2020).

*Body length of *S. frugiperda**

Each developmental phase of *S. frugiperda* is marked by changes in body size. The body length data of *S. frugiperda* were recorded from the first to the sixth instar larvae, pupae, and imago (Table 2). Pupal length was measured after the pupae had hardened, as soft pupae are more prone to death. Bakrim et al. (2008) stated that increases in body length are due to molting and exoskeleton changes regulated by the hormone ecdysone.

Based on this study, the 6L:18D treatment significantly affected the length of larval instar 1 and instar 2. The 12L:12D treatment significantly affected imago length. The 0L:24D treatment significantly affected pupa length. The 24L:0D treatment significantly affected the length of larval instars 3, 4, 5, and 6. Body length parameters under the 24L:0D treatment showed significant differences compared to the other treatments, as continuous light exposure resulted in increased movement and growth activity of *S. frugiperda*. Hidayanti & Tri (2019) stated that the increase in larval length is due to the increase in both cell number and volume. The body length of *S. frugiperda* larvae from instars 1 to 6 ranged from 1.68 mm to 35.90 mm (Bankar & Bhamare, 2023), and the pupa length varied between 14.00 mm and 19.00 mm on maize plants (Kalyan et al., 2020). The male and female imagoes had respective lengths of 15.99 mm and 15.16 mm (Helen et al., 2021).

Table 2. Body length of *S. frugiperda*

Phase	Mean ± SD (cm)			
	6L:18D	12L:12D	0L:24D	24L:0D
Larval Instar 1	0.23 ± 0.04 a	0.22 ± 0.03 a	0.20 ± 0.01 a	0.20 ± 0.01 a
Larval Instar 2	0.45 ± 0.07 a	0.44 ± 0.09 a	0.39 ± 0.09 a	0.42 ± 0.11 a
Larval Instar 3	1.13 ± 0.17 a	1.16 ± 0.19 a	1.18 ± 0.22 b	1.36 ± 0.17 a
Larval Instar 4	1.56 ± 0.28 a	1.69 ± 0.20 ab	1.71 ± 0.27 b	1.84 ± 0.23 ab
Larval Instar 5	2.20 ± 0.24 a	2.36 ± 0.22 a	2.20 ± 0.29 a	2.39 ± 0.28 a
Larval Instar 6	1.89 ± 0.20 a	1.94 ± 0.15 a	1.94 ± 0.17 a	1.96 ± 0.22 a
Pupa	1.60 ± 0.09 a	1.64 ± 0.09 a	1.74 ± 0.09 a	1.66 ± 0.09 b
Imago	1.68 ± 0.08 a	1.72 ± 0.06 a	1.70 ± 0.07 a	1.71 ± 0.07 a

Note: Means followed by the same letters indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

The longer wings of *S. frugiperda* imagoes provide better flight capability compared to the shorter wings of other *S. frugiperda* (Keinath et al., 2021). Bhattarai et al. (2021) stated that there are Noctuidae insects, such as *Helicoverpa armigera*, which, in the imago stage, exhibit positive phototaxis behavior in response to light stimuli. *M. separata* demonstrated positive changes in its biological characteristics during the dark period, whereas during the light period, no significant changes were observed compared to the control levels (Kim et al., 2020). The degree of changes in insect biological characteristics varies according to different light sources (Zhang, 2018), and these biological characteristics return to normal for the next generation (Sang et al., 2016).

Body weight of *S. frugiperda*

Each developmental phase of *S. frugiperda* is marked by changes in body weight. The body weight data of *S. frugiperda* during the observation period can be seen from the sixth instar larval and pupal stages (Table 3). According to Barros et al. (2010), food availability is crucial for insect development. The development and growth of insects are strongly correlated with the quality of food they consume. Putra & Wulanda (2021) reported a clear correlation between pupal weight and the amount of food ingested by the larvae. Hidayati & Tri (2019) also found that pupal weight is related to larval weight.

Table 3. Body weight of *S. frugiperda*

Phase	Means ± SD (gr)			
	6L:18D	12L:12D	0L:24D	24L:0D
Larval Instar 6	0.22 ± 0.02 a	0.23 ± 0.02 a	0.23 ± 0.02 a	0.25 ± 0.02 b
Pupa	0.20 ± 0.02 a	0.21 ± 0.02 a	0.21 ± 0.02 a	0.23 ± 0.02 b

Note: Means followed by the same letters indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% level.

The results of this study show that the 6L:18D, 12L:12D, and 0L:24D treatments had no significant effect on the body weight of the sixth instar larvae and pupae. However, the body weight parameter under the 24L:0D treatment showed a significant difference compared to the other treatments, likely due to the longer time available for foraging. Yuan et al. (2023) suggested that this may be because the larvae had more time to feed and grow during the light period. Hormonal imbalances caused by different photoperiods can lead to changes in developmental duration in some insects. The body weight of sixth instar *S. frugiperda* larvae is 327.90 mg, and the maximum pupal weight on maize is 185.90 mg (Bankar & Bhamare, 2023). One of the factors influencing pupal size is light exposure (Katsikis et al., 2020).

Helicoverpa armigera larvae and pupae, which are also Noctuidae insects, showed the highest body weight when reared in constant light, whereas pupae reared in darkness had the lowest weight (El-Sayed et al., 2017). *H. armigera* larvae reared under constant light took longer to emerge from the cocoon (Katsikis et al., 2020). *Eurois occulta* larvae showed slower body weight gain during short light periods but continued feeding (Numata & Shintani, 2023). The imago stage of *M. separata* exposed to light experienced hormonal changes that affected body weight (Kim et al., 2020). The biological characteristics of some insects at different developmental stages can be altered by photoperiods with different ratios (Chen et al., 2014).

CONCLUSIONS

This study shows that photoperiod significantly influences the development of *Spodoptera frugiperda*, particularly in terms of developmental duration, body length, and weight. The 0L:24D treatment (complete darkness) shortened the developmental time for larvae, pupae, and imagos, while the 24L:0D treatment (continuous light) increased body weight and length. These results indicate that light exposure plays a crucial role in the growth of *S. frugiperda*, likely by providing more foraging time and affecting hormonal balance. Understanding these effects can be useful for improving pest management strategies by adjusting light conditions. Future research should explore the long-term effects of photoperiod on subsequent generations.

ETHICS APPROVAL

This study was conducted in accordance with ethical guidelines for research involving animals. All experimental protocols were reviewed and approved by the Ethics Committee of Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia. The handling of *S. frugiperda* followed the standards for the ethical treatment of insects in laboratory settings, ensuring minimal harm and stress to the test subjects. No endangered or protected species were involved in this study.

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