The effect of temperature and photoperiod on the time taken for a meal to pass through the gut, defecation and digestion in the last larval instar of *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract. *Spodoptera littoralis* (Boisduval) is a highly destructive and polyphagous insect pest of great economic importance. It develops throughout the year and the larvae are non-diapausing. Little work has been done on the time taken for food to pass through the gut of *S. littoralis*. Thus, this study on starving and well fed last instar larvae of *S. littoralis* aimed to determine the effect of temperature and photoperiod on the time taken for a meal to pass through the gut, defecation and digestion. The results indicate that it depended on temperature, photoperiod and hunger. The time that elapsed between a larva being fed and the production of the first faecal pellet, which is a measure of the time it takes for food pass to pass through the gut (SFP), differed significantly at different temperatures and photoperiods. The SFP was longer at 15°C than at 30°C. At 20°C and under a short photoperiod, SFP was very much shorter than under a long photoperiod (18L : 6D). In addition, at 20°C, the time to defecation was significantly longer under a photoperiod of 12L : 12D than under continuous light. In all the tests > 95% of the larvae produced four faecal pellets per meal. The digestibility values varied significantly in the different tests. This study offers new insights into the passage of food from the crop to the rectum at different temperatures and photoperiods. This might help in understanding the adaptability of *S. littoralis* and may also help in controlling this important pest.

INTRODUCTION

Spodoptera littoralis (Boisduval) is one of the most destructive agricultural insect pests in its subtropical and tropical range. It can attack numerous economically important crops throughout the year (CABI, 2022). It is a highly polyphagous insect feeding on cultivated plants belonging to 40 families and 87 species, such as vegetables, fruit and ornamental crops (Salama et al., 1970). The larvae damage the leaves, fruiting points and flower buds. It occurs throughout Africa and extends eastwards into Turkey and north into eastern Spain, southern France and northern Italy (Venturini, 1975; Sidibe & Lauge, 1977). The pest develops throughout the year, without undergoing diapause (Miller, 1976; Sidibe & Lauge, 1977). The minimum temperature for development of all the stages is 13–14°C. Tolerance to cold generally increases through the larval stages and is greatest in the pupal stage (Miller, 1977, Khanna, 2004). At 18°C, larvae take 34 days and at 36°C only 10 days to complete their development.

Insect development and survival at different temperatures are described by Sidibe & Lauge (1977), Baker & Miller (1974) and Ocete Rubio (1984). Depending on the climate, *S. littoralis* can complete from two to seven generations per year (Salem & Salama, 1985), so this species can develop at different temperatures and photoperiods. As temperature and photoperiod influence the life processes of insects, they may also affect the physiology of food consumption, food utilization and movement of food in the alimentary tract. The passage of food through an insects' alimentary tract is reported for some insects, but there are no studies on the time required for food to pass through the gut of *S. littoralis*, or of any other Noctuid species.

The insect's digestive system consists of long tube called the alimentary canal, which runs lengthwise through the body. The insect uses its digestive system to extract nutrients and other substances from the food it consumes. The food ingested enters the alimentary canal where it is processed as it moves towards the anus where undigested food, faeces, are defecated (Nation, 2008). Digestion is the process by which food is converted into nutrients, which the body uses for energy, growth and cell repair (Thomson & Holling, 1977; Nation, 2008). Plant material treated

with stomach 'poisons' (insecticides) ingested by this insect might decrease the rate of passage of food through its gut and help in developing more effective insect control programs. Other information related to insect feeding, e.g., time taken for food to pass through the gut, crop emptying rate, defecation rate and digestion can be also important. In addition, information on the movement of poisonous or normal food through the gut would be valuable for physiological, toxicological and ecological studies (Snipes & Tauber, 1937; Gelperin, 1966; Venturini, 1975; Thomson & Holling, 1977). Gäde et al. (1997) report that, many insects change their development and/or physiology in order to survive during periods adverse conditions. Although photoperiod and temperature are known to regulate this change, the underlying mechanism largely remains to be elucidated. Development and reproduction in insects are regulated to a large extent by juvenile hormones (JH) and ecdysteroids (Gilbert, 2013). The JH signalling pathway is involved in the photoperiodic regulation of larval moults, in a temperature-dependent manner. Therefore, the present study investigated the passage of food from the crop into and within the insect's midgut and finally to the rectum, at different temperatures and photoperiods. This study may provide information for insect toxicologists, particularly the time required for food to pass through the gut under normal conditions or after ingesting stomach poisons. It may also help in understanding the adaptability of *S. littoralis* to its environment and seasonal changes.

MATERIALS AND METHODS

Insects

The stock culture of *S. littoralis* was reared on a semi-artificial diet at 25 ± 2°C, 65 ± 5% RH and a photoperiod of 12L : 12D as described by Hegazi et al. (1977). The semi-artificial diet consisted of: kidney beans 160 grams, dried yeast 35 grams, nipagin 3.5 grams, ascorbic acid 3.5 grams, agar 13 grams, formaldehyde 2.5 ml, water (total) 700 ml. New insects were regularly collected from the field to reduce the effects of inbreeding.

Coloured diet "Experimental diet"

In this approach, a marker that does not adversely affect the last instar larvae was used. Gut transit time was estimated by tracking the passage of dyed diet through the gut, from mouth to anus, based on the appearance of dye in defecated faeces. This is inexpensive, rapid and easy to evaluate. The "dye": Brilliant crystal blue (C.C.I. No. 51010, SIGMA) at 0.125 g/L diet, was used. The dye was "dissolved" in 3 ml distilled water and stirred for 5 min, which resulted in an intense clear blue solution. Then the solution was mixed with the hot diet to obtain an end volume of 250 ml. Then the mixture was stirred for about 15 min at 50–55°C while the diet was still soft and left for about 2 h to solidify and become suitable as larval food. The solidified diet was cut into cubes, ca. 2 cm³ and placed in small clear plastic Petri dishes containing the test larvae.

Feeding experiments

To test the effect of temperature on speed of passage of food, time to defecation of the first coloured pellet, and egestion of a meal, four experiments were conducted. A large egg mass of *S. littoralis* was selected and divided into four sub masses. The four subcultures were each kept at either 15, 20, 25 or 30 ± 1°C under a photoperiod of 6L : 18D. To test the effect of photoperiod on

rate of passage of a meal, another set of four experiments were conducted, in which another large egg mass of *S. littoralis* was selected and divided into four sub masses. All egg masses were reared at 20 ± 1°C, but each was kept under either a day length of 6 : 18, 12 : 12, 18 : 6 or 24 : 0 (L : D). All subcultures were maintained at 70 ± 5% RH. In all cases, *Spodoptera* larvae were reared individually in clear plastic petri dishes (3.5 cm in diameter). Penultimate-instar larvae in the pre-moult stage were starved overnight and those that moulted within 3 h were divided into two sub groups: (a) newly moulted larvae, i.e, un-starved larvae that were fed the diet immediately after moulting, (b) newly moulted larvae that were not fed for 6 h, i.e, starved larvae. Larval moults were based on recording the presence of shed head capsules (Mironidis & Savopoulou-Soultani, 2008). Therefore, all larvae were at the same physiological state, size and age. The guts of the freshly moulted 6th instar larvae were almost without residual faecal material. Waldbauer (1962, 1964) was the first to point out the advantages of using starved freshly moulted insects in growth and feeding studies. The experiments on the effect of temperature and photoperiod on *S. littoralis* were carried out in incubators (Hann, Munden, Germany), each equipped with six fluorescent 30-Watt cool white fluorescent tubes. In all experiments, the light was switched on at 5 am. The light intensity during photo phase was approximately 1270 foot candles. Light intensity was measured with a Weston light meter (Weston Electrical Instrument Company, Newark, NJ). Fans were used for cooling the electronic equipment during the light cycle. All cabinets were located in temperature controlled rooms (Hegazi et al., 2017).

Meal and passage through the alimentary tract

S. littoralis larvae were individually provided with one full meal of the dyed diet. A meal for this insect is defined as that ingested by a hungry larva of *S. littoralis*, the time taken for the meal to pass through the gut (SFP) is the time between a larva being fed and defecation of the first coloured faecal pellet. Then the coloured diet was removed and replaced with uncoloured diet and if the time to the subsequent defecation of an uncoloured faecal pellet was the same as the previous SFP then the dye did not affect the amount of diet ingested. The total time from eating the coloured diet to the defecation of all of the coloured faeces of a full meal (EP) represents the egestion time for a full meal i.e., the time required to defecate all the undigested coloured waste. The time taken is an estimate of the total egestion period, (EP); the defecation of the residue of a full meal. Only newly moulted 6th instar larvae (0–2 h) and/or those newly moulted were starved for 6 h. (starved larvae) were tested. For each experimental condition, three sets of *S. littoralis* larvae were used. The first set was provided with dyed diet until the first coloured pellet was defecated and then with non-coloured diet until the last coloured pellet was defecated. Larvae of the second set were starved for 6 h and then treated in the same as the first set of larvae. The 3rd set of larvae were provided with dyed diet as a control. *S. littoralis* larvae were individually placed in plastic Petri dishes, with 20 larvae/trial and three replicates. EP is the time from eating coloured diet to defecation of coloured faeces, which ended when the first uncoloured faece was defecated. That is, it is the time taken to digest and defecate a full meal. The number of coloured faeces defecated was used to define the number of faeces produced/meal. The digestion time for a full meal is the time required for the coloured food to pass from the mouth to the anus. There were 60 replicates for each treatment with one larva per replicate. The larvae were allowed to feed and complete one meal. The experiments were terminated when the first uncoloured white faeces were defecated. In each test, the digestion time, fresh weight of first faeces, fresh weight of all the faeces and number of coloured
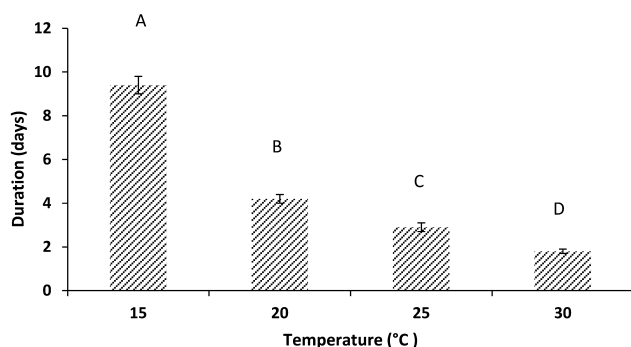
Fig. 1. Duration of development (days) of the last larval instar of *S. littoralis* recorded at different temperatures and a short photoperiod (6L : 18D). Letters above columns indicate significant differences between treatments (ANOVA, P < 0.05). Values are means ± SE.
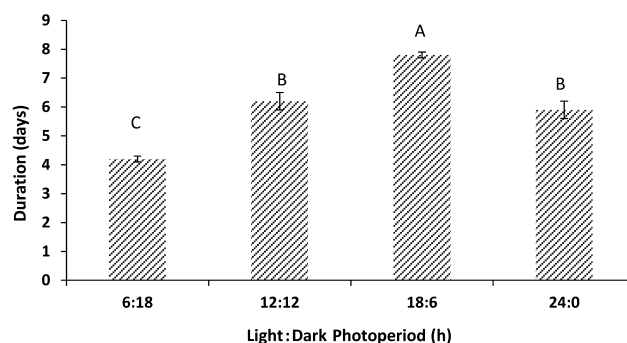

Fig. 2. Duration of development (days) of the last larval instar of *S. littoralis* recorded at different photoperiods at 20°C. Letters above columns indicate significant differences between treatments (ANOVA, P < 0.05). Values are means ± SE.

faeces / larva were recorded. Control larvae were left to continue growing, pupate and emerge as adults in order to determine the percentage mortality of the test larvae. The last instar larva of *S. littoralis* was selected because of its age and size, ease of handling, adaptability to laboratory conditions and ease of rearing on artificial diet.

Food utilization

Digestibility was measured in terms of dry weight. The initial dry weight of each larva was calculated from its weight and the mean percentage dry weight of ten similar larvae. The initial percentage dry weight of the diet was based on ten small samples that were oven-dried at 60°C. The dry weights of the diet ingested by the larvae were determined by multiplying the fresh weight by this constant. Since it was not possible to directly determine the dry weight of the larvae at the beginning of the experiments, the mean dry weight was estimated from samples of 10 newly moulted and 6 h starved newly moulted 6th instar larvae. The difference between the latter and the dry weight of the larvae at the end of the test is used as a measure of the increase in dry weight of a larva. Nutritional indices proposed by Waldbauer (1964) were used to determine the approximate digestibility (AD) of the diet for last instar larvae of *S. littoralis*.

Statistical analysis

Where appropriate, data were subjected to one-way (duration of larvae, speed of food passage and approximate digestibility) or two-way (interaction between temperature and photoperiod on starvation) analysis of variance to determine differences between means. Student's t-test was used for statistical analyses of data for

un-starved and starved larvae in different tests. Data are presented as means of number of faecal pellets, Spodoptera larvae or approximate digestibility ± SE.

RESULTS

Fig. 1 shows the effect of temperature (°C) on the duration of the last instar larva of *S. littoralis*, which the one way ANOVA revealed was significant. The duration of development decreased significantly with increase in temperature from 15 to 30°C (F = 226.2; df = 3, 36; L.S.D = 0.64; P < 0.05) and was 9.4 days at 15°C and 1.8 days at 30°C. Photoperiod significantly affected (F = 44.4; df = 3, 36; L.S.D. = 0.63; P < 0.05) the duration development of the last instar larvae kept at constant low temperature of 20°C (Fig. 2). The shortest duration (4.2 days) was recorded under the short photoperiod (6L : 18D) and the longest (7.8 days) under the long day photoperiod 18L : 6D at 20°C.

S. littoralis' digestive system is a long tubular tract called the alimentary canal, which runs lengthwise through the body. Food enters the alimentary canal via the mouth and exits via the anus. In the present study, the meal is that ingested by late instar larva with an empty gut (newly moulted = starved newly moulted larva). Ingested food travels in only one direction. The insect uses its digestive tract to extract nutrients and other substances from the food it consumes. Digestion takes time and undigested material is defecated. A larva produces as many as 4 faecal pellets

Table 1. Effect of different temperatures and a short photoperiod on SFP and EP of un-starved and starved 6th instar larvae of *S. littoralis*.

Temperature (°C)	Larvae	Defecation of faeces				Total faecal pellets	
		First faeces		Other faeces			
		Weight (mg)	*Time (h)	Weight (mg)	Time (h)	Defecation rate/ meal (No.)	EP (h)
15	Un-starved	1.76	8.0 ± 0.1 A	4.20	16.37	4.37	24.4 ± 0.3 A
	Starved	1.47	6.1 ± 0.1 a	3.74	12.43	4.12	18.8 ± 0.2 a
20	Un-starved	1.29	5.8 ± 0.1 B	3.78	7.91	4.13	13.7 ± 0.3 B
	Starved	0.93	4.0 ± 0.1 b	3.62	6.91	4.12	10.9 ± 0.2 b
25	Un-starved	1.04	5.1 ± 0.2 C	2.89	5.04	3.87	10.1 ± 0.1 C
	Starved	0.92	2.7 ± 0.1 c	3.17	6.31	4.12	9.0 ± 0.2 c
30	Un-starved	1.21	4.3 ± 0.1 D	4.25	4.07	3.75	8.4 ± 0.3 D
	Starved	0.86	2.5 ± 0.1 c	3.90	5.43	3.75	8.1 ± 0.1 c

* Speed of food passage. Values in rows with the same uppercase (un-starved) or lower case (starved) letter do not differ significantly (ANOVA; P > 0.05). Values are means ± SE.
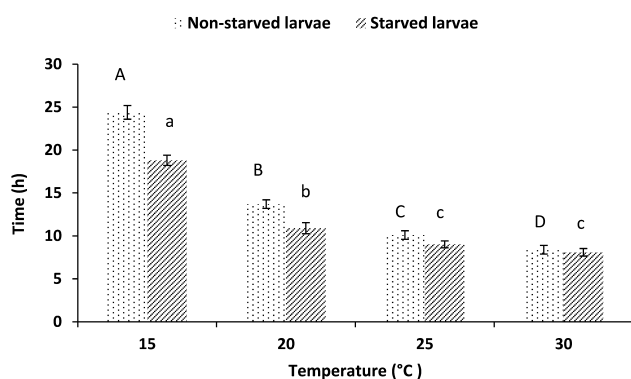
Fig. 3. The time (in h) required for the defecation of a full meal by starved and un-starved last instar larvae of *S. littoralis* (EP) kept at different temperatures. Columns with the same uppercase or lowercase letter do not differ significantly (ANOVA; P > 0.05). Values are means ± SE.
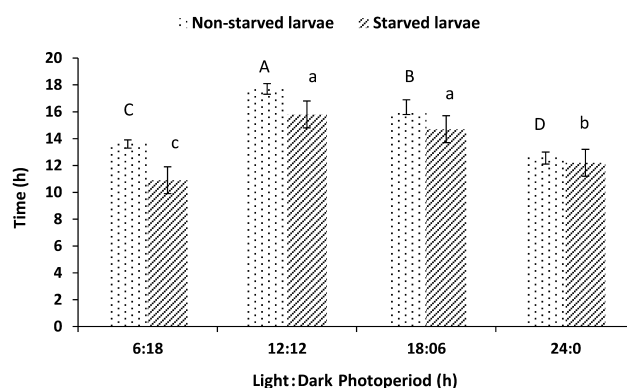

Fig. 4. The EPs recorded for starved and un-starved last instar larvae of *S. littoralis* at 20°C and different photoperiods. Columns with the same uppercase or lowercase letter do not differ significantly (ANOVA; P > 0.05). Values are means ± SE.

(defecation rate)/full meal (Tables 1, 2). All the larvae of *S. littoralis* fed the dyed diet subsequently successfully developed to the adult stage on a normal diet. Therefore, the dye is suitable for marking the diet as it is not toxic and does not stain the resulting pupae or adults. In addition, based on the control it did not affect the movement of the diet through the gut. Coloured diet passed through the tract in almost the same time as normal diet (data not shown). So, the dye is harmless, not absorbed by the larvae and the colour is visible in the faecal pellets. The time that elapsed between a larva being fed the dyed diet and defecation of the first coloured faecal pellet is defined here as the "speed of food passage" "SFP in h". In addition, the time to when all the coloured faeces were defecated is defined as the total time to digest a full meal (EP).

The effect of temperature on the speed of passage of food (time to the defecation of the first faeces, SFP) and time to digest a full meal (EP) (number of faeces/meal) for un-starved and starved last instar larvae of *S. littoralis* is presented in Table 1. The one-way ANOVA used to compare SFP recorded at different temperatures revealed it was significantly affected. The SFP differed significantly for un-starved (F = 191.9; df = 3, 36; L.S.D = 0.335; P < 0.05) and starved larvae *S. littoralis*: (F = 451.6; df = 3, 36; L.S.D = 0.22; P < 0.05). The time taken for food to

be digested and defecated (SFP) for un-starved larvae was 8.0 ± 0.1, 5.8 ± 0.1, 5.0 ± 0.1 and 4.3 ± 0.1 h at 15, 20, 25 and 30°C, respectively, and 6.1 ± 0.1, 4.0 ± 0.1, 2.7 ± 0.1 and 2.5 ± 0.1 h, for starved larvae. In addition, SFP differed significantly between un-starved and starved larvae at the same temperature, e.g., 15°C (t = 14.62, *P* < 0.05) and 20°C (t = 9.19, *P* < 0.05) or e.g., the same photoperiod, short (t = 9.4, *P* < 0.05) and 12L : 12D (t = 4.9, *P* < 0.05). Starvation significantly accelerated the speed of digestion even at the same temperature (Table 1).

EP differed significantly for un-starved (ANOVA, F = 280.2; df = 3, 36; L.S.D = 1.21; P < 0.05) and starved (ANOVA, F = 222.6; df = 3, 36; L.S.D = 0.94; P < 0.05) larvae at all the temperatures used (Fig. 3). At 15°C, it was 24.4 ± 0.3 h for un-starved and 18.8 ± 0.2 h for starved larvae. The transit time decreased significantly with increase in temperature from 15 to 30°C. EP at 15°C for un-starved differed significantly from that of starved larvae (t = 7.4, *P* < 0.05). Also, values varied significantly for starved and un-starved larvae at 20°C (t = 4.5, *P* < 0.05) and 25°C (t = 2.5, *P* < 0.05). Similar trends were recorded at 20°C and 25°C. However, there was no significant difference in the EP of un-starved and starved larvae at 30°C. Larvae regularly defecated 3.75 to 4.37 pellets per meal at the temperatures used. The effect of dif-

Table 2. Effect of photoperiod (at 20°C) on EP of un-starved and starved 6th instar larvae of *S. littoralis*.

L : D Photoperiod (h)	Larvae	Defecation of faeces				Total number of faeces	
		First pellet		Other pellets			
		Weight (mg)	*Time (h)	Weight (mg)	Time (h)	Defecation rate/ meal (No.)	EP (h)
6 : 18	Un-starved	1.29	5.8 ± 0.1 D	3.78	7.91	3.9	13.7 ± 0.1 C
	Starved	0.93	4.0 ± 0.2 c	3.62	6.91	4.0	10.9 ± 0.2 c
12 : 12	Un-starved	0.6	7.5 ± 0.2 B	1.55	10.23	3.8	17.7 ± 0.2 A
	Starved	0.7	6.5 ± 0.2 ab	1.65	9.28	3.6	15.8 ± 0.1 a
18 : 6	Un-starved	0.74	8.1 ± 0.1 A	1.73	7.68	3.1	16.1 ± 0.4 B
	Starved	0.67	6.7 ± 0.1 a	1.62	8.09	3.0	14.8 ± 0.1 a
24 : 0	Un-starved	0.68	6.2 ± 0.1 C	1.75	7.04	3.4	12.7 ± 0.4 D
	Starved	0.69	6.1 ± 0.1 b	1.65	6.64	3.3	12.2 ± 0.2 b

* Speed of food passage. Values in rows with the same uppercase (un-starved) or lower case (starved) letter do not differ significantly (ANOVA, *P* < 0.05). Values are means ± SE.

Table 3. Effect of temperature and L : D photoperiod (h) on the duration of development (in days) and mean ± SE AD% of the last instar larva of *S. littoralis*.

Temperature/ Photoperiod	Duration (days)	AD %	
		Starved	Un-starved
Temperature (°C), 6L : 18D			
15	9.4 ± 0.3 A	30.1 ± 0.6 C	28.2 ± 0.5 B
20	4.2 ± 0.1 B	35.9 ± 0.4 A	31.1 ± 0.6 A
25	2.9 ± 0.2 C	36.5 ± 1.0 A	30.1 ± 0.6 A
30	1.8 ± 0.1 D	33.1 ± 0.5 B	28.8 ± 0.5 B
L : D Photoperiod (h), 20°C			
6 : 18	4.2 ± 0.1 C	35.9 ± 0.4 A	31.1 ± 0.6 A
12 : 12	6.2 ± 0.3 B	33.8 ± 0.6 B	32.6 ± 1.1 B
18 : 6	7.8 ± 0.1 A	37.3 ± 0.5 A	36.9 ± 0.9 A
24 : 0	5.9 ± 0.3 B	31.8 ± 0.4 C	23.1 ± 0.6 C

For each set, means within a column with the same letter do not differ significantly (ANOVA, P < 0.05).

ferent temperatures on the approximate digestibility (AD) of the diet is presented in Table 3. The AD values recorded differed significantly at the different temperatures ($P <$ 0.05) for un-starved (ANOVA, F = 5.9; df = 3, 36; L.S.D = 1.9; P < 0.05) and starved (ANOVA, F = 23.6; df = 3, 36; L.S.D = 2.0; P < 0.05) larvae. The highest (31.1 ± 0.6) and lowest (28.2 ± 0.5) values of AD were for recorded for un-starved larvae reared at 20 and 30°C and it was 36.5 ± 1 and 30.1 ± 0.6, respectively, for starved larvae reared at 25 and 30°C.

The effect of photoperiod (at 20°C) on SFP and EP of un-starved and starved 6th instar larvae of *S. littoralis* is presented in Table 2. SFP of starved and un-starved larvae differed at the different photoperiods and temperatures used. Similarly EP differed significantly for un-starved (ANOVA, F = 41.9; df = 3, 36; L.S.D = 0.99; P < 0.05) and starved larvae (ANOVA, F = 34.9; df = 3, 36; L.S.D = 1.08; P < 0.05) at the different photoperiods used (Fig. 4). For un-starved larvae, the mean EP was, respectively, 13.7 ± 0.1, 17.7 ± 0.2, 16.0 ± 0.4 and 12.7 ± 0.4 at the photoperiods 6 : 18, 12 : 12, 18 : 6, and 24 : 0 (L : D). For starved larvae, SF was highly significantly shorter at the first three photoperiods. SFP at the different photoperiods used were, respectively, 10.9 ± 0.2, 15.8 ± 0.1 and 14.8 ± 0.1 h and differed significantly: 6L : 18D (t = 5.8, *P* < 0. 05), 12L : 12D (t = 4.6, *P* < 0. 05) and 18L : 6D (t = 4.2, *P* < 0. 05). It was longer for un-starved than starved larvae (Table 2). Larvae regularly defecated almost the same number of faeces per meal at each of the photoperiods used. There were no significant differences in EP recorded for starved (12.2 ± 0.2 h) and un-starved (12.7 ± 0.4 h) larvae kept under continuous illumination. While, there was significant difference in the SFPs of un-starved (5.14 h) and starved (6.04 h) larvae under continuous illumination. At 20°C, the AD values recorded under different photoperiods for un-starved (F = 45.9; df = 3, 36; L.S.D = 2.44; P < 0.05) and starved larvae (ANOVA, F = 26.9; df = 3, 36; L.S.D = 1.4; P < 0.05) differed. Values of AD for un-starved larvae were higher (36.9 ± 0.9%) under a long photoperiod than (23.1 ± 0.6%) under continuous illumination. For starved larvae the high-

est (37.3 ± 0.5%) and lowest (31.8 ± 0.4%) values were recorded under the same photoperiods. In most cases, the larvae defecated four faeces per meal in > 95% of the tests (Tables 1, 2).

DISCUSSION

Most of the studies on the effects of photoperiod and temperature on insects are mainly on the induction of diapause. Both are major factors determining the development of many insect pests (Eizaguirre et al., 1994; Fantinou et al., 1995; Capinera, 2008; He et al., 2017; Hegazi, et al., 2017). However, very little is known about the effect of photoperiod on non-diapausing insects, such as, *S. littoralis,* or adaptations by which insects synchronize their development with seasonal changes. There is a study on the effect of different photoperiods on the speed of development of the wasp, *Microplitis rufiventris*, a parasitoid of the larvae of *S. littoralis* (Hegazi & Führer, 1985). Under a short photoperiod (6L : 18D) this wasp completed its development more quickly than under both a long photoperiod 18L : 6D and continuous illumination 0L : 24D. Hegazi et al. (2021) report that the day lengths (0L : 24D; 6L : 18D; 12L : 12D; 18L : 6D and 24L : 0D) at constant temperatures (15, 20, 25, or 30°C) affect the speed of development of larvae of *S. littoralis*. The effect of photoperiod is more marked at 20°C than at 15°C or 25 and 30°C. At 20°C and a short photoperiod the development of larvae of *Spodoptera* was significantly shorter at the same temperature under a long photoperiod. The present work investigated the effects of a range of photoperiods and different temperatures on SFP, EP and AD. The rate of defecation of starved and un-starved larvae differed in all the tests. It is likely that the larvae increase in body mass faster under some photoperiods than others, which is supported by the higher values of AD recorded under long photoperiods. In addition, it is likely that larvae fed at night under the short photoperiod. Furthermore, different AD values associated with different photoperiods and temperatures affect the rate of development of larvae of *S. littoralis* and the population dynamics of this pest (Ruan & Wu, 2001). At 25°C, a full meal of a newly moulted 6th instar larva (186.6 ± 0.2 mg fresh wt.) weighed 263.5 ± 18.14 mg, the dry weight of which is 95.4 ± 0.07 mg. This is 1.4 times the body weight of a larva. In addition, larvae defecated 4 faeces the dry weight of which was 21.6 ± 0.8 mg. The incidence of defecation of starved and un-starved larvae differed at the different photoperiods and temperatures. The SFP of starved was faster than for un-starved larvae. SFP and EP might be of interest for future studies on using stomach poisons to control this pest (Snipes & Tauber, 1937). It is logical to suppose that temperature, photoperiod and starvation could differentially affect the peristaltic activity of the alimentary canal. Juvenile hormone (JH) is important in the control of insect development (Raabe, 1989; Nijhout, 1994; Gilbert, 2013). JH's are secreted by a pair of glands in the head known as the corpora allata (Wigglesworth, 1940) and maintain larval characteristics of the larvae of insects that make it possible for continued growth (Wigglesworth, 1964).

Ecdysone is a steroid hormone secreted by the prothoracic gland, which in its active form, stimulates metamorphosis and regulates moulting in insects. Nijhout & Williams (1974a, b) report that JH inhibits the secretion of the prothoracicotropic hormone (PTTH) by the brain of the last instar of *Munduca sexta* larvae. The release of PTTH is regulated by photoperiod for which the brain is the initiator (Ciemiokr et al., 1977; Gullan & Cranston, 2004; Johnson & Triplehorn, 2005) and serves to maintain development (Karp, 2021). Based on the above results, day length has significant effects on development and controls SFP and EP. So, day length may influence insects' basic physiological processes, such as metabolism and the nervous and endocrine systems. At low temperatures, the corpora allata, the prothoracic gland and JH biosynthesis may be sensitive to changes in day length. Insects, with very few exceptions, associate with several microbes during their life cycle (Lemoine et al., 2020). So, it is not only JH that plays an important role, but gut microbes may also play a pivotal role in food digestion. At low temperatures, the gut becomes more selective and most larval symbionts do not survive or become inactive, and this in turn may affect AD. It is not clear which factors elicit such variability in the effects of photoperiod and temperature on the development of larvae and what physiological mechanisms are involved.

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