

Original Article

Development, survival and morphometric parameters of boll weevil reared in different photoperiods

Desenvolvimento, sobrevivência e parâmetros morfométricos do bicudo-do-algodoeiro criado em diferentes fotoperíodos

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Abstract

Third instar larvae of the cotton boll weevil, *Anthonomus grandis grandis* (Boheman) (Coleoptera: Curculionidae) are offered to the parasitoid *Jaliscoa grandis* Burks, 1954 (= *Catolaccus grandis*) (Hymenoptera: Pteromalidae) for mass-rearing. Variations in the size of boll weevil larvae, produced on an artificial diet and reared in the laboratory, may be related to the luminosity of the environment. The objective of this study was to evaluate the development, survival, and morphometric parameters of cotton boll weevil larvae and pupae obtained from a rearing colony in the Entomology laboratory of Embrapa Algodão, fed an artificial diet under different photoperiods. The experimental design was completely randomized, with five treatments (photoperiods of 0:24, 10:14, 12:12, 14:10, and 24:0 (L:D)) and 260 replications. Each treatment consisted of 13 Petri dishes, with 20 boll weevil larvae per dish. The weight of boll weevil larvae and pupae and the duration of these stages were greater in the dark (00:24 hours) than in the other photoperiods (10:14 h, 12:12 h, 14:10 h, and 24:00 h), but survival was similar among photoperiods.

Keywords: *Anthonomus grandis grandis*, biology, development period, negative phototaxis.

Resumo

Larvas de terceiro ínstar do bicudo-do-algodoeiro, *Anthonomus grandis grandis* (Boheman) (Coleoptera: Curculionidae) são oferecidas ao parasitoide *Jaliscoa grandis* Burks, 1954 (= *Catolaccus grandis*) (Hymenoptera: Pteromalidae) para criação em massa. Variações no tamanho das larvas do bicudo-do-algodoeiro, produzidas em dieta artificial, para a criação de *J. grandis* em laboratório podem estar relacionadas à luminosidade do ambiente. O objetivo deste estudo foi avaliar o desenvolvimento, a sobrevivência e os parâmetros morfométricos de larvas e pupas do bicudo-do-algodoeiro, obtidas de uma colônia de criação do laboratório de Entomologia da Embrapa Algodão, alimentadas com dieta artificial em diferentes fotoperíodos. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos (fotoperíodos de 0:24, 10:14, 12:12, 14:10 e 24:0 (L:D)) e 260 repetições. Cada tratamento continha 13 placas de Petri com 20 larvas do bicudo-do-algodoeiro por placa. O peso das larvas e pupas do bicudo e a duração desses estágios foram maiores no escuro (0:24 horas) do que nos demais fotoperíodos (10:14 h; 12:12 h; 14:10 h e 24:00 h), mas a sobrevivência foi semelhante entre os fotoperíodos.

Palavras-chave: *Anthonomus grandis grandis*, biologia, período de desenvolvimento, fototaxia negativa.

1. Introduction

The introduced parasitoid *Jaliscoa grandis* Burks, 1954 (= *Catolaccus grandis*) (Hymenoptera: Pteromalidae) is used in the management of the boll weevil, *Anthonomus grandis grandis* (Boheman) (Coleoptera: Curculionidae), in Brazil. The reproductive potential and female-searching behavior of this parasitoid are high, as it locates and parasitizes weevil larvae inside flower buds, even at low host densities (Ramalho et al., 2000). This increases the importance of releasing this parasitoid to manage boll

weevil populations in cotton agroecosystems in Brazil (Ramalho and Malaquias, 2015).

Jaliscoa grandis can be reared in the natural or alternative hosts (Vanderzant and Davich, 1958; Vanderzant, 1965), but the choice of the latter depends on its cost and/or ease of rearing in the laboratory (Parra and Coelho Junior, 2022). The development of an artificial diet and mass production methods for the cotton boll weevil made it possible to multiply this parasitoid (Morales-Ramos et al., 2023). This natural enemy

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Received: May 29, 2025 – Accepted: September 16, 2025

Editor: Takako Matsumura Tundisi



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is mass-reared in third-instar larvae of the cotton boll weevil, encapsulated in cells modeled on parafilm sheets (Cate, 1987; Ramalho et al., 2000; Ramalho and Malaquias, 2015) or beeswax film (Aquino et al., 2000) and subjected to parasitism (Morales-Ramos et al., 2023). However, the weight, behavior, life cycle, and survival of immature boll weevils reared on an artificial diet differ from those of this pest collected from cotton squares fallen on the ground in cotton plantations (Vanderzant, 1965; Ramalho and Wanderley, 1996; Greenberg et al., 2005, 2008).

The body length and width, and the head capsule of boll weevil larvae collected in cotton squares and bolls are greater than those in the laboratory reared on an artificial diet under ambient light (Parrott et al., 1970; Monnerat et al., 2002). Under natural conditions, boll weevil larvae develop within cotton fruiting structures (squares and bolls), which provide a dark and protected environment throughout their immature stages. In contrast, artificial rearing systems typically expose larvae to constant artificial light during the photophase, even when partially embedded in the diet substrate. This discrepancy in light exposure between natural and artificial environments may affect key developmental and behavioral traits, representing a potentially confounding variable in experimental studies. Additionally, host size has been shown to influence sex ratio dynamics in boll weevil larvae, with reported female proportions of 70.6%, 45.1%, and 54.6% in large larvae, small larvae, and pupae, respectively (Greenberg et al., 1995). These findings highlight the need for greater standardization and control of environmental variables in laboratory rearing protocols.

The hypothesis tested in this study is that the size of cotton boll weevil larvae and pupae on an artificial diet in the dark (00:24 hours) is larger than that of those in other photoperiods (10:14 h, 12:12 h, 14:10 h, and 24:00 h). The objective was to evaluate the development, survival, and morphometric parameters of larvae and pupae of the cotton boll weevil reared on an artificial diet in different photoperiods.

2. Materials and Methods

2.1. Experiment location

The work was conducted at the Embrapa Entomology laboratory (7° 13'32"S latitude and 35° 54'19" W longitude) in the municipality of Campina Grande, Paraíba state, Brazil in 2024.

2.2. Obtaining the boll weevil

Specimens of the cotton boll weevil were obtained from a colony maintained in the Embrapa Cotton laboratory with an artificial diet (Monnerat et al., 2002) based on brewer's yeast, wheat germ, soy protein, Pharmamedia® (ADM, USA), concentrated cottonseed protein, Wesson salts, sugar, ascorbic acid, sorbic acid, methyl parahydroxybenzoate, vitamin solution, agar and distilled water (Vanderzant, 1965).

Adult weevils were transferred to plastic cages (20 x 15 x 10 cm) with the center of the lids and the bottom removed and replaced with a screen with 60 and 20 mesh, respectively, for aeration and passage of eggs and

excrement to another plastic container without a screen, of the exact dimensions, below the cage. The insects were kept in a climate-controlled room at 27 ± 2 °C and $60 \pm 10\%$ relative humidity and a photophase of 14:10 hours (L:D) until they laid eggs and 4,000 eggs were collected, washed, separated from feces, disinfested for 1 min in 0.3% benzalkonium chloride (Monnerat et al., 2002), inoculated in Petri dishes (60 mm diameter x 15 mm height), with the same diet, distributed in treatments and placed in climate-controlled chambers at 25 ± 2 °C and $60 \pm 10\%$ relative humidity for 48 hours until their larvae hatched. The bioassay was initiated with each plate divided by markings made with a fine-tipped atomic brush into eight equal parts for the random collection of larvae. The photophase was initiated at 07:00 A.M.

2.3. Development, survival and morphometric parameters of the boll weevil larvae and pupae in different photoperiods

The experimental design was completely randomized, with five treatments (photoperiods of 0:24; 10:14; 12:12; 14:10 and 24:0 hours (L:D)) and 260 replications. Each treatment consisted of 13 Petri dishes, with 20 boll weevil larvae per dish. Twenty weevil larvae were collected every two days to determine the duration and survival in the first, second, and third instars and that of larvae and pupae stages and to measure their morphometric parameters, being discarded after the evaluations.

The morphometric parameters, width of the head capsule (mm) and length and width of the body (mm), were determined using a digital stereomicroscope (Shenzhen May Kclong Technology Co. Ltd, Shenzhen, China) with 50x magnification and the hiView software for image analysis and data acquisition.

The weight of each larva, per instar, and of *A. grandis* pupae was determined on an AY220 analytical balance (Shimadzu Corporation, Columbia, MD, USA) with an accuracy of 0.0001 g.

2.4. Data analysis

The normality and the homoscedasticity of the residues of data on survival, development duration, and morphometric parameters of the first, second, and third instars and those of larvae and pupae of boll weevil were, respectively, verified using the Shapiro-Wilk and Bartlett tests. The data were then subjected to ANOVA, and the means were compared using the Tukey test ($P \leq 0.05$). Data with high variation coefficients (≥ 30) were transformed to the square root of $x + 0.5$ before statistical analysis and presented in the tables as untransformed means. The studies were performed using the Statistical and Genetic Analysis System (SAEG) of the Federal University of Viçosa (Ribeiro Junior, 2001).

3. Results

Survival in the first ($F_{4,8} = 3.63$, $P < 0.05$) and second ($F_{4,8} = 8.49$, $P < 0.01$) instars of boll weevil, with artificial diet, varied between photoperiods, but that of the third instar ($F_{4,8} = 1.33$, $P > 0.05$) and of the larval ($F_{4,8} = 1.00$, $P > 0.05$) and

pupal ($F_{4,8} = 1.00$, $P > 0.05$) stages did not. Survival in the first and second instars was higher in the photoperiods of 0:24 h and 10:14 h, and lower in the photoperiods of 12:12 h and 14:10 h, respectively (Table 1).

The duration of the first ($F_{4,39} = 3.45$, $P < 0.01$) and third ($F_{4,28} = 61.15$, $P < 0.01$) instars and of the larval stage ($F_{4,28} = 21.41$, $P < 0.01$) of boll weevil, with artificial diet, varied between photoperiods (Table 2), but that of the second instar ($F_{4,35} = 2.62$, $P > 0.05$) and the pupal stage ($F_{4,25} = 2.62$, $P > 0.05$) did not. The duration of the first instar was longer in the 10:14 h photoperiod, followed by the other longer photoperiods and shorter in the shortest (00:24 h) one, and that of the third and larval stages was longer in the shortest photoperiod (00:24 h).

The number of instars of boll weevil was three in all photoperiods (Table 3). The width of the head capsule in the first ($F_{4,63} = 8.36$, $P < 0.01$), second ($F_{4,39} = 16.63$, $P = 0.05$) and third ($F_{4,34} = 16.37$, $P < 0.01$) instars of boll weevil, with artificial diet, varied with the photoperiods (Table 3),

with higher values for the first in the 10:14, 12:12 and 14:10 hours than in the 00:24 and 24:00 hours. In the second instar, the width of the head capsule of boll weevil was similar between treatments. In the third instar, the width of the head capsule was greater in the 00:24 hour photoperiod and smaller in the 12:12 hour photoperiod. The growth rate varied with the photoperiods ($F_{4,46} = 11.29$, $P < 0.01$), following Dyar's rule (1980), with higher and lower rates in the 00:24 hour and 12:12 and 14:10 hour photoperiods, respectively.

The morphometric parameters of length, width and weight of the first ($F_{\text{length } 4,43} = 24.93$; $P < 0.01$), ($F_{\text{width } 4,34} = 21.62$; $P < 0.01$), ($F_{\text{weight } 4,43} = 351.10$; $P < 0.01$); second ($F_{\text{length } 4,43} = 24.93$; $P < 0.01$), ($F_{\text{width } 4,34} = 21.62$; $P < 0.01$), ($F_{\text{weight } 4,43} = 351.10$; $P < 0.01$) and third ($F_{\text{length } 4,43} = 24.93$; $P < 0.01$), ($F_{\text{width } 4,34} = 21.62$; $P < 0.01$), ($F_{\text{weight } 4,43} = 351.10$; $P < 0.01$) instars and pupae ($F_{\text{length } 4,43} = 24.93$; $P < 0.01$), ($F_{\text{width } 4,34} = 21.62$; $P < 0.01$), ($F_{\text{weight } 4,43} = 351.10$; $P < 0.01$) of boll weevil, originated from larvae fed with artificial diet, varied with the photoperiods,

Table 1. Survival (%) in the first, second and third instars and larval and pupal stages of *Anthonomus grandis* (Coleoptera: Curculionidae) on an artificial diet at $25 \pm 1^\circ\text{C}$, relative humidity of $60 \pm 10\%$ and photoperiods of 0:24; 10:14; 12:12; 14:10; 24:0 and 0:24 hours (L:D).

Stages	Instars	Photoperiod (L:D)	IN	Survival	FN
Larva		00:24	20	36.67 ± 3.33 a	08
		10:14	20	35.00 ± 4.16 a	07
		12:12	20	39.67 ± 3.33 a	08
		14:10	20	39.67 ± 3.33 a	09
		24:00	20	44.33 ± 6.96 a	09
	First	00:24	20	75.00 ± 4.00 a	15
		10:14	20	49.00 ± 11.37 ab	10
		12:12	20	53.67 ± 11.10 ab	11
		14:10	20	44.33 ± 6.96 b	09
		24:00	20	64.00 ± 7.00 ab	13
	Second	00:24	20	53.33 ± 6.67 b	12
		10:14	20	100.00 ± 0.00 a	10
		12:12	20	91.67 ± 8.33 a	10
		14:10	20	100.00 ± 0.00 a	09
		24:00	20	75.76 ± 12.33 ab	10
	Third	00:24	20	70.00 ± 15.28 a	08
		10:14	20	80.00 ± 20.00 a	07
		12:12	20	86.67 ± 13.33 a	08
		14:10	20	100.00 ± 0.00 a	09
		24:00	20	93.33 ± 6.67 a	09
Pupa		00:24	20	100.00 ± 0.00 a	08
		10:14	20	100.00 ± 0.00 a	06
		12:12	20	100.00 ± 0.00 a	08
		14:10	20	100.00 ± 0.00 a	09
		24:00	20	83.33 ± 16.67 a	07

Means followed by the same letter in the column within each stage or instar do not differ by Tukey's test ($p \leq 0.05$). Initial (IN) and final (FN) number of individuals.

Table 2. Duration (mean $\pm$ standard error) of the first, second and third instars and larval and pupal stages of *Anthonomus grandis* (Coleoptera: Curculionidae) on an artificial diet at $25 \pm 1^\circ \text{C}$, relative humidity of $60 \pm 10\%$ and photoperiods of 0:24; 10:14; 12:12; 14:10; 24:0 and 0:24 hours (L:D).

Stage	Instars	Photoperiod (L:D)	IN	Duration	NS
Larva		00:24	20	$17.11 \pm 0.11 \text{ a}$	09
		10:14	20	$13.14 \pm 0.55 \text{ b}$	07
		12:12	20	$12.50 \pm 0.42 \text{ bc}$	08
		14:10	20	$12.00 \pm 0.60 \text{ c}$	09
		24:00	20	$14.00 \pm 0.42 \text{ b}$	08
	First	00:24	20	$3.00 \pm 0.16 \text{ c}$	13
		10:14	20	$5.00 \pm 0.76 \text{ a}$	10
		12:12	20	$3.82 \pm 0.54 \text{ bc}$	11
		14:10	20	$3.33 \pm 0.29 \text{ bc}$	09
		24:00	20	$4.20 \pm 0.26 \text{ ab}$	15
	Second	00:24	20	$4.40 \pm 0.22 \text{ a}$	10
		10:14	20	$4.80 \pm 0.57 \text{ a}$	10
		12:12	20	$5.60 \pm 0.34 \text{ a}$	10
		14:10	20	$3.89 \pm 0.35 \text{ a}$	09
		24:00	20	$4.75 \pm 0.28 \text{ a}$	12
	Third	00:24	20	$9.67 \pm 0.17 \text{ a}$	09
		10:14	20	$3.43 \pm 0.30 \text{ c}$	07
		12:12	20	$3.25 \pm 0.54 \text{ c}$	08
		14:10	20	$4.78 \pm 0.36 \text{ bc}$	09
		24:00	20	$5.25 \pm 0.49 \text{ b}$	08
Pupa		00:24	20	$4.71 \pm 0.36 \text{ a}$	07
		10:14	20	$4.67 \pm 0.42 \text{ a}$	06
		12:12	20	$4.88 \pm 0.35 \text{ a}$	08
		14:10	20	$5.56 \pm 0.29 \text{ a}$	09
		24:00	20	$4.88 \pm 0.23 \text{ a}$	08

Means followed by the same letter in the column within each stage or instar do not differ by Tukey's test ($p \leq 0.05$). Initial (IN) and final (FN) number of individuals.

Table 3. Head capsule width (HCW) and growth rate (GR) in the first, second and third instars of *Anthonomus grandis* (Coleoptera: Curculionidae) (mean) fed with an artificial diet at $25 \pm 1^\circ \text{C}$, relative humidity of $60 \pm 10\%$ and photoperiods of 0:24; 10:14; 12:12; 14:10; 24:0 and 0:24 hours (L:D).

Treatment	Instar						XGR ²
	First		Second		Third		
	HCW ¹	GR	HCW	GR	HCW	GR	
00:24 h	0.76 ± 0.05b	-	1.31 ± 0.03a	1.89	1.97 ± 0.09a	1.52	1.70a
10:14 h	0.90 ± 0.05a	-	1.24 ± 0.01a	1.49	1.41 ± 0.03bc	1.14	1.32bc
12:12 h	0.98 ± 0.04a	-	1.17 ± 0.01a	1.20	1.33 ± 0.01c	1.48	1.19c
14:10 h	0.99 ± 0.05a	-	1.23 ± 0.01a	1.35	1.43 ± 0.05bc	1.16	1.26c
24:00 h	0.74 ± 0.04b	-	1.26 ± 0.05a	1.80	1.64 ± 0.06b	1.39	1.57ab

Means followed by the same lowercase letter in row¹ and column² do not differ by Tukey's test ($p \leq 0.05$); XRC = Mean growth ratio. (-) No data.

Table 4. Morphometric parameters, length, width and weight of first, second and third instar larvae and pupae of *Anthonomus grandis* (Coleoptera: Curculionidae) on an artificial diet at $25 \pm 1^\circ \text{C}$, relative humidity of $60 \pm 10\%$ and photoperiods of 0:24; 10:14; 12:12; 14:10; 24:0 and 0:24 hours (L:D).

Stage/instar	Photoperiod	Length (mm)	Width (mm)	Weight (mg)
Primeiro instar	00:24 h	$1.99 \pm 0.44\text{d}$	$1.06 \pm 0.23\text{c}$	$0.002 \pm 0.01\text{d}$
	10:14 h	$6.64 \pm 0.92\text{ab}$	$2.41 \pm 0.46\text{a}$	$0.021 \pm 0.02\text{a}$
	12:12 h	$7.46 \pm 0.21\text{a}$	$2.46 \pm 0.15\text{a}$	$0.020 \pm 0.00\text{a}$
	14:10 h	$4.29 \pm 0.76\text{c}$	$1.72 \pm 0.35\text{b}$	$0.009 \pm 0.00\text{b}$
	24:00 h	$5.79 \pm 0.81\text{bc}$	$2.11 \pm 0.36\text{ab}$	$0.006 \pm 0.04\text{c}$
Segundo instar	00:24 h	$6.51 \pm 0.93\text{a}$	$2.29 \pm 0.46\text{a}$	$0.009 \pm 0.01\text{c}$
	10:14 h	$7.05 \pm 0.91\text{a}$	$2.48 \pm 0.47\text{a}$	$0.024 \pm 0.03\text{a}$
	12:12 h	$7.34 \pm 0.29\text{a}$	$2.63 \pm 0.20\text{a}$	$0.019 \pm 0.02\text{b}$
	14:10 h	$7.55 \pm 0.42\text{a}$	$2.81 \pm 0.15\text{a}$	$0.022 \pm 0.00\text{ab}$
	24:00 h	$8.37 \pm 0.47\text{a}$	$2.78 \pm 0.28\text{a}$	$0.021 \pm 0.01\text{ab}$
Terceiro instar	00:24 h	$8.19 \pm 0.32\text{a}$	$2.97 \pm 0.14\text{a}$	$0.022 \pm 0.01\text{a}$
	10:14 h	$6.75 \pm 0.42\text{b}$	$2.65 \pm 0.24\text{ab}$	$0.019 \pm 0.01\text{b}$
	12:12 h	$5.26 \pm 0.30\text{c}$	$2.12 \pm 0.22\text{c}$	$0.016 \pm 0.00\text{c}$
	14:10 h	$7.52 \pm 0.41\text{ab}$	$2.73 \pm 0.22\text{ab}$	$0.021 \pm 0.01\text{a}$
	24:00 h	$6.49 \pm 0.54\text{bc}$	$2.46 \pm 0.26\text{bc}$	$0.012 \pm 0.03\text{d}$
Pupa	00:24 h	$7.81 \pm 0.29\text{a}$	$3.38 \pm 0.22\text{a}$	$0.021 \pm 0.01\text{a}$
	10:14 h	$7.26 \pm 0.17\text{ab}$	$2.91 \pm 0.14\text{b}$	$0.019 \pm 0.00\text{ab}$
	12:12 h	$6.91 \pm 0.20\text{c}$	$2.61 \pm 0.17\text{c}$	$0.019 \pm 0.02\text{b}$
	14:10 h	$7.08 \pm 0.19\text{bc}$	$2.66 \pm 0.15\text{c}$	$0.021 \pm 0.02\text{a}$
	24:00 h	$6.78 \pm 0.39\text{c}$	$3.15 \pm 0.31\text{ab}$	$0.016 \pm 0.02\text{c}$

Means followed by the same letter in the column within each instar do not differ by Tukey's test ($p \leq 0.05$).

with higher values in the 12:12 h and 10:14 h periods than in the 00:24 h period (Table 4). The length and width of the second instar larvae were similar between photoperiods, but their weight was, respectively, greater and smaller in the 10:14 h and 00:24 h photoperiods. The length, width, and weight in the third instar were greater in the 00:24 h photoperiod, with the values for the first two and the last parameters, respectively, lowest in the 12:12 h and 24:00 h photoperiods. The length and width of pupae were smaller in the 00:24 h photoperiod than in the 14:10 h and 12:12 h photoperiods. The weight of the pupa was greater in the 00:24 h and 14:10 h than in the 24:00 h photoperiod.

4. Discussion

Variations in the survival of first and second instar larvae of boll weevil, with artificial diet, under different photoperiods may be due to the blocking and/or reduction of the light beam by the thin layer of diet covering the neonatal larvae of this insect, although without effect on the more advanced instars. Larvae of these instars are larger, which increases their body exposure to light in different photoperiod regimes. Spectral transmission over one millimeter of diet decreased the passage of short

waves, reducing their incidence in early instar larvae of boll weevil (Harris et al., 1969). The greater survival of the first instar in the shortest photoperiod (00:24 h) and that of the second instar in the longest (10:14; 12:12 and 14:10 h) may be related to the morphophysiological differences between these negative phototaxic larvae, with the first being smaller, with more limited vision and responding to light stimuli differently (Perkins et al., 2008). This may also explain the similar survival rates of third instar larvae and the combined stages of larvae and pupae in different photoperiods.

Variations in the duration of the first and third instars and of the larval stage of boll weevil between photoperiods are probably due to the exposure of diets with weevil larvae to broad bands of visible radiation (LW) (463–600 nm) simulating long days, which may inhibit or stimulate the foraging and feeding of this insect by modulating the circadian rhythm of its enzymes secreted by the salivary glands (Harris et al., 1969; Field et al., 1999; Farnworth et al., 2018; Owens and Lewis, 2021). The longer duration of the first instar in the 10:14 h photoperiod, followed by the others, indicates an adverse effect of light incidence on neonatal larvae of the boll weevil, which may be related to their negative phototaxis (Wertman et al., 2018; Barros et al., 2019; Arruda et al., 2021) and, in addition, light can modulate the release of neuropeptides, such as the hatching hormone

(EH) in insects (Xu et al., 2011; Wadsworth et al., 2014). On the other hand, the increase in the duration of the third instar and of the larvae stage of the weevil in the shorter photoperiod (00:24 h) may be an adaptive mechanism to consume more food and to accumulate energy to form pupae (Scriber and Slansky Junior, 1981). This is necessary, as they complete their development, in nature, in the absence of light, inside cotton squares fallen to the ground or in bolls retained by cotton plants (Arruda et al., 2021). Similar results were reported for the immature stages of the boll weevil, showing shorter and longer development periods in both long and short photoperiods, within cotton flower buds (Greenberg et al., 2008).

The three instars of boll weevil larvae, in different photoperiods, indicate a lack of effect of this parameter in the induction of diapause in the weevil (Esperk et al., 2007; Sauders, 2012), but additional instars were reported under inducing conditions before or during the diapause (Esperk et al., 2007). Variations in the width of the cephalic capsule of the first and third instars of boll weevil fed an artificial diet are probably due to the negative phototaxis of these larvae (Wertman et al., 2018). Behavioral, genetic, and developmental evidence indicates that cryptic insects, with or without photoreceptor organs, can perceive light (Friedrich et al., 2011; Hustert and Mashaly, 2013; Tierney et al., 2015). The greater width of the head capsule of boll weevil in the first and third instars, respectively, in the longest (10:14, 12:12 and 14:10 hours) and shortest (00:24 hours) photoperiods may be related to differences in the feeding strategies of larvae of these instars (Lee et al., 2021). Larvae of this insect secrete compounds from the salivary gland or midgut, which are modified in the midgut differently in each instar of this insect, especially the regurgitated material (Moura et al., 2022). This effect may be due to light-induced changes in the circadian rhythm of the enzymes secreted by these glands (Field et al., 1999; Xu et al., 2011), which may also explain the higher growth rates of the second and third instars of boll weevil in the 00:24 hour photoperiod, following Dyar's rule and without additional instars.

Variations in the morphometric parameters of length, width and weight in the first, second and third instars and pupae of boll weevil, originated from larvae fed with an artificial diet, in different photoperiods can be attributed to morphophysiological differences between instars, with photoreceptor sensory organs less sensitive to light in neonatal larvae than in those of the final ones (Perkins et al., 2008; Despland, 2018; Vásquez-Ordóñez et al., 2020). This may partly explain the greater length, width, and weight of first instar weevil larvae in the 12:12 h and 10:14 h photoperiods than those of more advanced instars, which are more sensitive to light. On the other hand, the smallest and largest length, width and weight of the first and third instar in the dark (00:24 h) may be related to the period of these instars in this photoperiod.

In conclusion, the survival of larvae fed with an artificial diet and of boll weevil pupae was similar between photoperiods. Still, the duration of these immature stages was longer in the dark (00:24h). The width of the head capsule, in the last instar of boll weevil fed with an artificial diet, was greater in the dark (00:24h). The morphometric parameters length, width and weight of the body in the last

instar of boll weevil, with an artificial diet, were greater in the dark (00:24h) and similar in that of 14:10 h and the width of those in the photoperiod 10:14 h. The length, width, and weight of boll weevil pupae, with artificial diet, were greater in the dark (00:24h), the length and weight of pupae at 10:14 h, and the weight at 14:10 h. photoperiods. The hypothesis that cotton boll weevil larvae and pupae on an artificial diet in a dark environment (00:24 hours) are larger than those in other photoperiods (10:14 h, 12:12 h, 14:10 h, and 24:00 h) was confirmed. This may improve the quality of third-instar larvae of boll weevil reared in the laboratory to mass produce the parasitoid *J. grandis* with a higher proportion of females.

Acknowledgements

To the Brazilian agencies "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (Finance Code 001).

Data Availability Statement

Data is available upon request.

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