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#### **ORIGINAL**



# Biological aspects of *Carmenta foraseminis* (Busck) Eichlin (Lepidoptera: Sesiidae)

# Aspectos Biológicos de Carmenta foraseminis (Busck) Eichlin (Lepidoptera: Sesiidae)

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# **ABSTRACT**

This study characterized the biology of *Carmenta foraseminis* (Lepidoptera: Sesiidae) on cocoa and elucidated its life cycle under controlled conditions. Field observations were conducted in infested plantations in Juanjui, Rumisapa, and Tabalosos (San Martín, Peru). To determine the life cycle, 78 eggs collected from cocoa pods were used; newly hatched larvae were reared individually on a modified *Anticarsia gemmatalis* artificial diet. Larval instars were determined by measuring head-capsule width. Adults were diurnal; females oviposited mainly between 11:00 and 14:00 h on pods at 3-5 months of development. The mean number of eggs per pod was  $8,50 \pm 2,9$ . In the field, no adult feeding, courtship, or copulation was observed. At  $25 \pm 1^{\circ}$ C,  $71 \pm 1$  % RH, and a 14L:10D photoperiod, the egg-to-adult developmental time was  $56,35 \pm 2,7$  days. Egg incubation lasted  $7,58 \pm 0,51$  days. The larva had five instars with mean durations of  $5,15 \pm 0,36,5,03 \pm 0,19,6,06 \pm 0,23,5,12 \pm 0,33$ , and  $13,18 \pm 0,41$  days; the pupal stage lasted  $14,23 \pm 0,60$  days. Adult emergence occurred from 09:00 to 11:00 h; adult longevity in captivity was  $5,31 \pm 0,48$  days with a 1:1 sex ratio. These baseline parameters support artificial-diet rearing and provide a foundation for management research, including biological-control assays.

Keywords: Carmenta foraseminis; Cocoa; Oviposition; Artificial Diet; Head Capsule; Peruvian Amazon.

## **RESUMEN**

El objetivo fue caracterizar los aspectos biológicos de *Carmenta foraseminis* (Lepidoptera: Sesiidae) en cacao y elucidar su ciclo de vida bajo condiciones controladas. El trabajo de campo se realizó en plantaciones infestadas en los distritos de Juanjuí, Rumisapa y Tabalosos (San Martín, Perú). Para el ciclo biológico se emplearon 78 huevos colectados en frutos; las larvas se individualizaron y criaron en una dieta artificial basada en la formulación de *Anticarsia gemmatalis* (modificada). El número de estadios se determinó mediante la medición del ancho de la cápsula cefálica. Los adultos presentaron hábitos diurnos; las hembras ovipositaron principalmente entre 11:00-14:00 h sobre frutos de 3-5 meses de desarrollo. El número medio de huevos por fruto fue  $8,50 \pm 2,9$ . No se observaron, en campo, procesos de alimentación, cortejo ni cópula. A  $25 \pm 1\,^{\circ}$ C,  $71 \pm 1\,^{\circ}$  HR y 14L:10O, la duración del ciclo huevo-adulto fue  $56,35 \pm 2,7$  días. La incubación del huevo duró  $7,58 \pm 0,51$  días. La larva presentó cinco estadios con duraciones promedio de  $5,15 \pm 0,36;\,5,03 \pm 0,19;\,6,06 \pm 1.00$ 

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0,23;  $5,12 \pm 0,33$  y  $13,18 \pm 0,41$  días; la pupa duró  $14,23 \pm 0,60$  días. La emergencia de adultos ocurrió entre 9:00-11:00 h; la longevidad en cautiverio fue 5,31 ± 0,48 días con razón sexual 1:1. Estos resultados respaldan la cría en dieta artificial y proveen parámetros básicos para estudios de manejo (p. ej., ensayos con enemigos naturales).

Palabras clave: Carmenta foraseminis; Cacao; Oviposición; Dieta Artificial; Cápsula Cefálica; Amazonía Peruana.

#### **INTRODUCTION**

Cocoa (Theobroma cacao L.; Malvaceae) is a strategic crop for the Peruvian Amazon and, in particular, for the San Martín region. Among its limitations, the cacao borer, Carmenta foraseminis (Busck) (Lepidoptera: Sesiidae), stands out. This species was initially reported in Panama, boring into the seeds of Gustavia superba (H.B.K.) Berg (Lecythidaceae).(1) Other host plants recorded for Panama were Gustavia angustifolia (H.B.K) Benth (Lecythidaceae) and Eschweilera sp (Lecythidaceae). In the neotropical region of Venezuela, Colombia, Brazil, and Peru, cacao has been documented as the primary host. (2,3,4,5) It is currently considered an economically important pest for T. cacao cultivation in Venezuela, Colombia, and Peru, because its larvae cause perforations and promote the entry of moisture and pathogens that damage the fruit. (3,5,6,7,8,9,10,11,12,13) In the San Martín region, located in the high jungle of Peru, the pest has spread widely and high incidences have been recorded with damage aggravated by association with Phytophthora palmivora (E. J. Butler) E. J. Butler "brown rot" and Moniliophthora roreri (Cif & Par) "moniliasis," causing considerable damage to cocoa fruit. (14)

Previous studies on this species have primarily focused on morphology, types of damage, reproduction, and incidence. (3,5,8,13) However, critical gaps remain: (i) detailed characterization of larval stages based on the cephalic capsule for objective instar delimitation; (ii) documentation of oviposition behavior under natural conditions (time windows and selection of fruit phenological stage); and (iii) adaptation to artificial diet allowing standardized rearing in the laboratory. This last aspect is key, as cocoa fruits are perishable and make continuous rearing difficult, limiting basic ecology and biological control trials. (7,11,13)

In this context, the objective of this study was to describe oviposition behavior in the field and characterize the biological cycle of C. foraseminis under controlled conditions, using an artificial diet (derived from the modified Anticarsia gemmatalis formulation). The durations per stage were estimated, the number of instars was established based on the distribution of cephalic capsule width, and adult parameters (emergence, longevity, and sex ratio) were documented. These results provide reference parameters for monitoring and lay the groundwork for management trials, including the evaluation of biological control agents.

# **METHOD**

## Study area

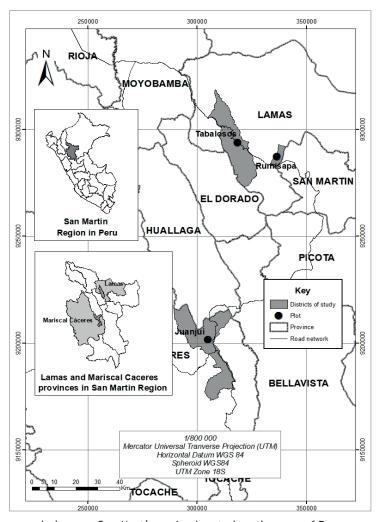
The study was conducted between 2018 and 2022. Biological data were recorded in three districts of the San Martín region, Peru: (1) Tabalosos, province of Lamas (UTM 318529, 9294007), altitude 641- m above sea level, average temperature 24,5°C, and annual precipitation 1444 mm; (2) Juanjuí, Mariscal Cáceres province (UTM 304947, 9201596), 577 m above sea level, 26,6°C and 1441 mm; and (3) Rumisapa, province of Lamas (UTM 336619, 9287333), altitude 340 m above sea level, with an average temperature of 25,2°C and annual precipitation of 1320 mm (figure 1). (15) All sites have cocoa plantations with 10 years of production and confirmed history of the pest.

The biological cycle study was conducted at the Insect Breeding Laboratory of the Faculty of Agricultural Sciences, National University of San Martín (University City, Morales District, San Martín Province), under controlled conditions as described in the corresponding subsection.

# Evaluation of biological aspects

Field observations were made to characterize the biology of C. foraseminis. The sessions were continuous from 6:00 a.m. to 7:00 p.m. for two weeks, and subsequently from 9:00 a.m. to 5:00 p.m. every month for one year. The following were recorded in the field: (i) the feeding activity of adults on flowers and fermented fruit fallen to the ground, both inside the plantations and in the surrounding vegetation; (ii) courtship and copulation behaviors; and (iii) oviposition behavior, including time of laying, phenological state of the host fruit, and number of eggs laid per female.

In addition, the sexual attraction of males was evaluated using traps baited with virgin females reared in the laboratory, used as a natural source of sex pheromones. Each trap consisted of a tube 15 cm long and 10 cm in diameter, with the inner walls coated with petroleum jelly to retain the insects. Inside, a smaller tube, 2 cm in diameter, wrapped in tulle, was placed, containing the virgin female. Fifteen traps per hectare were installed on cocoa plants to evaluate the attraction and capture of males.



**Figure 1.** Location of the sampled areas: San Martín region located on the map of Peru, map of the San Martín region showing the two provinces (Lamas and Mariscal Cáceres), and map of the districts (Tabalosos, Rumisapa, and Juanjui) where the study plots are located

# Evaluation of the biological cycle Artificial diet and preparation

<b>Table 1.</b> Composition of modified artificial diet for Anticarsia gemmatalis					
Components	Quantities	Modifications			
Kidney beans	37,50 g	Huasca beans			
Wheat germ	30,00 g				
Soy protein	15,00 g	Soybean meal			
Casein	15,00 g	Powdered milk			
Brewer's yeast	18,75 g				
Vitamin solution	4,50 ml				
Ascorbic acid	1,80 g				
Sorbic acid	0,90 g				
Nipagin	1,50 g				
Tetracycline	56,50 mg	Chloramphenicol			
Formaldehyde	1,80 ml	Not used			
Agar	11,50 g				
Distilled water	600 ml				
Source: Greene et al. (16)					

An artificial diet based on the modified Anticarsia gemmatalis standard formulation was used (table 1). (16) The diet was prepared using a gas stove, where 37,50 g of beans were cooked in a pressure cooker in 1 000 ml of water for one hour. After cooking, the water was removed and the beans were left to cool at room temperature. The beans, proteins, and carbohydrates were then placed in a blender, and 500 ml of distilled water was added; the contents were blended. In a metal container, 100 ml of distilled water was boiled, and agar was added. The bean and agar mixture was immediately placed in a container and brought to a boil, stirring the contents with a spatula until homogenized. It was removed from the heat, put in an electric mixer, and allowed to cool to 65°C. Next, the anticontaminants and vitamins were added. It was removed from the mixer to distribute the diet into Petri dishes and allowed to solidify at room temperature before being stored in a refrigerator at 10°C.

## Breeding, environmental conditions, and handling

The biological cycle began with freshly laid eggs (n = 78) collected in the field. In the laboratory, the eggs were confined to an artificial diet in Petri dishes and incubated at a temperature of 25 ± 1°C, with a relative humidity of 71 ± 1 % and a photoperiod of 14L:10D. The hatched larvae were then placed individually in Petri dishes containing the artificial diet. The diet was renewed every 10 days.

#### **Determination of instars**

The number of instars was determined by daily measurement of the width of the cephalic capsule of each larva, using a stereomicroscope with a calibrated ocular micrometer (magnifications 1×, 3×, and 5×). Stage assignment was based on the frequency distribution of capsule width and observation of exuviae/moults.

## Pupa and adult stages

At the onset of pupation, individuals were removed from the diet and transferred to Petri dishes with moistened cotton; they were then placed in breeding boxes until emergence. Pupal duration and adult emergence time were recorded.

Adults (females and males) were kept in breeding cages and fed a sugar water solution. In captivity, reproductive processes (courtship, copulation, and oviposition) were evaluated, longevity was recorded, and the sex ratio was determined.

## **RESULTS**

# Evaluation of biological aspects

Under natural conditions, no feeding, courtship, or copulation behaviors were observed in Carmenta foraseminis; likewise, traps baited with virgin females failed to capture males. Females were observed most frequently between 11:00 a.m. and 2:00 p.m. on sunny days (n = 15) and exhibited rapid flight, similar to that of a wasp.

During oviposition, females hovered around the fruit, landed, and explored the surface with their ovipositor using lateral movements until they found cracks or depressions in the shell, where they laid their eggs. After each oviposition, they flew away briefly and returned to continue laying eggs.

The behavior of one female was continuously recorded; she laid 18 eggs in two fruits (9 eggs per fruit) over a period of 34 minutes. In general, females oviposited in fruits that were 3-6 months old, most frequently in fruits that were 3-5 months old. A preference for previously infested fruits with larvae developing inside was observed. The average number of eggs per fruit was 8,50 ± 2,9 (n = 40). The eggs were laid individually and in isolation; clusters of 2-3 eggs were found only occasionally.

# Biological cycle

The egg (figure 2A) is brown and hard in consistency, ovoid in shape, flattened dorsoventrally, with fine longitudinal striations, measuring on average 0,54 ± 0,03 mm in length and 0,41 ± 0,02 mm in width. And the average time to hatching was  $7,58 \pm 0,51$  days.

The larva goes through five stages according to the frequency distribution graph of the larval cephalic width (figure 3). The measurements of the developmental stages and cephalic capsule width for each stage are shown in table 2. The first-stage larva (figure 2B) is hyaline in color, with a light brown head, small hyaline setae on the body, and a light brown plaque on the dorsal side of segment T1. The larva is very active, moving quickly, and penetrates the diet, settling at the bottom of the plate to feed. The average duration was  $5,15 \pm 0,36$  days. In the second stage, it is bright white, the head remains light brown, and the mandible is dark brown. The setae on the body turn light brown, and the dorsal plate of segment T1 is more noticeable. The average duration was 5,03 ± 0,19 days. The larva in the third stage (figure 2C) retains the characteristics of the previous stage, with more noticeable setae. The brown color is more evident on the dorsal plate of segment T1, and two diffuse bands in the form of V-shaped lines are observed. It lasts an average of 6,06 ± 0,23 days. In the fourth stage, the larva is milky white in color, with the head remaining light brown and the jaw area dark brown. The setae

on the body are more developed. The dorsal plate of segment T1 becomes light in color, the V-shaped bands are brown, and it lasts an average of  $5.12 \pm 0.33$  days. The larva in the fifth stage (figure 2D) exhibits similar characteristics to those of the previous stage; the color changes from white to a yellowish hue, the dorsal plate of segment T1 becomes lighter in color, and the brown V-shaped bands are more pronounced. Finally, the larva stops feeding, builds a cocoon with silk covered with dietary residues, and begins the prepupal stage (figure 2E).

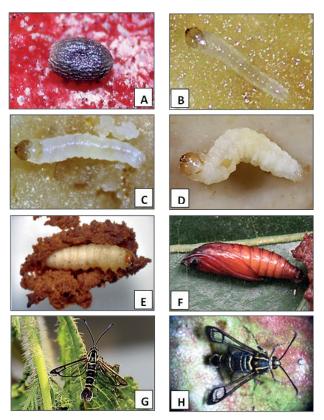


Figure 2. A) Carmenta foraseminis egg on cocoa, B) first instar larva, C) third instar larva, D) fifth instar larva, E) prepupa, F) pupa, G) male adult on leaf, H) female adult on cocoa fruit

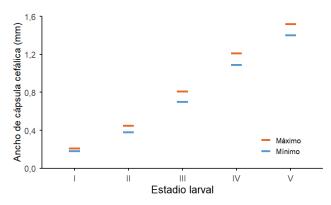


Figure 3. Frequency distribution of the width of the cephalic capsule of C. foraseminis larvae under laboratory conditions

<b>Table 2.</b> Measurements of stages, larval stages, cephalic capsule width, and survival during the study of the biological cycle of <i>Carmenta foraseminis</i> in the laboratory						
Stage	Stage	Length (mm)	Cephalic capsule (mm)	n	Survival rate (%)	
	Larva I	$1,80 \pm 0,41$	$0,20 \pm 0,00$	76	95	
	Larva II	$4,07 \pm 0,69$	$0,41 \pm 0,02$	64	80	
Larva	Larva III	$7,53 \pm 0,51$	$0,76 \pm 0,01$	62	77,50	
	Larva IV	11,97 ± 0,81	1,13 ± 0,03	59	73,75	
	Larva V	$14,83 \pm 0,83$	1,45 ± 0,01	57	71,25	
Pupa		-	-	57	100	

The pupa (figure 2F) is light brown in color, with prominent eyes. The antennae extend toward the ventral side, where the future wings are grouped. The abdomen has 10 segments and features dorsal lines with spines. The pupal period lasted  $14,23 \pm 0,60$  days. The adults broke out of the pupal exuviae, left the cocoon, and moved to the top of the cage to spread their wings.

# Adult and cycle duration

The egg-adult cycle lasted an average of 56,35 days. Adults emerged between 9:00 and 11:00 a.m. They are black in color, with two parallel yellow bands on the back of the thorax. The abdomen is black with yellow bands delimiting the segments. The male (figure 2G) has a tuft of black hairs at the end of the abdomen, and its wings are transparent with black venation. The female (figure 2H) has a thicker abdomen than the males. The average wingspan of females was  $15,33 \pm 0,52$  mm, and that of males was  $15,14 \pm 0,37$  mm. The average adult longevity was 5,31 ± 0,48 days, and the sex ratio was 1:1, as determined from 57 adults, comprising 30 males and 27 females. In captivity, the adults remained inactive and did not feed spontaneously; however, they ingested sugar solution when drops were placed near their proboscis.

## DISCUSSION

Under natural conditions, we did not record adult *C. foraseminis* feeding on sugar sources (flowers/nectar). Adult Sesiidae are rarely observed in the field, although some species are known to frequent flowers for feeding. (17,18) We were also unable to attract males with virgin females as a source of pheromones. In contrast Le et al. (19) identified sex pheromones capable of attracting Carmenta mimosa males (20) in the field, suggesting that a similar approach could be applied to this species.

We confirmed diurnal habits in C. for as  $e^{(9,21,22)}$  a typical pattern in the Sesiidae. (17,18,21,23,24,25) We did not observe mating or copulation in the field, which is common given the low detectability of this insect.<sup>(25)</sup> According to Carabelí et al. (22), mating in C. foraseminis could occur in the morning, after the emergence of adults, as occurs in Synanthedon exitiosa (Say). (23) In our study, C. foraseminis females in natural conditions oviposited between 11:00 a.m. and 2:00 p.m., and we did not observe any activity after that time. In contrast, S. exitiosa can begin oviposition in the morning and continue until dusk. (23)

The oviposition behavior observed, lateral exploration with the ovipositor and preference for cracks in rough fruit, coincides with previous reports for *C. foraseminis*<sup>(10,22)</sup> and with that described for *C. theobromae*, which lays eggs on the stems of *Psidium guajava* L. with growth cracks or cuts caused by anthropogenic action. (25)

C. foraseminis repeatedly lays its eggs in damaged fruit containing developing larvae. A similar case was observed in infestations of Synanthedon pictipes (G&R) and S. exitiosa, whose volatile compounds emanating from the damage caused by these Sesiidae further stimulated their oviposition. (26,27) The most severe infestations of *C. foraseminis* occurred in fruit at 3-5 months of phenological development, as observed by Cabezas et al. (28).

The average number of eggs per fruit is lower than that reported by Carabelí et al. (22) and Cubillos (10). C. foraseminis eggs are cryptic and almost invisible to the human eye(10), similar to those of C. theobromae. (25) The eggs are laid individually and distributed in isolation without a defined pattern, (22) and it is rare to find two to three eggs laid together. In contrast, in C. mimosa and C. theobromae, eggs may be laid individually or in groups. (24,25) The morphological characteristics of the eggs are similar to those described by Delgado et al. (3), Carabelí et al. (22), and Cubillos (10). In terms of size, they resemble the eggs of C. mimosa (20), are relatively larger than the eggs of C. theobromae<sup>(25)</sup>, and smaller than the eggs of Carmenta haematica (Ureta). The duration of the egg was similar to that reported by Carabelí et al. (22). The larvae begin to emerge between 5:30 p.m. and 6:30 p.m., a period that can extend into the night. In contrast, in C. haematica, emergence occurs at night. (25)

We confirm that C. foraseminis larvae go through five stages, as specified by Carabelí et al. (22). The morphological characteristics of the first-stage larva described in this study are similar to those described by Cubillos(11) and Carabelí et al.(22). Its length is 1,80 mm, relatively greater than that described by (22). In the final stage, the larva becomes photophobic as it approaches the end of its development and constructs a cocoon within which it pupates. (3,11,22)

The pupa is light brown in color, and its morphological details are described by Delgado<sup>(3)</sup>. The pupal period recorded in this study, at 25  $\pm$  1°C and 70  $\pm$  1 % RH, was 14,23 days, which is more extended than the 12 days reported by Carabelí et al.  $^{(22)}$  at 24,5  $\pm$  2  $^{\circ}$  C and 70  $\pm$  5 % RH. Morillo et al.  $^{(29)}$  report 12,78 days for C. theobromae at  $28 \pm 5$ °C and  $70 \pm 15$  % RH, and Forno et al. (24) indicate a period of 19 days for C. mimosa at a temperature of 26°C during the day and 21°C during the night and 55 % RH. the duration of the pupal stage varies with temperature; at higher temperatures, the pupal period of C. haematica decreased considerably from 22,6 days at 26°C to 15,5 days at 30°C. (25)

Adults are black with yellow banded patterns and transparent wings. (1,3) A peculiarity that should be highlighted is their relative resemblance to a Vespidae wasp, a characteristic common in Sesiidae. (20,30) Adults of C. foraseminis, under laboratory conditions, emerged between 9:00 and 11:00 a.m., while Herrera et al. (9) found a peak between 11:00 a.m. and 12:00 p.m. In general, several genera of Sessidae, including the genus

*Carmenta*, emerge in the morning hours, both in natural conditions and in captivity. (23,24,29,31) The sexual ratio of C. *foraseminis* was 1:1, as reported by Cubillos<sup>(11)</sup>. In contrast, Herrera et al. (9) indicate a sexual ratio of 3:4. The average adult lifespan of C. *foraseminis* under laboratory conditions was 5,31 days, which is very similar to the report by Carabelí et al. (22), who describe an average of 5,04 days. Other *Carmenta* species had identical lifespans. (24,29)

We did not observe courtship, copulation, or oviposition under laboratory conditions. However, there is evidence of reproductive success in Sessidae under captive conditions. (24,25,32,33) The low activity and sparse spontaneous feeding observed are consistent with the short adult life of these moths and the tendency of adults not to feed. (34) reproduction in captivity likely requires sensory cues (host volatiles, substrate structure, light spectrum) that are absent under standard conditions.

## **CONCLUSIONS**

Carmenta foraseminis has a well-defined biological cycle under controlled conditions, with an average duration of 56,35 days from egg to adult, passing through five larval stages. Females exhibit diurnal habits, ovipositing mainly between 11:00 a.m. and 2:00 p.m. on cocoa fruits 3 to 5 months into development, with an average of 8,5 eggs per fruit. The species did not feed or mate in the field, and its rearing on an artificial diet based on modified *Anticarsia gemmatalis* proved viable for laboratory studies. These results provide essential information for understanding the biology of this pest and constitute a solid basis for future integrated management programs with the potential to optimize sustainable biological control strategies in cocoa production systems in the Peruvian Amazon.

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#### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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