



Life Cycle of *Chilo zacconius* Blensynski (Lepidoptera: Crambidae) on Artificial Diet in The Laboratory

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Abstract

The African striped stemborer *Chilo zacconius* (Blensynski) is a major pest of rice in Nigeria. The life cycle of the African striped stemborer *C. zacconius* was assessed under laboratory conditions. The egg incubation period was 5.08 ± 0.19 days, while the mean duration of the I, II, III, IV, V instars were 4.97 ± 0.14 , 6.73 ± 0.28 , 8.13 ± 0.38 , 9.00 ± 0.46 , 8.00 ± 0.31 days respectively. Pupal duration was observed in 6.00 ± 0.19 days while the average emergence of the adult was 3.42 ± 0.15 days. Fecundity rate of the striped stem borer ranged from 80-150 eggs. The adult life span of the stem borer ranged between 3 to 4 days while the total life cycle is completed within 40 to 65 days. The morphometrics of all stages were also recorded accordingly. Additional research is still required to determine the optimal artificial diet for the mass rearing of *C. zacconius*, as this will enhance the development of tailored strategies to mitigate its harm while fostering the sustainability of rice farming.

Keywords: Artificial diet, *Chilo zacconius*, Life stage duration, Rice, Morphometrics.

INTRODUCTION

Rice is a common cereal and staple food that sustains over half of the world's population, globally (Muthayya *et al.*, 2014). It holds significant economic value as a cash crop while also serving as a dietary mainstay for millions of people (Munira and Roy, 2012; Hussain *et al.*, 2015). Recently, significant increase has been recorded in rice production sector in Nigeria, according to Sasu (2022), the production of paddy rice in Nigeria was estimated at 8.3 million metric tons while the milled rice production simultaneously was estimated at 5.4 million metric tons in 2022, solidifying the current status of Nigeria as the foremost producer of rice on the African continent (Danbaba *et al.*, 2019; Sasu, 2022; Sasu, 2023). Although rice production in Nigeria has significantly increased, there is a shortfall in production as the recent surge in demand for over 7 million metric tons of milled rice in Nigeria continues to outpace supply. The increase in rice production observed is due to

the inclusion of investors in rice production systems, increased cultivation, increased land availability, and intense cropping cycle integrated into the rice production systems in Nigeria. Despite this huge effort, the yield of rice remains abysmally low with a production estimate at 2400 kg/ha in contrast to China's impressive output of a 5,987.0 kg/ha (CEICDATA, 2023). Insect pests have been implicated as a major cause of yield loss in rice fields. Infestations by insects are grouped based on the part of plant they attack which includes the bud borers, plant hoppers, panicle feeders, root feeders, grain suckers and stemborers (Heinrich and Barrion, 2004; Ogah and Nwilene, 2017; January *et al.*, 2020; Togola *et al.*, 2020).

The rice stem borers remains a major menace in rice fields globally, they are explicit in their damage in rice tillers, during the vegetative and reproductive phases causing dead heart in young leaf shoots and white head symptoms in the panicles respectively. One of the major rice stem borers in Nigeria is the African striped stem borer *Chilo zacconius* Bleszynski, a crambid moth which remains a predominant rice stemborer in West Africa (Heinrich and

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Barrion, 2004). They are among the major rice stemborer species reported to have occurred in previous years in humid forest and savanna zones according to Akinsola (1990). The geographical distribution of *C. zacconius* has been documented in Benin, Cameroon, Côte d'Ivoire, Ghana, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, and Sierra Leone (Heinrich and Barrion, 2004).

Chilo zacconius is a pernicious stemborer that operates surreptitiously, causing substantial reductions in crop productivity within rice fields. According to Imolehin and Ukwugwu (1992) rice fields in Ibadan, Nigeria was reportedly wiped off due to the infestations by *C. zacconius* along with the white stemborer *Maliarpha separetella* and the pink stem borer *Sesamia calamistis*. The African striped stemborer *C. zacconius* undergo complete metamorphosis with only the larval stage inflicting intrinsic damage in rice plants. They feed on the young shoots of the rice tillers during the vegetative phase and the infested young tiller shoot dries out and turns brown hence termed 'dead heart'. However, rice plants may recover from this damage by compensating with newer growth of tiller shoots due to the active growth and meristematic division of the apical portion of the leaves at the vegetative stage of development (Dale, 1994; Pathak and Khan, 1994; Heinrich and Barrion, 2004).

This is in contrast to the damage observed during the reproductive phase, during this phase, the larva feeds and lodge within the tillers at the upper inter nodes with closer proximity to the panicles. The larva of *C. zacconius* have preference for the soft/succulent and nutrient dense portion of the rice tillers. Their boring activities goes on without notice subsequently interrupting the flow of nutrients to the panicles which bears the rice seeds. The panicles ends up empty and chaffy without any filling or partially filled if attacked during the grain filling stage. The indicative symptom at this stage is the uniformly characteristic beige color of the panicle termed "whiteheads". The disruption of nutrient supply within the tillers as a result of the feeding activities by *C. zacconius* may occur at any stage in the reproductive cycle, including heading/panicle initiation, leading to abortion of emerging panicles or during grain filling, impeding the milk filling in seeds (Dale, 1994; Ogah and Nwilene, 2017). The damage by *C. zacconius* at the reproductive phase is completely irreversible and cannot be

compensated for by the rice plants, this impacts the paddy yield negatively during harvest (Adewoye *et al.*, 2021). Unfortunately, there are no physical early warning signs for prompt control as symptoms are seen when the damage has been done. However, Wan *et al.* (2022) have recently unveiled a hyper-spectral imaging technique to discern the extent of infestation by striped stem borers, thereby underscoring the gravity of the damage inflicted on rice paddies by these pests.

Thus, in order to combat the obnoxious pest *C. zacconius*, the management relies exclusively on synthetic insecticides, which not only harm non-target organisms but also prove insufficient due to the intrinsic characteristics of the larval stage of *C. zacconius*. Few but significant efforts have been made on developing resistant rice varieties against this stem borer, more research needs to be done in order to understand the bioecology of *C. zacconius*. In this research, we assessed the various stages of the life cycle of *C. zacconius*, from egg to adult stage, and explored the larval development on artificial diet. The life cycle of *C. zacconius* involves distinct stages from egg to adult, each with its own unique characteristics and ecological implications. By examining the life cycle of *C. zacconius*, we will understand the behavior, reproduction, and potential control in future research.

MATERIALS AND METHODS

Collection of Eggs of *Chilo zacconius*

The eggs of *C. zacconius* were collected from the upper midrib of the rice leaves, sterilized with distilled water, and allowed to dry (Peddakasim *et al.*, 2018). Eggs were counted and transferred into a collecting tube of 15mls and plugged with damp cotton wool to prevent dehydration, the tubes were carefully set aside under normal laboratory conditions at a temperature of $28 \pm 5^\circ\text{C}$ and relative humidity of $72 \pm 5\%$ with a photoperiod of 12 h light and 12 h darkness (12L: 12D), the eggs were carefully monitored daily till larva emerged. The incubation period was indicated as the number of days it takes for eggs to hatch into the first instar larva.

Artificial Diet Preparation for larval growth of *C. zacconius*

To ascertain the morphometrics, developmental, and reproductive biology of *C. zacconius*, a wheat germ-based diet was formulated with minor alterations and supplemented with linseed oil to mitigate wing

malformation (Mc Morran 1965;Grisdale, 1973). The formulation was compounded at the Entomological Research Laboratory of the University of Lagos (Table 1).

Table 1: Composition of wheat germ-based diet for larval rearing (2 liters)

S/NO	INGREDIENTS	QUANTITY (g/ml)
1	Agar	34.72g
2	Casein	70 g
3	Sucrose	70 g
4	Wesson's salt	20 g
5	Distilled Water	1720 ml
6	Toasted wheat germ	61.39 g
7	4 M KOH	10 ml
8	Linseed oil	10 ml
9	Choline chloride	2 g
10	Vitamin solution	20 ml
11	Methyl paraben	5g
12	Gentamicin	4.2g
13	Ascorbic acid	8g

Source: Mc morrans (1965) and Grisdale, (1973)

Diet Preparation Procedure

All ingredients were weighed accordingly and mixed except Agar which was prepared separately. The amount of water needed was also portioned into two, with 800mls kept in the freezer to make ice. Agar was weighed and subsequently kept aside while 300mls of water was allowed to boil on a cooker for agar mix preparation. This was stirred continuously into hot water to prevent lumps while making it into a viscous liquid. The Agar mix was transferred immediately into a Blender, after which 800g of frozen distilled water (ice cubes) was added to cool the temperature of the mixture; this is to prevent denaturing of the vitamins added to the dry mix (Hervert *et al.*, 2016). The other portion of the diet (dry diet) was added and mixed thoroughly together in a blender. The diet was poured immediately before it starts to set into sixty 20 ml plastic cups, while the remaining content was poured into a storage container (500g) and kept in the refrigerator for future use. Fresh ones were made after one month and the previous remains were disposed off. The plastics were punched with a needle to prevent

condensation in the container while the larva feeds. A clean fork was also used to create horizontal lines across the diet to make the diet accessible for consumption. Diet was changed every 5 to 7 days for freshness to prevent possible infections among the larvae. A reservoir of the infested tillers were inoculated on the parent plants with the eggs of *C. zacconius* and set aside for development.

Morphometrics of Larva and Pupa of *Chilo zacconius*

Newly emerged larvae of *C. zacconius* were introduced into 20 ml plastic jars containing 5g of wheat germ-based artificial diet. Twenty larvae were used per batch and replicated thrice. They were arranged in the rearing unit under normal laboratory conditions at a temperature of $28 \pm 5^\circ\text{C}$ and relative humidity of $72 \pm 5\%$ (12L:12D), and were examined daily to check for the presence of exuvia which indicated new instar, data was taken from the first ten larvae to shed their exuvia. The total larval period was terminated by the onset of pupation (Peddakasim *et al.*, 2018).

Larval length and weight: From each diet-containing jar, the first 5 larvae to emerge were measured using a metric ruler and recorded the length, in millimeters, additionally, their weight was recorded per instar using a digital weighing scale (Model: SF-400C3) and were subsequently recorded per instar.

Larval Period: This is indicated by the number of days from the days the eggs hatched into 1st instar larva till the larvae transformed into a pupa. The average larval period was deduced from the first ten larvae to pupate.

Pupal period: The newly moulted pupa were kept on a damp filter paper placed inside a circular container measuring 10 cm by 7 cm. They were monitored daily for emergence into adults while the morphometrics and duration was subsequently documented.

Pupal length and weight: Out of the pupae kept for adult emergence observation, five matured pupae were taken randomly, measured, and weighed respectively.

Adult Longevity: This is the life duration for an adult moth before dying, this was also noted and recorded accordingly.

Total developmental period: The total developmental period was calculated by adding the incubation period, larval period, pupal period, and adult developmental days.

Fecundity: The average number of eggs laid all through the adult stage of the female (5) were calculated and recorded (Neupane and Subedi, 2019). Data were analysed using OriginPro software. Each experiment was replicated three times and values were expressed as mean and standard error.

RESULTS

Collection of eggs from the field

Eggs of *C. zacconius* collected from the upper surface of the rice leaves were covered with strands of white hairs with an average cluster of about 150 eggs. They appeared pale yellow and were rinsed and kept in a tube covered with damp wool for larval emergence. The eggs hatched within 4-6 days with the emergence of the larvae.

Morphometrics of *Chilo zacconius*

Larvae were later transferred from the tubes unto the diet for feeding and growth assessment. The larvae of *C. zacconius* were observed to be cream in color with five pink purple bold stripes on the dorsal part. The arrangement of the crochet on the abdominal legs of the larva were prominent in the third to

fifth instar larva; a major morphological identification key among larval rice stem borers (Meijerman and Uleberg, 1996) The larval morphometrics investigated in this study revealed that weights from the first instar larva to the fifth instar larva ranged from $0.01 \pm 0.00\text{g}$ to $0.43 \pm 0.06\text{g}$ while the length from the first instar larva to the fifth larva ranged from $4.97 \pm 0.14\text{ mm}$ to $15.26 \pm 0.19\text{ mm}$ (Table 2) Significant mortality was recorded throughout the larval developmental phases while using the wheat based diet.

The color of pupa was brown, with a lighter shade apparent in the head and thoracic area during its initial pupal phase. As it approached maturity, the brown color darkens gradually to a near- dark brown uniformity with the abdominal area which has a darker shade of brown color. On average, the pupa measured $13.10 \pm 0.35\text{mm}$ in length and weighed $0.67 \pm 0.04\text{ g}$ (Table 2). The adult moth of *C. zacconius* appears pale yellowish with streaks of black dots at the base of the fore wings while the hind wing is white and translucent. The male adults were observed to be shorter in length and lower in weight as compared to the female adult of *C. zacconius* which measured approximately $9.63 \pm 0.21\text{mm}$ $0.59 \pm 0.01\text{g}$ for the male while the female measured $11.77 \pm 0.32\text{ mm}$ in length and $0.72 \pm 0.03\text{g}$ in weight respectively (Table 2).

Table 2: Morphometrics of *Chilo zacconius*

Developmental stages	Length (mm)		Weight (g)	
	Range	Mean \pm SE	Range	Mean \pm SE
First instar larvae	3.1 - 4.4	3.74 \pm 0.07	0.00 - 0.01	0.01 \pm 0.00
Second instar larvae	5.3 - 7.8	6.31 \pm 0.13	0.02 - 0.03	0.02 \pm 0.00
Third instar larvae	6.7-9.8	8.03 \pm 0.16	0.10 - 0.93	0.28 \pm 0.16
Fourth instar larvae	9.2- 13.5	11.02 \pm 0.21	0.17 - 0.21	0.19 \pm 0.01
Fifth instar larvae	13.2-16.7	15.26 \pm 0.19	0.25 - 0.54	0.43 \pm 0.06
Pupae	11 – 15	13.10 \pm 0.35	0.40 - 0.80	0.67 \pm 0.04
Male adult	8 – 11	9.63 \pm 0.21	0.53 - 0.65	0.59 \pm 0.01
Female adult	10 – 14	11.77 \pm 0.32	0.47- 0.89	0.72 \pm 0.03

Duration of growth stages

The mean developmental period from egg to adult was 51.33 ± 2.10 days (Table 3). The average egg duration was 5.08 ± 0.19 days while the first instar larval duration was 4.97 ± 0.14 , the fourth instar duration was 8.00 ± 0.31 days. Also, The average duration of the pupal phase was 6.00 ± 0.19 , while Adult duration was 3.42 ± 0.15 days. Estimated larval period was 36.83 ± 1.57 while the egg to adult

developmental period was 51.33 ± 2.10 (Table 3). The male and female adult mate within 24 hours, while the female adult lays eggs in four to five batches of about 25 eggs per batch laid per single female in the oviposition cage under laboratory conditions. The mean fecundity observed in this study was estimated at 130.22 ± 8.48 eggs per lifetime (Table 3). The schematized life cycle is presented in Fig 1(a-e).

Table 3: Developmental stages of *Chilo zacconius*

Stages of development	Characteristics	Range(days)	Mean ± SE
Egg	Egg incubation period	4 – 6	5.08 ± 0.19
Larva	First instars	4 – 6	4.97 ± 0.14
	Second instar	5 – 9	6.73 ± 0.28
	Third instar	6 – 10	8.13 ± 0.38
	Fourth instar	7 – 13	9.00 ± 0.46
	Fifth instar	6 – 10	8.00 ± 0.31
Pupae	Pupal period	5 – 7	6.00 ± 0.19
	Adult longevity	3 – 4	3.42 ± 0.15
Adult fecundity	Number of eggs	80 – 150	130.22 ± 8.48
Larval developmental period	Total larval period	28 – 48	36.83 ± 1.57
Total life cycle	Egg to adult developmental period	40 – 65	51.33 ± 2.10

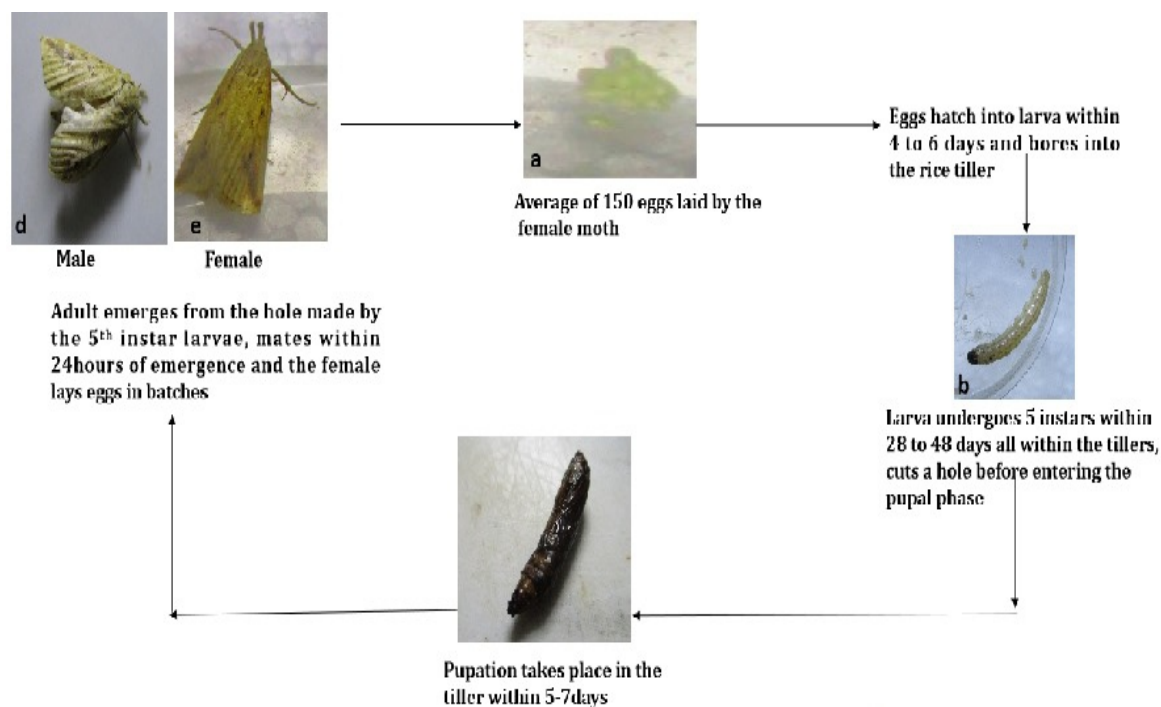


Plate 1a-e: Schematized life cycle of the African striped stemborer *Chilo zacconius*

DISCUSSION

African striped stem borer *Chilo zacconius* undergoes complete metamorphosis in rice fields with all developmental stages occurring on or within the host plants. The laying of eggs at the upper side of the leaves covered with strands of thin material and in masses is consistent with the reports of Dale (1994) and Breniere (1976) who made similar reports of the presence of overlapping eggs on the upper side of the rice leaves. From our laboratory investigation, mature female *C. zacconius* were observed to lay egg in clusters of 20 to 25 eggs, with a total range of 80 to 150 eggs per individual. This finding contrasts slightly with prior laboratory research conducted by Bordat and Pichot (1978), in which they estimated the number of egg masses produced by *C. zacconius* females at 250-350. However, subsequent reports have indicated that the overall quantity of egg masses may differ across populations under varying environmental conditions (Akinsola and Agyen-Sampong, 1984; Akinsola, 1990). Furthermore, the deposited eggs of *C. zacconius* were observed to have a pale yellow colour which emerges into first instar larvae within 4 - 6 days of development, corroborating Breniere's (1976) and Akinsola's (1975) prior observations regarding both color and duration between the egg and first instar larva. There are no concise data available regarding the morphometrics of *C. zacconius* larvae from previous studies. The pupa of *C. zacconius* at maturity appeared dark brown with distinctive four-pointed crest on its abdominal region while the head and the thoracic region had a lighter shade of brown when newly molted. This darkens towards maturity. Pupal and adult parameters observed in this study were consistent with the reports of the laboratory studies of Breniere's (1976). The life cycle duration of *C. zacconius* ranged between 40-65 days from our study which mirrors that of *C. partellus* and *C. infuscatellus* whose life cycles span between 34-66 days and 29-44 days respectively (Panchal and Kachole, 2013; Arunkumara *et al.*, 2018; Peddakasim, *et al.*, 2018; Kumar *et al.*, 2019).

The use of artificial diets for larval rearing have been reported to be successful and could also pose various challenges to many Lepidopteran larvae (Ngomane *et al.*, 2022; Wei *et al.*, 2023). The inadequacy of the wheat-based diet formulation have been implicated from different reports and may have stemmed from dietary sensitivity or an imbalanced

composition (Saranya and Samiayyan, 2017; Ngomane *et al.*, 2022). During this study, it was observed that *C. zacconius* larvae could barely sustain their development up to the second instar stage on the wheat-based diet, due to heightened mortality observed post moult. Some larvae of *C. zacconius* displayed an aversion to feeding on the wheat-based diet and instead gravitated towards perforated lids in their rearing cups. Hence, we included some field populations with similar morphometrics features during growth in the laboratory. The associated mortality aligns with the reports of Hervet *et al.* (2016) who further advised more research into modifying dietary compositions to improve larval survival on artificial diet. Hence, further research is required to ascertain more local nutrient requirements needed for the successful mass rearing of *C. zacconius* with lesser mortality in the laboratory.

CONCLUSION

Considering the duration of growth of *C. zacconius* as observed in this study, overlapping cycles within the rice fields is evident and further shows their destructive tendencies during the vegetative and reproductive phases in rice plants. This study has provided basic information that can be integrated into the management of *C. zacconius* in future research.

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