

Assessment of the Influence of Artificial Dietary Supplements on Aspects of Biology of Adult Cocoa Moth, *Ephestia Cautella*

Oyedokun, A.V^{1,2}, A.A., Omoloye², and F.A., Okelana¹

¹Entomology Section, Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria.

²Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.

Abstract: Access of adult insects to food supplement will affect the intrinsic life history traits. Artificial diets are important for mass culture of insects for research and other purposes. Therefore, the effects of sugar diet on the aspect of longevity of adults, fecundity of female and hatchability of eggs of cocoa moth, *Ephestia cautella* W. (Lepidoptera: Pyralidae) were investigated in the laboratory at ambient temperature $27 \pm 2^\circ\text{C}$, $70 \pm 5\%$ relative humidity and 12:12 hour photoperiod. Prior to mating, homogenous cultures of *E. cautella* reared on cocoa powder artificial diet were exposed to: water without sugar (WWS), sugar solution at 5% –Sa, sugar at 10% –Sb and no water, no sugar (NSNW)- control. The result showed a significant increase ($P > 0.05$) in the pre-oviposition period of Sa (3.17 ± 1.80) > Sb (2.42 ± 1.18) > WWS (2.08 ± 0.79) > NSNW (1.75 ± 0.75). The incubation period was 4.7 days, 4.0 days and 4.6 days and 3.2 days in WWS, Sa and Sb respectively. Fecundity ranged between 21.95 ± 7.82 in mated male X mated female to 131.05 ± 31.73 in virgin female treated to 10% sugar solution X virgin male without treatment. Female longevity significantly reduced in virgin female with 10% sugar solution (3.30 ± 0.24 days) than in virgin female without treatments (7.40 ± 0.17 days) and mated female treated to 5% sugar solution (7.40 ± 0.21 days). However, there was no significant difference ($P > 0.05$) in the longevity of virgin male without treatments (9.75 ± 0.23 days), mated male without treatments (9.00 ± 0.10 days) and virgin males treated to 5% sugar solution (9.45 ± 0.71 days). Diets and other supplements can be modified so as to suppress and/or improve the intrinsic life history traits in insects, which can be harnessed in mass rearing of insects, as well as a non-chemical technique in pest management.

Keywords: Artificial diets; *Ephestia cautella*; Fecundity; Life history; Longevity; Preoviposition.

I. Introduction

The Cocoa moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) is a major pest of stored cocoa (*Theobroma cacao* L.) beans in Nigeria and other countries exporting or importing the cocoa beans. It causes quantitative and qualitative economic damage in stored cocoa beans and other stored products. It has a worldwide distribution, occurring both in the tropical and temperate climates of the world, attacking grains, nuts, dried fruits and a great variety of other stored products [1]. *E. cautella* is a cosmopolitan insect pest and notably a leading storage pest of cocoa in the cocoa producing and cocoa importing countries of the world (<http://www.oardc.ohio-state.edu/cocoa/storage.htm>). Many pyralid moths were reported to have non-feeding adults [2]. *E. cautella*, a typically indoor pest of stores and warehouses and has a non-feeding adult with functional mouthpart modified for sucking liquid [3, 4, 5]. Food quality and availability will affect the life-history investment in any organisms [6]. Access to food supplements by larvae of insects affects the life-history investment strategies in reproductive traits in adults [5]. Whereas, access to drinking water after mating and during adulthood in cocoa moth increased longevity in males and females and also resulted in the total number of fertilized eggs [3]. Water sources are attractive to free-flying adult males and females of *E. cautella* [7, 8, 5]. Stored-product moths and beetles are often used as model species in laboratories for life-history and mating-strategy studies in insects [9, 10, 11]. These insects are cosmopolitan, are able to inhabit indoor facilities and are adapted to live on abundant food commodities with either good or poor quality [4, 12]. The cocoa moth's natural food media (cocoa beans, dried fruits, nuts, grains etc) require low moisture content for proper storability; adults should therefore be highly dependent on water for reproductive investments such as gonads development and gamete production, in connection to adult moth's attractiveness to water [7, 8]. This study predicted that the longevity of adults will be greatly influenced by one-time access to water prior to mating, which will positively affect the intrinsic fecundity rate and eventual larvae emergence (percentage hatchability) of *E. cautella*. In this study, it was also predicted that if supplements like sugar at different concentrations (5% and 10%) are added to the drinking water provided to newly-emerged male and female adults prior to mating, the life-history investment strategies in reproductive traits (longevity, fecundity and hatchability) will be affected positively. Therefore, the objective of this study was to evaluate the use of water and sugar solutions as supplements to alter the intrinsic reproductive investments of *E. cautella*, which may serve as an impetus for

dietary control- a non- chemical control- and management strategy for this economic pest of cocoa and other stored products.

II. Materials and Methods

2.1 Experimental station

This study was carried out in the Entomology Laboratory of the Cocoa Research Institute of Nigeria, Headquarters, Ibadan (longitude 3.85° N and latitude 7.22° E) at ambient temperature $27 \pm 2^{\circ}\text{C}$, $70 \pm 5\%$ relative humidity and 12:12 hour photoperiod.

2.2 Insect culture

Cultures of *E.cautella* reared on dried cocoa beans collected from the cocoa store of the Cocoa Research Institute of Nigeria, (CRIN), Ibadan that had been kept in storage for more than a year were used for this experiment. Adults (≤ 1 day old) that emerged from this natural cocoa culture were paired (male + female) in cages (18.5cm top diameter X 13.5cm base diameter X 19.5cm height) and the first instar larvae that emerged from the laid eggs were transferred immediately into cocoa powder artificial diet bowls (14cm diameter X 6cm height) using soft paint brush that had been moistened in 50% alcohol in distilled water, to attain a homogenous culture. The cultures were maintained in a rearing room of the Entomology Laboratory of CRIN at $27 \pm 2^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity (r.h.) and 12:12 hour photoperiod until they pupated. Pupae were collected into separate cages, and were kept until adult emergence. Emerged adults were sexed into males and females through the morphological distinguishing features of the insect (*E.cautella*), kept in separate cages (18.5cm X 13.5cm X 19.5cm) for 1 hour at ambient temperature and relative humidity before being transferred into the treatment cages. All experiments were conducted under these conditions.

2.3 Experimental design

The experiment was designed to assess the effects of the treatments on the life history traits of *E. cautella* via the pre-oviposition period of adult females, fecundity rate, incubation period and total eggs hatched. Also, the longevity of adult males and females as influenced by the treatments: No Water No Sugar (NWNS) access - Control; Water without Sugar (WwS) access – Treatment 1; Sugar Solution at 5% (Sa) access – Treatment 2 and Sugar Solution at 10% (Sb) access - Treatment 3 before and after mating in adult *E. cautella* was evaluated. Each treatment was replicated ten times in a Completely Randomized Design.

2.4 Water and Sugar access

Hydrophilic absorbent cotton BP wool of 0.5g each was soaked in 10ml distilled water for 30 seconds, drained out with hand that was covered with sterile rubber gloves to prevent contamination as well as to retain appreciable quantity of water and/or sugar solution in the cotton wool. The drained cotton wool was placed centrally in a sterile Petri dish (9cm diameter) made by Whatman Schleicher & Schuell (No 1001 090) and placed in the plastic experimental cage (18.5cm X 13.5cm X 19.5cm) prior to the introduction of the virgin adults into the cages. Newly-emerged adults (≤ 1 day old each) were placed singly in each plastic experimental cage of (18.5cm X 13.5cm X 19.5cm) containing the water and/or sugar solutions- soaked cotton wool for 12 hours (7 am – 7 pm) before pairing the male and female for mating. The singly – placed adults were observed every 30 minutes to be certain that they sucked from the soaked cotton wool. The same procedure was followed for the 5% and 10% sugar solution treatments. The mated males were subjected to the same treatment after first mating while the mated females were subjected to the treatment after laying for three days. This methodology was adapted from [13] with some modifications.

2.5 Mating experiment

Pairs of the ≤ 1 day old emerged adults (virgin male and virgin female) were placed in a plastic rearing cages (18.5cm X 13.5cm X 19.5cm) following treatments of water and sugar solutions at different levels prior to mating. The control was neither with sugar solution nor water. The pairs were observed for mating activity every 45 minutes for 10 hours. The mated pairs, after mating were separated and transferred into different cages for oviposition (female) and longevity (male and female) studies. The laying females were transferred into new cages every day after laying for three days before being subjected to the treatments again, while the mated males were subjected to the treatment and kept in separate cages to observe their longevity. In another experiment, different crosses of virgin and mated adults without the water and sugar solution treatments were observed via; virgin male X virgin female, mated male X virgin female, virgin male X mated female and mated male X mated female, to determine their fecundity rate and percentage hatchability of adult female of *E. cautella*. This experiment was replicated ten times. Similarly, the fecundity and percentage hatchability of the virgin females treated to water alone, 5 % sugar and 10 % sugar solutions and mated with virgin males without treatments access were also observed.

2.6 Eggs-laying and hatching

Pre- oviposition periods were recorded for each treatment pair and the control. Each day, the eggs laid were counted using hand lens (Eschenbach Germany Pat. Mag.X5) and were transferred into Petri dishes lined with black filter paper while the laying females were transferred into new cages for subsequent laying. Eggs laid per female were counted daily till each female died. The total number of eggs laid per female was counted and the eggs laid per day were kept separately in Petri dishes lined with black filter paper and incubated at ambient temperature and relative humidity for 12 days after which the unhatched eggs were considered unviable. Incubation period (days) of eggs for each treatment and the control were recorded, and the mean number of eggs that hatched per batch were computed and expressed as the percentage hatchability per female for each treatment.

2.7 Statistical analysis

All the data collected on the treatment effects on longevity of male and female, total number of eggs laid/female, egg incubation period and the total number of eggs hatched were subjected to one way analysis of variance and the means were separated using Tukey's Studentized Range (HSD) of SPSS for Windows Version 17.0.

III. Results

3.1 Incubation period

The influence of treatments on the incubation period of the laid eggs showed that there were significant variations based on the kind of treatment apportioned to the adult *E. cautella* prior to mating (Fig. 1). Eggs laid by adult cocoa moths with NWNS treatment had the longest incubation period when compared with the eggs laid by adults subjected to other treatments. The incubation periods following the treatments were between 3.17 days in 10% sugar solution (Sb) and 4.67 days in no sugar, no water (NWNS) treated adults (Fig. 1). 10% sugar treatment of adults resulted into reduced incubation period of the eggs with about one day while treatment with water alone reduced the incubation period with about 0.67 days – about 16 hours. Meanwhile, there was about 2 hours reduction in the incubation period of adults treated to 5% sugar when compared with NWNS treatment.

3.2 Longevity Studies

Table 1 shows the longevity of adult *E. cautella* following different treatments. There were significant differences ($P > 0.05$) in the mean longevity (days) of both adult male and female of *E. cautella* following treatment with water and sugar solutions at 5% and 10%. The mean longevity of adult male and female were 9.75 ± 0.23 and 7.40 ± 0.17 days respectively while longevity of the mated male and female were 9.00 ± 0.10 and 5.60 ± 0.11 days respectively. Longevity of virgin male and female treated to water alone was 6.15 ± 0.44 and 5.00 ± 0.26 days respectively, which was significantly different ($P > 0.05$) from the longevity of virgin male and female treated 5% sugar solution (9.45 ± 0.71 and 8.50 ± 0.69 days respectively). Similarly, significant decrease ($P > 0.05$) in the longevity of virgin adult male and female of *E. cautella* following treatment with 10% sugar solution was recorded, when compared with virgin male and female that was not subjected to such treatment. Longevity of mated male and female following treatment with water, 5% sugar solution and 10% sugar solution. There were no significant differences ($P > 0.05$) in the longevity (days) of virgin male, mated male, and virgin male treated with 5% sugar. Likewise, no significant differences ($P > 0.05$) was recorded in the mean longevity of mated female, virgin male treated with 10% sugar and mated male treated with water alone.

3.3 Fecundity and Hatchability

The mean fecundity and mean hatchability as well as percentage hatchability of *E. cautella* under different treatments of the adult female is shown in Table 2. The mean fecundity of virgin female mated with virgin male was 88.45 ± 28.09 and the hatchability was 68.55 ± 30.19 (77.5%), which was significantly different ($P > 0.05$) from mean fecundity, mean hatchability and percentage hatchability of eggs of virgin female mated with already mated male that was 30.65 ± 10.92 , 20.80 ± 9.66 (67.86%) respectively. There was a significant reduction ($P > 0.05$) in the mean fecundity and mean hatchability of adult female of *E. cautella* when either virgin male or mated male was crossed with already mated female (Table 2). Treatment of virgin female of *E. cautella* to water, 5% sugar solution, and 10% sugar solution significantly altered the intrinsic fecundity and hatchability of adult female of *E. cautella* and the eggs laid respectively. Female adults treated to water alone had the mean fecundity rate of 74.35 ± 20.10 and female adults treated to 5% sugar solution had mean fecundity rate of 84.35 ± 20.10 , which were not significantly different (at 5% confidence interval) from the fecundity rate of virgin male crossed with virgin female. There were significant differences ($P > 0.05$) in the mean hatchability of the adults (virgin male x virgin female) and the adult female (virgin) treated to water and 5% sugar solution prior to mating with virgin male. Adult female treated to 10% sugar had a mean fecundity rate and mean hatchability of 131.05 ± 31.73 and 85.90 ± 18.88 respectively, which were significantly different

($P > 0.05$) from all other treatment levels. Virgin female *E. cautella* treated to 10% sugar solution prior to mating recorded a significantly ($P > 0.05$) higher mean number of laid eggs (131.05 ± 31.73) and mean hatchability of 85.90 ± 18.88 (65.54% of the total eggs laid).

IV. Discussion

From this study, it was found that access of adult cocoa moth (*E. cautella*) to drinking water and sugar solutions at different concentrations significantly altered its intrinsic reproductive input and/or output either positively or negatively, in relation to longevity of adults, fecundity of adult females, incubation periods of laid eggs and percentage hatchability of the laid eggs which suggests that a slight change in the nutritional ecology of the adult *E. cautella* was capable of influencing its intrinsic life history traits. Although adult *E. cautella* has non-feeding adults with functional mouthparts that are used to drink liquids [3, 4, 5], access to drinking water prior to and after mating during adulthood in almond moths (cocoa moths) increased longevity in males and females, and also resulted in an increase in the total number of fertilized eggs [3]. This study had a contrary result to what [3] reported on the effects of water on longevity of both virgin and mated adults (male and female), as access to water significantly ($p = 0.05$) reduced the longevity of adults of *E. cautella* treated before and after mating. Access of adult *E. cautella* to water and sugar at different concentrations affected the reproductive investment of *E. cautella*, in that water access reduced the lifespan (longevity) of both virgin and mated adults (male and female) of *E. cautella* but resulted in higher percentage hatchability of eggs laid by adult female, which may be due to availability of adequate quantity of water required for egg formation during terminal growth phase, which turned out to include the osmotic uptake of water [14] and/or might be due to swelling of the follicle cells as well as oocyte [15], resulting into increases in cytoplasmic concentrations of both potassium and chloride ions that contributed to osmotic swelling [16]. This study agrees with earlier report that female *E. cautella* that received water as adults showed decreased longevity if mated with a male that also had access to water as an adult, indicating a negative effect of water if received by both males and females [5]. Also, the result of this study was contrary to the prediction on longevity that access to water and sugar solution at different concentrations would influence longevity positively, because virgin adult male and female of cocoa moth that had access to drinking water had the shortest longevity when compared with virgin adult male and female without access to water and/or sugar solutions. Though, this study did not investigate the metabolism and enzymatic activities in the adults exposed to water alone and/or sugar solutions (5% and 10%), perhaps this reduced longevity might be due to the increased metabolic process of the reserved energy for sustenance in the adults after the treatment, which water might have aided to release. Longevity in mated adult females that had access to 5% sugar solution was not significantly different from virgin female adults without access to water. This might be as a result of the advantage that many female insect species have by obtaining extra nutrients and water from the males through mating [17, 18]. Whereas, this study supported earlier findings on the effects of access to water prior to mating of the female adults of cocoa moth as the treatment influenced fecundity, which resulted in a significantly higher number of fertilized eggs and the resultant first instar larvae that emerged from such eggs [3] as against the female adults that had access to sugar solutions (5% and 10% concentrations). Adult females treated to 10% sugar solution resulted in highest number of eggs laid but with a low number of first instar larvae emergents which may suggest that the metabolic process of the ingested sugar had altered the intrinsic viability of the laid eggs in which the enzymatic activities on the ingested sugar had suppressed the natural fertility of the eggs or maybe there was a trade-off of egg viability with digestion of the ingested sugar, even though this study did not investigate the physiological effects of these treatments on the intrinsic reproductive biology of adult *E. cautella* for now. However, many carbohydrates especially sugars, are powerful feeding stimulants and are major source of energy for most insects especially during flight but different carbohydrate utilization depends on the ability of insects to hydrolyze polysaccharides [19]. Although some insects are known to have an absolute requirement for a specific carbohydrate in their diet, many others do not have these requirements, a functional carbohydrate sometimes may appear as non-functional if it is not ingested effectively [20, 21, 22]. For all insects, carbohydrate is a very important fuel source. Many carbohydrates, especially sugars are powerful feeding stimulants [22]. This study showed that sugar which is a powerful phagostimulant has some physiological influences on the reproductive inputs and outputs of *E. cautella* at different concentrations as expected in this study, which significantly altered the total number of eggs that hatched into first instar larvae (Table 1). Different carbohydrates utilization depends on the ability of insects to hydrolyze mono and polysaccharides. Some store products insect pests like the genera *Tenebrio*, *Ephestia* and *Oryzaephilus* require carbohydrate source of up to 70% (example in *Tenebrio*) to reach maturity and shortage of which will alter their development [19]. Consequently, the result of this study showed that sugar solution at concentrations (5% and 10%) negatively affected the total number of first instar larvae that emerged even when the total number of eggs laid was significantly higher at 10% sugar treatment (Table 1), the eventual mean number of larvae that emerged from such eggs were lower compared with virgin male and female crossed together without such treatment, and with females treated to water only. This suggest that sugar solutions at such

concentrations has the potency of reducing the intrinsic viability of the eggs of the insect and thereby offering a kind of ovicidal ability that needed to be exploited.

V. Conclusion

Control of *E. cautella* in makeshift stores, storage bins, warehouses and shipping cargoes had been mainly through the use of synthetic insecticides over the years; and cocoa being hygroscopic in nature absorbs these chemicals which eventually increase the pesticide residue in the cocoa beans. Therefore, this study has demonstrated the potentials of water and sugar solutions at 5% and 10% to trap and/or alter the intrinsic reproductive investments of *E. cautella* adults in warehouses in the producer countries prior to shipping to destination countries. This is an IPM approach that may be combined with some other eco-friendly options in managing this economic insect pest of stored cocoa beans. Further studies on the effective usage of the readily-available materials like water with or without sugar to trap flying insects need to be explored, with respect to warehouse conditions.

Table 1: Longevity of adult *E. cautella* following different treatments

Treatment	Longevity $\bar{x} \pm SE$
Virgin male	9.75 ± 0.23g
Virgin female	7.40 ± 0.17ef
Mated male	9.00 ± 0.10g
Mated female	5.60 ± 0.11cd
Virgin male wt. water	6.15 ± 0.44 cde
Virgin female wt. water	5.00 ± 0.26bc
Virgin male wt. 5% sugar	9.45 ± 0.71g
Virgin female wt. 5% sugar	8.50 ± 0.69fg
Virgin male wt. 10% sugar	5.80 ± 0.14cd
Virgin female wt. 10% sugar	3.30 ± 0.24a
Mated male wt. water	5.65 ± 0.11cd
Mated female wt. water	3.55 ± 0.11ab
Mated male wt. 5% sugar	8.70 ± 0.11fg
Mated female wt. 5% sugar	7.40 ± 0.21ef
Mated male wt. 10% sugar	8.40 ± 0.21fg
Mated female wt. 10% sugar	6.60 ± 0.11de

N = 10 / treatment. Means following the same letter(s) in a column are not significantly different (P > 0.05) following Tukey's Studentized Range (HSD)

Table 2: Fecundity and Hatchability of *E. cautella* at different mating and treatment levels

Treatment	Mean fecundity $\bar{x} \pm SD$	Mean Hatchability $\bar{x} \pm SD$	Percentage Hatchability
Virgin Male X Virgin female	88.45 ± 28.09b	68.55 ± 30.19b	77.50
Mated male X Virgin female	30.65 ± 10.92a	20.80 ± 9.66a	67.86
Virgin Male X mated female	22.40 ± 5.61a	13.65 ± 5.88a	60.94
Mated male X mated female	21.95 ± 7.82a	13.30 ± 3.81a	60.59
Female(wt 5% sugar) X virgin male	84.35 ± 20.10b	47.95 ± 18.29b	64.49
Female (wt 10% sugar) X virgin male	131.05 ± 31.73c	85.90 ± 18.88c	65.54
Female (wt water alone) X virgin male	74.35 ± 20.10b	57.95 ± 10.22ab	77.94

N = 10 treatment. Means following the same letter in a column are not significantly different (P > 0.05) following Tukey's Studentized Range (HSD).

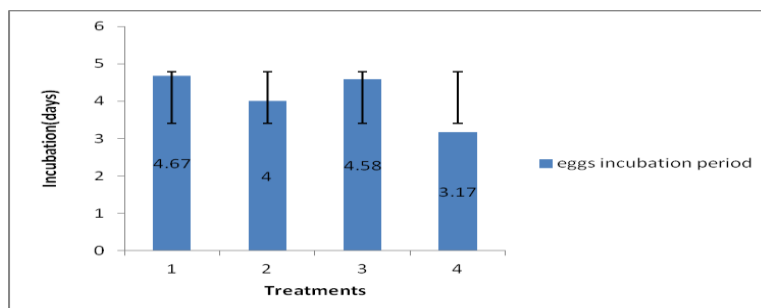


Figure 1: The mean incubation period of the eggs at different levels of treatment

On the horizontal axis, 1 - No water, no sugar, 2 – Water without sugar, 3 – Sugar at 5% conc., 4 – Sugar at 10% conc. On the vertical axis are the incubation periods in days.

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