Assessment of damage caused by *Ephestia cautella* (Walker) to stored cocoa beans

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ABSTRACT

Ephestia cautella is one of the most common storage pests of stored cocoa beans. When left uncontrolled it can cause extensive damage to stored dried cocoa beans. Increased Free Fatty Acid levels (FFA) affect the fat structure and reduce the hardness of cocoa butter. Insects have been found to be a contributory factor to the increased levels of FFA. Inadequate studies have been done exclusively on the influence of *E. cautella*. The study was to evaluate extent of damage of *E. cautella* and its impact on the FFA levels, Experiments were conducted under temperature and relative humidity ranges of 27 °C – 36.5 °C and 40 – 85 percent, respectively, in the Insectary building of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and Quality Control Division of COCOCBOD, Tema, Ghana. Thirty-six mini sacks, each containing 400 g of cocoa beans stackes were grouped into four, and placed in a transparent cage measuring 65 cm length × 65 cm width and 75 cm high. Each group had a pile up stack of three, placed side by side, totaling nine mini sacks per group. Twenty newly emerged *E. cautella* were released into the cage and damaged assessed monthly up to a period of 4 months. The damage caused included significant decrease in weight loss and increase in Free Fatty Acid levels to stored cocoa beans over a period of 4 months. The mean percentage damage to cocoa beans by *E. cautella* were 10.31 and 29.05 in the first and fourth months, respectively, with corresponding percentage weight losses of 1.21 and 6.53. There were high levels of FFA in damaged beans caused by *E. cautella* as compared to the controlled beans. The FFA levels in the controlled beans were all below 1 percent, whilst the FFA levels in *E. cautella* infested beans were above the threshold of 1.75 percent aside month 1. Thus, *E. cautella* infestation caused increased levels of FFA within stored cocoa beans. There was a strong positive correlation among insect numbers monthly, percentage damage, weight loss and free fatty acid.

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Introduction

Cocoa (*Theobroma cacao* L.) is an economic commodity, which serves as a direct source of income to most economies especially in the developing countries. In Ghana, it is the backbone of the economy and represents the most significant export crop (Tracy, 2009), accounting for 8.2 percent of the country's GDP and 30 percent of the total export earnings (Gain, 2012). However, insect infestation and chemical residues in cocoa beans are the major challenges confronting the cocoa industry in Ghana (Bateman, 2010). The beans are susceptible to attacks by numerous insect pests, which can cause serious economic and nutritional loss. According to Dharmaputra *et al.* (2000), insect feeding results in loss of weight of cocoa beans, hence, reducing its commercial

and nutritional value. *E. cautella* is the main storage pest of cocoa in the tropics, as well as one of the most common insects that infest stored cocoa beans in Ghana (Moermans *et al*., 1998).

The larvae is as an internal feeder which cause damage to the inner parts of the beans, however, it can also cause external damage, that can be easily observed by the presence of holes on beans, faeces, silk webbing and dead bodies. *E. cautella* larvae cause extensive damage to cocoa beans by feeding and producing extensive webbing and cocoons between adjacent surfaces (Hill & Waller, 1999), thereby, destroying the nutritional composition of beans and, subsequently causing moldiness. The sale of dry cocoa beans lately has assumed a scientific dimension, and much emphasis is placed on the content of free fatty acids (FFA). This is as a result of factors including insect infestation as one, other than biochemical factors that may be responsible for the increases in FFA levels in stored cocoa beans (Jonfia-Essien & Navarro, 2012).

Increase in FFA levels causes rancidity of the cocoa butter (EEC, 1990; Belitz *et al*., 1998). High FFA levels in cocoa beans reduces the commercial value of the chocolate. For the quality of cocoa butter, the percentage FFA level per the directive 73/241/EEC ought not to exceed the maximum content of 1.75 percent oleic acid equivalent in cocoa butter (EEC, 1973). The International Cocoa Standards (ICS) regards cocoa bean with any form of insect infestation as defective and of less value (Anon, 1970).

According to the recommendations of a FAO committee on cocoa grading, Grade I cocoa should not contain more than a total maximum of 3 percent by count of insect damage, germinated and flat beans and grade II cocoa not more than a total maximum of 6 percent, anything above this is to be rejected as substandard cocoa, that can be marketed only under special contract (FAO, 1969). This means that there should be zero tolerance for insect infestation.

Jonfia-Essien & Navarro (2012) reported on the damage of *Lasioderma serricorne* (Fabricus), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens) infestation on dry cocoa beans for 9 months. As their numbers built up the content of FFA levels of the cocoa beans also increased above the acceptable limit. However, there is inadequate information on the assessment of damage caused by *E. cautella* over a period of time to standard fermented cocoa beans in storage. The study, therefore, evaluated the damage and impact of *E. cautella* alone on the content of FFA levels on stored cocoa beans over a period of 4 months.

Materials and methods

The experiments were conducted at the Entomology laboratory of the Department of Crop and Soil Sciences of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The temperature and relative humidity ranges were 27 °C to 36.5 °C and 40% to 85%, respectively. The Laboratory analyses were carried out at the Quality Control Division (QCD) of COCOCBOD, Tema, Ghana.

Source of experimental materials

Ephestia cautella adults used for this experiment were cultured from a laboratory stock culture in the Insectary of the Department of Crop and Soil Sciences, KNUST. Cocoa beans were provided by the Warehouse of Quality Control Division (QCD) of COCOBOD – Kaase (Kumasi) for the study. The beans were put in an EVERmed (ULF 440 W PRO 2) freezer at –80 °C for 6 days to kill all stages of insect that might be present. The beans were spread out in the laboratory to equilibrate to ambient temperature. The measured moisture content of the cocoa beans was 10 percent. All damaged or defective beans were removed and only undamaged beans were used for the experiments.

Preparation of materials

Newly acquired jute sacks for storing cocoa were sowed into mini sacks (22 cm × 14 cm).

The mini jute sacks were sterilized in an oven at 105 ºC for 10 min to kill all stages of any insect that might be in the sacks. Transparent cages, 65 cm length × 65 cm width and 75 cm high, were constructed, all the sides were covered with muslin cloth to ensure good ventilation and to prevent insects from escaping. Four hundred grams of dried cocoa beans were weighed into each mini sack and sealed by sowing all sides with nylon thread. Thirty-six mini sacks of cocoa beans were stacked into four groups. Each group had a pile up stack of three, placed side by side, totalling nine mini sacks per group. These were then placed in a cage and each group were separated from each other with narrow spaces in-between.

Experiment 1. Twenty pairs (0 - 1 day old) of *E. cautella* adults were collected with an aspirator early in the morning and transferred into the transparent cage.

Assessment of damage

Damage caused by *E. cautella* to cocoa beans was determined or assessed by the presence of hole(s), faeces and silk webbing. The set-ups were left undisturbed for 4 months. After the 4th month of storage, three replicates of mini jute sacks from one group were selected horizontally (one top, one middle, one bottom) and brought out from the cage. Each mini sack was opened and emptied into two serially arranged sieves of mesh sizes 0.71 mm and 2.00 mm. After sieving, the dead and live larvae were counted. The frass produced were weighed. Cocoa beans were also separated into damaged and undamaged. The damaged beans were with holes and other physical damage. Each category of beans from each sack was counted and weighed.

The percentage of damaged beans was calculated as follows:

$$\text{Percentage of damaged beans} = \frac{(\text{number of damaged beans}}{\text{total number of beans})} \times 100$$

The percentage weight loss was calculated as follows:

$$\textit{Percentage weight loss} = \frac{(UNd\text{-}DNu\ )}{(U\ (Nu+Nd))} \times 100$$

where:
U = weight of undamaged beans
D = weight of damaged beans
Nu = Number of undamaged beans
Nd = Number of damaged beans

(Source: Boxall, 1986)

Experiment 2. Damage caused by 10 unsexed first instar larvae of E. cautella to cocoa beans

Ten unsexed first instar larvae of *E. cautella* were carefully placed in 400 g cocoa beans in mini jute sacks. The jute sacks were sealed and placed in the transparent cage similar to the above. The 24 sacks were stacked in four groups and each group had 6 sacks of cocoa beans. The set-up was closely monitored till an adult emerged indicating the complete life cycle of the insect. The damage caused by 10 unsexed larvae of *E. cautella* to 400 g of stored cocoa beans was assessed for three months, using the procedure as described above. Four sacks were randomly sampled from each group after every month.

Experiment 3. Determination of Free Fatty Acid (FFA) in cocoa beans attacked by E. cautella.

Fifty grams each of the damaged and undamaged beans were put in 9 cm Petri dishes in an oven (105 °C) for 1 h. The beans were manually deshelled and the nibs were grinded using a blender to increase the surface area for extraction. Five grams of the grinded samples were placed in thimbles. The initial weights of each 500 ml round bottom flasks were determined and each flask filled with 260 ml of petroleum benzene. The thimbles with grounded beans were placed in Soxhlet apparatus. The fat was extracted in the Soxhlet apparatus using AOAC (2005) method 963.15. After 2 h, samples were removed and were grinded with sand. Samples were placed back in the Soxhlet apparatus for another 2 h for the extraction of fat.

The petroleum benzene was evaporated using the rotary evaporator, leaving the fat extracted in the round bottom flask. The fat in the round bottom flask was dried in an oven at a temperature of 105 °C for 1 h to evaporate the remaining petroleum benzene. The weight of the flask and the extract were recorded. Then 50 ml of 1:1 diethyl Ether/Ethanol was added to each flask containing fat. The solution was titrated against 0.1M NaOH using phenolphthalein indicator and swirled constantly until faint pink colour appeared and the end point was noted. The FFA contents were computed in triplicates according to the official method 42-1993 (IOCCC, 1996).

The formula used was:

$$\% \text{ FFA} = \frac{(2.82 \times F \times V \times C)}{M} \times 100\ \%$$

where;
2.82 = molecular mass of oleic acid
F = factor of NaOH used (0.085)
V = volume of NaOH used (Titre value)
C = concentration of NaOH used for titration (0.1)
M = mass of the extracted fat (M2 – 1), where M1 = mass of conical flask before extraction, M2 = mass of conical flask after extraction.

Statistical analysis

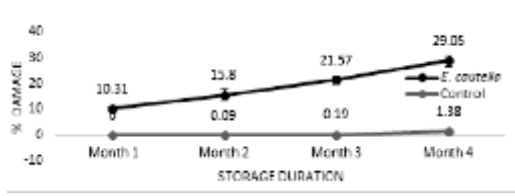
The data was analysed using analysis of variance (ANOVA) with Genstat 12th edition. The means were separated using Least significant difference (LSD) at 5 percent level of probability.

Results

Damage caused by E. cautella to stored cocoa beans

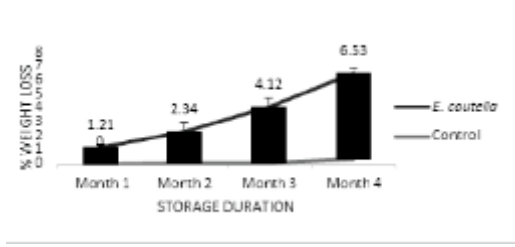
There were significant differences ($P < 0.05$) in the percentage damage caused by *E. cautella* between the months of storage (Fig. 1). The damage caused increased as the storage period prolonged. There was no significant difference ($P > 0.05$) between the damage recorded for the control beans during the storage. There were significant differences ($P < 0.05$) in the weight loss between months of storage.

The percentage weight loss caused by *E. cautella* increased with the storage period. The highest percentage weight loss caused was 6.53 which occurred in the fourth month (Fig. 2). There were significant differences ($P < 0.05$) in the mean numbers of *E. cautella* between the months of storage (Fig. 3). There were strong positive correlations between insect numbers and percentage damage, weight loss and free fatty acid (Table 1). As insect numbers increased during the storage period, percentage damage, weight loss and free fatty acid also increased.



LSD (0.05) = 3.64
The error bars represent standard error of means (SEM)

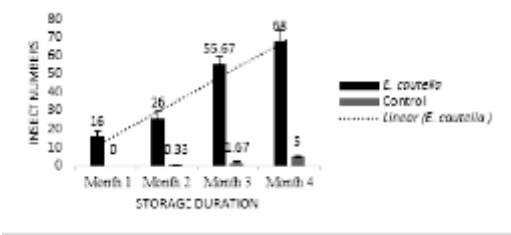
Fig. 1. Mean percentage damage caused by *E. cautella* to stored cocoa beans



LSD = 1.02

The error bars represent standard error of means (SEM)

Fig. 2. Mean percentage weight loss caused by *E. cautella* to stored cocoa beans



LSD (0.05) = 9.40
The error bars represent standard error of means (SEM)

Fig. 3. Mean numbers of *E. cautella* within the sacks of stored cocoa beans

TABLE 1
Correlation among monthly insect numbers, percentage damage, weight loss and free fatty acid caused by E. cautella

Storage duration (Days)	*Correlation coefficient (R) Percentage damage*	*Percentage weight loss*	*Percentage FFA*
30	0.964**	0.853*	0.927**
60	0.999**	0.957**	0.951**
90	0.978**	0.922**	0.991**
120	0.997**	0.993**	0.984**

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed)
Six samples were used for each parameter

Damage caused by 10 unsexed first instar larvae of E. cautella to cocoa beans

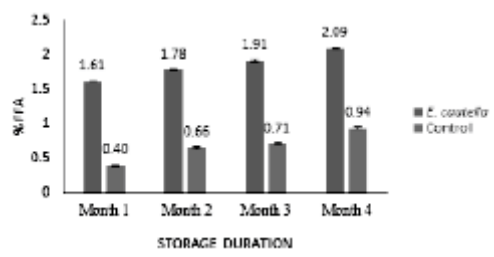
Table 2 shows the damage, weight loss caused and frass produced by 10 unsexed first instar larvae to cocoa beans for 3 months storage. There were significant differences ($P < 0.05$) between months for damage caused, weight loss and frass produced.

TABLE 2
Cumulative damaged caused by 10 unsexed first instar larvae of Ephestia cautella to cocoa beans

Months	*Mean±SE*		
	% Damaged	% Weight loss	Frass produced (g)
1	2.94±0.37	0.47±0.04	0.50±0.16
2	8.46±0.56	1.35±0.23	1.72±0.27
3	14.64±1.15	2.45±0.33	3.15±0.23
LSD (0.05)	2.47	0.74	0.73

Determination of free fatty acids (FFA) levels of cocoa beans infested with E. cautella

The FFA levels increased significantly ($P < 0.05$) during the storage period, the lowest levels recorded in the first month and the highest in the fourth month. There were significant differences ($P < 0.05$) between the mean percentage levels of free fatty acids in cocoa beans infested with *E. cautella* and the control (Fig. 4).



LSD (0.05) = 0.02
The error bars represent standard error of means (SEM)

Fig. 4. Mean percentage FFA levels of cocoa beans infested by *E. cautella*

Discussion

The significant increase in percentage damage caused by *E. cautella* from 10.31 % in the first month to 29.05 % in the fourth month during the storage period indicated that as the storage period increased, insect numbers also increased, resulting in more damage. This confirms the report of Amuh (1961) that there was a correlation between the degree of infestation and the length of storage period. The feeding activity of the larvae resulted in loss of weight of the cocoa beans. Also the increase in the percentage weight loss during the storage duration was related to the increase in percentage damage, confirming the report of Dharmaputra *et al*. (2000). The insect numbers within the 400 g mini jute sacks of stored cocoa beans included immature stages of *E. cautella* which ranged from 16 in the first month to 68 in the fourth month (Fig. 3). These were mostly larvae that had emerged from the cocoa beans. A cut test on the damaged samples of cocoa beans revealed that 82% had different stages of developing larvae within.

The percentage damage and weight loss caused by 10 unsexed first instar larvae to 400 g of stored cocoa beans was significant by the end of the third month. By extension, if the damage caused to 400 g of cocoa beans is extrapolated to 1 bag (64 kg) of cocoa beans, the cumulative percentage damage cause for the third month will be 0.092 % which is below the total maximum of 3 % acceptable insect damaged beans for grade one cocoa beans as recommended by an FAO committee (FAO, 1969). Notwithstanding, damage would build up if the cocoa beans are not treated periodically against pests before exporting.

The percentage FFA levels for the control experiments were below 1%. And that for the damaged beans ranged from 1.61 to 2.09 % from the first to fourth month, respectively (Fig. 4). Jonfia-Essien & Navarro (2012) reported that the free fatty acid (FFA) content is expected to be less than 1.0% to meet the acceptable level of 1.75% in cocoa butter extracted from the dry cocoa beans. *Ephestia cautella* damage to stored cocoa beans in the second month is enough to increase the FFA levels above the acceptable percentage of 1.75% oleic acid, per the directive of 73/241/EEC for quality cocoa butter (EEC, 1973). The results confirms the report of Jonfia-Essien & Navarro (2012) that insect damage to the cocoa beans in storage resulted in mustiness, leading to mould formation and the breakdown of fat to FFA in the beans. The significant increase in FFA levels of infested beans as compared to the control beans during storage suggests that *E. cautella* infestation increases the levels of FFA in cocoa beans other than biochemical factors that may be responsible. *Ephestia cautella* alone pierced and caused serious infestation to cocoa beans and subsequently this increased the levels of FFA. This is in contrast to report by Jonfia-Essien (2001, 2004) that *Lasioderma serricorne* and *Araecerus fasciculatus* pierce the shell of beans to provide entrance for *E. cautella.*

Conclusion

Ephestia cautella caused significant damage to cocoa beans within four months of storage period. The percentage FFA levels from the second month of storage of *E. cautella* infestation were above the recommended 1.75 percent, making the product unwholesome for cocoa butter processing. However, FFA levels increased over the storage period even without infestation, but it was minimal and far below the accepted levels. Good storage management must be practiced if cocoa beans are stored in the warehouse for longer periods before export and should be kept free of pests, especially *E. cautella*.

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