Impact of Cocoa Bean (*Theobroma cacao* L.) Packaging on the Presence of Insect Populations during Conservation

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ABSTRACT

This study assessed the impact of four different types of cocoa bean packaging on insect development during conservation, has been studied. Specifically: bags made of Jute (SJ), polypropylene (SP), and plastic polyethylene film envelopes either in jute (SPJ) or polypropylene (SPP) bags. The aerothermal characteristics of the storage warehouse and changes in cocoa bean water content were documented. Furthermore, the way in which insects developed in the packaging was analyzed through of indices of wealth, of specific diversity, and fairness. Furthermore, the evolution of insects in the stocks has also been studied through the determination of indices of wealth, of specific diversity and of fairness. Over a 51 week conservation period, the average warehouse temperature was 28.59 ± 1.53 with an average relative humidity of 70.90 ± 8.40 %. Cocoa bean water content of packaging SPJ (5.82 ± 0.60 %) and SPP (5.89 ± 0.65 remained significantly lower than the standard 8 %, while those of SJ (8.76 ± 1.21 %) and SP (8.92 ± 1.20 %) were above the standard. Fifteen insect species were detected (four primary pests and 11 secondary pests) in the SJ (N = 1082 and S = 14) and SP (N = 1294) and S = 13) packaging, with high numbers of individuals and species, in addition to high diversity and fairness indices. In conclusion, this study shows that polyethylene plastic film is the most effective at maintaining the physical integrity of conserved cocoa beans.

Key words: Cocoa beans insects, conservation, water content, diversity.

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INTRODUCTION

In the Côte d'Ivoire, the national production of cocoa (*Theobroma cacao* L.) is steadily growing. In 1965, the Côte d'Ivoire produced 113,300 t cocoa; however, today it is the leading nation, producing an estimated 1,650,000 t in 2012 (Braudeau, 1969; FAOSTAT, 2012). Thus, the cocoa culture is an important sector of the Côte d'Ivoire economy, because it contributes to approximately 31 % of the nation's export earnings and contributes 10 % to the Gross Domestic Product (GDP) annually (anonymous 1, 2013). Yet, even though the Côte d'Ivoire produced the most cocoa, the quality requires improvement. Of concern, the trade losses generated by the poor quality of cocoa are valued at more than 20 billion CFA francs per year for producers (Anonymous 2, 2009). The low quality of cocoa, such as the Marchand brand, is due to the storage-conservation step.

The Côte d'Ivoire has a hot and humid climate. This phenomenon results in a high risk of cocoa beans absorbing moisture from the humid environment during long-term storage (Barel & Irié-Bi, 1988). A major consequence of this situation is the development of insects. Cocoa packages that are sampled before export may contain a single individual (in larval or adult form), which may lead to the rejection of an entire batch (Doumbia & Kouassi, 2009). Insects tend to encounter cocoa beans in the fields, and then be incorporated within the packaging and conservation or storage structures (Lavabre, 1970; Appert, 1985; Verstraeten *et al.*, 1996). While jute bags are recommended for packaging of Marchand cocoa beans, producers tend to preferentially use polypropylene woven bags. Unfortunately, both types of bags contain interstices (tiny spaces) that ensure the beans are in contact with the surrounding environment. This issue might result in moisture resumption, promoting the development of insects.

To prevent insect development, several structures based on modified atmospheres have been constructed and trialed in West Africa (including Ghana), Latin America, and Asia. Most of these structures are made of plastics that are specifically designed to create a highly hermetic environment (Navarro *et al.*, 1984; 1993; 1995; Varnava & Mouskos, 1997; Navarro, 2006; 2007; Sabio *et al.*, 2006; Jonfia-Essein *et al.*, 2010). These studies support the importance of determining the effectiveness of the packaging currently used for cocoa beans in Côte d'Ivoire. This study aimed to assess how four different types of cocoa bean packaging influenced the development of insects during long-term storage. The timing of appearance of insects and the number of individuals from different species will be monitored (diversity), in addition to following the phases of development during storage. The results are expected to show the optimal packaging type to minimize insect development and help enhance the quality of cocoa beans as an important economic export of the Côte d'Ivoire.

MATERIALS AND METHODS

Biological material

The biological material was composed of a mixture of cocoa beans purchased from three (3) producers in the village of Yobouekro (subprefecture of Yamoussoukro), which is located on the Yamoussoukro-Bouafle axis. The beans were fermented under banana leaves for six (6) days, and then dried on cement in the sun for five (5) days.

Packaging equipment

The packaging material included jute bags (60 kg), woven polypropylene bags (50 kg), and a polyethylene plastic packaging (length: 100 cm; width: 75 cm; thickness: 0.080 mm) sold by traders in Yamoussoukro. The bags and plastic packaging were been resized to have a capacity of 500 g cocoa beans. Double thickness of plastic packaging was used to make it more airtight.

Types of packaging

Four (4) types of cocoa bean packaging (or lots) were tested as follows (Figure 1):

• beans packed directly in jute bags (SJ batch);

- beans packed directly in Polypropylene woven bags (SP batch);
- beans packed in plastic polyethylene film hermetically sealed by a thermo-welder and protected by a jute bag (SPJ batch);
- beans packaged in plastic polyethylene film hermetically sealed by a thermo-welder and protected by a Polypropylene woven bag (SPP batch).







SPJ





Figure 1: The four types of cocoa bean packaging.

(SP = Polypropylene bag; SJ = Jute bag; SPJ = Plastic film + Jute bag; SPP = Plastic film + Polypropylene bag)

For each of the four types of packaging, 75 bags (numbered 1 to 75) were used containing 500 g dried cocoa beans.

Study of the conservation

The batches of the four different packaging were arranged on pallets (1 m x 70 cm) and were placed in a store room (at ambient temperature) on the farm of the Higher School of Agronomy (ESA) of the Institut National Polytechnique Felix Houphouet Boigny (INP-HB) of Yamoussoukro (RCI).

Sampling

The first two samples were collected after an interval of three (3) weeks, after which samples were collected at regular 15-day intervals over a 51-week period. At each sampling event, three bags were selected at random.

Evolution of aerothermal parameters

The evolution of air temperature and relative humidity during conservation was recorded daily using a thermohygrographe from MAXANT (93100 MONTREUIL, FRANCE).

Evolution of cocoa bean water content

Cocoa bean water content was determined from a 5 g sample of cocoa powder dried at 103 °C \pm 2 °C for 16 h \pm 15 min in a Memmert oven (International Standard Organisation ISO 2291, 1980) FAO, 1981). The moisture content of the beans was expressed as a percentage of the dry mass.

Identification and inventory of insects

All of the collected bags were analyzed in the Laboratory of Zoology and Entomology of Department of Formation and Research Agriculture and Animal Resources (DFR-ARA) to identify and inventory the insect populations that were present in the various packages. The samples were filtered using a sieve mesh of 5 mm, to collect all adult insects and larvae. The collected insects were identified under a binocular magnifier (x 40), with the help of various identification keys (USARS, 1986; Mills, 1990; Delobel & Tran, 1993; Alzouma *et al.*, 1994; Fleurat-Lessard, 1982; Darracq, 2004; Ngamo & Hance, 2007; Destia, 2009).

Frequency of Occurrence

After identification, the frequency of occurrence (C) of each insect war determined according to the formula of Dajoz (2000), in which:

$$C(\%) = 100 \times \frac{P_i}{p}$$
 (1)

where Pi is the number of times that a species occurs and P is the total number of observations. This formula delineates five (5) classes of frequency of occurrence: ubiquitous species (C = 100 %), persistent species (50 % < C < 100 %), frequent species (25 % < C < 50 %), intermittent species (5 % < C < 25 %), and rare species (C < 5 %).

Relative Abundance

The relative abundance (Ar) was calculated according to the formula of Zaime & Gautier (1989), in which:

$$A_r(\%) = 100 \times \frac{N_i}{N}$$
 (2)

where N_i is the number of individuals of a given species and N is the total number of individuals of all species combined. This formula defines four (4) classes of relative abundance: very abundant species (Ar = 10 %), quite abundant species (5 % < Ar < 10 %), abundant species (1 % < Ar < 5 %), and species of low abundance (Ar < 1 %).

Insect species richness and diversity

The Shannon index (H') takes the number of taxa encountered in an environment into account. This index is influenced by species richness and dominant species. It is calculated according to the following formula (3) (Daget, 1976):

$$H' = -\sum_{i=1}^{S} \frac{n_i}{N} \times \log_2\left(\frac{n_i}{N}\right) \tag{3}$$

where n_i is the number of individuals of a given species and N is the total number of individuals of all species combined. This index measures species diversity. This index is zero when there is only one species, and its value is at a maximum when all species have the same abundance (H'_{max} = log2 (S)), where S is the total number of species.

Equitability (E) (or Regularity) measures the equitable distribution of species (i.e. species evenness). This index infers population balance (Daget, 1976), in which:

$$E = \frac{H'}{H_{max}} = \frac{H'}{\log 2(S)}$$
(4)

where S is the total number of species, H' is the Shannon diversity index, and H'max is the maximum Shannon diversity index. E tends to be zero when one taxon dominates a population, and is equal to 1 when all taxa have the same abundance.

Moth-eaten beans

Fifty beans were selected at random from each bag and inspected to determine the percentage of moth-eaten beans during the conservation period.

Data Analysis

The evolution of cocoa bean water content and aerothermal parameters were subject to statistical analysis. For the evolution of cocoa bean water content during conservation, analysis of variance (ANOVA 5 %) was used to determine whether there was a significant difference among the four lots (SJ, SP, SPJ, and SPP). The smallest significant difference (LSD) at the 5% threshold was used to determine at what level of significance this difference is located. Variability in aerothermal parameters in the storage warehouse during the conservation period were analyzed by the coefficient of variation (CV).

RESULTS

Evolution of aerothermal parameters

The average annual air temperature during the study period (March 2012 to April 2013) was 28.59 ± 1.53 °C. The highest and lowest average air temperatures in the store were in December 2012 (32.43 ±1.15 °C) and July 2012 (26.30 ±1.25 °C), respectively (Figure 2). The average relative air humidity was 70.90 ± 8.40 % during the study period. The lowest average air humidity was recorded in January (56.00 ±11.03 %), February (60.33 ± 1.75 %), and March 2013 (67.01 ± 3.35 %), while the highest value was recorded in November 2012 (95.66 ± 2.44 %) (Figure 2).

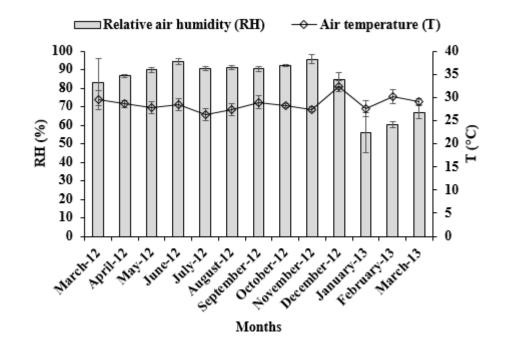


Figure 2: Average variation in air temperature and relative humidity in the store during the study period.

Analysis of the coefficients of variation showed that the variability of the average relative humidity (11.84 %) was almost equivalent to double that of the air temperature (5.36 %) (Table 1).

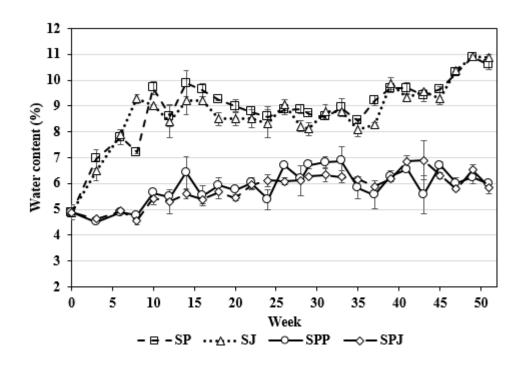
T (° C)	RH (%)
28.59±1,53	70.90±8,40
5.36	11.84
	28.59±1,53

Table 1: Statistical analysis of the aerothermal parameters

T = Air temperature ; HR = Relative air humidity

Water content

The water content in cocoa beans of the four packages showed two patterns of evolution; the former was represented by SJ and SP, while the latter was represented by SPJ and SPP (Figure 3). During the 51-week conservation period, the beans in the SJ and SP packages resumed moisture progressively over time. The water content of SJ and SP changed from $4.88 \pm 0.30\%$ to $10.59 \pm 0.16\%$ and $10.86 \pm 0.11\%$, respectively. In contrast, the water content of SPJ and SPP beans remained relatively stable, changing from 4.88 ± 0.30 per cent to $5.85 \pm 0.24\%$ and $5.98 \pm 0.10\%$, respectively. Throughout the study, the water content of cocoa beans in the SP and SJ packages remained significantly higher than those of SPP and SPJ. In the first weeks, the water level was 9 % in the first group versus 6% in the second group (Table 2), with this difference being confirmed as significant by ANOVA. In contrast, there was no difference between the two (2) packages within each group (Table 2).



SP = Polypropylene bag; SJ = Jute bag; SPJ = Plastic film + Jute bag; SPP = Plastic film + Polypropylene bag.

Figure 3: Evolution of bean moisture content over time.

Conditioning types of beans	Water	
Conditioning types of beans	content (%)	
Beans packed in jute bags (SP)	8,92 ± 1,20a	
Beans packed in polypropylene bags (SJ)	$8,76 \pm 1,21a$	
Beans packed in polypropylene bags placed in a jute bag (SPJ)	$\textbf{5,82} \pm \textbf{0,60b}$	
Beans packed in polypropylene bags placed in a polypropylene bag (SPP)	$5{,}89\pm0{,}65b$	

Table 2: Average moisture content (%) of the packages during the 51 weeks of conservation

In the "Water content" column, averages followed by the same letter are not significantly different at the 5 % threshold.

Inventories of detected insects

Insects were only detected in the SJ and SP packages. In total, 16 species of insects were detected, of which 15 were identified and 1 could not be identified (Table 3). The detected species generally belonged to three (3) orders of insects. Based on the mode of feeding, the most important insects were primary (four (4) Coleoptera) and secondary (10 Coleoptera and one (1) Lepidoptera) pests (Table 3).

Frequency of Occurrence

The data analysis shows the presence of four (4) constant species [*Tribolium castaneum* (C = 86 %), *Carpophilus dimidiatus* (C = 74 %), *Araecerus faciculatus* (C = 54 %), and *Cryptamorpha despardenisi* (C = 52 %)], two (2) frequent species [*Oryzaephilus surinamensis* (C = 36 %) and *Lasioderma serricorne* (C = 26 %)], four (4) accessory species [*Ephestia cautella* (C = 12 %), *Palorus subdepressus* (C = 12 %), *Carpophilus hemipterus* (C = 12 %), and *Urophorus nitidus* (C = 6 %)], and five (5) rare species [*Rhyzopertha Dominica* (C = 4 %), *Acanthoscelides obtectus* (C = 2 %), *Oryzaephilus surinamensis* (C = 2 %), *Ahasverus advena* (C = 2 %), and *Cryptolestes pusillus* (C = 2 %)] (Table 3, Figure 4).

Out of the total insect population that was detected, three (3) highly abundant species were detected [*Carpophilus dimidiatus* (Ar = 37.58 %), *Cryptamorpha despardenisi* (Ar = 33.46 %), and castaneum beetle (Ar = 10.44 %)], one (1) quite abundant species [*Araecearus faciculatif* (Ar = 7.41 %)], four (4) abundant species [*Carpophilus hemipterus* (Ar = 3.41 %), *Heteroptere* (Ar = 2.57 %), *Oryzaephilus surinamensis* (Ar = 2.02 %), and Palorus subdepressus (Ar = 1.01 %)], and 8 species with low abundance [Lasioderma serricorne (Ar = 1.01 %)], and 8 species with low abundance [Lasioderma serricorne (Ar = 1.01 %)], and 8 species with low abundance [Lasioderma serricorne (Ar = 1.01 %)].

0.97 %), Silvanus proximus (Ar = 0.38 %), Ephestia cautella (Ar = 0.38 %), Urophorus nitidus (Ar = 0.13 %), Ahasverus advena (Ar = 0.08 %), Rhyzopertha Dominica (Ar = 0.08 %), Acanthoscelides obtectus (Ar = 0.04 %), and Cryptolestes pusillus (Ar = 0.04 %)] (Table 3, Table 4).

Order	Family	Species	Trophic level	Relative abundance (%)	Frequency of occurrence (%)
Coleoptera	Anobidae	Lasioderma serricorne		0.97	26
	Anthribidae	Araecerus faciculatus	 _ RP _	7.41	54
	Bruchidae	Acanthoscelides obtectus	_ KP _	0.04	2
	Brosthrychidae	Rhyzopertha Dominica		0.08	4
	Tenebrionidae	Tribolium castaneum		10.44	86
		Palorus subdepressus	 RS	1.01	12
	Nitidulidae	Carpophilus dimidiatus		37.58	74
		Carpophilus hemipterus		3.41	12
		Urophorus nitidus		0.13	6
	Silvanidae	Cryptamorpha despardenisi		33.46	52
		Oryzaephilus surinamensis		2.02	36
		Silvanus proximus		0.38	2
		Ahasverus advena		0.08	2
	Cucujidae	Cryptolestes pusillus		0.04	2
Lepidoptera	Pyraldae	Ephestia cautella		0.38	12
Heteroptera	nd	Nd	Nd	2.57	22

Table 3: Inventory of insects detected during the study

Nd = not determined; *RP*: primary pest; *RS*: secondary pest.

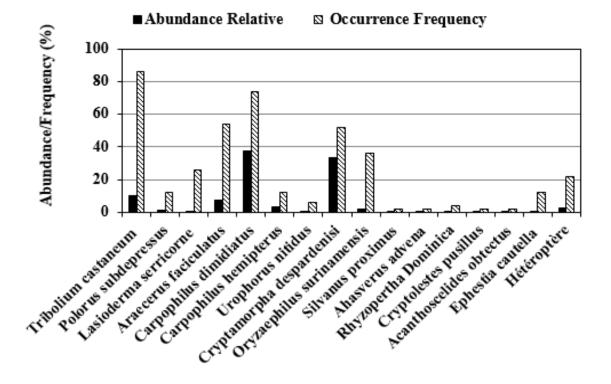


Figure 4: Enumeration of insects: relative abundance and frequency of occurrence.

Ecological Indices

Both SJ (S = 14 and N = 1294) and SP (S = 13 and N = 1082) packaging had an almost equal species richness index, Similar results were also obtained for the species diversity and fairness indices (Table 4).

	Bean packaging				
Measured Parameters	SJ	SP	SPJ	SPP	
Number of Individuals	1082	1294	0	0	
Number of Species	14	13	0	0	
Species Diversity	2,066	1,996	-	-	
Equitability	0,543	0,539	-	-	

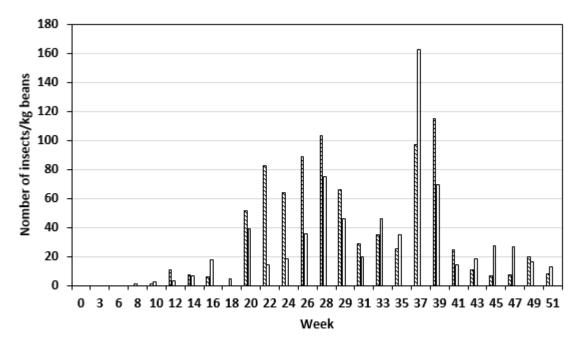
Table 4: Ecological indices in relation to the type of cocoa bean packaging

 $Polypropylene \ bag; \ SJ = Jute \ bag; \ SPJ = Plastic \ film + Jute \ bag; \ SPP = Plastic \ film + Polypropylene \ bag.$

Evolution of insect adults and larvae

During the 51-weeks study period, insect pests began to appear from the eighth week in the SJ and SP packaging, whereas they did not appear at all in the SPP and SPJ packaging. A greater number of adult insects were counted compared to that of larvae (Figures 5 and 6).

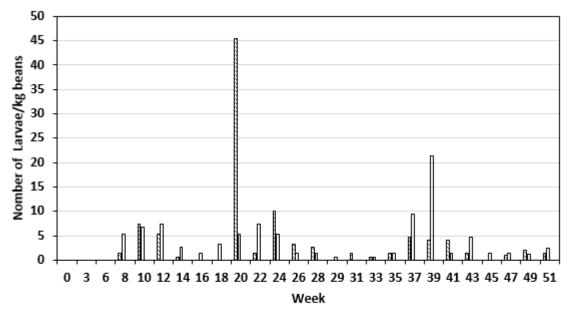
From the 18th week, there was an overall increase in adults, peaking at 155 and 99 adults/kg beans in the SP and SJ packaging, respectively. After this week, the number of individuals sharply decreased until the 35th week, down to 38 and 53 adults/kg beans in the SP and SJ packaging, respectively (Figure 5). A similar trend was detected for larvae in the SP and SJ packaging, but at lower numbers, except for SP, with high numbers being detected in the 20th week (68 larvae/kg beans) (Figure 6).



SP □SJ ■SPP BSPJ

SP = Polypropylene bag; SJ = Jute bag; SPJ = Plastic film + Jute bag; SPP = Plastic film + Polypropylene bag.

Figure 5: Evolution in the number of adult insects in relation to cocoa bean packaging.



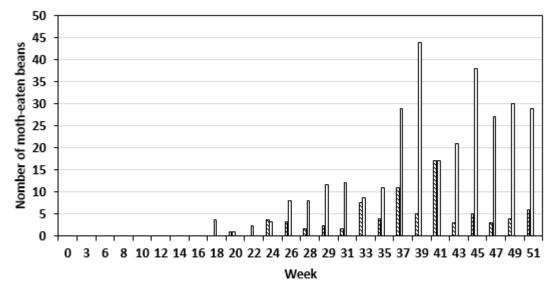
SP □SJ ■SPP ■SPJ

SP = Polypropylene bag; SJ = Jute bag; SPJ = Plastic film + Jute bag; SPP = Plastic film + Polypropylene bag.

Figure 6: Evolution in the number of larvae in relation to cocoa bean packaging.

Evolution in the number of moth-eaten beans

The evolution in the number of moth-eaten beans (beans perforated by insects) was determined to quantify the presence of insect pests. Moth-eaten beans were detected from the 18th and 20th week onwards for the SJ and SP packaging, and not at all for the other packaging. The number of moth-eaten beans evolved in the same way as that of insect pest numbers, and ranged from 3 to 23 for SP and 3 to 36 for SJ (Figure 7).



⊠SP □SJ ■SPP ■SPJ

SP = Polypropylene bag; SJ = Jute bag; SPJ = Plastic film + Jute bag; SPP = Plastic film + Polypropylene bag.

Figure 7: Evolution in the number of moth-eaten beans in relation to cocoa bean packaging.

DISCUSSION

During this 2-month study, the average air temperature was 28.53 ± 1.53 °C. While this temperature is appropriate for cocoa bean conservation, it also promotes insect development. The mean development thresholds of insects range between 13 °C and 41 °C (Cruz, 1988; Debolel and Tran, 1993; Verstraeten *et al.*, 1996; Hayma, 2004). However, to prevent insect development, cocoa beans must have a water content of less than 8 %, which is the accepted standard value (ICCO, 1996). This recommended maximum rate (8 %) for cocoa bean storage is often defined as the water content that must be balanced with a relative air humidity of 65 to 70 %, below which, insect development is arrested (Cruz, 1988; Hayma, 2004; Barel, 2013). The initial water content of the packaged beans in the current study (prior to the onset of conservation) was 4.88 %. To maintain this level, a relative air humidity of between 40 and 60 % is required. However, the average relative humidity in the store was 70.90 ± 8.40 % below the port areas (80 to 85 %). This

observation supports that documented by Barel (2013) and Cruz (1988), who found that the relative air humidity in the store was lower than that outside the store. Thus, the rapid increase in the water content of beans stored SJ and SP may be explained by moisture resumption, due to the beans being highly hygroscopic. Both jute bags and woven polypropylene have interstices that promote the direct contact of beans with the ambient air (Burle, 1962; Barel, 1995; Kebe *et al.*, 2005; Dodemont, 2007; Barel, 2013).

Unlike the SP and SJ packaging, the average water content remained stable in the SPJ and SPP packaging, at around 6 %. This result demonstrates that the plastic polyethylene film slow down moisture resumption by beans. The film served as a barrier against the exchange of beans with ambient air, preventing moisture resumption and providing good conservation material, despite the unfavorable climatic conditions (Asiedu, 1991). The absence of insect pests in the SPP and SPJ packaging may also be explained by an increase in CO_2 content within the plastic film during conservation. These results are similar to work on controlled and modified atmospheres, such as SuperGrainbagTM (Challot *et al.*, 1979; Guenot et al., 1976; Navarro *et al.*, 1984; 1993; 1995; Varnava and Mouskos, 1997; Navarro, 2006; Sabio *et al.*, 2006; Jonfia-Essein *et al.*, 2010). These results also support that moisture resumption in the SP and SJ packaging triggered insect development (Barel, 2013). The evolution of air temperature and relative humidity show that the environmental conditions of the store facilitate water resumption by certain types of packaging, leading to optimal conditions for insect pest development.

Out of the 16 insect species collected by the current study, 15 were identified as belonging to Coleoptera and Lepidoptera, while one (1) unidentified species belonged to Heteroptera. These results are consistent with those obtained by several authors (Fleurat-Lessard, 1982 and 1994; 1985; Ratnadass and Sauphanor, 1989; Mills, 1990; Delobel and Tran, 1993; Alzouma *et al.*, 1994; Darracq, 2004; Destia, 2009), demonstrating that most insects of stored commodities primarily belong to the orders of Coleoptera and Lepidoptera, followed by other orders. Out of the 14 beetles encountered and identified, four (4) were primary pests (*L. serricorne, A. faciculatus, A. obtectus, R. dominica*) and 10 were secondary pests (*T. castaneum, P. subdepressus, C. dimidiatus, C. hemipterus, U. nitidus, C. despardenisi, O. surinamensis*, with *S. Proximus, A. advena and C. pusillus*) that are of worldwide economic importance (Delobel and Tran, 1993). In addition, most of these species occur in warm regions. The appearance of these insects (in adult and larval forms) from the eighth week in the current study correlates well with

the moisture content of the cocoa beans at this time. Specifically, until the eighth week, the moisture levels of cocoa beans in SJ and SP packaging were around 9 %, which exceeded the 8 % standard. In addition, the high percentage of the secondary pest, *T. castaneum*, may be explained by the presence of broken beans in different packages.

The observed similarity in the indices of insect species wealth, diversity, and fairness in the SJ and SP packages confirms that these two packaging types have a similar effect on cocoa bean quality. However, SJ contained more moth-eaten beans than SP packaging. This difference may be explained by the fact that SJ bags had larger openings compared to SP bags, enabling the escape of larvae and adult insects.

CONCLUSION

This study demonstrated that the aerothermal conditions (air temperature and relative humidity) of Côte d'Ivoire stores are relatively constant across months, with values potentially promoting insect development. Packaging cocoa beans in polyethylene films combined with jute or polypropylene bags, rather than using these bags alone, protects against insect development and could enhance cocoa bean quality and economic value.

Thus, in total, 16 species of insects which 15 have been identified and 1 unidentified. Among the identified species *Tribolium castaneum*, *Carpophilus dimidiatus*, *Araecerus faciculatus* et *Cryptamorpha despardenisi* were constants.

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