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Triple-bagging of cowpeas within high density polyethylene bags to control the cowpea beetle *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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ABSTRACT

Laboratory and on-farm trials were carried out to determine the effectiveness of cowpeas triple-bagging with heavy-grade polyethylene to control the cowpea weevil, *Callosobruchus maculatus* (F.), the main storage pest of cowpea, *Vigna unguiculata*, Walp, in West Africa. In the laboratory bruchids numbers and seed damage were significantly reduced when storing cowpeas within 2 layers High Density Polyethylene (HDPE) bags of at least 80 μm wall thicknesses. This thickness considerably reduced oxygen concentration in the bag after 5 days of storage and inhibited insect development. However late instar larvae and pupae were less affected by low oxygen concentration. On-farm storage trials with 2 layers HDPE 50 kg capacity bags tightly sealed and placed in an additional woven nylon bag (triple bag) was effective in controlling the bruchids for 7 months. Moreover, seed damage (<7%) and grain germination were not significantly affected (>89%). These findings allow optimizing the triple-bagging technology with readily local manufactured and affordable bags for long duration cowpea storage.

1. Introduction

In West Africa post-harvest grain storage is a recurrent constraint for cowpea (*Vigna unguiculata* (Walpers)), the most important indigenous African grain legume. At harvest, 1–5% of cowpea seeds are infested by the most prominent species, *Callosobruchus maculatus* F. (Sanon et al., 2005). Even this relatively low initial infestation rate can lead to 80–100% damage of unprotected seeds after six months of storage (Ouédraogo et al., 1996; Dabiré, 2001). As a result, this has led farmers to sell their cowpeas at low harvest-time prices in October and November, whereas the most profitable prices extend from February to September (Oumarou, 1998; Langyintuo, 2003; Langyintuo et al., 2003). Therefore prolonging the time cowpeas can be stored, preferably without the use of pesticides, can help to alleviate poverty among farmers.

Airtight grain storage is an ancient proven technique for controlling stored-product insect pests (Hyde et al., 1973; De Lima, 1990). This technique is based on the principle of low oxygen environment to inhibit insect survival and reproduction. When tightly sealed the storage system prevents air from entering or leaving. The respiration process of insects, fungi and grain involves intake of oxygen and liberation of carbon dioxide, which leads to lower oxygen and higher carbon dioxide level in the sealed storage system where aerobic respiration is restricted further.

Hermetic storage of cowpeas in metal drums containers and triple-bagging was successful in controlling *C. maculatus* (Seck et al., 1996; Murdock et al., 2003). With triple-bagging, the cowpea seeds are sealed in a series of 2 heavy-grade polyethylene plastic bags. It is a quite simple method which can be used for cowpea storage. The triple-bagging technology was disseminated in Cameroon in the 90's (Murdock et al., 2003). The triple bag consisted of 2 layers of polyethylene bag which were expected to be as hermetic as possible and both included in a protective polypropylene woven bag. However, because farmers were lacking appropriate polyethylene bags, so they often added insecticides or combined insecticides with solar heating (Moussa, 2006). The triple-bagging technique can be easily adopted by farmers since heavy-grade polyethylene bags allowing low oxygen permeability are available and affordable. Furthermore, polyethylene bag storage is a flexible technique that fits with the West Africa commodities trade (Genest et al., 1990).

We evaluated local manufactured High Density Polyethylene (HDPE) bags with walls thickness ranging from 50 μm to 100 μm. After this first experiment a prototype triple bag was developed and tested at farm scale using large quantities of cowpeas for long duration storage. The current study is part of research carried out within the Purdue Improved Cowpea Storage (PICS) project to

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optimize the triple-bagging technology for cowpea storage in West and Central Africa (www.ag.purdue.edu/ipia/pics).

2. Materiel and methods

2.1. Study environments

The laboratory study was conducted under the prevailing conditions of the *Centre de Recherches Environnementales et Agricoles de Kamboinse* in central Burkina Faso. The average temperature and relative humidity during the study were 32 °C ± 2 and 49% ± 4 r.h. On-farm storage trials were carried out from October 2007 to May 2008 in three localities; (i) Donsin in central Burkina Faso, (ii) Dori in North Burkina Faso, and (iii) Tougan in Northwest Burkina Faso. Climatic parameters fluctuated during the storage period in all of the 3 localities (Fig. 1). Temperature tended to decrease from October to February but remained 1–2 °C higher in Dori and Tougan. Relative humidity decreased in all the sites from October to March and slightly increased in April and May.

2.2. Source of the cowpea seeds and the insect for laboratory studies

Cowpea grain used for this study was of the landrace variety *Moussa*, which has white, smooth, and irregular seeds. The grain was purchased soon after harvest from farmers around the *Kamboinsé* District. About 100 kg of grain was screened and sorted to eliminate insect eggs, seed weevils, and grain with holes. The sorted grain was then put into a freezer at −18 °C for 1 week to destroy any remaining insects before the seed was used for insect rearing and laboratory trials. Seeds were not treated with insecticides before the trials.

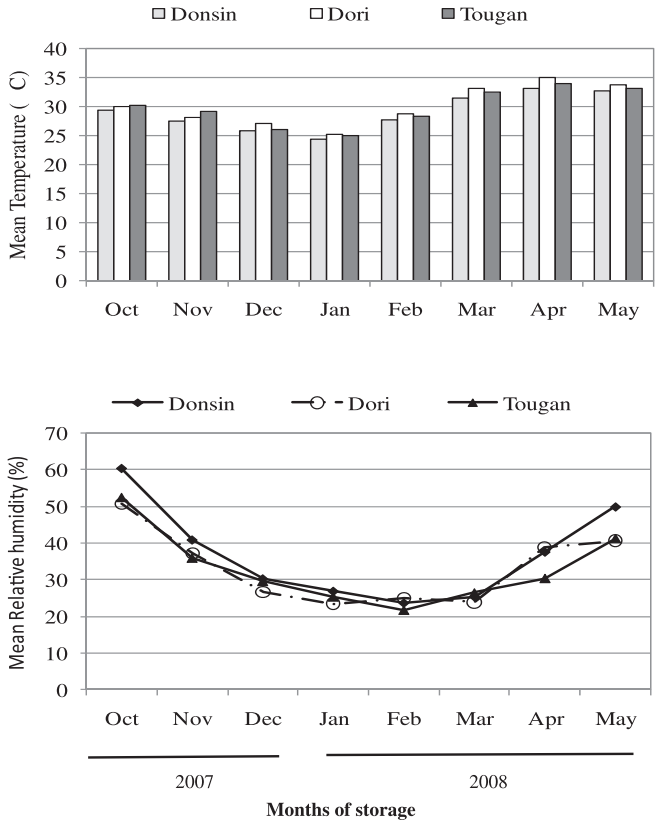

Fig. 1. Temporal variations in mean temperature and relative humidity in the 3 sites of experiments during on-farm storage trials from October 2007 to May 2008.

The *C. maculatus* strain used in this study was obtained from insects that emerged from cowpea seeds collected around the *Kamboinsé* District in October 2007. Newly emerged adults were placed in rearing containers (Plexiglas, 18 × 11 × 4 cm) with healthy cowpea seeds on which females laid their eggs. New generations of insects that emerged after 20–32 days, depending on the climatic conditions (Sanon and Ouedraogo, 1998), were used in these experiments. Insects were reared under the previously described laboratory conditions.

2.3. Characteristics of polyethylene bags used

High density polyethylene bags of five (5) different wall thicknesses (50 μm, 60 μm, 80 μm, 90 μm, 100 μm) manufactured by FASOPLAST™ in Ouagadougou, Burkina Faso were tested for cowpea storage. Polyethylene low density plastic bags of 25 μm thickness commonly used by farmers in Burkina Faso were used as control. Data comparing gas permeabilities of low and high density polyethylene have shown that oxygen permeability was lower through high density polyethylene film (Wang et al., 1998). Moreover, gas permeability could be related to the polyethylene film thickness (Gholizadeh et al., 2007).

Prototype bags of 192 mm × 165 mm and 110 mm × 80 mm were used for laboratory experiments. For the on-farm trials, triple bags of 50 kg capacity were used. Each triple bag was made of 2 high density polyethylene bags (111 cm × 60 cm × 80 μm) and both included in a polypropylene woven bag (110 cm × 61 cm).

2.4. Development of C. maculatus inside polyethylene bags of different thicknesses and layers under laboratory conditions

These tests were carried out with small prototype polyethylene bags of 192 mm × 165 mm of the aforementioned thicknesses. The experiment was set up with one or two layer of polyethylene bags of each of the different thicknesses, in a Randomized Complete Block with 4 replications and 12 treatments. The treatments consisted of five plastic thicknesses (50 μm, 60 μm, 80 μm, 90 μm, 100 μm) with one layer and the same thicknesses with 2 layers. Commonly low density polyethylene bags of 25 μm with one or two layer were used as control. Each bag was filled with 700 g healthy seeds and artificially infested by adding seeds bearing 24 bruchid eggs and seeds bearing 24 individuals of each of the four instars larvae and 5 pairs of adult *C. maculatus*. This corresponds to an introduction of 130 individuals which ensures an infestation rate close to the 5% natural infestation (Ouédraogo et al., 1996). Each bag was compressed to remove the maximum of air, tightly sealed and placed in muslin cloth of 350 mm × 220 mm. This cloth trapped the bruchids in case of perforation of the polyethylene bags. The oxygen concentration of each bag was measured with a MOCON® portable O_2/CO_2 analyzer 5 days after bagging the cowpea. This duration seems to be necessary before critical levels of O_2 occur in hermetic storage containers (Calderon and Navarro, 1980; Villiers et al., 2007).

After 7 months of storage (October 2007 − May 2008) the bags were opened and the numbers of dead and alive *C. maculatus* adults were counted. Three random samples of 100 grains were removed from each bag to determine the number of grains with holes. The number of holes on each polyethylene bag was also recorded.

2.5. Effect of triple-bagging on C. maculatus adults, eggs and larval stages under laboratory conditions

For this experiment the cowpea was stored within bags made of 2 layers HDPE (110 mm × 80 mm) of 80 μm wall thickness placed in a muslin cloth. In order to determine the effect on adult, ten pairs of

newly emerged adult *C. maculatus* were placed in bags, each containing 50 healthy cowpeas. We set up a control treatment with ten pairs adults kept in Petri dishes. The insects were kept in the bags or Petri dishes and the number of dead insects was daily recorded until all the insects were died in the double-layer polyethylene bags and in the control. There were four replicates for each treatment.

To study the effect on *C. maculatus* immature stages we first obtained eggs by exposing cowpea seeds to *C. maculatus* females (flightless form) for a 24-h oviposition period. The seeds bearing only one egg were selected and either used for experiments or monitored under laboratory conditions in several sets of cultures to obtain post-embryonic stages. Under the rearing conditions, the post-embryonic developmental stages were obtained 7–8 days after oviposition for LI larvae, 9–10 days for LII larvae, 12–13 days for LIII larvae, 15–16 days for LIV larvae, and 17–18 days for pupae. For each immature stage, 50 seeds bearing 100 individuals were sorted out and mixed up with 100 g of healthy seeds and sealed in the triple bag. For each development stage, 2 batches were used and 4 replications were set up. A batch of seeds placed in Petri dishes stand for the control. The bags were placed on the bench in the laboratory. Hatching was observed with a binocular microscope 1 week after exposure (Ouédraogo et al., 1996). Hatched eggs of *C. maculatus* can be readily distinguished from unhatched eggs because the former are usually filled with shavings from the seed and turn a milky white as the larvae burrow into the seed (Sanon and Ouedraogo, 1998). For post-embryonic mortality the bags were monitored until the total emergence of the first adult generation within a period of 15 days (Ouédraogo et al., 1996). The mortality of each immature stage was computed by mining the number of emerging insects from the total number of insects emerging from the control.

2.6. Development of C. maculatus population under long-term triple-bagging under laboratory conditions

The same type bags of the previous experiment were used again. We introduced in the inner bag 100 g grains of cowpea (artificially infested by adding 40 *C. maculatus*) to fill the bag completely without air pocket. The inner bag was securely tied and shut at the neck and the surrounded bag was also tied and shut in the same way. The polyethylene bags were covered additionally with a muslin cloth. A batch of 160 bags was used in this experiment. The bags were placed on the bench in the laboratory for 10 months under the prevailing ambient laboratory conditions (mean temperature was 32 °C ± 2 and relative air humidity 49% ± 4).

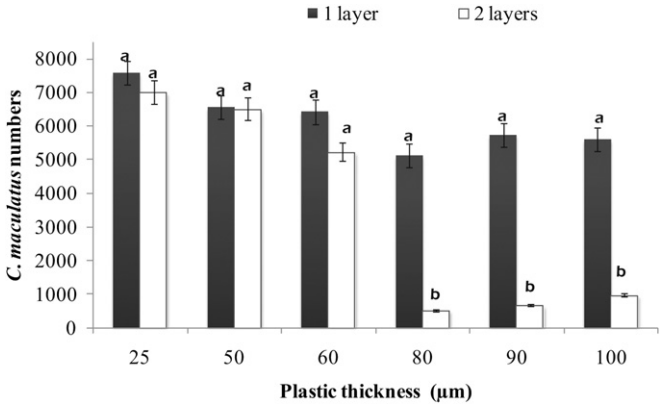

Fig. 2. Influence of polyethylene thickness and number of layers on the numbers of *C. maculatus* (means ± SE) developing on cowpea after 7 months of storage under laboratory conditions. Means were compared by a SNK test at the 5% level, with different alphabetic letters indicating significant differences.

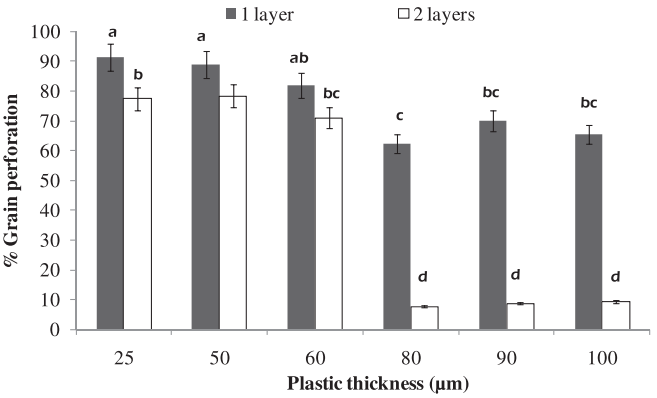

Fig. 3. Influence of polyethylene thickness and number of layers on cowpea perforation (means ± SE) by *C. maculatus* after 7 months of storage under laboratory conditions. Means were compared by a SNK test at the 5% level, with different alphabetic letters indicating significant differences.

Every couple of weeks, 8 bags were randomly selected and emptied and the cowpeas removed to collect and count the dead and living *C. maculatus* adults.

2.7. Effectiveness of triple-bagging in controlling C. maculatus population under on-farm storage conditions

Field-infested cowpeas (i.e., cowpeas that had not been sorted to remove insects) were used for this experiment. The seeds were stored in 50 kg capacity HDPE triple bags of 80 µm wall thickness, i.e. two layers of polyethylene bags were covered additionally with woven nylon bag. Each triple bag was tightly sealed and was kept unopened for 7 months. The trials were conducted in three districts, Dori, Donsin and Tougan. In each district the trial involved 150 farmers selected randomly in 30 villages (5 farmers per village). The bags were placed in storage facilities that were not exposed to light. The farmers monitored the stored seeds for 7 months, from October 2007 to May 2008. In each village a control with polyethylene low density (25 µm) commonly used bags was set up. The bags were monthly visually inspected for potential damage. Before the beginning of the experiment 500 g cowpea seeds were randomly taken from 10 farmers in each district to evaluate the bruchids initial infestation by counting the dead and living insect emerging during 21 days of survey (Sanon et al., 2005). A subsample of 100 seeds was randomly selected and evaluated for seed perforation. After 7 months of storage, the bags were opened for the first time and samples of 500 g cowpea seeds were randomly

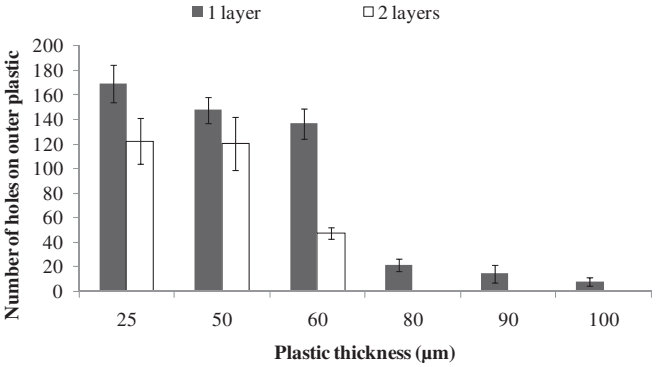

Fig. 4. Influence of polyethylene thickness and number of layers on outer plastic perforation (means ± SE) by *C. maculatus* developing on cowpea under laboratory conditions.

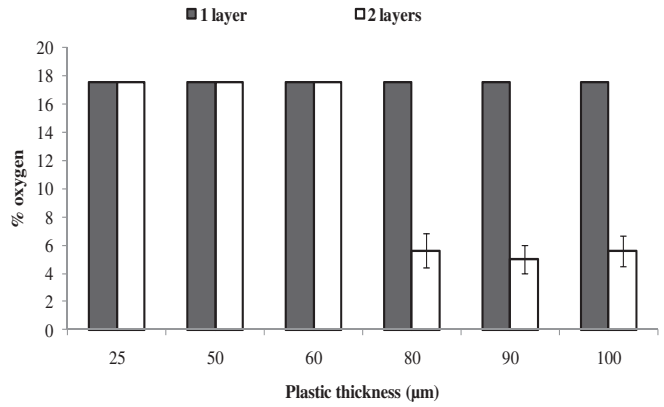

Fig. 5. Influence of polyethylene thickness and number of layers on oxygen concentration (means ± SE) measured after 5 days of cowpea storage under laboratory conditions.

selected from 10 farmers in each district for the same observations. A comparison ratio (R) between the bruchid numbers at the beginning and the end of storage was computed by dividing the final number of insect by the initial number of insects collected in each sample. An effective bruchid control must yield R values close to 1. Germination tests were conducted on five sets of 50 seeds randomly selected from the 500 g taken from each bag; the grains were placed on a moistened filter paper and examined daily for germination.

2.8. Data analysis

Data on the influence of polyethylene layers and thicknesses were subjected to a multivariate analysis of variance with polyethylene layers as independent variable and their thickness as covariate, using SAS version 8 software (PROC GLM, SAS Institute, 2001). When MANOVA were significant, means were separated by the Student Newman–Keuls test at the 5% level. The effects of long-term bagging on the *C. maculatus* population and immature survival in laboratory were determined by a regression analysis.

3. Results

3.1. Development of C. maculatus inside polyethylene bags of different thicknesses and layers under laboratory conditions

Bruchid numbers and seed damages were significantly reduced when storing cowpea with 2 layers HDPE bags of 80–100 µm wall thickness (Figs. 2 and 3). When using one layer of polyethylene, the insects were able to perforate all the bags regardless of the thickness (Fig. 4). But 2 layers of polyethylene of 80–100 µm thickness prevent outer layer perforation. In that case the oxygen concentration in the bags after 5 days of storage was below 6% compared to the 17.5% recorded at the beginning of storage (Fig. 5). MANOVA revealed a high significant influence of polyethylene layers and their thicknesses on bruchid numbers, seed damages, bag perforation and oxygen content after 5 days of storage (Table 1).

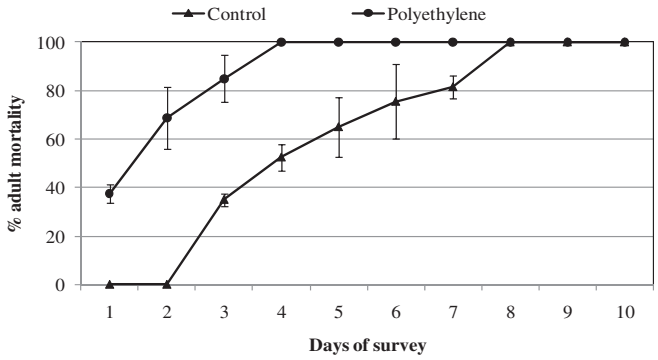

Fig. 6. Temporal variations of *C. maculatus* adult mortality (mean ± SE) inside polyethylene bags of 80 µm double layers under laboratory conditions.

3.2. Effect of triple-bagging storage on C. maculatus adults, eggs and larval stages under laboratory conditions

A total mortality of *C. maculatus* adults was recorded after 4 days of storage in the double-layer polyethylene bags while only 52.5% of insects died in the control (Fig. 6). Total mortality of insects was noticed in the control after 8 days. A significant mortality of *C. maculatus* eggs and post-embryonic stages was recorded in polyethylene bags compared with the control ($P < 0.05$; Table 2). However the fourth instars larvae and pupae were significantly less affected and as a consequence exhibited a higher survival (Table 2). The regression analysis revealed a negative linear correlation between *C. maculatus* developmental stages and mortality recorded in polyethylene bags ($y = -2.76\,x + 98.7$; $R^2 = 0.52$; $F_{(1,\,22)} = 23.71$, $P < 0.0001$).

3.3. Development of C. maculatus population under long-term triple-bagging in laboratory conditions

During the lab storage experiment, bruchid population slightly increased up to 8 weeks of cowpea storage inside the polyethylene bags. At this period, up to 20 individuals/bag on the average was recorded (Fig. 7) and the increase in bruchid population was very low and fitted to a logarithmic curve trend ($y = 2.5\,\ln(x) + 13.6$; $R^2 = 0.44$). The bruchids collected during each sampling date were mainly dead and after 28 weeks of storage living bruchid adults were no longer recorded in the polyethylene bags.

3.4. Effectiveness of triple-bagging in controlling C. maculatus under on-farm storage conditions

After 7 months of triple-bagging the bruchid numbers per sample were very low regardless of the location. A comparison ratio close to 1 was recorded, meaning that the *C. maculatus* population

Table 1
Multivariate analysis of polyethylene thicknesses and layers influence on bruchid numbers, seed damages, polyethylene perforation and oxygen content measured 5 days after bagging.

	Total insect numbers			Seed damages (grain perforation)			Polyethylene perforation			Oxygen content		
	Df	*F*	*P*	*Df*	*F*	*P*	*Df*	*F*	*P*	*Df*	*F*	*P*
Thickness	1, 44	140.31	<0.0001	1, 44	225.96	<0.0001	1, 44	140.32	<0.0001	1, 44	75.29	<0.0001
Layer	1, 44	8.35	<0.01	1, 44	11.71	<0.005	1, 44	3.45	0.070	1, 44	21.67	<0.0001
Thickness × Layer	1, 44	44.72	<0.0001	1, 44	3.83	0.056	1, 44	33.70	<0.0001	1, 44	75.29	<0.0001

Table 2
Influence of polyethylene of 80 μm double layers on the survival of *Callosobruchus maculatus* eggs, larvae and pupae. Means followed by the same letters did not significantly differ based on a SNK test at the 5% level. Uppercase letters were used for comparison within rows and lowercase letters within column.

Mean mortality (% ± SE)			
Developmental stages	Polyethylene double layers	Control	
Eggs of 24h	92.00 ± 0.82 ab A	4.75 ± 1.93 B	
1st instar Larvae	93.00 ± 2.04 ab A	7.25 ± 2.06 B	
2nd instar larvae	92.00 ± 2.54 ab A	7.25 ± 2.06 B	
3rd instar larvae	88.25 ± 3.81 ab A	6.75 ± 2.50 B	$P < 0.05$
4th instar larvae	81.25 ± 3.71 b A	4.75 ± 0.50 B	
Pupae	85.00 ± 2.48 b A	4.75 ± 2.06 B	
	$P < 0.05$	$P > 0.05$	

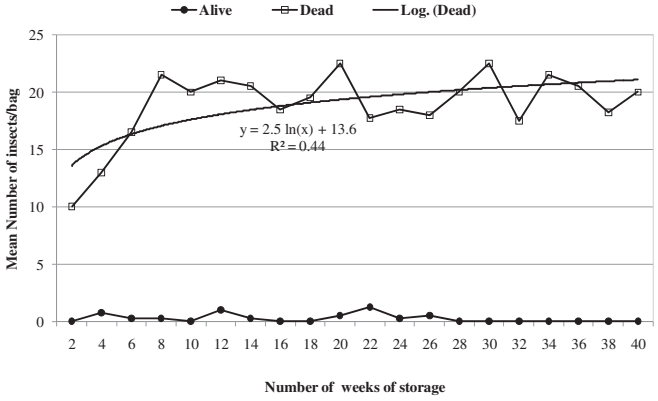

Fig. 7. Temporal variations of dead and alive *Callosobruchus maculatus* mean numbers in storage experiment using 2 pieces of HDPE bags of 80 μm wall thickness included in a muslin bag under laboratory conditions.

was inhibited (Table 3). At the end of the storage the percentage of grains with perforation did not differ from the beginning of the storage ($P > 0.05$). Moreover the viability of the seeds was not significantly affected by triple-bagging (Table 3).

4. Discussion

Hermetic storage of grain to prevent insect damage is difficult to achieve with traditional materials. Metal drums and glass containers can be used but are often too expensive and difficult to handle. Triple-bagging was originally developed in Cameroon (Murdock et al., 2003) but large scale adoption failed because appropriate and affordable bags were not locally available. This study aims at identifying the best triple bag ensuring cowpea hermetic storage to prevent bruchids damages.

In laboratory trials we highlighted that 2 layers of HDPE bags of at least 80 μm wall thickness are necessary to reduce significantly the number of bruchids emerging from the seeds. Even wall thickness superior to 80 μm can be perforate by the insect when used in only one layer. Like all aerobic organisms, development and survival of insects is strongly correlated with oxygen concentration (Dobie et al., 1991). Since the bag is perforated when using one layer, the oxygen concentration increases and allows the development of the bruchids population.

The on-farm trial confirmed the laboratory results. Triple-bagging of cowpeas for 7 months with 2 pieces of HDPE bags of 80 μm wall thickness placed in an additional woven nylon bag tightly sealed was effective in controlling *C. maculatus* population. Moreover the seeds were kept undamaged and viable. Similar result was reported for long-term storage under hermetically sealed containers for controlling insect pest of several commodities (Bartali et al., 1990; Donahaye et al., 1991; Santos, 2006; Bartosik et al., 2008).

The double 80 μm thickness HDPE layer is considered to have a low permeability to oxygen (Wang et al., 1998). In such environment the oxygen level dropped sharply in the storage system. The role of low oxygen concentration in causing mortality of stored-product insect in hermetic storage was reported by Bailey (1965). Some containers allow oxygen concentration depletion to less than 3% within 3 days of storage (Moreno-Martinez et al., 2000; Quezada et al., 2006). In that case the insect was entirely and rapidly eliminated. In our experiment the oxygen concentration dropped by 6% after 5 days of storage and presumably may remain at a relatively low level for the 7-months storage period. This enables adult to continue living for a few days in the triple-bagging environment and lay eggs before dying. However the low oxygen concentration affected all the immature stage of *C. maculatus* particularly eggs and earlier instars larvae. Consequently, the triple-bagging was effective in reducing new emerging insects at the beginning of storage. In long-term laboratory experiment, after 26–28 weeks of storage, any further living adult was not recorded. The occurrence of up and down peaks between 8 and 40 weeks of storage may be related to the disturbance of insect population structure induced by hypoxia and also related to the relatively long (20–25 days in the experimental conditions) post-embryonic development time (Sanon and Ouedraogo, 1998). Although *C. maculatus* 4th instars larvae and pupae proved to be less affected by hypoxia, few of them will develop to adult stage. Similar results were reported on *C. maculatus* by Margam (2009).

Hermetic storage is reported to increase carbon dioxide concentration. The concomitant synergistic effect of oxygen depletion and CO_2 accumulation for insect control was demonstrated (Calderon and Navarro, 1979, 1980). These findings lead to use modified atmosphere for disinfestations of commodities (Mbata and Reichmuth, 1996; Ofuya and Reichmuth, 2002). However, former feeding behavior studies showed clearly that it is the level of O_2, not that of CO_2, which is mainly responsible for the cessation of active feeding of *C. maculatus* immatures leading to the death (Margam, 2009).

Triple-bagging is a low cost-effective system for cowpea storage in Africa. However the sustainability of this technology needs to be monitored. Since insects were able to develop resistance to hypoxia (Donahaye, 1992) there is a need to further investigate on the

Table 3
Influence of triple-bagging on the mean number (±SD) of *C. maculatus* at the beginning (I) and the end (F) of storage, the comparison ratio of *C. maculatus* numbers (R), and the rates of perforation and germination of cowpeas after 7 months of storage in 3 sites of Burkina Faso. Means within a column are compared using the Student-Newman–Keuls Test. Different alphabetic letters indicate significant differences at $P < 0.05$.

C. maculatus per 500 g cowpeas					Impact on grain quality	
Sites	Storage period	Initial number (*I*)	Final number (*F*)	$R = F/I$	Mean grain perforation (% ± SE)	Mean grain germination (% ± SE)
Dori	Beginning	22.2 ± 3.1 b	495.8 ± 17.1 b	22.33	75.8 ± 4.7 a	92.2 ± 7.2 a
	End		27.3 ± 5.9 c	1.23	4.9 ± 0.6 b	89.6 ± 5.2 a
Donsin	Beginning	47.7 ± 10.8 a	860.6 ± 45.8 a	18.04	70.0 ± 6.9 a	89.2 ± 8.4 a
	End		35.2 ± 4.9 c	0.73	6.3 ± 1.9 b	92.4 ± 5.9 a
Tougan	Beginning	48.1 ± 7.0 a	893.4 ± 16.7 a	18.57	72.2 ± 5.3 a	94.2 ± 5.9 a
	End		37.9 ± 7.4 c	0.78	5.5 ± 2.1 b	89.8 ± 6.6 a

strategy developed by late instars larvae and nymphs to survive to the low oxygen concentration. Donahaye (1992) was able to select a *Tribolium castaneum* (Herbst) strain resistant to the low oxygen concentration after exposure for 40 generations to modified atmosphere of 0.5% O_2. Since the triple-bagging technology is being deployed at large scale in West Africa the insect could develop resistance even if the pressure for selection to low oxygen concentration is lower.

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