

Triple-Layer Hermetic Storage: A Novel Approach Against *Prostephanus Truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus Zeamais* (Mot) (Coleoptera: Curculionidae)

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Abstract

Studies were carried out under laboratory conditions of $32 \pm 2^\circ\text{C}$ and 58-88% r.h. to determine the effectiveness of the triple-layer hermetic bag against the Larger Grain Borer, *Prostephanus truncatus* (Horn) and the maize weevil, *Sitophilus zeamais* (Mot) on stored maize. Three storage bags (Triple-layer hermetic bag, Jute and Polypropylene) and three maize varieties (Obatanpa, Abrodenkye and Kamangkpong) widely cultivated in Ghana were used in the study. A factorial experiment was conducted involving 5 kg of each maize variety with moisture content between 12.5-14%. These were stored in the various bags and a destructive sampling of 54 bags (27 bags for each insect species) was done each month for a 6 month storage period. Percentage damage, weight loss and mean development periods of insect numbers were determined. Mean grain damage and weight loss were significantly different ($p < 0.001$) in the different storage bags. The triple-layer hermetic bag recorded the least mean weight loss of 2.94% while jute and polypropylene bags recorded higher mean values of 19.55% and 23.65% respectively. *Prostephanus truncatus* caused the highest mean weight loss of 17.19% while *S. zeamais* caused 10.57%. The high yielding improved variety (Obatanpa) was the most susceptible with a mean weight loss of 16.75% while Kamangkpong recorded the least susceptibility to both insect species with a mean weight loss of 11.09%. Abrodenkye recorded 13.55% weight loss. Moisture content and insect numbers were significantly different ($p < 0.001$) in the various bags. It is therefore recommended that the triple-layer hermetic bag should be used for quality preservation of maize.

Keywords: *Prostephanus truncatus*; *Sitophilus zeamais*; Triple-layer hermetic bag; Polypropylene; Jute

Introduction

Maize production in Ghana

In Sub-Saharan Africa, maize is one of the most important grain staples for agricultural income and caloric intake, accounting for nearly 20% of the plant-based food supply [1].

About 1.6 million hectares of maize is cultivated annually in Ghana, 80% of which is owned by smallholder farmers [2]. Average annual production increased from 141,000 metric tons in 1983 to 533,000 metric tons in 1993 [3] and is currently 1,871,700 metric tons [4]. The current value of maize production is approximately US \$ 400,000,000 a year (late 2008 values) [4].

The problem of insects in relation to food storage

Stored maize is attacked by 20 different species of insect pests including the maize weevil, *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) and the Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) [5]. Insect pest damage to stored grain results in major economic losses to farmers throughout the world [5]. These losses are diverse and intense, and it is estimated that approximately one-third of the world's food crop is damaged or destroyed by insects during growth, harvest and storage. In Ghana, about 20% of annual maize and cowpea production are lost to insects [2].

Apart from the actual nutrient losses, kernels damaged by insects may be contaminated with dangerous levels of aflatoxins [6]. Additionally, there is contamination by dead beetles, pupae, frass and larval cocoons, some of which contain highly dangerous substances. For example, integuments of *S. granarius* L. have been found to contain

various carcinogenic compounds such as ethyl, methyl and methoxy quinines which are heat resistant and therefore cannot be destroyed by boiling or baking [6].

The widespread use of synthetic chemicals has led to some serious problems, including development of resistant insect strains [7,8]. Even with appropriate chemicals, storage of maize for six-months may still result in dry weight losses of 7.5% and depress grain market value by 27% [9]. However, many farmers resort to the application of inappropriate and dangerous non-grain insecticides which pose great health concerns [10]. Thus, effective low-cost, non-chemical grain protection technologies have the potential for tremendous impact in Africa [2].

Justification

Millions of rural farmers in Africa produce maize, but completely lack access to improved post-harvest storage technologies, including the most basic storage structures or technical assistance for effective

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storage. This situation forces small producers to sell their maize at the time of harvest, with the disadvantage of low market prices. Later, they buy it back for their own consumption at inevitably higher prices. Therefore, storage alternatives must be found to minimize the qualitative and quantitative maize grain losses, preferably without the use of pesticides [11].

Hermetic storage in metal drums is known to provide good control of all storage insect pests including the Larger Grain Borer [12]. However, the high initial cost of drums in some areas and the tendency for people to use them for other purposes such as water storage limits their use in rural grain storage. Plastic bags provide a cheaper alternative but insects tend to perforate the bags, even if the grain is fumigated initially. As hermetic storage works on the principle of using up the available oxygen in an airtight container, considerable damage may already be caused by the LGB before the pest dies [2].

Triple-layer hermetic bags have been used to control cowpea bruchids, *Callosobruchus maculatus* (F) [13] on cowpea, *Dinoderus spp* and *P. truncatus* on cassava chips [14] with very promising results. However, little is known about the effect of the triple layer bags on LGB in stored maize, hence, the need for this research. The goal of this study was to evaluate the effectiveness of the multi-layer hermetic bags for the protection of stored maize against infestation by *P. truncatus* (Figure 1a and 1b) and *S. zeamais* (Figure 2).

Materials and Method

Experimental site and source of maize varieties

The study was conducted in Wa, Techiman and at the African

Regional Postgraduate Program in Insect Science (ARPPIS) laboratory of the University of Ghana, Legon, in the Upper West, Brong-Ahafo and the Greater Accra Regions of Ghana, respectively from June, 2011 to June, 2012. The maize used for the study was purchased from farmers during the harvesting period (August-September, 2011) at Wa and Techiman. Obatanpa is an improved variety that is given out to farmers by the Ministry of Food and Agriculture (MOFA) while Abrodenkye and Kamangkpong are local varieties that have been passed down to the inhabitants of Wa and Techiman by their forefathers. The three maize varieties used for the study had moisture content ranging between 12.5 and 14% and are the most commonly cultivated varieties in the three major geographical regions of Ghana (Savannah north, Middle forest belt and the Coastal evergreen south). Also, they have been reported to be high yielding, very nutritious, easy to cultivate and mature earlier than other varieties.

Characteristics of the bags used

The triple-layer hermetic bags were manufactured by Bio-plastics (a local manufacturer of plastic and rubber products in Ghana) and supplied by the Forum for Agricultural Research in Africa, (FARA). Each triple layer bag consists of two plastic bags (made of polyethylene) put inside a third bag made of woven polypropylene to give additional protection and strength. The bags were 100 µm thick and measure 34×62 cm in width and length, respectively.

Ordinary bags made of polypropylene and jute sacks were bought from Madina market in Accra for the experiment. The polypropylene bags were used as whole bags whereas the jute were cut and sewn into 5 kg sizes; the jute sacks are not readily available in good condition and so farmers tend to buy second hand bags which had been earlier used for the storage of cocoa beans. It was for this reason that the few good ones obtained had to be cut and neatly sewn in order to obtain the required quantities.

Culturing of experimental insects

A parent stock of adult *P. truncatus* was obtained from the entomology laboratory of the Savannah Agricultural Institute (SARI), Nyankapala, Tamale in the Northern Region and the Plant Protection Regulatory Services Directorate of the Ministry of Food and Agriculture (PPRS/ MOFA) at Pokuase in Accra, while *S. zeamais* was collected from infested maize stock at the Madina market, Accra. These were both reared on whole maize grains in a controlled environment. Culture conditions of $28 \pm 2^\circ\text{C}$, 65% relative humidity and 12L: 12 D photo regime [15,16] were used. The insects were placed in a plastic bowl covered with a nylon mesh and left under the sun for three hours so that insects infested with mites would die. About 100 unsexed adults were introduced into a glass jar containing 500 g of sterilized maize. The grains were sterilized in a refrigerator for 24 hours and in an oven at 40°C for six hours [16]. After two weeks of oviposition, the parent adults were removed using an aspirator and killed by freezing. This ensured the emergence of same age progeny for use in establishing the main culture with subsequent re-culturing every two weeks. By this, insects of the same cohort were always available for the experiment.

Laboratory experiments

Effectiveness of the triple-layer bag against *P. truncatus* and *S. zeamais*: This experiment compared the effectiveness of the triple-layer hermetic bag with two other conventional storage bags (Jute and Polypropylene; popularly known as ‘fertilizer sack’ in Ghana). Three unsterilized maize varieties (Obatanpa, Abrodenkye and Kamangkpong) were divided into three groups, that is, A, B and C,

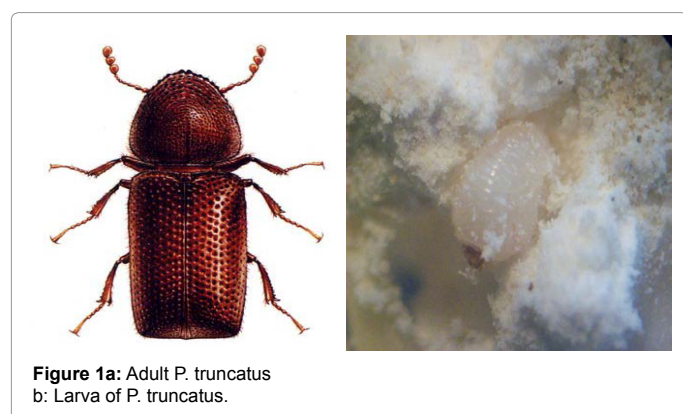


Figure 1a: Adult *P. truncatus*
b: Larva of *P. truncatus*.

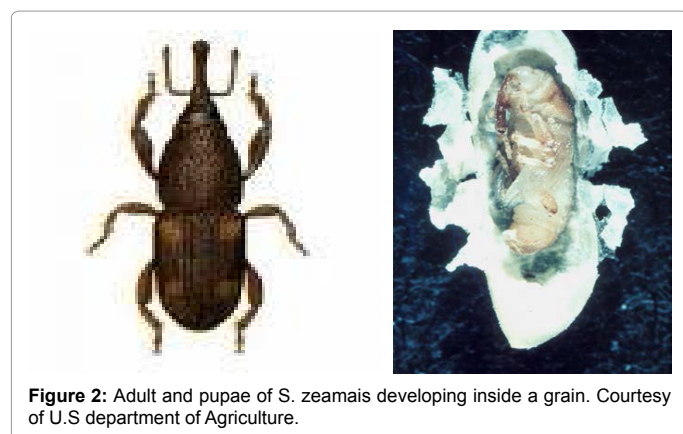


Figure 2: Adult and pupae of *S. zeamais* developing inside a grain. Courtesy of U.S department of Agriculture.

respectively. These groups had three replicates each, that is, A1, A2, A3, B1, B2, B3 and C1, C2, C3. Each of these maize samples weighing about 5 kg was put into each triple layer hermetic bag and replicated 3 times. Unsterilized maize was used to simulate the actual practices used by farmers during storage. Fifty unsexed LGBs from the culture were introduced (using camel hair brush) into each of the maize samples. The bags were pressed gently to take out all the available air present in each of them. When enough air was expelled, the bags were quickly tied with plastic ropes and stored for six months. The same was done for the polypropylene and jute sacks which served as the controls.

Another set of the same maize varieties (A, B, and C) with three replicates in each sample was set up for the *S. zeamais*. Fifty unsexed *S. zeamais* were introduced into them as in above and stored hermetically for six months. In all, there were 324 experimental units of sample bags. These were stored under room conditions to simulate farmers' actual storage conditions. Destructive sampling was done monthly (i.e. 54 bags per month; 27 bags for each of the two insect species and 3 replicates of each type of bag) for 6 months of storage to determine the grain loss. Plates 1 and 2 shows part of the experimental setup.



Plate 1: A section of the experimental setup.



Plate 2: Researcher inspecting the setup.

Determination of percentage damage, weight loss and moisture content: Two different sets of experiments were conducted; each for the *P. truncatus* and *S. zeamais*. Each set was sampled after one month (4 weeks), two (8 weeks), three (12 weeks) up to the sixth (24 weeks) month of storage. At each sampling occasion, the contents of each experimental bag were sieved using the set of USA standard sieve series (Nos. 10 to 35 mm). Adult insects, frass and grains were collected separately. After sieving, each grain sample was divided into sub-samples by the cone and quarter method. These sub-samples were used for the determination of the moisture content, percentage damage, weight loss and the germination potential.

Determination of percentage damage: A random sample of 100 grains of each variety was taken from each storage bag using the cone and quarter method. Bored grains were separated from whole grains and their numbers counted. This was done monthly and repeated three times for each sub-sample and the value of means taken. The percentage damage was calculated using the formula described by Adams and Schulten [17], and Duna [18]:

$$(\%) \text{ Damaged grains} = \frac{\text{No. of bored grains}}{\text{No. of bored grains}} \times 100$$

Assessment of weight loss: Maize grain loss bioassay was conducted to determine the damage caused by *P. truncatus* and *S. zeamais* to maize stored hermetically and to serve as basis for determining the effectiveness of hermetic triple bagging technology. The Thousand grain mass (TGM) method described by Boxal [19] was used to determine dry-weight loss. A sample of 1 kg of each maize variety was taken and sieved to remove all unwanted material and to obtain a working sample. A sub-sample of the working sample was used to determine the moisture content. The moisture content was determined five times and the mean value recorded. The remaining sample was accurately weighed and the number of grains counted. This was also repeated five times.

$$\text{The TGM was calculated using the formula: } \text{TGM} = \frac{10W(100 - MC)}{N}$$

Where W = weight of sample N = number of grains in sample and MC=moisture content

This was done before storage and repeated five times monthly for six months for each maize variety stored in the three different bags. At the end of each month, the percentage weight loss was determined using the formula, % (weight loss) = $\frac{(M1 - Mx)}{M1} \times 100$

Where M_1 = the TGM of grain at the start of storage.

M_x = the TGM of grain at the time x (i.e. 1, 2 or 6 months).

Analysis of results

Microsoft Excel 4.0 package was used for all statistical calculations. Where necessary, the data was transformed using either:

(a) for insect count: $x^1 = \log_{10}(x)$ for insect population [16]

(b) for percentages: $x^1 = \arcsine(P)^{1/2}$, where $P = \frac{x}{100}$

An analysis of variance was performed on the transformed data with homogeneity of variance. Fisher's Protected LSD was used to separate the means.

Results

Grain damage and weight loss

The levels of grain damage were significantly different ($p < 0.001$)

among the storage bags. After 6 months of storage, the triple-layer hermetic bag recorded the least damage of 26.57% while the highest was 57.03% in the jute bag. Weight losses were highly significant ($p < 0.001$) between the bags. The triple-layer hermetic bag recorded the least mean weight loss of 2.94% while polypropylene recorded the highest mean value of 23.65% with jute recording 19.55%. When stored in the triple-layer hermetic bag, *P. truncatus* caused a mean weight loss of 4.73% while *S. zeamais* recorded 1.56%. In the polypropylene bag, *P. truncatus* caused the highest mean weight loss of 27.92% while *S. zeamais* caused 15.81%. In the case of the jute sacks, *P. truncatus* recorded 23.58% while *S. zeamais* recorded a mean loss of 19.65% (Figure 3). Damage and weight loss increased within the first 2 months. Generally, the triple-

layer hermetic bag was observed to reduce grain damage and weight loss as the storage period progressed (Figures 4 and 5).

Highly significant differences ($p < 0.001$) were recorded among the maize varieties in terms of weight loss. Kamangkpong recorded the least mean weight loss of 11.09% while Obatanpa recorded the highest mean weight loss of 16.75%. Abrodenkye recorded 13.55%. The results also indicated highly significant ($p < 0.001$) difference in the bag-insect-variety interactions. However, there was no significant difference in the bag-insect interaction. Figure 6 shows the effect of each insect species on each maize variety in the different storage bags. Regardless of the insect species and the maize variety being infested in storage, the triple-layer hermetic bag reduced grain weight loss better than the polypropylene and jute sacks (Figure 6). Plates 3 and 4 show the effect of the two insect species on the various storage bags.

Moisture content

Variations in moisture content were significantly different ($p < 0.05$) among the various storage bags. There was highly significant difference ($p < 0.001$) in the bag-month interaction. The mean moisture content increased gradually with time from month 1 to 2, reduced a little and remained fairly constant in months 3 and 4, then increased slightly from month 5 to 6 at a constant rate (Figure 7). The least mean moisture content of 13.31% was recorded in the jute bag in the 3rd month while the highest mean moisture content of 15.56% was recorded in the hermetic bag in the 5th month (Figure 7). However, there was no significant difference in the interaction between the bag and variety.

Number of live insects after storage

The number of live insects was significantly different ($p < 0.05$) in the various storage bags. The least mean number of live insects was observed in the hermetic bag, which recorded an average of 7 *P. truncatus* in one case and 7 *S. zeamais* in another. The highest mean number of live insects was recorded in both the jute and polypropylene bags (Figure 8). The polypropylene bag recorded an average of 820 live *P. truncatus* and 412 live *S. zeamais* while the jute sack recorded an average of 827 live *P. truncatus* and 411 live *S. zeamais* (Figure 8). There was a highly significant difference ($p < 0.001$) in the number of live insects between the two insect species and the length of storage.

There was highly significant difference ($p < 0.001$) in the bag-insect interaction. Figure 8 shows the numbers of each insect species recorded per kilogram of maize in each storage bag after the 6 months period. Highly significant differences ($p < 0.001$) existed in the bag-insect-variety interaction (Figure 9) and the interaction among insect-variety-month (Figure 10).

Discussion

Damage and weight losses

Grain damage includes scarification of the pericarp and of the periphery of the endosperm, eating out of the germ, partial or complete consumption (hollowing out) of the kernel [18]. The damage and weight losses caused by the tunneling and feeding activities of adult insects were observed to have increased gradually in the jute and polypropylene bags as the storage period progressed while a reduction was rather experienced in the triple-layer hermetic bags. This observation confirms the findings of Murdock et al., Navarro et al., De Bruin, Villers et al. Donahaye et al. [13,20-23] who all reported little or no damage to hermetically stored grain.

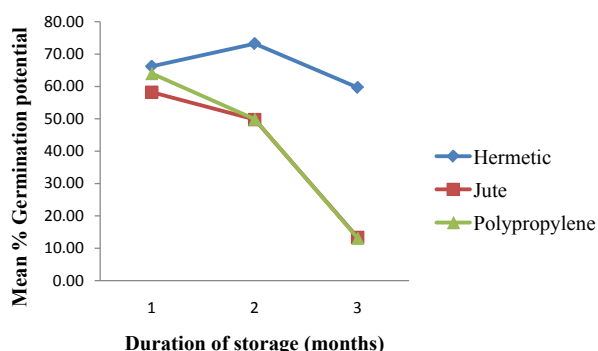


Figure 3: A graph showing the viability of grains infested with *S. zeamais* and stored in different bags over a 3 month period.

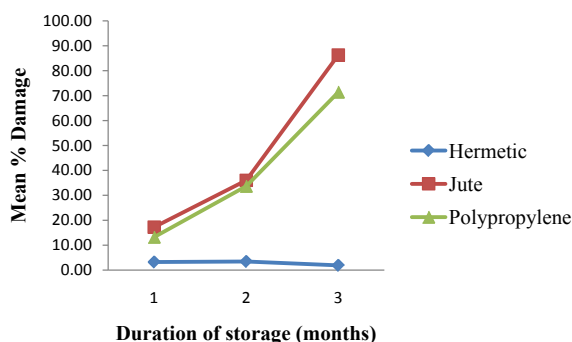


Figure 4: A graph showing the damage caused by *S. zeamais* in different bags over a 3 month period.

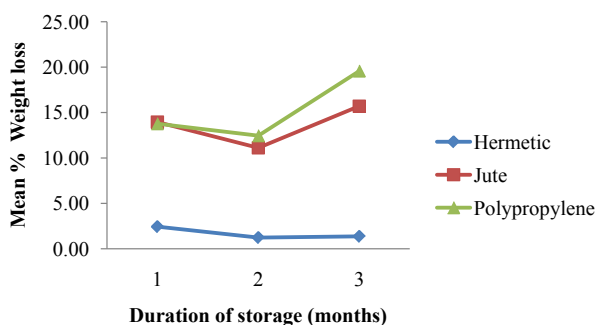


Figure 5: A graph showing the weight loss in percentage caused by *S. zeamais* in different bags over a 3 month period.



Plate 3: Several exit holes created by *P. truncatus* in the jute and polypropylene bags.



Plate 4: Effect of LGB on the polypropylene and hermetic bags after 6 mo.

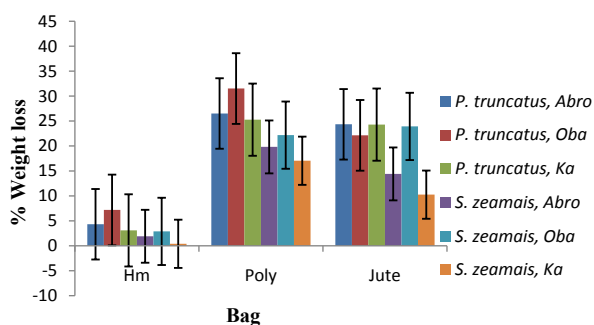


Figure 6: Weight loss by each insect species on the maize varieties in different bags.

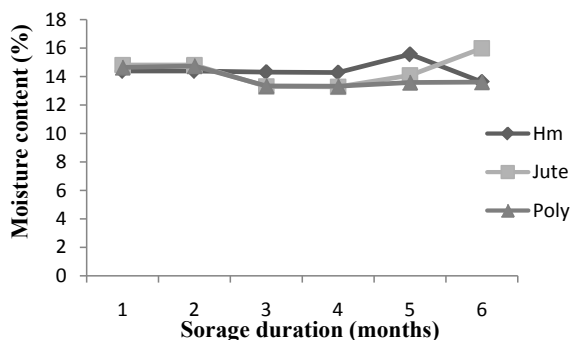


Figure 7: Moisture content in the various bags during a 6 month period.

The fact that there was no significant difference in the bag-insect interaction means that, the bags were not contingent on the insect species in terms of grain damage and weight loss. Thus the triple-layer

hermetic bag did not favour one insect species over the other. In other words, hermetic bag used in controlling *P. truncatus* can also be used against *S. zeamais*.

Greater damage and weight loss were also recorded in grain infested with *P. truncatus* than those infested with *S. zeamais*. This was not unexpected considering the fact that *P. truncatus* has been widely reported by many scientists to be the most destructive insect pest of contemporary times [2,24].

The huge losses recorded in the conventional storage bags (jute and polypropylene) was due to the benign environmental conditions

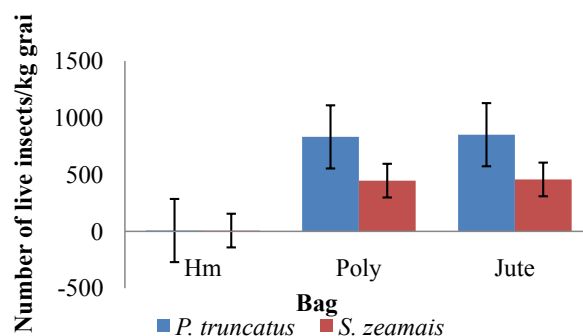


Figure 8: Mean number of live insects after 6 months of maize storage in various bags.

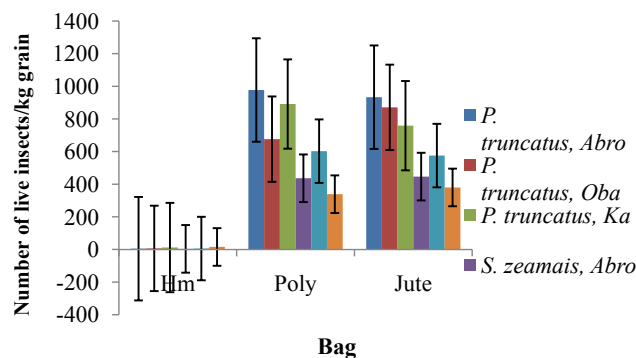


Figure 9: Mean number of live insects in each maize variety stored in different bags.

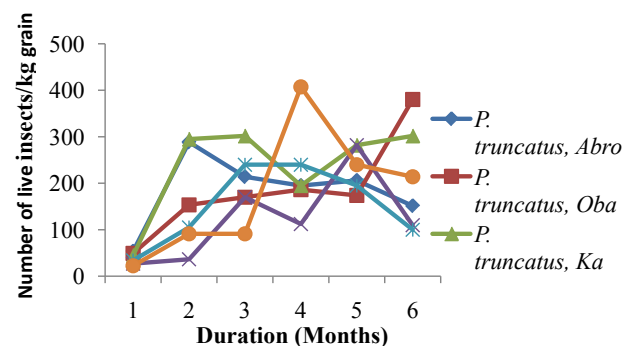


Figure 10: Mean number of live insects per maize variety stored for six months.

coupled with the ease of exchange of air (O₂) between the stored product environment and the surrounding environment. This is seriously lacking in the triple-layer hermetic bags due to its air tightness hence the inability of the insects to survive, reproduce and cause damage and weight loss [13,25]. Similar results were obtained from studies on cowpea in Niger, Burkina Faso, Benin, Northern Ghana and other West African countries [13,26,27].

The nature of the maize varieties i.e. texture, grain hardness, density among others could be attributed to the observed damage and weight losses since the degree of damage by the two insect pests was very minimal in all the maize varieties stored in the triple-layer hermetic bags throughout the storage period, while in the conventional storage bags (jute and polypropylene), all maize varieties were reduced to fine powder and rendered not even fit for animal consumption.

From the amount of grain powder produced by the different maize varieties in the jute and the polypropylene bags, the high yielding improved variety (Obatanpa) suffered greater damage and weight loss than the two local varieties (Kamangkpong and Abrodenkye). This was expected, as Obatanpa had soft pericarp and endosperm texture which is easy to bore through and tunnel. Regardless of the insect species and maize variety been infested in storage, the triple-layer hermetic bag performed far better than the polypropylene and jute sacks with regards to grain damage and weight loss. The triple-layer hermetic bag is effective and can be used to control *P. truncatus* and *S. zeamais* on stored maize in farmer's stores.

Moisture content

The results of this experiment indicated fluctuations in moisture content with duration of storage. The fact that there was no significant difference in the bag-variety interaction means that the storage bags are not contingent on the maize variety in terms of moisture content. Regardless of storage methods, the moisture content increased after four weeks of storage from a mean of 14.0% at start of the experiment to a mean of 14.81%. However there was little change in moisture content with the prolonged storage of 6 months.

The jute and polypropylene bags indicated a fall in moisture content after the second month and this remained almost constant until the fourth month. The hermetic bag experienced almost constant moisture content from the month 1 to the fourth month. This confirms the results of Jonfia-Essien et al. [28] who apparently reported a similar trend with cocoa beans storage in hermetic cocoons.

The sharp rise in moisture content by the triple-layer hermetic bags could be attributed to the temperature (heat) fluctuations experienced in the fifth month (February). During this period also known as harmattan, day time is always characterized by very hot weather while the evening and morning are very cold, hence condensation can take place and increase the moisture content [5].

Number of live insects after storage

This study revealed very few surviving insects in the triple-layer hermetic bags while several hundreds were recorded in the jute and polypropylene bags. This could be attributed to the O₂ depletion in the triple-layer hermetic bags. Due to respiratory activities of the insects and the grain itself, O₂ is used up leading to a buildup of CO₂ causing insect asphyxiation.

All the *P. truncatus* and *S. zeamais* beetles introduced into the triple-layer bags died at the end of the fifth month. Thus, the triple-layer hermetic environment provided an effective control of the insect

population in conformity to the findings of other researchers such as Jonfia-Essien et al. [28].

In the case, of the jute and polypropylene bags, there is easy exchange of air between the stored product environment and the surrounding atmosphere hence O₂ is always available for the continuous survival and consequent reproduction of the beetles. This was also evident in the large number of dead insects recorded in the jute and polypropylene due to natural mortality.

Conclusion and Recommendations

In conclusion:

1. All three maize varieties were susceptible to *P. truncatus* and *S. zeamais*. The high yielding improved variety, Obatanpa was the most susceptible or preferred by the pests while Abrodenkye and Kamangkpong were the least susceptible. Susceptibility appears to be related to the ability of the insects to bore into the grains and the powder produced resulting in weight loss.
2. It is possible to protect maize against *P. truncatus* and *S. zeamais* using the triple-layer hermetic bag.
3. The triple-layer hermetic bag is more cost-effective than the jute and polypropylene bags.
4. Some parasitoids were also observed in the conventional storage bags; but these could not prevent the insects from causing further damage.

From these results, it can be observed that the triple-layer hermetic bag has the potential to control *P. truncatus* and *S. zeamais* in stored grain. It can therefore contribute significantly to an integrated control of these two destructive insect pests.

Recommendations

Further studies should include investigations on effects of climate and increased temperatures on the effectiveness and longevity of the three layer hermetic bags. The development of biodegradable triple-layer bags that combines different defense mechanisms with other desirable traits such as resistance or tolerance against rodents and other biotic and abiotic factors should also be considered.

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