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# Evaluation Of Zerofly® Hermetic Storage Bags For Protection Of Maize Against Insect Pests In Ghana

A. Bosomtwe, E. A. Osekre, G. V. Bingham, G. P. Opit

**Abstract:** High post-harvest loss of maize due to stored product insect pests remains a food security challenge in Ghana. This field study evaluated the effectiveness of a novel technology, ZeroFly® Hermetic storage bags with different inner liners, to protect maize against infestation by *Sitophilus zeamais* Motschulsky and *Prostephanus truncatus* Horn. The study was carried out in the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Ghana, during the period September 2017 to March 2018. Maize pre-fumigated with Phostoxin® was stored in 50-kg ZeroFly® Hermetic storage bags. Experimental treatments were 20-µm High Density Polyethylene (HDPE) inner liner with oxygen absorber, 20-µm HDPE inner liner without oxygen absorber, 80-µm Polyester and Ethylene Vinyl Alcohol (EVOH) inner liner with oxygen absorber, 80-µm EVOH inner liner without oxygen absorber, 50-µm Charter NEX high barrier inner liner with oxygen absorber, 50-µm Charter NEX medium barrier inner liner with oxygen absorber, and untreated maize-filled 50-kg polypropylene bags without oxygen absorber (control). Maize-filled 50-kg polypropylene bags without oxygen absorber were used as Control. The percent insect damaged kernels on number basis (%IDKnb) recorded in the 20-µm HDPE liners and polypropylene bags were > 15% compared to < 1.8% recorded in the other treatments. Weight losses in the 80-µm EVOH and 50-µm Charter NEX high and medium barrier liners were < 0.35%. Aflatoxin levels were below the 15 ppb threshold. The results of the study showed that, ZeroFly® Hermetic storage bags with 80-µm EVOH inner liner and 50-µm Charter NEX high and medium barrier inner liners protected stored maize against *S. zeamais* and *P. truncatus* over the 6-month storage period.

**Index Terms:** Bagged grain, hermetic liners, mycotoxin, post-harvest loss, stored product insect

## 1 INTRODUCTION

Maize (*Zea mays* L.) is a staple food in Africa and its contribution to food and income security of households of smallholder farmers is substantial [35], [7]. In Ghana, maize is produced on about one million hectares of land and accounts for 50-60% of total cereal production. Even though the crop is produced throughout the country, the middle and southern sectors constitute the main production areas with 84% of production volume, with the remaining 16% grown in the northern sector of the country [26]. In 2018, an estimated 2.2 million tons of the commodity was produced in Ghana and annual rate of growth of 8.75% was achieved over the years (<http://knoema.com/atlas/Ghana/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production>). The total maize production in Ghana is done by about 70% of small holder farmers. Significant post-harvest losses of maize due to pests and mycotoxins attack occur in developing countries [11]. Global food losses and food waste report by FAO indicated that, more than 50% of the losses occur during production, post-harvest handling and storage [15]. In Ghana, stakeholders estimate that about 40% and 20-30% of harvested maize are lost to insect pests during the minor and major seasons, respectively [33].

Inefficiencies in post-harvest handling and storage systems are critical factors to food commodity losses and threaten the survival of millions of people living in food-deficit regions in the world [23]. However, use of appropriate and effective grain storage materials can result in reduced insect pests' activity in stored grains hence mitigating losses. Most small-scale farmers in Africa find traditional storage technique such as maize cribs, bamboo storage structures, pots, bag storage and warehouses economically feasible to construct because they are made of locally available materials [14], [1]. The current preferred grain storage technique in most developing countries is bag storage [24], [13]. Nonetheless, post-harvest loss of grains caused by insect pests and fungi (mycotoxins) is pervasive across Africa, and there is urgent need to find solutions in order to ensure food safety and security. Hermetic storage bags mostly have high density polyethylene inner liners with low permeability to air exchange. Respiration of grains, insects and other living organisms in the bag depletes oxygen and increases carbon dioxide to levels that inhibit growth and development [27]. Storage of grains in hermetic bags to minimize losses is being promoted in sub-Saharan Africa [7], [13]. Several workers [22], [4], [5], [6] have worked on hermetic storage bags for the protection of various commodities against storage insect pests in Ghana but the investigation have concentrated on the Triple-Layer hermetic storage bags. Hermetic storage bags are designed in varying capacities suitable for the storage needs of smallholder farmers [29]. They are simple and cost less compared to other hermetic storage structures [38]. The deltamethrin-incorporated polypropylene storage bag (ZeroFly® storage bag) produced by Vestergaard SA, Lausanne, Switzerland, is a new technology that [34] found to have potential to reduce post-harvest grain losses caused by stored product insect pests in bagged grains. Another type of bag, the deltamethrin-incorporated polypropylene hermetic storage bag (ZeroFly® Hermetic storage bag) is hypothesized to provide even better protection [36]. The effectiveness of

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hermetic bags depends largely on the inner liner which ensures airtightness. To ensure airtightness and prevent insect damage of the bag liners, the Purdue Improved Crop Storage Bag (PICS bag) has two inner liners. The ZeroFly® Hermetic storage bag (hereafter referred to as ZFH bag) comprises a single inner liner with an outer deltamethrin-incorporated polypropylene fabric. Therefore, there is the need to ensure that the inner liner of the ZFH bag is tough and robust enough to resist insect damage to ensure airtightness, and hence hermetic condition. The present study sought to determine the effectiveness of different types of inner liners used inside ZFH bags for protection of stored maize against infestation by two key insect pests, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). The objective of this study was to determine the robustness of different inner liners to withstand insect damage or puncture and ensure airtightness of the hermetic bags

## 2 MATERIALS AND METHODS

### 2.1 Study Site

The experiment was carried out in the Entomology laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The study spanned the period September 2017 to March 2018 (six-month period). The experiment was maintained under temperatures of  $30 \pm 2^\circ\text{C}$  and relative humidity of  $65 \pm 5\%$ .

### 2.2 Maize

White maize variety "Obatanpa" was used for the study. Maize was purchased from a single farmer in Ejura (a maize growing community in the Ashanti region of Ghana) to ensure uniformity. The maize was dried to a moisture content of ~13% (PHL moisture meter) using the solar biomass hybrid dryer (SBHD) [2], [9] and then fumigated using Phostoxin® tablets (Shenzhen Baocheng Chemical Industry CO., Ltd, Shenzhen, P. R. China) to ensure that the maize used for the study was free from all life stages of insect pests before the set up.

### 2.3 Pre-Experiment Disinfestation

Maize was disinfested by fumigating with Phostoxin®. Sixteen Phostoxin® tablets were placed in a stack of forty-two 50 kg bags (mini sacks). The recommended application is 30 tablets for 28.32 m<sup>3</sup>; one tablet produces 25 ppm of phosphine (hydrogen phosphide or PH<sub>3</sub>) in 28.32 m<sup>3</sup> (<http://www.researchfumigation.com/msds/weevil-cid-applicator-manual.pdf>). The volume of the stack of forty-two 50 kg bags was 14 m<sup>3</sup> (4 m × 3.5 m × 1 m). Therefore, 16 tablets were required for the 14-m<sup>3</sup> stack. One tablet was placed per m<sup>2</sup> (of the 4 m × 3.5 m area) and two tablets were placed in the center. The stack was then covered with a gas-tight tarpaulin to ensure gas leakage was as minimal as possible. The fumigation lasted five days after which the tarpaulin was removed and the room aerated for three days. Seven days after the removal of the tarpaulin, the fumigated maize was used to set up the treatments. Assuming complete decomposition and no leakage, the estimated highest concentration of PH<sub>3</sub> in the fumigated stack is 809 ppm. This was calculated based on the fact

that 1 tablet in 28.32 m<sup>3</sup> produces a concentration of 25 ppm of PH<sub>3</sub> gas. Using the formula  $C_1V_1 = C_2V_2$ , the concentration of PH<sub>3</sub> gas in 14 m<sup>3</sup> from 1 tablet and consequently, from 7 tablets was calculated as 809 ppm, assuming complete decomposition and no leakage from the stack

### 2.4 Treatments

In this study, Experimental treatments were 20-µm High Density Polyethylene (HDPE) inner liner with oxygen absorber (treatment 1), 80-µm Polyester and Ethylene Vinyl Alcohol (EVOH) inner liner with oxygen absorber (treatment 2), 20-µm HDPE inner liner without oxygen absorber (treatment 3), 80-µm EVOH inner liner without oxygen absorber (treatment 4), 50-µm Charter NEX high barrier inner liner with oxygen absorber (treatment 5), 50-µm Charter NEX medium barrier inner liner with oxygen absorber (treatment 6), and untreated maize-filled 50-kg polypropylene bags without oxygen absorber (control) (treatment 7).

### 2.5 Description of the EVOH and Charter NEX Inner Liners

The 80-µm liner is made of Polyamide (nylon), Polyester and Ethylene Vinyl Alcohol (EVOH) with TIE glue layers. It is a 9-layer bag with < 1.5 cc/m<sup>2</sup>/24 h oxygen transmission rate (OTR). The 50-µm Charter NEX high and medium barrier liners are made with polyamide as barrier resins. Both liners have the same thickness but different barrier properties. High barrier (CNS-151.32) looks or feels thicker with low transparency with OTR at 6 cc/m<sup>2</sup>/24hr. However, the medium barrier feels thinner and has relatively good transparency, with 100 cc/m<sup>2</sup>/24hr OTR. The liners and the ZeroFly® storage bags were obtained from Vestergaard Frandsen's local distributor in Nigeria (Turner Wright Nigeria Limited 15, Adenekan Salako Close, Ogba, Lagos, Nigeria), whereas polypropylene bags (distributed by Bentrionic Productions, P. O. Box KS 14318, Kumasi, Ghana) were obtained from a local market in Kumasi.

### 2.6 Set up of Treatments

Disinfested maize was transported to KNUST in bags provided by the maize producer (farmer). The six 50-kg bags in each treatment were filled with the disinfested maize. Two sachets of AGELESS® oxygen absorber (MITSUBISHI CHEMICAL GAS COMPANY, INC) were added to treatments 1, 2, 5 and 6. Six 50-kg maize-filled bags were assigned to each treatment. Bags in each treatment were exclusively on one wooden stack to prevent maize in bags from absorbing moisture from the floor. The pallets were 2 meters apart from each other. Altogether, there were forty-two 50-kg maize-filled bags used in this experiment. Two hundred 2 to 5-day old live adult *S. zeamais* and one hundred 2 to 5-day old live adult *P. truncatus* were introduced into each of the 50-kg bags by placing batches of 50 and 25 of *S. zeamais* and *P. truncatus*, respectively, into various sections of the maize-filled bags and then sealing the inner liner and the outer woven deltamethrin-incorporated polypropylene fibre. Only 100 adult *P. truncatus* were introduced into each bag due to difficulty in getting more than 100 per bag. The insect-infested maize was put in the inner liners of the ZFH bags as well as in the polypropylene bags. Insects used for

infestation were obtained from a stock culture maintained in the insect laboratory of the Department of Crop and Soil Sciences, KNUST.

## 2.7 Sampling and Data Collection

Initial sampling was conducted at the start of the study after which monthly ones were undertaken as described below for six months. During monthly sampling, bags were numbered 1 - 6 and pieces of paper were also numbered similarly. Pieces of paper were folded and placed in a container, mixed up, and one was randomly picked to determine which bag in each stack (treatment) got selected for sampling and data collection. Bags in the various treatments were destructively sampled in each month (maize bag sampled each month for each treatment for data collection was not returned to stock) (destroyed). It should be noted that because of the difficulty encountered in obtaining multiples of 8,400 adult *Sitophilus zeamais* and 4,200 adult *Prostephanus truncatus* for set-up of the study in replicates and with the study objective of determining the actual number of insects that would be added to the number initially introduced into maize in each 50 kg bag which thus required emptying the whole 50 kg bag sampled per treatment in each month for insect count, there was no replication for the monthly data collected.

### 2.7.1 Determination of Moisture Content (MC)

The moisture content of maize in each bag sampled was taken using the PHL-IL (USDA-ARS) moisture tester (hereafter referred to as PHL moisture meter). The meter was inserted in the maize 3 min after which the moisture content (MC) (%), temperature (°C) and relative humidity (r.h) (%) were recorded. Three different recordings were taken for each bag. Mean values were later calculated for moisture content, temperature and relative humidity.

### 2.7.2 Grain Sampling

A 1.2-m open-ended trier (grain probe) (Seedburo Equipment, Chicago, IL) was used to sample maize from randomly selected bags; these were the same bags MC was recorded. Samples were taken from three different locations of each bag with extreme caution to avoid puncturing the inner liner. Samples from each bag were mixed thoroughly in a basin to ensure homogeneity. A sub-sample of 250 g was weighed using a dial spring scale (CAMRY, Yongkang, China) then the percent insect damaged kernels on number basis (%IDKnb) and percent weight loss (%WL) were estimated as described below

### 2.7.3 Estimation of Insect Damaged Kernels on Number Basis (IDK)

The 250 g maize sample was poured on a tray and all kernels with insect-made hole(s) were separated from the undamaged ones using a hand lens (10X) and the number of damaged kernels was recorded. The damaged kernels were weighed using an electronic weighing balance (Mettler Toledo, Batch No. PB302, Capacity: 0.2-310 g). Per cent IDK on number bases was calculated using the formula below:

$$\text{Per cent IDK (number basis)} = \frac{\text{Number of IDK}}{\text{Total number of kernels}} \times 100$$

### 2.7.4 Weight Loss

Percentage weight loss as a result of insect damage was determined using the count and weigh method of [21], [10].

$$\text{Weight loss (\%)} = \frac{[(W_u \times N_d) - (W_d \times N_u)]}{W_u \times (N_d + N_u)} \times 100$$

Where,  $W_u$  = Weight of undamaged grain,  $N_u$  = Number of undamaged grain,  $W_d$  = Weight of damaged grain, and  $N_d$  = Number of damaged grain.

### 2.7.5 Number of Discolored Grain per 100 Grains

One hundred kernels were randomly selected from the 250 g sample and inspected using a hand lens with 10X magnification. Discolored grains were counted and rated using the [29] method for rating discoloration of grains.

### 2.7.6 Extraction of Insects from 50-kg Bags

One bag of maize in each treatment was randomly selected monthly. The whole bag of 50 kg of maize sampled from each treatment was sifted using U.S. Standard #10 (2-mm openings) and #25 sieves (0.71-mm openings) (Dual Manufacturing Co., Franklin Park, IL) to recover insects. Live and dead insects recovered from the maize were counted. After sieving the insects out, bags were taking out and not returned to stock (destructive sampling).

### 2.7.7 Mycotoxin Level Determination

Before the different bags were filled with maize, 5-kg of grains were randomly sampled from different spots of the heaped grains (approximately 2,100 kg), mixed thoroughly and 1-kg was sub-sampled for testing. After six months of storage, 250 g of maize was sampled from one bag in each treatment for mycotoxin analyses. Initial and final mycotoxin (aflatoxin and fumonisin) analyses were performed using Romer Labs AgraStrip® Total Aflatoxin Quantitative Test procedure (A) and Romer Labs AgraStrip® Quantitative Total Fumonisin (FUM) Test procedure (B) (Romer Labs®, Inc. Union, MO, USA). In both tests, sample grinding, extraction, solute preparation and test procedures were done in accordance with the manufacturer's instructions (Romer Labs Methods, romerlabs.com).

### 2.7.8 Seed Viability

Seed viability test was performed on samples taken before and after the storage. Before storage, 5-kg of maize was obtained as previously explained and 1-kg portions were sub-sampled into Ziploc bags for the initial viability test whereas 250-g portions were sub-sampled for the terminal viability test. At the end of the study, 250-g of maize was randomly sampled from each treatment for the viability test. Germination test was conducted by soaking a filter paper in a Petri dish (115 x 18 mm) using 15 ml of water and placing 100 randomly selected seeds on the paper. For each treatment, the procedure was replicated four times. The Petri dishes were randomly placed on tray and kept at ambient conditions ( $30 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  r.h.) for seven days, after which percentage seed germination was computed using the formula:

$$\text{Percentage germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds planted}} \times 100$$

### 2.7.9 Insect-Made Holes in Bags

ZeroFly® Hermetic bag selected for sampling in each month was examined for number of holes made by insects in the inner liners. Maize was poured from the inner liners.



The empty liners were then lifted and using hand lens (10X) number of insect-made holes in them were counted.

## 2.8 Data Analyses

Data could not be subjected to analysis of variance (ANOVA) because there was no replication of treatments in this experiment. Therefore, data for response variables of single bags sampled in each treatment over the 6-month storage are reported.

## 3 RESULTS

### 3.1 Moisture Content, Temperature and Relative Humidity in Maize Storage Bags

Moisture content of maize in the 20- $\mu$ m HDPE liners with or without oxygen absorber increased from an initial mean of  $14.4 \pm 0.1\%$  to  $15.8 \pm 0.3\%$  at the end of six months of storage. The mean MC recorded in the 80- $\mu$ m EVOH liners with or without oxygen absorber ranged between  $14.5 \pm 0.6\%$  and  $13.5 \pm 0.2\%$ , while that of maize in the 50- $\mu$ m Charter NEX high and medium barrier liners moved from an initial of  $15.2 \pm 0.1\%$  to  $14.3 \pm 0.1\%$ . Moisture content of maize in the polypropylene bag decreased from an initial  $15.0 \pm 0.1\%$  to  $13.5 \pm 0.2\%$  (Fig. 1). Initial mean temperature in all bags was  $26.6 \pm 0.1$  OC (Fig. 1). However, in most months, temperature increases in bags with the 20- $\mu$ m HDPE liner and PP bags were higher than that of the 80- $\mu$ m EVOH liners, the 50- $\mu$ m Charter NEX high and medium barrier liners. The highest temperatures of  $34.5 \pm 0.50$ C and  $34.3 \pm 0.0$ C were recorded in the 20- $\mu$ m HDPE liner with or without oxygen absorber, respectively, in the fifth month after storage (February); however, temperatures decreased in the sixth month (March) to  $30.9 \pm 0.6$  and  $29.3 \pm 0.1$ OC. With the exception of the fifth month when  $30.7 \pm 0.4$ OC was recorded in the 80- $\mu$ m EVOH liner without oxygen absorber, maize temperatures in the 80- $\mu$ m EVOH liner, the 50- $\mu$ m Charter NEX high barrier and medium barrier liners were below 29OC throughout the storage period. At the end of six months of storage, r. h. in the 20- $\mu$ m HDPE liners with or without oxygen absorber ranged between  $71.9 \pm 0.6\%$  and  $83.3 \pm 1.0\%$ ; this r. h. range was comparatively higher than the  $65.5 \pm 1.6\%$  to  $78.5 \pm 0.6\%$  range recorded in the 80- $\mu$ m EVOH, the 50- $\mu$ m Charter NEX high and medium barrier liners. The PP bags had the lowest r. h. ranging between  $61.8 \pm 1.3\%$  and  $77.2 \pm 0.6\%$  (Fig. 1).

### 3.2 Number of *S. zeamais* and *P. truncatus* in 50-kg ZeroFly® Hermetic and Polypropylene Bags

Live adult *S. zeamais* in maize in the ZeroFly® bags with 20- $\mu$ m HDPE liner with or without oxygen absorber (Treatment 1 and 3) increased substantially in the fifth month (February) (this was the month with the highest numbers) from the initial 200 in respective treatment bag to 5,837 and 6,296. Similarly, 7,152 adult *S. zeamais* were retrieved from the PP bag in February. Maize in the ZeroFly® bags with 80- $\mu$ m EVOH liner with or without oxygen absorber (Treatment 2 and 4), 50- $\mu$ m Charter NEX high barrier liner (Treatment 5) and 50- $\mu$ m Charter NEX medium barrier liner (Treatment 6) had no live *S. zeamais* after the second month of storage (November) (Table 1). Generally, dead *S. zeamais* were high in the 20- $\mu$ m HDPE liner with oxygen absorber, (range, 0–1803) and without

oxygen absorber, (range, 0-1862). A range of 0–1084 was found in the PP bags compared to 0–459 collected from the rest of the treatments (Table 1). In the case of live *P. truncatus*, the number started increasing in the 20- $\mu$ m HDPE liner with or without oxygen absorber two months after storage (November) from the initial 100 adults introduced in each bag (Table 2). The number of adults ranged from 25 to 516 in the 20- $\mu$ m HDPE liner with oxygen absorber; the range in the 20- $\mu$ m HDPE without oxygen absorber was from 29 to 413 adults. In the PP bags, number of live *P. truncatus* increased from 246 to 524 adults in the period from the second month of storage (November) up to the fifth month (February); the number reduced in the sixth month to 397 adults. In the 80- $\mu$ m EVOH liner with or without oxygen absorber, 50- $\mu$ m Charter NEX high and medium barrier liners, no live *P. truncatus* were found throughout the storage period (Table 2). The number of dead *P. truncatus* was higher in the 80- $\mu$ m EVOH liner and in the 50- $\mu$ m Charter NEX high and medium barrier liners than in the 20- $\mu$ m HDPE liners and PP bags after the first month of storage. However, at the end of six months of storage, number of dead insects was generally higher in the 20- $\mu$ m HDPE liners and PP bags than the other treatments.

### 3.3 Per cent Insect Damaged Kernels by Number (%IDKnb) and Weight Loss (%WL) per 250 g Maize

From an initial mean of 1.0%, %IDKnb increased to 15.9% and 17.8%, in the 20- $\mu$ m HDPE liner bags with oxygen absorber and 20- $\mu$ m HDPE liner without oxygen absorber, respectively, five months after storage. For the 80- $\mu$ m EVOH liner with or without oxygen absorber, 50- $\mu$ m Charter NEX high and medium barrier liners, %IDKnb did not exceed 1.8% throughout the storage period. For the PP bags, %IDKnb reached the highest recorded level of 40.8% by the sixth month of storage (Fig. 2). For %WL, there was an increase from an initial 0.1% to 1.2% and 1.0% in ZeroFly® bags with 20- $\mu$ m HDPE liner with or without oxygen absorber, respectively, after five months of storage. Weight losses recorded in the 80- $\mu$ m EVOH liner with or without oxygen absorber, 50- $\mu$ m Charter NEX high and medium barrier liners were less than 0.4% during the 6 months of storage. In the PP bags, %WL increased in the first 3 months after storage from 0.1% to 1.6%, but there was a drastic increase to 12.4% by the fourth month of storage and further increase to 15.7% in the sixth month (Fig. 2).

### 3.4 Number of Discolored Grains per 100 Grain in ZeroFly® Hermetic and Polypropylene Bags

Number of discolored kernels were greater (1–46) in 20- $\mu$ m HDPE liner with oxygen absorber and 1-51 in without oxygen absorber. There were fewer than 3 per 100 kernels that were discolored in the 80- $\mu$ m EVOH liners and 50- $\mu$ m Charter NEX high and medium barrier liners at the end of the storage period (Table 3). For the PP bags, number of discolored grains ranged between 1 and 4.

### 3.5 Total Aflatoxin and Fumonisin Levels in Maize in ZeroFly® Hermetic and Polypropylene Bags

Mean total aflatoxin levels of  $64.9 \pm 4.3$ ,  $344.7 \pm 15.3$  and  $59.1 \pm 5.5$  ppb were recorded in maize in 20- $\mu$ m HDPE liners with or without oxygen absorber and the PP bags,

respectively, after 6 months of storage. Total aflatoxin levels of maize in the 80- $\mu$ m EVOH liner without oxygen absorber and 50- $\mu$ m Charter NEX high barrier liner were  $14.2 \pm 0.1$  and  $12.3 \pm 1.0$  ppb, respectively, at the end of the storage period. Maize in 80- $\mu$ m EVOH liner and 50- $\mu$ m Charter NEX medium barrier liner with oxygen absorber contained aflatoxin levels of  $15.9$  ppb  $\pm 0.6$  and  $15.7 \pm 0.8$  ppb, respectively. In Ghana, aflatoxin threshold for maize is 15 ppb [18]. Total fumonisin levels in maize in the 20- $\mu$ m HDPE liners with or without oxygen absorber were  $19.1 \pm 0.9$  and  $95.9 \pm 3.8$  ppm, respectively; in the PP bag, fumonisin levels of maize at the end of 6 months of storage was  $20.9 \pm 0.4$  ppm. Total fumonisin levels of maize in the 80- $\mu$ m EVOH liners, 50- $\mu$ m Charter NEX high and medium barrier liners were below 4 ppm (Table 4).

### 3.6 Number of Insect-Made Holes in Different Types of Inner Liners of ZeroFly® Bag

The 20- $\mu$ m HDPE liners had insect-made holes ranging from 2-114 depending on the level of infestation. In contrast, the 80- $\mu$ m EVOH liner, 50- $\mu$ m Charter NEX high and medium barrier liners had no insect-made holes during the 6 months. In the PP bags, insect-made holes ranging from 28-302 were found after the second month of storage (Table 5).

### 3.7 Viability of Maize

After 6 months, percent germination levels of maize stored in the 80- $\mu$ m EVOH liners with or without oxygen absorber (Treatment 2 and 4), 50- $\mu$ m Charter NEX high barrier liner (T5) and 50- $\mu$ m Charter NEX medium barrier (T6) liners were  $69.7 \pm 0.9\%$ ,  $74.7 \pm 2\%$ ,  $72.7 \pm 0.7\%$ , and  $72.0 \pm 1.5\%$ , respectively. The germination level at the start of the experiment was  $73.6 \pm 0.9\%$ . However, germination of maize in the 20- $\mu$ m HDPE liners with or without oxygen absorber (T1 and T3) and PP bags (T7) was  $< 15\%$  (Table 6).

## 4 DISCUSSION

Grain MC is critical for safe storage of any commodity in view of its effects on biological agents such as insects and fungi in the storage environment. Apart from the bags with 20- $\mu$ m HDPE liners for which grain MC increased over the storage period, all other treatments had a decrease. The increase in grain moisture content in the 20- $\mu$ m HDPE liners was possibly due to insect damage to liners which allowed entry of air thereby leading to increase in insect population and the accompanying MC. According to [16], respiration by insects, molds and grains results in production of heat which promotes water vapor production and increase in MC of grains. Expectedly, MC and r.h. were low in the PP bags because PP and jute bags which have relatively large openings in their fabric allow sufficient exchange of moisture between grains and ambient air [17]. Hermetic condition causes oxygen depletion and upsurge in carbon dioxide which affect feeding, reproduction and survival of insects and microorganisms [28]. Based on data from the present study, greater numbers of live adult insects of both *S. zeamais* and *P. truncatus* were recorded in maize in the ZeroFly® bags with 20- $\mu$ m HDPE liner with or without oxygen absorber and in the PP bags. This observation could be as a result of relatively higher air exchange between bags and ambient environment in bags with

punctured liners caused by insect activity. Sufficient air entry into bags due to punctures cause insects to survive and reproduce thereby causing more damage to the liners. Interestingly, it was observed that insect numbers in the ZeroFly® bags with 20- $\mu$ m HDPE liners correlated positively with the number of tears and punctures in the liners. In a similar study, [39] found that low oxygen affected total egg production and reduced progeny emergence. [17] reported that farmers lamented about the fact that hermetic bags tear easily and raised it as a major concern negating the effectiveness of hermetic bags for maize storage. The 80- $\mu$ m EVOH liner, 50- $\mu$ m Charter NEX high and medium barrier liners did not suffer any insect damage or punctures throughout the storage period. The 80- $\mu$ m EVOH liner, 50- $\mu$ m Charter NEX high and medium barrier liners provided the necessary hermetic condition needed to kill insects that were introduced whereas, the outer deltamethrin-incorporated PP bag prevented infestation from external sources. When compared to the 80- $\mu$ m EVOH and 50- $\mu$ m Charter NEX high and medium barrier liners, the 20- $\mu$ m HDPE liners appeared to be more prone to puncture because they are thinner. Punctures in the liner compromise the hermetic system which results in increased air exchange thereby facilitating increased survival and reproduction of insects; this then results in more puncture holes. Results of this study are similar to those of [31] who reported strong correlations between number of holes observed in hermetic grain bags and insect numbers, mortality rate and losses, and concluded that holes in the liner negatively affected the effectiveness of hermetic storage bags. *Prostephanus truncatus* is known to have extraordinary capabilities to bore through different kinds of materials including plastics [8], [7]. According to [32] an ideal hermetic storage structure including an inner liner should have high integrity in terms of structural strength and should be air-tight or provide reasonable impenetrability in order to minimize or eliminate the effects of ambient atmosphere on insect activities, and that these intrinsic qualities that assure effective storage differ with product type. According to [27], when insects are enclosed in limiting oxygen conditions, they die out of desiccation because they are unable to generate the water needed to maintain vital life processes. The different materials used in manufacturing the liners may have accounted for their varying tensile strength. The holes created by insects in the 20- $\mu$ m HDPE liner raise concerns about the durability and dependability to protect stored maize against insects. The increase in %IDKnb in the 20- $\mu$ m HDPE liner is a result of high numbers of live *S. zeamais* and *P. truncatus* that were present and the fact that they are internal feeders. According [19], internal feeders are more destructive because their larvae feed inside infested grains, and when they exit grains as adults leave highly visible exit holes which ultimately increases the %IDKnb. Data from this study showed that after just 2 months of storage, %IDKnb in the 20- $\mu$ m HDPE liner and in the PP bag had exceeded 5%, which is the acceptable threshold set by the Ghana Standards Authority for consumption and commercial purpose [18]. However, in the 80- $\mu$ m EVOH liners and the 50- $\mu$ m Charter NEX high and medium barrier liners, %IDKnb was less ( $< 1.8\%$ ) throughout the storage period. It was surprising that a weight loss of  $< 1.3\%$  was recorded in the ZeroFly® Hermetic bags with 20- $\mu$ m HDPE liners

similar to that recorded in the bags with 80- $\mu$ m EVOH liner, given the higher %IDKnb. Typically, higher IDK would be expected to be associated with higher weight loss as was the case of > 15% weight loss recorded in the PP bags at the end of 6 months of storage. [34] also reported maize weight loss of 11.88% when maize was stored in PP bags for 6 months. Per cent discolored grains were higher ( $\geq 4\%$ ) in the 20- $\mu$ m HDPE liners with or without oxygen absorber in November (two months after storage), and as high as 46% and 51%, respectively, in February. This can be attributed to high MC and temperature caused by insect respiration. Additionally, limited aeration in the 20- $\mu$ m HDPE liners may have created a warm microclimate for mold production and the associated increased discoloration. According to [37] and [12], favorable temperature and r.h. promote insect multiplication and mold growth which result in grain discoloration. With reference to [30] grain discoloration rating method, maize in the 80- $\mu$ m EVOH liners and 50- $\mu$ m Charter NEX high and medium barrier liners had  $\leq 2\%$  discolored grains and hence qualify to be rated as good. The high aflatoxin levels in the 20- $\mu$ m HDPE liner and polypropylene bag can be attributed to high MC and temperature in these bags, which result from insect activities. [3] noted that MC and temperature are the two critical environmental factors that promote growth and development of mold in maize. Total aflatoxin levels in the 20- $\mu$ m HDPE liner and PP bag were at least three times the acceptable threshold of 15 ppb [18], which means that maize in these bags is not wholesome for consumption. In relation to fumonisin levels, it was < 2.5 ppm in the 80- $\mu$ m EVOH liners and 50- $\mu$ m Charter NEX high and medium barrier liners, and below the safe threshold of 4 ppm [18]. Higher fumonisin levels recorded in maize in the 20- $\mu$ m HDPE liners and PP bags (16.3 – 95.9 ppm) render the maize unwholesome. Percentage germination of maize in 80- $\mu$ m EVOH and 50- $\mu$ m Charter NEX high and medium barrier liners was high and close to the initial (73.6  $\pm$  0.9%). This could be due to limited or no grain damage resulting from complete mortality of insects and lower number of discolored grains in these treatments. Generally, germination percentage reduced marginally compared to the initial. The germination results of this study compare favorably with those of [25], who indicated that insect population size and the storage period were the factors that had the highest influence on seed germination in their work on *S. oryzae* in corn. According to [20], internal feeders mostly prefer feeding on the embryo because it is rich in protein which inevitably compromises the physiological qualities of seeds.

## 5 CONCLUSION

The results of this study showed that, ZeroFly® Hermetic storage bags with 80- $\mu$ m Ethylene Vinyl Alcohol (EVOH) inner liners and 50- $\mu$ m Charter NEX high and medium barrier inner liners protected stored maize against *S. zeamais* and *P. truncatus* by preventing damage to the inner liners by these insects by killing artificially introduced insects. The bags also maintained aflatoxin and fumonisin levels in maize within safe range for consumption. These imply that the 80- $\mu$ m Ethylene Vinyl Alcohol (EVOH) inner liners and 50- $\mu$ m Charter NEX high and medium barrier inner liners are suitable for use as inner liners for the ZeroFly® Hermetic storage bag for protection of stored

maize against *Sitophilus zeamais* and *Prostephanus truncatus*. Thus the ZeroFly® Hermetic storage bag with these inner liners can be used alongside the Triple-layer hermetic bags (PICS) by farmers to store grains.

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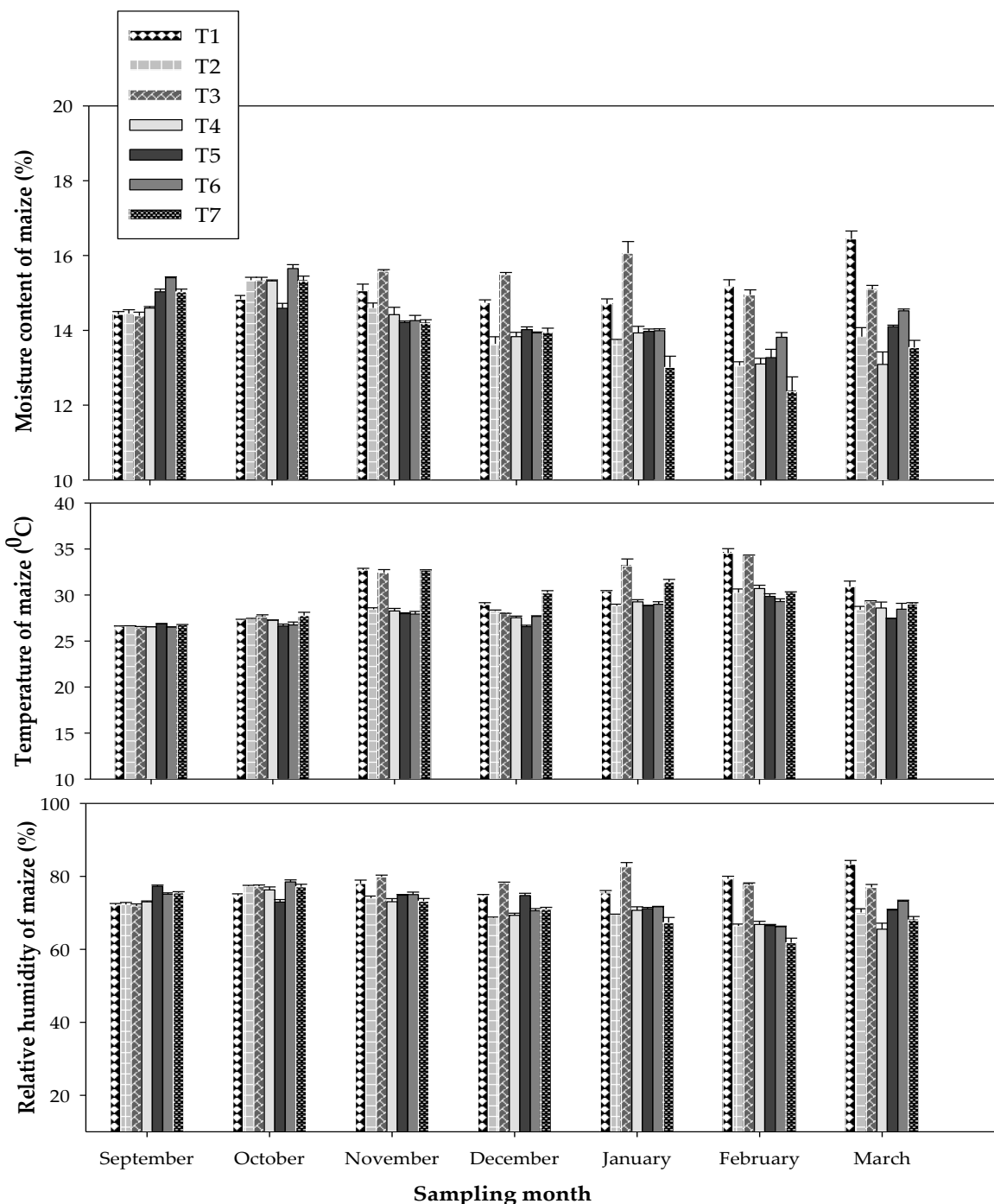
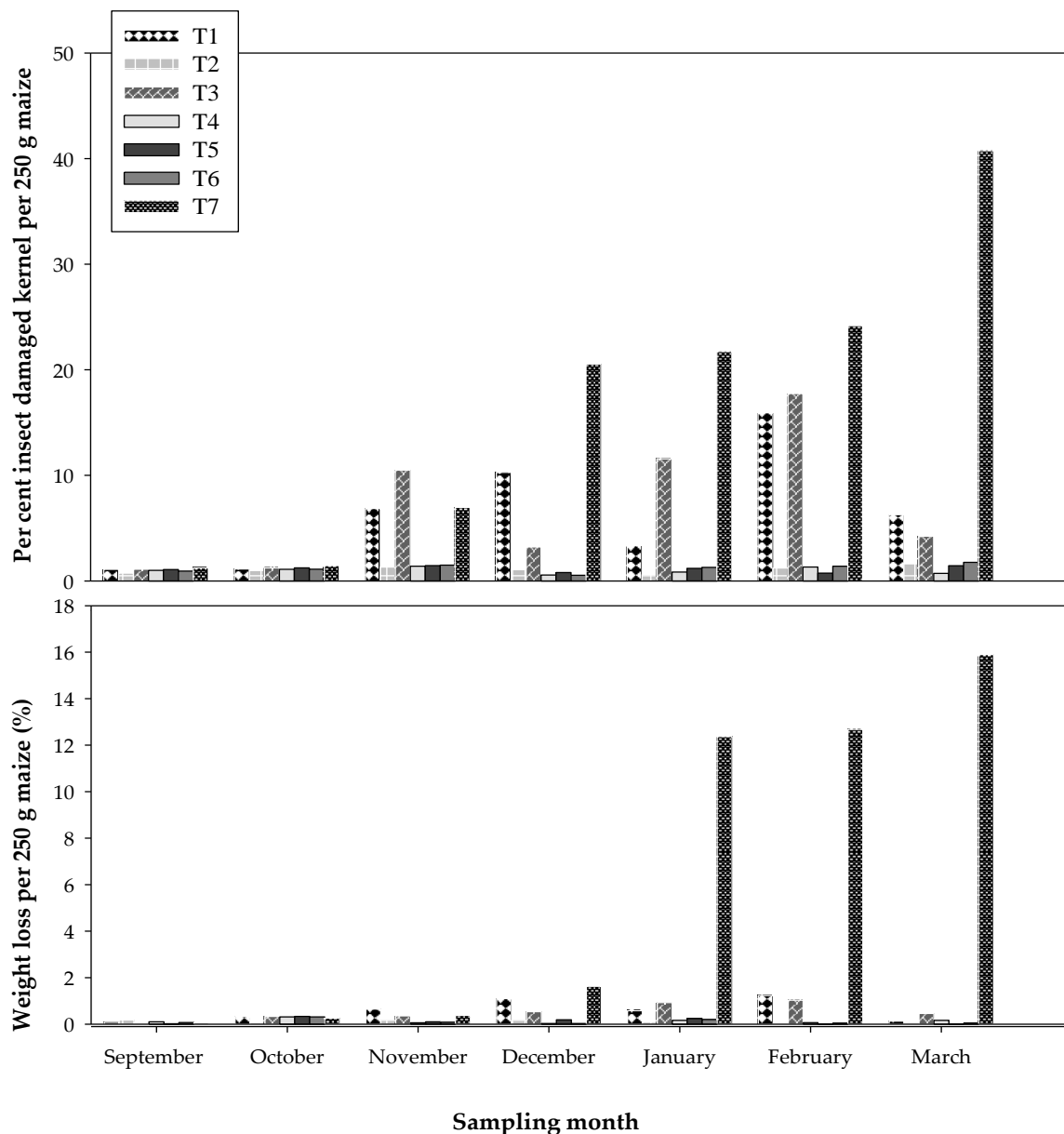


Fig. 1. Moisture content, temperature and relative humidity of maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period in a study in Kumasi, Ghana.

T1= Maize-filled 50 kg ZeroFly® bag with 20-µm HDPE liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm EVOH liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm HDPE liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm EVOH liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm Charter NEX high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm Charter NEX medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag



**Fig. 2.** Insect damage kernel on weight basis and weight loss in maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period in a study in Kumasi, Ghana

T1= Maize-filled 50 kg ZeroFly® bag with 20-µm HDPE liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm EVOH liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm HDPE liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm EVOH liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm Charter NEX high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm Charter NEX medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 1**

*Number of live and dead Sitophilus zeamais in maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period.*

Treatment	Sampling month													
	September		October		November		December		January		February		March	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
T1	200	0	84	248	1653	132	3092	774	378	429	5837	1803	2155	1508
T2	200	0	0	234	7	170	0	91	0	317	0	235	0	222
T3	200	0	99	205	3211	216	377	249	1272	493	6296	1862	1186	346
T4	200	0	4	283	1	173	1	459	0	430	0	333	0	212
T5	200	0	2	259	0	189	2	236	0	221	0	322	0	223
T6	200	0	0	315	8	179	2	114	0	256	0	347	0	301
T7	200	0	227	178	2337	216	4423	946	6826	801	7152	1084	6043	731

T1= Maize-filled 50 kg ZeroFly® bag with 20- $\mu$ m HDPE liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80- $\mu$ m EVOH liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20- $\mu$ m HDPE liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80- $\mu$ m EVOH liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50- $\mu$ m Charter NEX high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50- $\mu$ m Charter NEX medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 2**

*Number of live and dead Prostephanus truncatus in maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period.*

Treatment	Sampling month													
	September		October		November		December		January		February		March	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
T1	100	0	24	60	516	78	148	52	25	157	477	168	337	80
T2	100	0	0	86	0	74	0	47	0	94	0	93	0	79
T3	100	0	26	65	195	101	29	20	169	143	413	189	368	67
T4	100	0	0	89	0	96	0	59	0	116	0	88	0	91
T5	100	0	0	91	0	92	0	50	0	91	0	106	0	89
T6	100	0	0	98	0	89	0	38	0	102	0	87	0	99
T7	100	0	72	13	246	21	389	154	400	125	524	154	397	70

T1= Maize-filled 50 kg ZeroFly® bag with 20- $\mu$ m liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80- $\mu$ m liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20- $\mu$ m liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80- $\mu$ m liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50- $\mu$ m high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50- $\mu$ m medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 3**

*Number of discoloured grains in maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period.*

Treatment	Sampling month							
	September	October	November	December	January	February	March	
T1	1	1	5	5	4	37	46	
T2	1	0	1	1	1	1	2	
T3	1	1	4	6	33	51	14	
T4	0	1	1	1	1	2	3	
T5	0	0	1	1	1	2	1	
T6	1	1	1	1	0	2	2	



T7	1	1	3	2	3	4	4
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T1= Maize-filled 50 kg ZeroFly® bag with 20-µm liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 4**

*Total aflatoxin and fumonisin levels (Mean ± SEM) in maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners for a six-month period*

Treatment	Aflatoxin (ppb)	Fumonisin (ppm)
Initial	3.6 ± 0.1	< 0.3 ± 0
T1	64.9 ± 4.3	19.1 ± 0.9
T2	15.9 ± 0.6	1.4 ± 0.2
T3	344.7 ± 15.3	95.9 ± 3.8
T4	14.2 ± 0.1	0.9 ± 0.1
T5	12.3 ± 1.0	1.1 ± 0.1
T6	15.7 ± 0.8	2.4 ± 0.4
T7	59.1 ± 5.5	20.9 ± 0.4

T1= Maize-filled 50 kg ZeroFly® bag with 20-µm liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 5**

*Number of holes in different types of ZeroFly® inner liner over a six-month storage period*

Treatment	Sampling month						
	September	October	November	December	January	February	March
T1	0	2	12	10	4	87	28
T2	0	0	0	0	0	0	0
T3	0	0	19	4	32	114	12
T4	0	0	0	0	0	0	0
T5	0	0	0	0	0	0	0
T6	0	0	0	0	0	0	0

T1= Maize-filled 50 kg ZeroFly® bag with 20-µm liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag

**Table 6**

*Percentage germination (Mean ± SEM) of maize stored in polypropylene bags and ZeroFly® bags with different types of inner liners over six months.*

Treatment	% germination
Initial	73.6 ± 0.9
T1	14.6 ± 1.5
T2	69.7 ± 0.9
T3	12.7 ± 1.8
T4	74.7 ± 2.0
T5	72.7 ± 0.7
T6	72.0 ± 1.5
T7	7.6 ± 0.9

T1= Maize-filled 50 kg ZeroFly® bag with 20-µm liner + oxygen absorber; T2 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner + oxygen absorber; T3 = Maize-filled 50 kg ZeroFly® bag with 20-µm liner - oxygen absorber; T4 = Maize-filled 50 kg ZeroFly® bag with 80-µm liner - oxygen absorber; T5 = Maize-filled 50 kg ZeroFly® bag with 50-µm high barrier liner + oxygen absorber; T6 = Maize-filled 50 kg ZeroFly® bag with 50-µm medium barrier liner + oxygen absorber; T7 = Maize-filled 50 kg polypropylene bag