



Field evaluation of the long-lasting treated storage bag, deltamethrin incorporated, (ZeroFly[®] Storage Bag) as a barrier to insect pest infestation



Sulochana Paudyal^a, George P. Opit^{a,*}, Enoch A. Osekre^b, Frank H. Arthur^c, Georgina V. Bingham^d, Mark E. Payton^e, James K. Danso^b, Naomi Manu^b, Evans P. Nsiah^f

^a Dept. of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK, 74078-3033, USA

^b Dept. of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

^c USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502, USA

^d Vestergaard SA, Place St. Francois 1, 1003, Lausanne, Switzerland

^e Department of Statistics, Oklahoma State University, 301 MSCS Building, Stillwater, OK 74078, USA

^f PENS Food Bank, P. O. Box 143, Ejura- Ashanti, Ghana

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ABSTRACT

The deltamethrin incorporated polypropylene (PP) bag, ZeroFly[®] Storage Bag, is a new technology to reduce postharvest losses caused by stored-product insect pests. Maize was pre-fumigated and used for the following treatments: ZeroFly bags filled with untreated maize, PP bags filled with maize treated with Betallic Super (80 g pirimiphos-methyl and 15 g permethrin per liter as an emulsifiable concentrate (EC)), and PP bags filled with untreated maize (control). The experiment was conducted from February–August 2015, at four sites in different locations of the Middle Belt of Ghana. Moisture content (MC), number of live and dead insects, insect damaged kernels (IDK) and maize weight loss data were collected monthly. ZeroFly bags and Betallic treatment significantly reduced insect damage compared to the control treatment. ZeroFly bags were able to keep IDK levels below 5% for 4 months, but the levels increased to 5.2 and 10.2% by 5 and 6 months of storage, respectively. In the control, IDK increased significantly over time and reached 32% after 6 months. The ZeroFly bag was effective against *Sitophilus*, *Tribolium* and *Cryptolestes* species for 4 months. Mean weight loss of $\leq 3.68\%$ was recorded in ZeroFly bags during 6 months of storage whereas 11.88% weight loss occurred in the PP bags by 6 months of storage. Based on our results, ZeroFly bags were found to have potential for use in the reduction of postharvest grain losses in bagged grains. Maize may still have been infested during bagging hence ZeroFly bags were effective for storage for only 4 months. However, greater benefits of using ZeroFly bags are realized if insect-free grains or legumes are stored in bags.

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1. Introduction

Most of the calorie and protein needs of humans are obtained from cereal grains, grain legumes and oilseeds (Cordain, 1999). Maize (*Zea mays* L.) is one of the important cereal grains grown widely throughout the world. It is a major staple food in Africa (FAO, 2004; Tefera et al., 2011), and significantly contributes to household food security for smallholder farmers (Baoua et al., 2014). From 2008 to 2011, maize was planted on approximately

31.1 million ha of land in sub-Saharan Africa, and the average annual production during this period was 56.7 million tons (AGRA, 2014). However, due to poor storage techniques, maize producers in developing nations experience considerable losses after harvest (Giga et al., 1991; Boxall, 2001; Alonso-Amelot and Avila-Núñez, 2011).

Over the past few decades, postharvest loss estimation and measures to effect mitigation of these losses have been high on the international agenda (FAO, 1999; FAO and World Bank, 2011; Abass et al., 2014; Affognon et al., 2015). Despite international focus on postharvest loss issues, farmers in the developing nations still face significant losses. Estimates of postharvest losses vary in literature,

* Corresponding author.

E-mail address: george.opit@okstate.edu (G.P. Opit).

and global figures for losses of 9–40% are often quoted (Pimental, 2002; FAO and World Bank, 2011; Parfitt et al., 2010; Tefera, 2012; Hodges et al., 2014). Postharvest losses can arise from inappropriate storage techniques, deterioration by insect pests and rodents, high ambient temperature and high relative humidity (FAO and World Bank, 2011; Anankware et al., 2013; Abass et al., 2014). However, most of the postharvest losses result from damage caused by storage insect pests (Giga et al., 1991; Bett and Nguyo, 2007). Infestation of stored maize by insect pests may produce unpleasant odors, render the grain unfit for consumption, reduce nutritional content and grain quantity thereby leading to low market prices (Hill, 1990; Jood and Kapoor, 1992; FAO, 2004; Mboya, 2013). Furthermore, insects can facilitate entry of fungal spores into kernels or seeds by breaking the seed coat; they also disseminate of fungal spores, hence potentially contributing to increased production of mycotoxins that are carcinogenic and immunosuppressive to humans (Khan et al., 2016).

A number of stored-product insect pests, which ultimately cause quantitative and qualitative deterioration (FAO, 2009), can infest stored maize. Of the pests that infest maize, beetles and moths are the most important. *Sitophilus oryzae* (L.), the rice weevil, *Prostephanus truncatus* (Horn), the larger grain borer, *Tribolium castaneum* (Herbst), the red flour beetle, *Plodia interpunctella* (Hübner), the Indianmeal moth, *Sitotroga cerealella* (Oliver), the Angoumois grain moth, and *Rhyzopertha dominica* (F.), the lesser grain borer, are the most important pests of stored maize (USDA, 1986; Rees, 2004; Groot, 2004; Hagstrum et al., 2012).

Effective grain storage can reduce pest activity and can help secure food quality and quantity until the next harvest season. For storage of cereal grains and grain legumes, farmers in sub-Saharan Africa predominantly use traditional storage techniques such as open platforms, woven baskets, pots, mud rhombuses, maize cribs, bamboo storage structures, straw roofed storage structures, underground storage, bag storage and warehouses (FAO, 1994a; Adejumo and Raji, 2007). Because most of the aforementioned structures are made of locally available materials, smallscale farmers find them economically feasible to construct. Storing cereals in sacks such as jute or polypropylene bags is currently the most common storage technique (FAO, 1994a; Koono et al., 2007; De Groot et al. 2013). However, postharvest losses do occur in bagged commodities that are not subjected to insect pest management actions; losses of up to 60% have been reported in maize that is stored using traditional polypropylene bags (Costa, 2014).

Farmers in developed countries use grain protectants and fumigants to control insect pests of stored maize (White, 1995; Arthur, 1996; Zettler and Arthur, 2000). Some challenges to pesticide use are that resource-poor smallholder farmers in developing countries find the cost of pesticides prohibitive, thereby making them unaffordable, and these chemicals have been abused and misused by farmers in ways that pose risks to human health and the environment (Kamanula et al., 2011; Hodges et al., 2014). Additionally, insects develop resistance to chemical insecticides making them less effective (Opit et al., 2012). Therefore, a need exists for the development of reduced-risk approaches for managing insect infestations in bagged grain. Additionally, ways of scaling up reduced risk technologies are needed because farmers in developing countries have been too slow to adopt such technologies (USDA, 1986; Kaminski and Christiaensen, 2014).

In rural parts of Ghana, most maize produced by smallholder farmers is traditionally stored in jute or polypropylene bags (FAO, 1994a; Akramov and Malek, 2012; Anankware and Bornu-Ire, 2013). These bags often do not protect the grains against insect pests, leading to heavy losses. Reducing postharvest losses in bagged grain could increase smallholder farmers' income and contribute to increased food security. The need for a bag that

effectively mitigates infestation of grain stored in it led to the idea of insecticide-containing fabrics for storage bags. Several studies have documented evaluation of bags with insecticide-containing fabrics for the control of stored product pests (Parkin, 1948; Atkins and Greer, 1953; Muthu and Pingale, 1955); but the bags tested were not commercially available then and are still unavailable to date. The concept of insecticide-incorporated textile has been successful in mosquito nets for mosquito control to mitigate malaria (Barlow et al., 2001). Building on the concept of insecticide-containing fabrics for storage bags, Vestergaard SA, Lausanne, Switzerland has developed a deltamethrin incorporated polypropylene bag (ZeroFly® Storage Bag), which has great potential to reduce postharvest losses of cereal grains and grain legumes stored in it for use in developing countries (Anankware et al., 2014; Costa, 2014). The ZeroFly bag is designed to give protection to commodities by preventing the entry of insect pests, thereby facilitating preservation of cereal grains and grain legumes. However, there is little published data on field trials with the ZeroFly Storage Bag. Therefore, the objective of this study was to determine the effectiveness of the ZeroFly bag to protect maize from infestation by stored-product insect pests under field conditions in Ghana.

2. Materials and methods

2.1. study sites

This experiment was conducted in four warehouses, in three major maize growing areas located in the “middle belt” of Ghana, a transitional ecological zone of Ghana between the coastal and upland areas. The warehouses were in Ejura (located at a longitude 1°5' W and 1°39' W and latitude 7°9' N and 7°36' N) — two warehouses, Techiman (located at a longitude 1°42' 54 W and latitude 6°37'25 N) and Wenchi (longitude 2° 6' 0" W and latitude 7° 45' 0" N). The experiment was conducted during the period February to August of 2015, in the four warehouses (hereafter referred to as sites), two at different locations in Ejura, one in Techiman and another in Wenchi. Sites selected for the experiment were either near maize storage areas or near market areas where bagged maize was collected for the grain trade.

2.2. Treatments

There were three treatments assigned to each site. The first treatment was Betallic Super EC (Batch number Eas-13 g-13193, Hockley International Ltd, Manchester M22 5lb, UK)-treated maize of MC of 11–13% in 50-kg untreated polypropylene (PP) bags. Betallic Super EC is a pesticide that contains two active ingredients, 80 g pirimiphos-methyl and 15 g permethrin per liter as an emulsifiable concentrate (EC). According to the Betallic label, an application rate of 300 ml in 15 L of water for application on twenty 100-kg bags (maxi bags) of grain is recommended. In this study, 7.5 ml of Betallic was mixed with 0.38 L of water to treat each batch of 50 kg of clean, dry, insect pest-free maize. A hand sprayer (Bentronic pressure sprayer) of 2-L capacity was used to apply the insecticide. Therefore, a total volume of 135 ml and 6.84 L of Betallic and clean water, respectively, was applied on nine 100-kg bags (0.9 tonnes) of maize used in the Betallic treatment, at the four sites. Treated maize was thoroughly mixed using a wooden spade to ensure uniformity, and the treated maize was air-dried before being used to fill 50-kg untreated PP bags. Filled bags were sealed by sewing using thread and a needle, and the bags were then stacked on pallets at the four sites.

The second treatment was untreated maize in untreated 50-kg PP bags (control). Clean, dry, insecticide- and insect pest-free maize with a MC of 11–13% was used to fill untreated PP bags. No

insect pest-control measures were conducted in the control bags during the 6-month maize storage period; which was also the duration of the experiment.

The third treatment consisted of untreated maize of MC of 11–13% in the 50-kg deltamethrin (DM) incorporated PP bags [ZeroFly® Storage Bags (hereafter referred as ZeroFly bags). In this study, each of the three storage methods is referred to as a “treatment”, although each of them is not a treatment as defined in statistics. ZeroFly bags were obtained from Vestergaard Frandsen’s local distributor in Nigeria (Turner Wright Nigeria Limited 15, Adenekan Salako Close, Ogba, Lagos, Nigeria), whereas untreated polypropylene bags and Betallic Super EC (distributed by Bentronic productions, P. O. Box Ks 14318, Kumasi, Ghana) were obtained from a local market in Ejura, in the Ashanti region of Ghana. According to the producer’s label, the ZeroFly bag contains 3 mg/kg deltamethrin and the insecticide is incorporated into the PP fabric in such a way as to allow slow and controlled release of the active ingredient for at least two years.

2.3. Maize and its pre-experimental disinfestation

Ghana is a major maize producing African country, and most farmers grow white maize varieties (Ragasa et al., 2013). Therefore, a white variety of maize called “Obatanpa” was used for the experiment. Maize was purchased from a single local farm in Ejura to ensure uniformity of maize used in the experiment. The maize was cleaned, dried to a moisture content of 11–13% (John Deere moisture meter measurement (SW08120, Illinois, US)) and then fumigated using Phostoxin® tablets before the experiment was set up to ensure that the maize used was free from all life stages of insect pests. Twenty-six Phostoxin tablets were placed in a stack of seventy-two 50-kg bags (mini bags). Thirty tablets are recommended for 28.32 m³; one tablet produces 25 ppm of phosphine (hydrogen phosphide or PH₃) in 28.32 m³ (<http://www.researchfumigation.com/msds/weevil-cid-applicator-manual.pdf>). The stack of seventy-two 50-kg bags had a volume of 24 m³ (6 m × 4 m × 1 m). Therefore, 26 tablets were required for a 24-m³ stack. One tablet was placed per m² (of the 6 m × 4 m area) and the final two tablets were placed in the center to distribute the 26 tablets for the 24 m² base area. The stack was then covered with a gas-tight tarpaulin to ensure gas leakage was as minimal as possible. The estimated PH₃ concentration inside the stack after complete decomposition of the tablets was 764.71 ppm. The fumigation lasted 5 d after which the tarpaulin was removed. Fourteen days after the removal of the tarpaulin, the fumigated maize was used to set up the treatments referred to above.

2.4. Methodology

Maize was transported to each site in bags provided by the maize supplier. Filling of 50-kg bags for the three different treatments was done at each site, starting with maize and bags which were untreated (no pesticide in either the maize or bag). This was done to prevent insecticide cross-contamination among treatments through maize and/or bags. In each of the four sites, there were six sub-replicates for each treatment, i.e. six maize-filled bags were assigned to each treatment; each stack of six bags was on a separate pallet to prevent bags from absorbing moisture from the floor. The pallets were placed 4 m apart from each other. There were eighteen 50-kg bags per site. Additionally, five mouse and rat traps were placed in each site to minimize rodent damage to bags of stored maize — evidence of the presence of mice and rats had been seen during preparation of the sites for the experiment. The experimental design used was randomized complete block design (RCBD) with four replications and six sub-replicates for each treatment

replicate. Each site represented a replication.

2.5. Sampling and data collection

Bags were sampled at the start of the experiment, just after the experiment had been set up in February 2015. Monthly sampling was then conducted at the beginning of the month from March to August. Bags in each stack of six were randomly numbered 1–6 to facilitate sampling. The assignment of numbers to bags was by randomly picking numbered and folded pieces of paper, each with one of the six numbers written on, which had been shuffled in a small plastic container and accordingly assigning numbers to bags. Three bags from each treatment were randomly selected for sampling during each sampling event. The order of sampling for the three treatments, from first to last, was the control, ZeroFly bags and Betallic treatment. The rationale for the order of sampling was to avoid insecticide cross-contamination among treatments.

2.5.1. Moisture content (MC)

Moisture content in each bag sampled was assessed using two moisture meters. The bag selected for sampling in each treatment was opened by undoing the thread seal. A moisture meter developed by the USDA-ARS Center for Grain and Animal Health Research, Manhattan KS, referred to in this study as the PHL meter, was inserted in a bag of maize, left to stabilize over a 3-min period, and then the temperature (°C) and MC (%) readings were taken. Three different readings were taken from different positions for each bag and the average values were calculated. A John Deere moisture meter (Manufactured by agraTronix™; Moisture Check Plus™, USA for Deere & Company; Batch SW08120) was also used to measure the MC of maize in each of the bags data were collected using the PHL meter. Three measurements were taken and the average MC was calculated for each bag.

2.5.2. Grain sampling

A 1.2-m open-ended trier (grain probe) (Seedburo Equipment, Chicago, IL) was used to sample maize from bags. Three triers, one for each treatment, were used for sampling. Samples were taken from the center and two opposite sides near the inner surface of each bag. Samples from each bag were mixed thoroughly in a basin to ensure homogeneity. A sample of 250 g was then weighed out using a dial spring scale (CAMRY, Yongkang, China). The 250-g maize sample was placed in a labeled plastic bag and taken to the laboratory for data collection. The maize from each bag that remained in the basin, after the 250-g sample was weighed out, was put back into the bag from where the samples were taken; this was done immediately after all required data had been taken. Sampled bags were sealed by sewing using thread and a needle immediately after unused maize from the samples taken had been put back. To avoid insecticide cross contamination, hand gloves were changed after handling samples for each treatment. Floors in the different sites were always kept clean and mice traps and rat traps were replaced regularly.

For each of the 250-g samples collected, data on number of insects of each species, number of insect damaged kernels (IDK), percentage of IDK and weight of damaged and undamaged kernels was obtained. All samples were processed at Kwame Nkrumah University of Science and Technology Insectary (KNUST Insectary), located in the Department of Crop and Soil Sciences, Kumasi, Ghana. Percent weight loss due to insect damage was determined using the count and weight method (FAO, 1992) [% Weight loss = $\frac{(W_u \times N_d) - (W_d \times N_u)}{W_u(N_d + N_u)} \times 100$, where, W_u is the weight of undamaged grain, N_u is the number of undamaged grain, W_d is the weight of damaged grain and N_d is the number of damaged grain]. The

average number of kernels in a 250-g sample was 778 and out of those, the numbers of damaged and undamaged kernels were recorded.

Samples for determining levels of aflatoxin and fumonisin were taken 4 and 6 months after storage began. Three 250-g samples were obtained from three bags for each treatment, at each site; these were mixed and a 500-g sample was then weighed out and ground. A 50-g subsample was obtained from this. Two 10-g samples were obtained from the 50-g sample for determining aflatoxin and fumonisin levels. In summary, four 10-g samples were tested for each bag treatment and month, for both aflatoxin and fumonisin. Maize samples collected were stored in 17-L Koolatron® 12-V Compact Portable Electric Cooler (P75, Koolatron® Canada, Brantford, Canada) after the sampling, before being transported to the laboratory for testing. Samples were stored in a refrigerator until testing was conducted. For the determination of the levels of aflatoxin and fumonisin, Romer Labs AgraStrip® Total Aflatoxin Quantitative Test procedure (2014a) and Romer Labs AgraStrip® Quantitative Total Fumonisin (FUM) Test procedure (2014b), respectively, were used.

2.6. Statistical analyses

Statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Treatment effects were assessed using analysis of variance methods (PROC MIXED). A repeated measures model in a randomized complete block design was utilized, with site as the blocking factor and month as the repeated factor. An autoregressive covariance structure was used to model the correlations within treatment and across months. Analyses of the numbers of IDK and numbers of live and dead insects were conducted with the use of a square root transformation. A square root transformation was used to correct for heterogeneous variances and the lack of normality of the count response variable. The simple effects of treatment given month were assessed with protected planned contrasts (SLICE option in an LSMEANS statement). In the case of percent MC, IDK and weight loss, data analyses were conducted with the use of an arcsine transformation to stabilize variances but untransformed percentages are reported.

3. Results

3.1. Moisture content

For moisture content measured by the PHL or JD meters, there was no significant interaction between storage period and treatment (Table 1). Effects of storage period were significant for both

PHL and JD meters (Fig. 1; Table 1). For moisture content measurements taken on the same bags of maize at approximately the same time, there was a $\leq 2\%$ difference in the moisture content measured by the PHL and JD meters (Fig. 1) — here $\leq 2\%$ refers to MC units as opposed to either PHL or JD MC measurements being lower or higher by a percentage of 2. Moisture content measurements for the PHL moisture meter ranged from 11 to 13.9% (Fig. 1A), whereas those for the JD meter ranged from 13 to 14.8% (Fig. 1B).

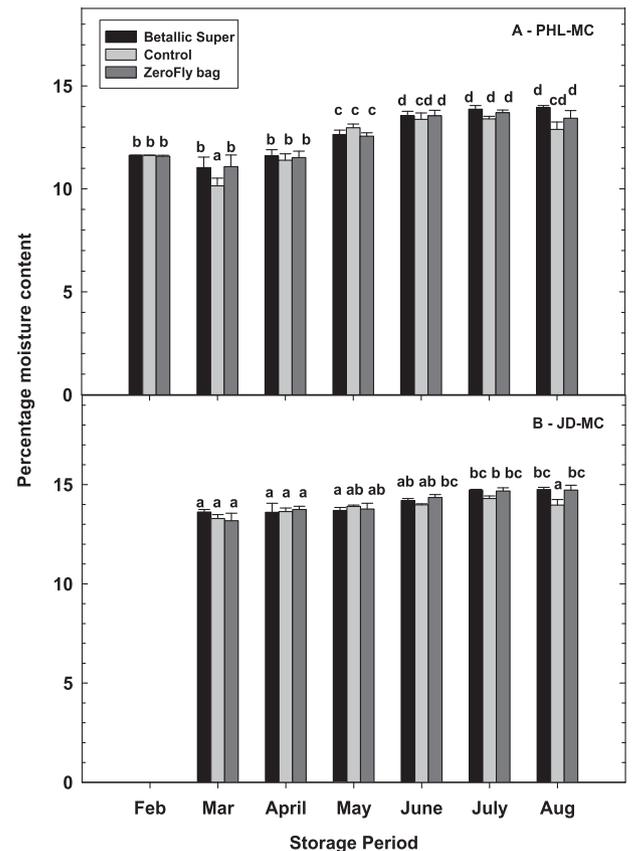


Fig. 1. Moisture content (%) (mean \pm SE) measured by PHL (A) and JD meter (B) of maize kernels in the Betallic Super-treated maize-filled PP bags (Betallic Super), untreated maize-filled PP bags (control) and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Table 1

ANOVA results for storage period and treatment for moisture content measured by both JD and PHL meters, number of insect damaged kernels, percentage of insect damaged kernels (%) and weight loss per 250-g samples from the Betallic, control and ZeroFly bag treatments.

Response Variable	Source	df	F	P
Moisture Content (PHL meter)	Treatment	2, 14.4	3.42	0.0609
	Storage period	6, 47	46.49	<0.0001
	Treatment \times Storage period	12, 47.8	0.89	0.5618
Moisture Content (JD meter)	Treatment	2, 8.47	1.96	0.1992
	Storage period	5, 35.4	12.57	<0.0001
	Treatment \times Storage period	10, 36.9	1.03	0.4412
Number of Insect Damaged Kernels	Treatment	2, 7.08	15.93	0.0024
	Storage period	6, 48.2	19.89	<0.0001
	Treatment \times Storage period	12, 48	5.00	<0.0001
Insect Damaged Kernels (%)	Treatment	2, 5.5	14.68	0.0062
	Storage period	6, 44.4	18.19	<0.0001
	Treatment \times Storage period	12, 44.5	4.78	<0.0001
Weight loss (%)	Treatment	2, 6.73	9.02	0.0125
	Storage period	6, 46.4	19.25	<0.0001
	Treatment \times Storage period	12, 46.3	5.22	<0.0001

3.2. Insect damaged kernels (IDK)

The interaction between treatments and storage period was significant for the number of IDK and percentage of IDK (Table 1). Our results show that the ZeroFly bag and Betallic treatments significantly reduced insect damage compared to untreated PP bags with untreated maize (control treatment). Mean numbers of IDK were not significantly different during the entire storage period in the Betallic treatment (Fig. 2). In the control treatment, IDK increased significantly from 11% per 250-g maize sample in June to 32% in August. However, in the case of ZeroFly bag treatment, numbers of IDK were not significantly different during the period from June to August; the number of IDK increased from 3% in May to 10% in August (Fig. 2).

3.3. Insect infestation level

3.3.1. *Sitophilus* spp.

There was significant interaction between storage period and treatment in relation to the number of live *Sitophilus* spp. (Table 2). There were no live adult *Sitophilus* in samples from the Betallic and ZeroFly bag treatments during the first 2 months of storage. In the Betallic treatment, numbers of live *Sitophilus* were not significantly different during the entire storage period (Fig. 3). In the case of the ZeroFly bag treatment, numbers of live *Sitophilus* in bags were not significantly different during the period February to July, but the number increased numerically to 15 at the end of the study in August. The number of live *Sitophilus* was significantly higher in

bags in the control treatment, where 32 insects were found in June, but the number decreased to 20 in August (Fig. 3A). Although, there was no significant interaction between storage period and treatment for dead *Sitophilus*, depending on the month, the numbers of dead insects were numerically or significantly higher in the ZeroFly bags than in the other two treatments (Fig. 3B). On average, 31 dead *Sitophilus* per sample were found in ZeroFly bags at the end of storage in August. These data indicate that ZeroFly bags are likely contributing to increased mortality of *Sitophilus* spp. but may not be killing the insects as quickly as the Betallic treatment.

3.3.2. *Tribolium* spp.

There was significant interaction between storage period and treatment for both live and dead *Tribolium* spp. (Table 2). The numbers of live *Tribolium* were not significantly different for samples from the ZeroFly bag and Betallic treatments in all storage periods; in both treatments, the mean number of insects per sample was below 1 during the 6 months of storage. In the control treatment, mean number of live insects increased significantly to 6.5 in June, but decreased to 1.7 and 2.1 in July and August, respectively; the July and August number of live *Tribolium* spp. were statistically similar to the number of insects in March (Fig. 4A). In all the treatments, dead *Tribolium* spp. numbers were below 2 (Fig. 4B).

3.3.3. *Cryptolestes* spp.

There was significant interaction between storage period and treatment for both live and dead *Cryptolestes* spp. (Table 2). Despite the lack of significant differences in the numbers of live insects in the Betallic and ZeroFly bag treatments, there were numerically more insects in the latter treatment (11.5 ± 6.48) than in the former treatment (0.8 ± 0.42) at the end of study (August) (Fig. 5A). In the control treatment, the numbers of live insects were significantly higher than in the other two treatments, and numbers increased significantly with storage time from 45 in July to 92 in August (Fig. 5A).

3.4. Grain weight loss

In relation to maize weight loss, the interaction between storage period and treatment was significant (Table 1). There were no significant differences among the three treatments during the first 3 months of storage. In the control treatment, percentage weight loss increased significantly from June to August when 11.9% weight loss occurred. No significant weight loss occurred in the Betallic treatment where weight loss did not exceed 1% during entire storage period of 6 months. In the ZeroFly bag treatment, there was no significant weight loss until August when 3.7% weight loss occurred (Fig. 6).

3.5. Aflatoxin and fumonisin levels

There was no significant interaction between storage period and treatment for aflatoxin levels ($F = 3.15$, $df = 2,9$, $P = 0.09$). Bag treatment had a significant effect on aflatoxin levels ($F = 6.24$, $df = 2,9$, $P = 0.02$) whereas storage period had no effect ($F = 3.31$, $df = 1,9$, $P = 0.10$). Aflatoxin levels in the ZeroFly bag, Betallic and control treatments were 7.3 ± 0.6 , 7.0 ± 0.5 , and 28.9 ± 8.4 ppb (1 ppb = 0.001 mg/kg), respectively. Levels of aflatoxin in the ZeroFly bag and Betallic treatments were similar, but were both lower than those in the control. In relation to fumonisin, storage period, treatment and the interaction between storage period and treatment were not significant ($F = 0.01$, $df = 1,9$, $P = 0.91$; $F = 1.4$, $df = 2,9$, $P = 0.30$; $F = 1.17$, $df = 2,9$, $P = 0.35$, respectively). Fumonisin levels in the ZeroFly bag, Betallic and control treatments

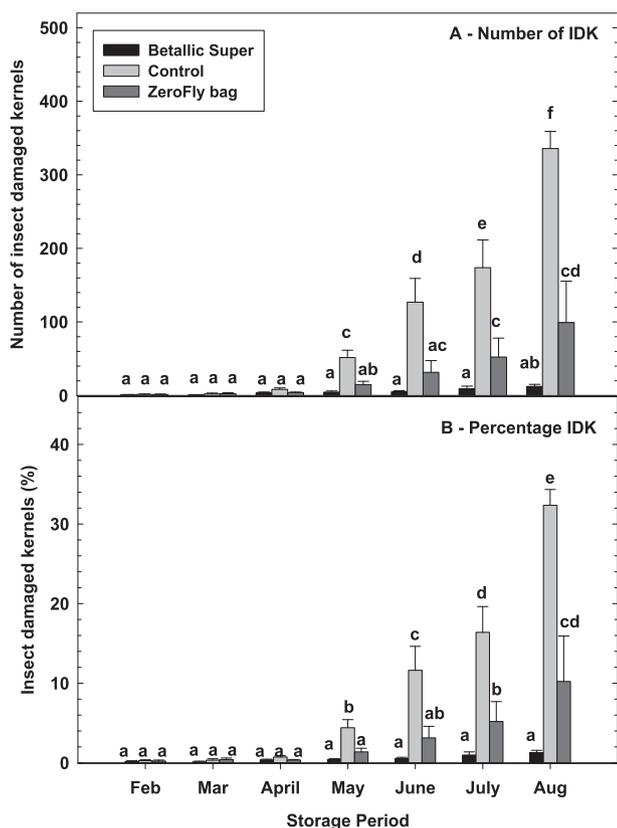
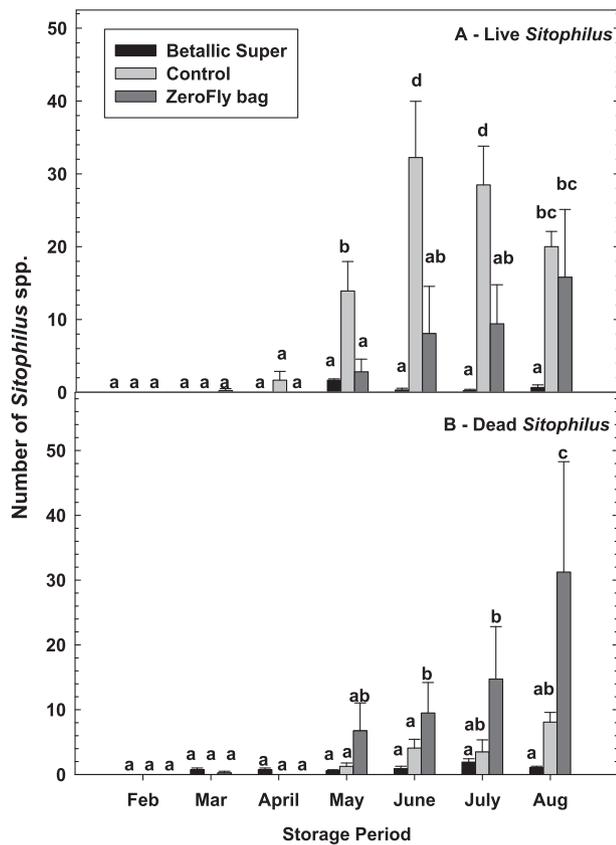


Fig. 2. Number (A) and percentage of insect damaged kernels (IDK) (B) (mean \pm SE) per 250 g of maize kernels in the Betallic Super-treated maize-filled PP bags (Betallic Super), untreated maize-filled PP bags (control) and ZeroFly[®] Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

Table 2ANOVA results for storage period and treatment for number of live and dead *Sitophilus* spp., *Tribolium* spp. and *Cryptolestes* spp. per 250-g samples from Betallic, control and ZeroFly bag treatments.

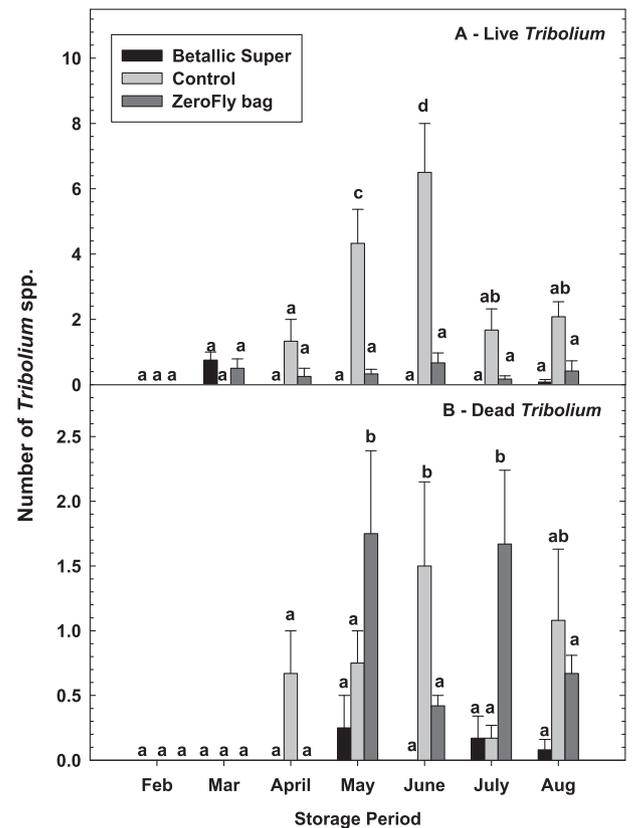
Insects	Source	Live insects			Dead insects		
		df	F	P	df	F	P
<i>Sitophilus</i> spp.	Treatment	2, 7.8	9.14	0.0090	2, 5.67	2.23	0.1933
	Storage period	6, 48.4	6.46	<0.0001	6, 40.5	6.06	0.0001
	Treatment × Storage period	12, 48.2	3.66	0.0006	12, 41.1	1.62	0.1230
<i>Tribolium</i> spp.	Treatment	2, 30.5	53.03	<0.0001	2, 5.76	6.57	0.0327
	Storage period	6, 50.1	9.26	<0.0001	6, 41	5.05	0.0006
	Treatment × Storage period	12, 50.7	9.25	<0.0001	12, 41.7	2.58	0.0116
<i>Cryptolestes</i> spp.	Treatment	2, 6.82	1.47	<0.2938	2, 4.58	3.81	0.1060
	Storage period	6, 48.9	9.09	<0.0001	6, 26.9	6.92	0.0002
	Treatment × Storage period	12, 48.4	6.16	<0.0001	12, 28.9	2.32	0.0318

**Fig. 3.** Numbers of live (A) and dead *Sitophilus* spp. (B) (mean ± SE) per 250 g of maize in Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control) and ZeroFly® Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

were 1.6 ± 0.6 , 1.2 ± 0.5 , and 2.7 ± 0.8 ppm, respectively.

4. Discussion

Based on our data, maize moisture content at the start of the storage period (February) was 11–13%, a MC level considered safe for storage of maize (FAO, 1994b, 2011). However, moisture content increased over the storage period, in all treatments, and was significantly higher after 6 months of storage in August. The MC levels in August were 13–14.8%. It is possible that insects infesting maize contributed to increase in MC, but other factors may have played a bigger role in the increase given that the Betallic treatment had low insect infestation. Increase in MC levels may have been due

**Fig. 4.** Numbers of live (A) and dead (B) *Tribolium* spp. (mean ± SE) per 250 g of maize in Betallic Super-treated maize-filled PP bag, untreated maize-filled PP bag (control) and ZeroFly® Storage Bag (ZeroFly bag). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

to the influence of ambient relative humidity — relative humidity in the Middle Belt of Ghana increases during the period February–August (Jarvis et al., 2014). Moisture content is an important physiological variable that always needs to be considered for successful grain storage and should be monitored regularly; drying the maize to 12–13% MC prior to storage helps to reduce deterioration and microbial growth (FAO, 2011; Costa, 2014). Moreover, sufficiently dried grain and good storage conditions can delay the treatment of grains with pesticides; additionally, high moisture content in grain can reduce the performance of pesticides (FAO, 1994a). The commercially available JD moisture meter costs $\geq \$250$ in Ghana and is too exorbitant for smallholder farmers in developing countries. Conversely, the PHL meter is a relatively

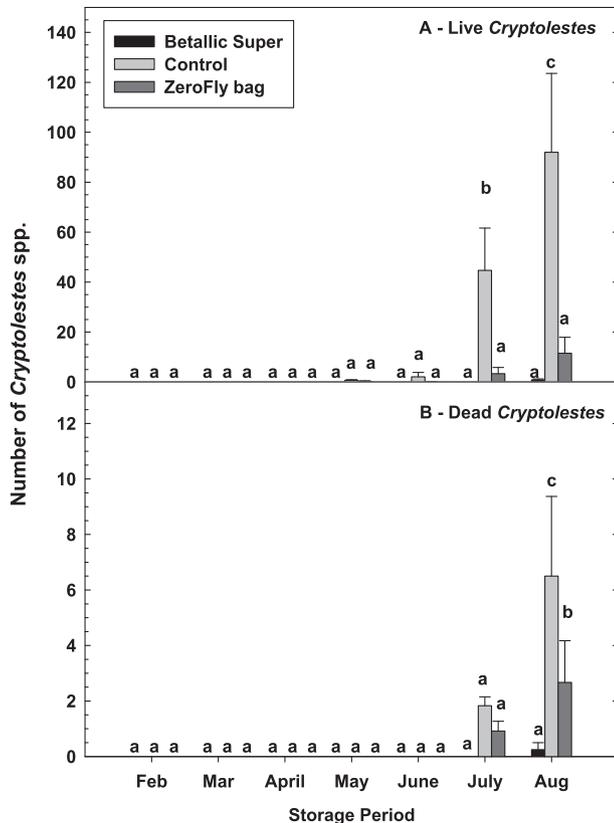


Fig. 5. Numbers of live (A) and dead (B) *Cryptolestes* spp. (mean \pm SE) per 250 g of maize in Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control) and ZeroFly[®] Storage Bags (ZeroFly bags). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

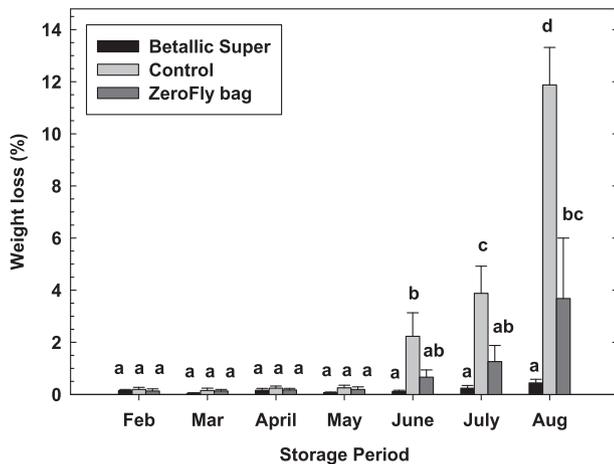


Fig. 6. Percentage weight loss (mean \pm SE) per 250 g of maize obtained from Betallic Super-treated maize-filled PP bags, untreated maize-filled PP bags (control) and ZeroFly[®] Storage Bags (ZeroFly bag). Maize was sampled at the start of storage (February) and at monthly intervals thereafter for six months. Means followed by different lowercase letters are significantly different ($P < 0.05$).

low cost (\$50–\$70) moisture meter produced by the USDA-ARS, Center for Grain and Animal Health Research, Manhattan, KS. The PHL meter gives MC measurements using both temperature and relative humidity of air surrounding the grain (Opit et al., 2014). Based on our data, there was approximately a 2% difference in the measurements of the PHL and JD meters.

The Ghana Standards Authority (GSA) thresholds for aflatoxin and fumonisin in maize are 15 ppb and 4 ppm, respectively — these levels are the national permissible levels for maize (Ghana Standards Authority, 2013). Based on our data, levels of aflatoxin in the ZeroFly bag and Betallic treatments were below 15 ppb (7.3 and 7.0 ppb, respectively). In the control treatment, the levels were above the threshold level (29 ppb). The levels of fumonisin were below the GSA threshold in all treatments. Fumonisin levels in the ZeroFly bag, Betallic and control treatments were 1.6, 1.2, and 2.7 ppm, respectively. It is very likely that the high levels of aflatoxin in the control treatment were a result of high insect infestation levels (Hell et al., 2000).

One of the variables the Ghana Standards Authority uses to grade maize is the percentage of insect-damaged maize kernels, i.e. percentage IDK (Ghana Standards Authority, 2013). The percentage IDK threshold accepted by the Ghana storage industry, wholesalers, retailers and consumers is 5%. Although other factors such as diseased, discolored, broken, stained, germinated and shriveled kernels are used to determine grades and acceptability of maize, a level >5% IDK results in maize getting rejected (Ghana Standards Authority, 2013). In the current study, percentage IDK increased significantly with storage time in the control treatment and levels of 11, 16 and 32% per 250-g maize samples were found in June, July and August, respectively. These values are far higher than the 5% threshold referred to above. In contrast, the Betallic treatment was able to keep percentage IDK levels well below 2% during entire storage period. In the ZeroFly bag treatment, IDK levels were below 5% until June (3.1%), but levels increased to 5.2 and 10.2% in July and August, respectively.

Based on our data, the ZeroFly bag and Betallic treatments were highly effective in suppressing insect infestation and damage levels compared to the control treatment (untreated PP bag). The common way of storing maize in Ghana is using untreated PP bags that contain untreated maize (the negative control treatment in the present study). In the control treatment, the number of *Sitophilus* spp., *Tribolium* spp. and *Cryptolestes* spp. were generally significantly higher than in other two treatments.

The Betallic treatment was most effective at suppressing stored-product insect pest levels. The ZeroFly bag was quite effective up to 4 months of storage. After 4 months of storage, the insect levels (*Sitophilus*, *Tribolium* or *Cryptolestes*) in the ZeroFly bag increased significantly and resulted in percentage IDK levels > 5%. The increased infestation levels in the ZeroFly bags after 4 months of storage seem to indicate that insects (eggs and/or larvae) that may have survived the phosphine fumigation before maize was bagged may not contact the surface of the ZeroFly bag enough for their population levels to be significantly reduced. For stored-product insects to be killed by the ZeroFly bag fabric, they need to contact the fabric for long periods of time (≥ 24 h) (Paudyal et al., 2016). It is also likely that the repeated sealing and unsealing of the ZeroFly bags during grain sampling may have created breaches in the deltamethrin barrier that allowed easy access of insects into the bags. Anankware et al. (2014) showed that the ZeroFly bag causes 100% mortality of *S. zeamais* after 48 h of exposure to the ZeroFly bag fabric. This may indicate that ZeroFly bags are likely contributing to increased mortality of insects but are not doing so quickly enough to result in the same kind of control observed in the Betallic treatment.

In the present study, mean weight losses of $\leq 0.44\%$ and $\leq 3.68\%$ were recorded in Betallic and ZeroFly bag treatments, respectively, during the 6 months storage period, compared to losses of 2.23, 3.88 and 11.88% in June, July and August, respectively, in the control PP bag treatment. Data from this study corroborate those from Costa (2014) who found that postharvest losses (including weight losses) of 59 and 54% were recorded in maize stored for 90 d in

traditional PP bags in field experiments conducted in Uganda and Burkina Faso, respectively. However, losses in ZeroFly bags were 2.7% and 2.4% after 90 d of storage in Uganda and Burkina Faso, respectively. This implies that, in sub-Saharan Africa, severe and significant postharvest losses due to insects occur in maize stored in PP bags without any control measures.

From observation during our study, the ZeroFly bag appeared to act as a barrier to insects from outside (Fig. 7). Observations during visits to field sites showed that insects were not seen crawling on the outside surface of ZeroFly bags (Fig. 7A). However, large numbers of insects were usually observed crawling on the surfaces of PP bags in the control treatment (Fig. 7B). The fact that the effectiveness of the ZeroFly bag results mostly from preventing insect entry into bags means maize (grains) put into bags at the start of storage needs to be insect-free to avoid damaging insect levels developing inside bags during storage. Additionally, the bags must retain their physical integrity during the storage period (no holes, proper resealing each time after the bag is opened) to effectively protect the grain in bags from insect infestation. Based on the need for insect-free grain during filling of ZeroFly bags, it is our recommendation that commercial aggregators and large-scale farmers who are better equipped to properly disinfect grain before bagging use these bags. Commercial aggregators and large-scale farmers in the developing countries can afford the cost of fumigation or any other effective insecticide in order to keep the commodities clean and insect-free before storage in ZeroFly bags. Smallholder farmers in developing countries usually have neither the knowledge nor the resources required for good disinfection of grain before bagging.

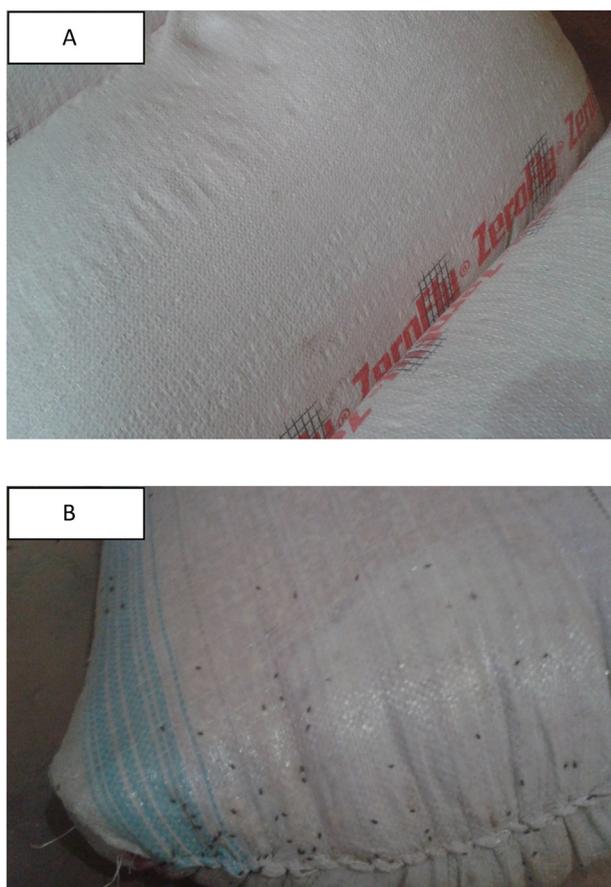


Fig. 7. Pictures of a ZeroFly bag with no live insects on the surface (A) and a polypropylene bag with live insects on the surface (B).

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