Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality

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Abstract

A large-scale study was conducted to assess which of the five most accessible hermetic storage devices on the Kenyan market fulfill the needs of smallholder farmers by positively impacting three major areas of concern: insect infestation, grain quality, and mycotoxin ( aflatoxin and fumonisin) contamination. Efficacy of two hermetic silos (plastic and metal) and three hermetic bags (PICS, GrainPro’s GrainSafe™, and Super Grain) was directly compared to current maize storage in polypropylene (PP) bags under local environmental conditions using representative storage volumes during a 6-month storage period. Impact of maize grain stored at typical (~15%) and recommended (< 13.5%) moisture levels and potential efficacy losses through frequent interruption of the underlying hermetic principals was assessed. Hermetic storage significantly reduced the increase in aflatoxin compared to PP bags regardless of the moisture level of the grain. An < 5% per month aflatoxin increase was achieved by three of the five devices tested: Metal silo, PICS and GrainSafe™ bag. A strong correlation between grain moisture, storage time and aflatoxin development was found in PP bags, but not in any of the hermetic devices. The same result was not obtained for fumonisin development in stored maize. The rate of Fumonisin increase was similar in all tested devices, including the polypropylene bags, and conditions. The periodic opening of the hermetic devices had no significant effect on the efficacy of the hermetic devices but the repeated disturbance of the PP bags led to a significant increase in aflatoxin levels. The maize weevil Sitophilus spp. was most commonly found with a total incidence of 72%. Grain storage under hermetic conditions reduced insect infestation, grain weight loss and discoloration. However, maize storage above recommended moisture levels led to a distinct odor development in all hermetic devices but not the PP bags. Hence, proper grain drying is a prerequisite for maize storage in airtight conditions.

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1. Introduction

Maize consumption is a primary avenue through which humans in Africa become exposed to mycotoxins, toxic fungal metabolites (Shephard, 2008). Kenyan maize consumption is estimated at 98 kgs per person and year, with higher volumes consumed in rural areas (Nyoro et al., 2004). Mycotoxin contamination of maize intended for human and animal consumption in Sub-Saharan Africa, caused by aflatoxin- and fumonisin-producing fungi, imposes a major health concern (Kimanya, 2015; Wild and Gong, 2010). In 2004, an outbreak of acute human aflatoxin-poising (acute aflatoxicosis) in the Eastern Province of Kenya attracted worldwide attention (Centers for Disease Control and Prevention, 2004). Here, consumption of maize highly contaminated with aflatoxins was found responsible for the human fatalities (Azziz-Baumgartner et al., 2005). The extremely high aflatoxin levels were caused by a novel aflatoxin-producing fungal species endemic almost exclusively in the outbreak regions (Probst et al., 2014, 2011, 2007). The continuing Kenyan aflatoxocoses epidemic, claiming human lives each year, demands ongoing attention until realistic management strategies, both pre- and post-harvest, are in effect.

Aflatoxins (namely aflatoxins B1, B2, G1, and G2) are a group of naturally occurring secondary metabolites produced by members
of the fungal species Aspergillus Section Flavi. The most common aflatoxin, aflatoxin B1, is a genotoxin known to be carcinogenic and teratogenic for both humans and animals (McKean et al., 2006; Wang and Tang, 2004). It is the only mycotoxin classified as a Group 1a human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2002). Mycotoxins can cause acute poisoning and liver cancer. Chronic, long-term exposure also contributes to stunting and growth impairment in children in affected populations, and ultimately predisposes children to other infectious diseases (Gong et al., 2004; International Agency for Research on Cancer, 2015). A survey of blood samples for Aids Indicator in Kenya conducted by the U.S. Centers for Disease Control and Prevention (CDC) in 2007 established that around 80% of the samples had detectable levels of Aflatoxin B1. Exposure was similar across all ages, genders, and socioeconomic status, however, the highest levels were detected in the Eastern Province of Kenya, the epicenter of the recent aflatoxicoses outbreaks (Yard et al., 2013).

Fumonisins (namely fumonisins B1, B2, and B3) are a group of naturally occurring secondary metabolites mainly produced by members of the fungal genus Fusarium, mainly F. verticillusoides and F. proliferatum (Picot et al., 2010). Fumonisins were first reported after an outbreak of leukoencephalomalacia in horses fed with moldy maize in South Africa (Sydenham et al., 1990). These toxins compromise the health of humans (e.g. neural tube defects in newborns and growth retardation in children) and animals (e.g. leukoencephalomalacia) by interfering with sphingolipid, phospholipid and fatty acid metabolism (Gelineau-van Waes et al., 2005; Kimanya et al., 2010; Missmer et al., 2006; Wild and Gong, 2010). Fumonisin B1 is possibly carcinogenic to humans, and it is likely associated with esophageal cancer. Therefore it was classified by IARC as a Group 2B carcinogen (International Agency for Research on Cancer, 2002). Fumonisin contamination is frequently overshadowed by the increased attention towards aflatoxins, but has recently been studied more frequently, including in Kenya (Alakonya et al., 2009; Mutiga et al., 2015; Wild and Gong, 2010).

In most of Africa, crops are grown and managed by small-scale farmers. Maize is used as a source of family income, and personal consumption. To sustain family needs, maize is also frequently purchased from local markets (Hell and Mutegi, 2011). Farmers in more southern areas of Africa (including Southern Tanzania) only have one rainy season and therefore harvest usually occurs under dry conditions. Kenya has two rainy seasons and maize is often harvested just before the next rain event, forcing farmers to harvest when the grain is still with high moisture content (20–30%). Following harvest, maize is often sun-dried by leaving the cobs directly on the ground for 5–7 days and then stored in a granary. In many areas, harvest is also the time when farmers prepare the land for the next planting season before the rains set in, therefore leaving the recently harvested maize unshelled until the planting is completed. The later shelled grain is placed on the ground under the sun for another 5–7 days, then treated with insecticide and stored in polypropylene bags. Farmers believe that the 5–7 day drying period is adequate for the maize to dry to the recommended grain moisture level (~13.5%) for safe storage. However, in most instances this is not the case and, in general, under farmer’s drying practices grain is stored with a moisture content around 15%. This moisture content is generally acceptable for grading purposes, since the stored volumes are sufficiently small for the moisture to equilibrate with the environment in devices that allow gas exchange (non hermetic) such as the favored polypropylene bags. Most smallholder farmers name rats and insects, specifically infestation by the maize weevil (Sitophilus spp), as their major post-harvest storage concern. Mycotoxin contamination is generally considered a minor problem, since farmers are unable to detect it. Additionally, grain quality parameters, such as grain blemishes, discoloration and holes caused by insect activity, are important for Kenyan farmers as they negatively affect marketability and sale of the grain.

In recent years, there has been increasing promotion of hermetic grain storage devices in Asia and Africa (De Groote et al., 2013; Njoroje et al., 2014). Hermetic, or airtight, storage prevents grain moisture loss and limits gas exchange; consequently, modifying the internal atmosphere in the device. If used correctly, the depletion of oxygen creates a hostile environment for aerobic microorganisms and insects, possibly eliminating the use of pesticides. However, many factors will dictate the success of hermetic storage, e.g. impact of the hermetic principle by frequent opening of devices to remove grain. In the current study, two silos (metal and plastic silos) and three bags (Purdue Improved Crop Storage [PICS] Triple Layer hermetic bag, GrainSafe™ bag, Grain Pro Super Grain bag) were identified based on hermetic properties but also availability on the Kenyan market and price. Silos and GrainSafe bags are bulk storage devices, consequently the initial cost is higher than for smaller (usually 90 kg) storage bags. The metal silos are made out of galvanized aluminum and can be fabricated by local artisans at varying storage capacity (~144 US$/350 kg silo). Plastic silos are made out of heavy duty reinforced plastic and also come in a variety of shapes and sizes. They are locally produced in Kenya by Ken-tainers (~92 US$/350 kg silo). GrainSafe bags are produced by GrainPro (imported to Kenya duty free) and sold at ~190 US$/bag. GrainSafe bags are made out of a patented plastic technology and need to be placed on an elevated platform to allow grain removal from a downspout. PICS and Grain Pro Super bags are smaller bagging devices with an average filling capacity of 90 kg. PICS bags were initially developed by Purdue University to improve cowpea storage in West Africa (Murdock et al., 2012). The bags utilize a multilayer technology (one outer polypropylene bag and two inner linings of high density polyethylene) to enable airtight storage. PICS bag are produced locally by Bell Industry in Kenya and sold at ~2.50 US$/bag (Baoua et al., 2013). The Grain Pro Super Bag, produced by GrainPro, also relies on multilayer technology (one outer polypropylene bag and one inner lining of high density polyethylene) (Baoua et al., 2013). Farmers have the option to purchase the inner lining separately (~2.50 US$) and place it in a locally purchased polypropylene bag (0.50 US$/bag).

The Storage and Drying for Aflatoxin Prevention (AflaSTOP) project works to ensure that businesses operating in Africa are able to provide improved storage devices to smallholder farmers. Farmer acceptance of any new storage system relies on heavy advertisement and training, mouth-to-mouth propaganda, initial cost and lifespan of the device. Therefore, the efficacy of any storage technology needs to be compared to and assessed as superior than current farmer practices by addressing most areas of storage concerns. Most of the hermetic devices on the market have been studied independently using single variable, such as effects of hermetic storage on insect survival or aflatoxin contamination (De Groote et al., 2013; Ndegwa et al., 2015; Njoroge et al., 2014; Tefera et al., 2011; Williams et al., 2014). However, a thorough comparative investigation of hermetic storage has to be conducted using multi-variable analyses to investigate complex interactions between variables, e.g. aflatoxin/fumonisin development as a function of insect infestation.

The current study sought to determine if any of the five here described hermetic storage devices are able to: 1) impede post-harvest mycotoxin contamination (both aflatoxin and fumonisin); 2) withstand a frequent opening, and therefore an interruption of the hermetic principle, without loss of their efficacy; 3) reduce insect infestation, and 4) limit storage induced grain quality losses.
(grain weight loss, grain discoloration). The study also investigated whether different grain moisture affected the results (maize below 13.5% moisture content or between 13.5 and 14.5% moisture content). Also, to determine the performance of the hermetic devices under different climatic conditions (two different regions within the Eastern Province of Kenya) and different grain moisture.

2. Materials and methods

2.1. Storage devices

Five hermetic storage devices, readily available for the Kenyan market, were tested for their ability to impede mycotoxin contamination, control insect infestation and limit grain quality deterioration in stored maize (Table 1): 1) Metal Silo (350 kg storage capacity); 2) Plastic Silo (350 kg storage capacity); 3) GrainSafe™ Bag (1000 kg storage capacity); 4) PICS Triple Layer hermetic storage bag (90 kg storage capacity); and 5) Grain Pro Super Grain Bag (GP) (90 kg storage capacity), which is inserted inside a locally manufactured polypropylene bag. Traditional maize storage in polypropylene bags (PP, 90 kg storage capacity) was used as a control (Table 1).

2.2. Sources of aflatoxin contaminated maize used for storage experiments

The maize grain used in this study was locally grown by smallholder farmers in the districts of Meru and Makueni of Kenya. Maize from the two growing regions was kept separate at all times, but the process for acquisition and processing was the same for both areas, as described below. Maize was harvested by the farmers in February and March 2014, stored on the cob, later shelled and dried under the sun by the participating farmer. Grain was not fumigated or otherwise treated with pesticides. To identify contaminated maize, bags of shelled maize grain were sampled on-site (at the grower's farm) and tested for aflatoxin content using Neogen's Reveal® Q+ fully quantitative lateral flow tests and the associated Reveal AccuScan (Neogen Europe Ltd., United Kingdom). Contaminated maize grain with aflatoxin levels above 10 ppb were purchased immediately. Approximately 25.6 metric tons from each region (294 bags, average bag weight 86.94) was acquired to fill each tested device to its full capacity. To ensure homogenization and that all devices have similar degrees of mycotoxin levels, all grain was initially intermixed and piled on tarpaulin. The pile of grain was thoroughly mixed for multiple days using an industrial seed mixer. Grain moisture content was monitored before and after the mixing with a Cimbria Super Pro moisture meter (Cimbria Unigrain Ltd., Denmark). Grain from the two regions was kept separate.

2.3. Treatments and experimental design

In order to test whether the storage devices can control post-harvest aflatoxin contamination at moisture levels achieved by farmers drying practices (~15%), as compared to the legal and recommended moisture content for stored maize (~<13.5%), the homogenized grain was divided evenly into two piles. The first pile was adjusted to a grain moisture content below 13.5% (Dry Treatment), the second pile to a grain moisture content between 14% and 15% (Wet Treatment). Mobile grain dryers were used to dry the grain (Agrex, Italy).

Duplicated experiments were carried out in two Kenyan districts, Meru (agro-ecological zone – moist transitional) and Makueni (agro-ecological zone – dry mid-altitude), located in the Eastern Province of Kenya. Locations differ in altitude, have varying ambient temperature and relative humidity ranges.

The storage experiments were conducted from May to November (Meru) and June to December (Makueni). Each storage device was tested in both locations using both grain moisture treatments. Experiments were arranged in a randomized complete block design (RCBD) with six replicates. A repeated measurement design was integrated into the RCBD to assess treatment differences within a storage device. Each block consisted of one commercially available store in a small trading outpost. Six stores were chosen per district and treated as replicates. Stores were chosen based on similar physical properties (brick/rock walls, concrete floor, corrugated galvanized iron roofing) and location. Stores were locked and safeguarded to prevent theft of contaminated maize grain.

Data loggers recording relative humidity and temperature on an hourly basis were placed in the center of each store. To achieve randomization in each store, a tarpaulin was placed on the floor, with the center of the tarpaulin matching the center of the store. Devices were allocated randomly within each store. Maize grain from either treatment was added to each device in each block and each district. Devices were filled to capacity. Once all devices were filled, they were sealed. The 350 kg metal silos were smaller than anticipated and capacity was reached at 312 kg of grain, whereas the plastic silos held 358 kg of maize. The GrainSafe bags were filled with 780 kg and all other bags (PICS, Super Grain, and PP) with 98 kg of maize grain.

To determine whether opening and closing the bags every month had an effect on the underlying hermetic principle of the storage device, two bags were used for each 98 kg bagged replicate (PICS, Super Grain, and traditional PP bags). A frequent opening of devices is also expected by farmer’s who remove grain frequently for personal consumption or sale. Bags were labelled as ‘A’ for bags sampled monthly and ‘B:’ for bags sampled at initial set-up and at the end of the storage period.

2.4. Evaluation parameters and sampling protocol

Grain was stored in the storage devices for 6 months. The following parameters were assessed at initial set-up and monthly thereafter: Mycotoxin (aflatoxin and fumonisin) content, insect incidence (live and dead insects) and activity (% damaged grain), as well as odor development, grain discoloration, moisture and weight loss. The same sampling order was followed every month for the ‘A’ bags while ‘B’ bags were sampled only at the beginning and the end of the storage period. One composite sample of approximately 2 kg consisting of various sub-samples taken with a 1.5 m long compartmentalized grain probe (Cimbria Unigrain Ltd., Denmark) was obtained from each device each month. To collect samples from bagged devices, bags were lifted off the pallet and placed on the floor, the grain probe was inserted three times down the middle of the bag, twice along the sides, and once on each diagonal. This procedure was repeated until the 2 kg sample was collected. In the silos, the probe was inserted through the inlet. The probe was inserted straight down to collect the sub-samples up to three times. The probe was also inserted at an approximately 30–40° angle across the device to collect up to four times. In the case of the GrainSafe, the top zipper was undone and the probe was inserted directly straight down and once in the center and in each corner to obtain one sub-sample from each position. Additionally, sub-samples were obtained by inserting the probe diagonally from each top corner across the device and pushed down towards their opposite bottom corner. Devices with outlets also had approximately 250 mgs collected from each outlet. Visual inspection of the grain was conducted immediately and any occurrence of live insects was recorded on the sample label. To accommodate the repeated measurement analyses, four 2 kg composite samples were taken per device and replicate at the beginning and the end of the
storage period. The four samples were analyzed independently. The grain probe, and all associated sampling devices were thoroughly sanitized with a 65% ethanol solution between each sampling to avoid cross contamination among devices. Samples were stored in double Ziploc bags, which were labeled and sealed. Samples were delivered to the laboratory, placed in the refrigerator, and kept at 4°C until further processing.

### 2.5. Sample processing and grain quality parameters

#### 2.5.1. Sample processing and insect evaluation

Samples were analyzed in the same order as they were taken. Using a Cimbria Super Pro moisture meter (Cimbria Unigrain Ltd., Denmark), the moisture content of the sample was recorded. To evaluate insect infestation, insects were separated from the maize grain and foreign matter using a 3 mm and followed by a 2 mm sieve mesh size, which are smaller than the maize grain. All insects collected from the 2 kg sample were identified and total numbers recorded. The sample was then thoroughly mixed and a 110 g subsample was collected for grading purposes (% damaged and discolored grain). The remaining maize was ground using a laboratory mill (Romer Series II™ Mill, Romer Labs Diagnostic GmbH, Austria). Ground maize was mixed thoroughly and a 110 g subsample was removed for mycotoxin analysis.

### Table 1

<table>
<thead>
<tr>
<th>Storage technology</th>
<th>Characteristics</th>
<th>Storage capacity/and estimated price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal silo</td>
<td>Produced by local artisans. Made out of aluminum and placed on a pallet.</td>
<td>200 to 1000 kg/$144</td>
</tr>
<tr>
<td>Plastic silo</td>
<td>Produced by Kentainers (local). Made out of heavy duty reinforced plastic and placed on a pallet.</td>
<td>~350 kg/$92</td>
</tr>
<tr>
<td>GrainSafe™ Bag</td>
<td>Produced by Grain Pro and imported into Kenya duty free. Made out of patented plastic technology and placed on a custom frame.</td>
<td>800 to 1300 kg/$190 frame can be made locally</td>
</tr>
<tr>
<td>PICS Triple Layer hermetic storage bag</td>
<td>Originally introduced to West Africa by Purdue University and now manufactured in Kenya by Bell Industries. Placed on a pallet.</td>
<td>~90 kg/$2.50 per bag</td>
</tr>
<tr>
<td>Grain Pro Super Bag (GP)</td>
<td>Produced by Grain Pro and imported into the country duty free. Made out of patented plastic technology. Needs to be placed inside another bag and on a pallet</td>
<td>~90 kg/$2.50 each (plus an additional PP bag at about $0.50)</td>
</tr>
<tr>
<td>Polypropylene (PP) bag</td>
<td>Control: Traditional storage bag made of polypropylene. Non-hermetic, and widely available and used.</td>
<td>~90 kg/$0.50 per bag</td>
</tr>
</tbody>
</table>
2.5.2. Mycotoxin analyses

Aflatoxin and fumonisin analysis was carried out using Neogen’s Reveal® Q+ fully quantitative lateral flow tests and the associated Reveal AccuScan according to manufacturer’s instructions (Neogen Europe Ltd., United Kingdom). Quantitative results were recorded in parts per billion (µg/kg) for total aflatoxin content, and parts per million (mg/kg) for total fumonisin content. Mycotoxin baselines were established at the beginning of the storage experiment using six independent samples per device/treatment/location and changes were recorded monthly thereafter.

2.5.3. Grain weight loss

Grain weight loss in storage is caused by loss of both mass and moisture. Moisture loss is caused mainly by evaporation as the grain equilibrates with the environmental conditions. Additionally, insects feeding on the grain leads to grain breakage (insect damage grain) and grain holes (insect-holed grain). To determine grain weight loss, the grain was separated into categories, undamaged and damaged. Grain in the damaged category was additionally separated according to cause of damage. Weight loss was calculated based on the weight of the damaged categories compared to the weight of the same number of whole undamaged kernels.

2.5.4. Discolored grains and odor development

- The discoloration of grain is an important grading factor used both by the formal and informal trading sectors in Kenya. Maize with higher degrees of discoloration have a discounted price in the market, therefore, it is important to assess the effect of storage in hermetic devices on the coloration of the grain. Additionally, farmers relate discoloration with aflatoxin content. The number of discolored grains was quantified monthly, starting at the end of the 6-month storage period going backwards to assess in which month the discoloration started. Odor development is a great concern in airtight storage devices since it could indicate the presence of anaerobic microorganisms. The odor severity at time of sampling was ranked based on a scale: 0 – no unusual odor; 1 - slight odor, disappearing completely during sampling; 2 – distinct odor, decreasing slightly during sampling; and 3 – extreme odor.

2.6. Statistical analyses

The efficacy of the different hermetic devices on all the variables under study, including aflatoxin, fumonisin, insect infestation and grain quality was examined by Analysis of Variance (ANOVA), while the effect of storage time in the devices for all the variables in study was analyzed by Regression Analysis. The infestation of several species of insect and its effect in grain quality and toxin contamination were also analyzed by correlation and regression analyses. Additionally, the effect of store locations, grain moisture, and regions on all the variables were also subjected to analysis of variance. All statistical analyses were performed using the statistical software SAS (SAS Institute, Inc., Cary, NC, USA). Preliminary data analyses including histograms and box-and-whisker plots were conducted to detect possible outliers on all study variables. These were all further subjected to analysis of variance (ANOVA), correlation analyses. The increase of both aflatoxin and fumonisin over time was also examined via simple linear regression analysis and non-linear regression analysis. ANOVA was performed by the General Linear Models (GLM) procedure. The means of all the variables in study (aflatoxin and fumonisin content and increases, insects, grain quality, etc.) for locations, devices and time (months) were subjected to Tukey’s HSD (honest significant difference) to determine significance among means. Region, grain moisture (dry and wet) treatments, and comparison of aflatoxin and fumonisin content and increase from the start (month 0) to the end (month 5) were further subjected to pairwise Least Square Means t-tests adjusted by the Tukey-Kramer (α = 0.05) method. Regression Analyses were performed with SAS using the REG Procedure (Proc Reg) for linear regression and the NLIN Procedure (Proc Nlin) for non-linear regression. The linear least squares regression method was used for linear regression modeling and the Gauss-Newton method was used for the non-linear regression modeling.

3. Results

3.1. Aflatoxin development in stored maize

3.1.1. Moisture and aflatoxin content of maize grain at the beginning of the storage period

The initial average moisture level of the maize purchased in Meru was 18% and was dried down to 12% for Dry Treatment and 14% for Wet Treatment. The initial average moisture level of the maize purchased in Makueni was 14% and was dried down to 13% for Dry Treatment and kept at 14% for Wet Treatment. There was no significant change in moisture content at any point during the six-month storage period in the hermetic devices. However, grain moisture of maize stored in the traditional PP bags naturally adjusted to 12.2–12.8% in both treatments.

Maize was naturally contaminated with high levels of aflatoxins in both regions (Table 2). There were no significant differences in the average total aflatoxin content of maize used for Dry Treatment compared to Wet Treatment in Meru (1293 ppb versus 1265 ppb) and Makueni (515 ppb versus 488 ppb) (Table 2). Both the initial aflatoxin and moisture content showed that the homogenization protocol was successful. There was no significant difference in the rate of aflatoxin increase as a function of moisture content between treatments.

3.1.2. Aflatoxin development as a function of location

The influence of the regional location of the storage device on the rate of aflatoxin increase was investigated. Locations differ in both altitude (Makueni 1132 m and Meru 1711 m) and climate during the storage period with higher average ambient temperature and lower relative humidity for Makueni (20.6°C and 64%) compared to Meru (15.5°C and 71%). Both regions experienced precipitation during the storage period from October to December with an average precipitation of 115.6 mm in Makueni and 125.5 mm in Meru. Average rainfall days in Makueni; 232 mm and an average of 17.5 average rainfall days in Meru. However, from June to September precipitation were much lower in both regions (Makueni: 1.8 mm with an average of 3.5 days with precipitation; Meru: 62.8 mm and 8.7 average days with precipitation). The different environmental and geographic conditions did not significantly influence the post-harvest development of aflatoxins in the different storage devices tested.

3.1.3. Aflatoxin development as a function of storage devices and moisture treatments

At the start of the storage experiment, aflatoxin content of maize grain was not significantly different among devices and between treatments within each tested location (Table 2, Lowercase letter). Increase in aflatoxin content was recorded for all devices during the course of the 6-month storage period in both regions, with increases being significantly greater in maize stored in traditional PP bags. In Meru, the average total aflatoxin increase in control PP bags was 116% in the Dry Treatment and 117% in the Wet Treatment (Table 2). Overall, the rate of aflatoxin increase in PP bags was significantly higher than in any of the other devices. Hermetic
Table 2
Aflatoxin content (ppb) and % increase by device and moisture treatment in Meru and Makueni, Kenya.

<table>
<thead>
<tr>
<th></th>
<th>Beginning of storage</th>
<th>End of storage</th>
<th>Total aflatoxin increase (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meru</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control: PP</td>
<td>1919 a, B</td>
<td>4134 a, A</td>
<td>177 a</td>
</tr>
<tr>
<td>PICS</td>
<td>1729 a, B</td>
<td>2226 b, A</td>
<td>17 b</td>
</tr>
<tr>
<td>GrainSafe</td>
<td>2031 a</td>
<td>2122 b</td>
<td>11 b</td>
</tr>
<tr>
<td>GP bag</td>
<td>1883 a, B</td>
<td>2165 b, A</td>
<td>14 b</td>
</tr>
<tr>
<td>Plastic silo</td>
<td>2005 a</td>
<td>2285 b</td>
<td>20 b</td>
</tr>
<tr>
<td>Metal silo</td>
<td>1973 a, B</td>
<td>2383 b, A</td>
<td>25 b</td>
</tr>
<tr>
<td><strong>Wet Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control: PP</td>
<td>1867 a, B</td>
<td>4121 a, A</td>
<td>116 a</td>
</tr>
<tr>
<td>PICS</td>
<td>1997 a, B</td>
<td>2410 b, A</td>
<td>27 b</td>
</tr>
<tr>
<td>GrainSafe</td>
<td>1895 a, B</td>
<td>2178 b, A</td>
<td>14 b</td>
</tr>
<tr>
<td>GP bag</td>
<td>1949 a, B</td>
<td>2349 b</td>
<td>23 b</td>
</tr>
<tr>
<td>Plastic silo</td>
<td>2111 a</td>
<td>2195 b</td>
<td>15 b</td>
</tr>
<tr>
<td>Metal silo</td>
<td>1968 a</td>
<td>2208 b</td>
<td>16 B</td>
</tr>
</tbody>
</table>

| **Makueni**             |                      |                |                             |
| Control: PP             | 515 a, B             | 2981* a, A     | 491* a                      |
| PICS                    | 543 a                | 519 c          | 3 c                         |
| GrainSafe               | 504 a                | 524* c         | 4 c                         |
| GP bag                  | 541 a, B             | 874* b, A      | 73* b                       |
| Plastic silo            | 518 a, B             | 1013* b, A     | 101* b                      |
| Metal silo              | 467 a                | 551 c          | 9 c                         |
| **Wet Treatment**       |                      |                |                             |
| Control: PP             | 440 a, B             | 2645* a, A     | 424* a                      |
| PICS                    | 521 a                | 579 b          | 15 b                        |
| GrainSafe               | 488 a                | 464* b         | 0 b                         |
| GP bag                  | 480 a, B             | 610* b, A      | 21* b                       |
| Plastic silo            | 492 a                | 463* b         | 0* b                        |
| Metal silo              | 506 a                | 575 b          | 14 b                        |

a The average percent of increase presented in these columns might not be equal to the percentage increase given by the average aflatoxin of the baseline and the end of storage because it corresponds to the average of individual increases of all replicates in each treatment, which is presented for statistical analysis purposes. Devices with same letter are not significantly different. Treatments (Dry and Wet) with asterisks are significantly different at the indicated device. Different capital letters indicate significant differences between the beginning and the end of the storage period at the indicated device.

3.1.4. Aflatoxin development as a function of opening the storage devices monthly

The frequent opening of PP, PICS and Super Grain (GP) (‘A’) bags had no significant effect on the increase of total aflatoxin content compared to bags never opened until the end of the storage period (‘B’ bags) in all treatments and locations. However, the total aflatoxin increase was significantly higher (P = 0.05) in the traditional PP bags compared to PICS and Super Grain (GP) bags in both treatments (Table 3).

In Makueni, the total aflatoxin content increase in the traditional PP bags was also significantly greater compared to PICS and Super Grain (GP) bags, whereas the never opened (‘B’) bags had significantly less aflatoxins than the frequently opened (‘A’) bags. The different treatments did not have a significant effect in PP (‘B’) bags kept sealed until the end of the storage period. In general, aflatoxin levels were not affected by frequent opening of PICS bags in any treatment, but there was a significantly lower rate of increase in unopened PICS bags compared to unopened Super Grain (GP) bags in the Dry Treatment. Interestingly, a frequent opening of Super Grain (GP) (‘A’) bags had significantly lower rate of aflatoxin development with a total increase of 73% compared to the unopened (‘B’) bags with a 142% increase, but only in maize from the Dry Treatment (Table 3). The unopened Super Grain (GP) (‘B’) bags had a higher number of insect penetration holes than the Super Grain (GP) (‘A’) bags.

3.2. Fumonisin development in stored maize

There was no significant difference in fumonisin content among devices, locations or treatments at the start of the storage experiment. The average fumonisin content of the maize at the start of the storage period was 1.5 ppm (Dry Treatment) and 1.6 ppm (Wet Treatment) in Meru; 1.6 ppm (Dry Treatment) and 1.7 ppm (Wet Treatment) in Makueni (Table 4). The average fumonisin increase in traditional PP storage bags during the 6-month storage period was 13% for maize stored at moisture contents below 13% and 10% for grain stored at higher moisture content in Wet Treatment (Table 4). The 6-month increase in fumonisin content was similar between all devices, including the traditionally used PP bags, within the tested regions (Table 4). The only significant change in fumonisin levels occurred within the Super Grain (GP) bags and plastic silo in the Dry Treatment and metal silo in the Wet Treatment, but only in Makueni. Significant differences in fumonisin increases between treatments were found in PICS bags and the plastic silos, where levels of fumonisin increases were significantly lower in maize from the Wet Treatment. There was no significant difference in the increase in fumonisin levels between the never opened bags (B bags) and the frequently opened bags (A bags) indicating that the interruption of the hermetic principles did not influence fumonisin levels (data not presented).
Insect infestation in stored maize

3.3. Insect infestation in stored maize

At the start of the storage experiment, there was a significantly higher presence of insects in maize obtained from Makueni than from Meru; 75% versus 6% of samples contained living insects, respectively. All hermetic devices significantly controlled insect infestations with no significant increase in any insect numbers between set up and the end of the storage experiment independent of insect species or initial population densities. Insect development in PP bags was analysed to assess regional species distribution patterns, population densities and growth using current farmer storage methods (Fig. 3). On average, less than 1 insect per 2 kg sample was found at the beginning of the experiment in Meru and 11 insects per 2 kg sample in Makueni. These numbers significantly increased to 127 and 389 insects per 2 kg sample by the end of the 6-month storage period. In both districts, the maize weevil Sitophilus spp. was the most common with a total incidence at the end of the storage period of 78% (Makueni) and 65% (Meru). The second most noticeable insect species was Tribolium castaneum (red flour beetle), with an incidence of 22% (Makueni) and 17% (Meru).

At the start of the storage experiments, the percentage of samples with viable insects in Makueni: 72% Sitophilus zeamais (maize weevil), 4.1% T. castaneum (red flour beetle), 1.8% Sitotroga cerealella (Anjoumois grain moth), 0.15% Prostephanus truncatus (larger grain borer), and 0.02% Trogoderma granarium (Khrapa Beetle). Rhyzopertha dominica (lesser grain borer) and Orzyaephilus surinamensis (Saw toothed beetle) were not found at set-up but during later sampling. The average monthly increase of insects are reported in Fig. 2.

The percentage of samples with viable insects in Meru: 5% Sitophilus spp, 0.5% T. castaneum, and 0.02% O. surinamensis (see Fig. 3). S. cerealella, P. truncatus, T. granarium and R. dominica were not found at set-up but during later sampling.

Some insect populations did increase significantly over the course of the 6-month storage period (Fig. 2).

3.4. Aflatoxin development as a function of insect population, moisture and time

Using correlation and multiple linear regressions analyses, aflatoxin contamination in traditional PP bags was investigated as a function of insect population, moisture, and time. In Meru, a positive relationship was found between aflatoxin content and insect and moisture content in traditional PP bags: Sitophilus spp (r = 0.70, P > r < 0.0001, n = 80) and T. castaneum (r = 0.56, P > r < 0.0001, n = 80). In Makueni, relationships were only found with moisture content (r = −0.72, P > r < 0.0001, n = 81) of maize stored in traditional PP bags: Sitophilus spp (r = 0.71, P > r < 0.0001, n = 81), and T. castaneum (r = 0.70, P > r < 0.0001, n = 81).

There was a negative correlation between moisture and time (r = −0.61, P > r < 0.0001, n = 81) and moisture and aflatoxin development (r = −0.50, P > r < 0.0001, n = 81) and a positive correlation between time and aflatoxin (r = 0.68, P > r < 0.0001, n = 81) in traditional PP bags but not in any hermetic device. For PP bags, the multiple regression analyses indicated that aflatoxin contamination increases mainly as a function of time when analysis is performed by treatments and regions (Makueni dry Y = 441 + 503 × month, r² = 0.85, P > F < 0.0001, df = 38, Meru dry Y = 2472 + 314 × month, r² = 0.72, P > F < 0.0001, df = 37), Makueni Wet Treatment Y = 401 + 436 × month, r² = 0.76, P > F < 0.0001, df = 41) and Meru Wet Treatment (Y = 2151 + 402 × month, r² = 0.78, P > F < 0.0001, df = 41). However, when analysis is performed by treatments regardless of region then grain moisture also is a factor influencing aflatoxin along time in the Dry Treatment (Y = 17402 + 287 × month − 1274 × moisture; r² = 0.60, P > F < 0.0001, df = 76), but not in the Wet Treatment (Y = 1276 + 419 × month; r² = 0.44, P > F < 0.0001, df = 83). Furthermore, the negative correlation between the moisture and aflatoxin content of the grain indicated that aflatoxin contamination increased as moisture content decreased.

3.5. Grain quality parameters important for farmers

3.5.1. Grain weight loss

Grain weight loss in storage is caused through either natural transpiration or insect damage.
There was no significant change in the moisture content of maize grain stored in hermetic devices over the storage period. However, grain moisture levels in traditional PP bags dropped below 13.5% within the first 3 months of storage with an average of 12.1% in the Dry Treatment and 11.4% in the Wet Treatment. Grain damage through insect activity was assessed over time. There was no significant difference in grain weight loss during the entire storage period among the hermetic device. However, the average grain weight loss of the storage devices in Makueni (3.87) was significantly higher than in Meru (1.09) ($P < 0.0001, df = 748$). This is consistent with the much higher population of insects in Makueni and therefore their impact on the grain. There was no significant effect of grain moisture content ranging from 12 to 15% and the development of insect populations as indicated by both Variance Component Analysis and Analysis of Variance.

### 3.5.2. Discolored grain

- The number of discolored grains was assessed at the start of the storage period and compared to the number of discolored grains at each sampling date. In general, all devices experienced a slow but significant increase in discoloration over time, independent of treatments and region. In Meru, the total number of discolored grains increased by 2.3% (Dry Treatment) and 1.7% (Wet Treatment). Here, the increase was lowest in the metal silo (<1% in the Wet Treatment) and Super Grain (GP) bags (1% in the Dry Treatment) compared to the traditional PP bags (3% in the Dry Treatment and 14% in the Wet Treatment). Furthermore, traditional PP bags discoloration increased earlier in storage in the Wet Treatment; however, at the end of the 6-month storage period differences between treatments were not significant.

- In Makueni, the total number of discolored grains increased by 1.7% (Dry Treatment) and 3.4% (Wet Treatment). Increases in discolored grains ranged from 2% (PIGS, metal and plastic silos, and traditional PP bags) to >5% (GrainSafe and Super Grain (GP) bags) in the Wet Treatment and from >1% (PIGS bag) to 4% (metal and plastic silos and traditional PP bags) in the Dry Treatment. Discoloration in traditional PP bags was significantly faster in the Wet Treatment compared to the Dry Treatment until the fourth month of storage, but by the end of the sixth month of storage the differences were not significant.

Pearson correlation analysis between discoloration and aflatoxin content within the traditional PP bags indicated a weak correlation, but not conclusive. However, correlation in grain from the Wet Treatment was stronger in Meru ($r = 0.69, n = 42, P > F < 0.0001$) compared to Makueni ($r = 0.4, n = 42, P > F = 0.0078$). In comparison, the correlation was a little weaker compared to the traditional PP bags.
Oryzaephilus surinamensis (O.s., Saw toothed beetle), Tribolium castaneum (T.c., red flour beetle), Rhizopertha dominica (R.d., lesser grain borer), Oryzaephilus surinamensis (O.s., Saw toothed beetle), Sitostroga cerealella (S.c., Anjo- 
mois grain moth) in maize stored for six month in traditional polypropylene (PP) bags. Maize was sampled (2 kg composite sample) at the start of storage and at monthly intervals thereafter for six months.

4. Discussion

Aflatoxin contamination of maize can be broadly divided into two phases based on crop maturity, and for a successful aflatoxin management both stages of contamination should be considered (Cotty et al., 2008; Pitt et al., 2013). The first contamination phase occurs during crop development, pre-harvest, and has been linked to hot and dry growing days, plant stress and insect damage (Cotty and Jaime-Garcia, 2007). During this stage, biological control technology, based on atoxigenic isolates of A. flavus, have been proven successful to mitigate aflatoxin contamination in the field and are currently marketed or been developed for many African countries (Atehnkeng et al., 2016; Augusto et al., 2013; Bandyopadhyay et al., 2016). The second contamination phase occurs after crop maturation, around harvest and or during storage, and is favored by rain, high humidity and warm temperatures (Cotty et al., 2008; Jaime-Garcia and Cotty, 2003; Jaime et al., 2013). Poor storage conditions and improper grain drying have been linked to this contamination cycle (Maina et al., 2016; Pitt et al., 2013). Moisture content of stored grain has been an area of concern in regards of mycotoxin contamination. It is assumed that drying the maize to below 13.5% will naturally reduce aflatoxin contamination and that storage of maize grain below 18% naturally prevents fumonisin increases (Munkvold et al 1994). Our current results indicate that drying alone does not prevent the increase of either mycotoxin. For example, the average fumonisin increase in traditional woven polypropylene storage bags during the 6-month storage period was 13% for maize at moisture content below 13% and 10% for grain stored between 14 and 15% moisture content. However, our current results indicate that storing maize hermetically will reduce the rate of increase of aflatoxin contamination but not of fumonisin contamination. Additionally, repeated opening of the hermetic bags for 6 month did not affect moisture content in the grain or impede efficacy of limiting aflatoxin increase. Similar results were found for a frequent opening of PICS bags in a study conducted by Tubbs et al. (2016).

The significantly higher level of aflatoxin in the unopened Super Grain (GP) bags in Makueni compared to the bags opened monthly is possibly explained by the corresponding higher number of insect penetrating holes in the unopened bags than the opened bags. The higher number of holes would allow more air to pass into the bags allowing a higher rate of Aspergillus growth. It is possible that disturbing the bags once a month dislodged boring insects and therefore the number of penetrating holes in the opened bags were less. The plastic silo also failed to control aflatoxin in Makueni. When we finalized the trials we carefully looked over each plastic silo, we found that two of these silos had small holes in the plastic molding which would have meant the devices were not hermetic and these two devices had aflatoxin levels similar to the unopened PP bags. Improved manufacturing could address this problem.

The significantly higher levels in the aflatoxin levels in the PP
opened bags compared to the unopened bags in Makueni is unexplained. At set up the aflatoxin levels between treatments were the same as were the insect profiles, the insect profiles were statistically similar in the final rounds (In Meru they were similar for Wet Treatment). High correlation between insect populations and aflatoxin contamination does not imply causation of aflatoxin development by the insects. Since the environment in the stored grain was conducive for both fungal (Aspergillus) growth and insect reproduction, a positive correlation between the levels of aflatoxin and insect numbers is expected. Furthermore, positive relationship between aflatoxin, moisture content and insects was found in Meru, but not in Makueni where positive relationship was only between aflatoxin and moisture content and aflatoxin continued to increase even when moisture levels decreased, implying that the increasing numbers of insects did not noticeably affect the grain moisture levels. In this comparison, 10% of the maize was sampled in the opened bags, whereas 5% of the maize was sampled in the unopened bags. However, no significant difference between opened and unopened PP bags was found in Meru, indicating that it is unlikely simple sampling ratios is a factor. Insect damage for the Wet Treatment grain in Meru was not significantly different to Wet Treatment in Makueni indicating high levels of infestation, yet there was no significant difference in Meru between opened and unopened PP bags. Therefore, insects are unlikely to be the simple explanation why unopened PP bags in Makueni had significantly lower aflatoxin levels. Possibly in opening the PP bags once a month, lifting them on and off the pallet, and re arranging the grain through sampling, Aspergillus propagules were moved around and propagated more vigorously on uninfected maize, which was more obvious in Makueni because of its significantly higher rates of aflatoxin increase. Or perhaps the Aspergillus strains in Makueni propagate more strongly when disturbed.

Smallholder farmers in Kenya know about the danger of aflatoxins, less so about fumonisin, in their harvested product but since the contamination process is invisible, reducing insect damage and maintaining grain quality parameters are considered of higher importance. Insect damage is currently addressed by the use of post-harvest fumigants or pesticides. This is an area of concern since post-harvest fumigation/pesticide protocols are not strictly regulated and impose a risk for human health. While the traditional woven polypropylene bags are widely available and low in acquisition cost, the ongoing need for fumigants imposes a susceptibility of the user to increasing insecticide prices, tainted products that may require higher application rates than anticipated and/or impeding insect resistance developments. Eliminating the use of pesticides through the use of hermetic storage would remove associated costs and greatly increase personal safety.

The proper post-harvest handling of maize is an important factor in preventing storage related problems. These problems can lead to substantial loss of marketability and consequently reduced income to acute endangerment of human and animal health. Inadequate storage conditions worsen these problems. The here presented study was largely conducted to assess which of the five most commonly accessible hermetic storage devices on the Kenyan market fulfill the needs of smallholder farmers by addressing three major areas of concern: insect infestation, grain quality, and mycotoxin contamination. The goal of the study was to simultaneously test new and existing hermetic storage devices suitable for smallholder farmers to store maize at moisture content up to 15 percent using locally produced and naturally contaminated maize grain stored at volumes and grain moisture contents achievable by smallholder farmers. Results of the here presented study showed that the PICS bag, Grain Pro Grain Safe, and the metal silo performed similarly well in reducing insect infestation, hindering mycotoxin increases and keeping grain quality high. However, an acceptance of any new storage devices will only be possible if benefits from adopting the method (e.g. significantly reduced insect infestation) will outweigh disadvantages perceived by farmers (e.g. cost and lifespan of the device). These benefits maybe immediate (e.g. improved grain quality and reduced insect infestation) or invisible (reduced accumulation of mycotoxins).

To assess acceptance of these devices on a farmer (end-user) level, financial and non-financial criteria were applied as a secondary evaluation fundament in a separate experiment, which is out of the scope of the present manuscript. These criteria included: 1) Acquisition cost: Most small-scale farmers have low levels of wealth/savings, lack of credit availability and high cost of credit when it is available. Thus, high cost items are more difficult to find demand in the market; 2) Cost Over Useful Life: This applies only to storage devices which have significantly different useful lives and thus is a mechanism to normalize this difference so that a relevant comparison can emerge; 3) Prevention of insect and/or rodent damage: The ability to resist insect and rodent damage is important since these can be significant sources of post-harvest losses; 4) Ease of integration with existing storage devices/techniques: Given the target market, a device that offers familiarity with current practices is preferred as to maximize acceptance and minimal changes to current routines; 5) Other Risk factors and additional (hidden) costs: Complexity of usage: higher complexity increases the risk that the full benefits of the device will not be obtained; Spending Flexibility: uncertainty of yearly income in relation to financing a new device year by year (PP bag or PICS bag) or advance money on loan to pay for more expensive device (Grain Pro bulk bag or silo); Reparability: Ability and or ease to repair any damage that occurs to the device; Maintenance: Amount of repairs expected to keep the device effective; Durability: Resistance to damage (that leads to replacement/repair costs). Using our findings three hermetic devices reduced aflatoxin increases in storage to below 5% per month in both locations; metal silo, PICS bag and GrainSafe, at the same time these devices prevented insect infestation, moisture loss and reduced the rate of grain discoloration. Taking all of these criteria into consideration, the initial acquisition cost is one of the most important deciding criterion for farmers. Here, both GrainPro GrainSafe and metal silo seem unrealistic alternatives for most smallholder farmers due to the high initial cost. Even if one made the assumption of credit availability over the life of these devices, the interest costs would outweigh the expected economic benefits of storage in all but extreme cases (i.e. years where market prices drastically shot up right after harvest). From an “operational” viewpoint, the metal silo has very positive attributes due to its durability; however, these positives may not outweigh the high initial cost. The metal silo also appears attractive because it is ‘rat proof’, however research shows that maize stored in PICS bags in clean stores is rarely affected by rats (Ndewga et al.). Given that farmers already store in PP bags which are easily penetrated by rats their concern related to PICS bags and rats may focus more on damaging an expensive asset rather than concerns of losses to rats which they already experience.

Flexibility, or optionality in economic jargon, is very important for agricultural producers given the risks and variabilities in production yields and market prices. For this factor, the metal silo is the least attractive device, followed by the GrainPro’s GrainSafe because farmers are not able to reliably forecast their yearly profits and are likely to be hesitant to invest future income. Overall, PICS bags, from a lower initial cost to the elimination of pesticide needs, are most promising to penetrate the Kenyan market and be accepted by local farmers. It seems quite feasible that increased demand for the PICS bag could lead to production scale economies that could tilt the pure economics to the favor of the PICS and away from the traditional woven polypropylene bag.
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