EFFECT OF SOLAR DISINFESTATION AND PURDUE IMPROVED CROP STORAGE BAGGING IN THE CONTROL OF *PROSTEPHANUS TRUNCATUS* HORN IN MAIZE

A dissertation submitted in partial fulfillment of the requirements for the degree of Masters of Science in Food Safety and Quality.

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree/Research in any other university.

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To my dear daughter Nelly Kanyi, my beloved parents James Njoroge Waweru and Nellius Kanyi Njoroge.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

PICS	Purdue Improved Crops Storage
icipe	International Centre for Insect Physiology and Ecology
DAAD	German Academic Exchange Service
LGB	Larger grain borer
FAO	Food and Agricultural Organization
EU	European Union
RUP	Restricted Use Pesticide
DDT	dichlorodiphenyltrichloroethane
ELISA	Enzyme-linked immunosorbent assay
RH	Relative Humidity
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
PP	Polypropylene
SEM	Standard Error of the Mean
wwb	wet weight basis
LT	lethal temperature
CRSP	Center for Research in Security Prices
USAID	United States Agency for International Development
ANOVA	analysis of variance
ANCOVA	analysis of covariance and variance
GHF	Gas hermetic Fumigation
VHF	Vacuum Hermetic Fumigation
CABI	Centre for Agriculture and Biosciences International

PHL	Post Harvest Losses

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ABSTRACT

This work demonstrates how an integrated approach can be used for controlling Larger Grain Borer (*Prostephanus truncatus*) in maize (*Zea mays L.)* using solar disinfestation followed by hermetic storage in Purdue Improved Crop Storage (PICS) bags. PICS bags work by sealing grains in an airtight environment for long-term pest-free storage. In order to provide safe food with no inputs of chemical pesticides, the combination of solar disinfestation and the PICS bagging is likely to be a promising integrated approach for controlling *P. truncatus.*

The first experiment of this work was the heat disinfestation experiment. The aim of this experiment was to come up with a simple way of reducing *P. truncatus* populations as an adjunct to hermetic storage of maize grains in PICS (Purdue Improved Crop Storage) bags. The oven tests were supposed to lay the basis for large scale disinfestation of maize grains using solar energy. As a result it would eventually be determined if solar disinfestation and hermetic storage can be combined to form an effective hurdle technology to prevent postharvest losses of maize due to insect damage especially *P. truncatus*. As such, this describes an integrated approach towards the management of *P. truncatus.*

Maize infested with adult insects of *Prostephanus truncatus* was used to study the heat tolerance of *P. truncatus* so as to lay the basis for solar disinfestation of maize prior to storage. The experiments were carried out in a Memmert (B54 Scwabach, Western Germany) air oven for different time-temperature combinations. The time-mortality data was first subjected to analysis of covariance and variance (ANCOVA) to determine the effect of time, temperature and the interaction of time and temperature on the mortality of *P. truncatus.* Time had no significant effect ($p > 0.05$) and as a result one-way ANOVA was done for each exposure time separately. The oven tests showed a critical temperature of 60˚C as essential to effect a near complete kill of

98.5 % mortality for an exposure period of 90 min. Exposure to higher temperatures of 65 and 70˚C of 60 min and 30 min was able to achieve 98 % and 100 % mortality respectively. Complementary log-log transformation (logistic regression) analysis was done as well and the lethal dose values were calculated to determine differences in lethal temperature (LT) values among the four exposure times (30, 60, 90 and 120 min). The LT_{95} for an exposure time of 120 min was 55.3˚C while for 60 min it was 61.3˚C. Longer exposure times resulted in further significant reduction in lethal temperature values.

Preliminary solar disinfestation experiments showed that it was possible to achieve up to 60˚C on a sunny day with temperatures of at least 26˚C between 11:30 a.m. and 2:30 p.m. when the sun is hottest. Therefore, it was concluded that heat disinfestation is an effective low-cost alternative to maize grain fumigation to control *P. truncatus* in Sub-Saharan African.

The second experiment aimed at comparing the performance of hermetic PICS bags to woven polypropylene bags in the storage of maize for 6 months. It has been established that hermetic post-harvest maize storage can effectively control maize weevil, *Sitophilus zeamais*, which can be responsible for up to 50 % damage to stored maize grain. Its use eliminates the need for toxic and expensive chemicals. Therefore, laboratory experiments with *Prostephanus truncatus* Horn which causes more severe damage were carried out. Maize infested as well as non-infested with *P. truncatus*, was stored for 6 months under hermetic (PICS bags) and non-hermetic (woven polypropylene (PP bags) conditions. Grain moisture content (m.c.) at the beginning of the experiment in January 2012 ranged from 12.30 to 13.31 % and the weight loss damage was 0%. Under hermetic conditions, after six months' storage gas composition levels were at 6.82, 7.68 and 9.34% and CO_2 level had risen to 13.52, 12.75 and 9.88 %. Insect counts were low in the PICS bags, 2 ± 1 insects per 125 g sample; and very high in the woven bags, 52.00 ± 9.85 live *P.*

truncatus, 68.67±9.07 *P. truncatus* larvae and 74.33±12.10 *S. zeamais* per 125 g sample in the worst case. Germination capacity did not change much for the PICS bags but decreased greatly for woven bags up to 12% in the worst case. Losses were also significantly different for the maize stored in the PICS bags and the woven bags with losses as high as 47.66±4.59 % recorded for the woven bags.

In terms of effectiveness in storing maize without damage, after six months of storage, PICS bags appeared to be better compared to PP bags. As there is no difference between the PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3), it is likely that PICS bags do not need any integration with solar disinfestation before performing. More studies are needed to confirm the results of the present study.

Hermetic storage is an effective low cost-effective system for grain produced in the rural areas of developing countries.

Keywords: Maize (*Zea mays L.), Prostephanus truncatus*, heat disinfestation, hermetic storage, Purdue Improved Crop Storage (PICS) bagging

CHAPTER ONE

1.0. INTRODUCTION

The Larger Grain Borer (LGB), *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), is a serious pest of farm stored maize (Golob, 2002; Vowotor *et al.,* 2005) and dried cassava (Hodges *et al.,* 1995). It is an indigenous pest of stored maize in Central America and was accidentally introduced to farms in East Africa (Tanzania) in the late 1970s and West Africa (Togo) in the early 1980s (Dick, 1988). Spreading out from Tanzania and Togo, the pest is now officially recorded in 16 African countries, namely Benin, Burkina Faso, Burundi, Ghana, Guinea-Conakry, Kenya, Malawi, Namibia, Niger, Nigeria, Rwanda, South Africa, Tanzania, Togo, Uganda, and Zambia (Farrell, 2000). In addition to maize stored on the farm, grain stored in large quantities, bags or bulk, are also subject to infestation by *P. truncatus* (Dales and Golob, 1997). Due to this widespread concern of the problem of *P. truncatus,* a variety of control measures are used to contain the problem.

Current control measures for the *P. truncatus* include chemical insecticides, fumigation and biological control using *Teretrius nigrescens* Lewis (Coleoptera: Histeridae), a predator of *P. truncatus* (Richter *et al*., 1997; Hell *et* al., 2006). These methods are expensive and unaffordable to small-scale farmers in developing countries. This has culminated into increased postharvest cereal losses due to *P. truncatus* particularly maize.

Maize (*Zea mays L.),* is the most important cereal crop grown in Sub-Saharan Africa and contributes significantly to food security for her people. However, recurrent adverse weather conditions coupled with field pests and diseases contribute negatively to yields (Mugo *et al.,* 2002). Loss and deterioration of available maize resources in storage further add to the problem.

Storage pests further reduce the amount of crop that ultimately becomes available for consumption. These losses significantly affect the availability of the staple food to people if control measures are not applied. Despite heavy losses incurred in storage, very little attention has been given to research on stored product pests in general and that of maize in particular until recently. Technologies have been developed to reduce the impact of field pests and diseases (Langyintuo, 2004), but storage pests, such as *Prostephanus truncatus* remain a problem. The storage insect pest, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is the most destructive insect pest on stored maize in many parts of Africa (Farrell *et al.,* 1996).

P. truncatus infestation has been reported to contribute to up to 80% post-harvest losses on shelled maize after six months of storage (Singano *et al.,* 2007). It has also been reported that *P. truncatus* destroyed up to 30% of cob stored maize within a period of 3-6 months (Helbig, 1995; Singano and Nkhata, 2004). In intense cases of infestation, the stored maize can practically be completely destroyed; resulting in total loss of the staple food (Singano and Nkhata, 2004).

In Sub- Saharan Africa, maize production is undertaken by resource-poor farmers with little or no control measures against *P. truncatus* during storage. In addition, there are public concerns over the continuous application of synthetic pesticides in protection of stored product. Available literature on the control of *P. truncatus* in maize stores indicates that major emphasis has been on the use of insecticides. The increasing incidence of insecticide resistance (Perez- Mendoza, 1999) and environmental concerns about the use of chemical insecticides calls for the adoption of alternative sustainable pest management strategies.

Restrictions due to the adverse effects of pesticide residues in food and the environment have resulted in the imposition of strict limitations on pesticide registration by regulatory agencies.

Consumer demand for chemical-free and insect contamination-free products increased the attention to the application of non-residue organic technologies for the protection of stored grain.

1.1. Problem Statement

Maize, a staple food in sub-Saharan Africa has been reported to undergo high postharvest losses (PHL). Post harvest losses in maize are mainly due to storage insect pests, *Prostephanus truncatus* and *Sitophilus zeamais,* which cause up to 30 % loss (Likhayo *et al.,* 2004). Other causes of PHL in maize include mechanical damage, insect infestation, fungal pathogens, and lack of PH handling systems, among others. Postharvest maize insect infestation is a major constraint to food security and income generation in Sub-Saharan Africa because of significant yield losses and grain quality degradation, especially food safety of the grain (Abebe *et al.,* 2009). In the past, *P. truncatus* infestation was often a less serious problem because farmers cultivated traditional varieties which, although low yielding, were generally more resistant to attack by insects (Golob, 2009). However, the introduction of high-yielding maize grain varieties has resulted in increased storage losses as these varieties are usually more susceptible to *P. truncatus* damage (Golob, 2009). *P. truncatus* begins attacking the mature crop in the field. New hybrids are more susceptible as the cob is not completely covered by its protective sheath. During harvesting they are transferred from the field to the grain stores. Maize grains that are adversely damaged by *P. truncatus* are more susceptible to the growth of aflatoxin producing fungi. As *P. truncatus* bores through the maize grains, it releases starch. The hygroscopic nature of starch allows imbibition of water from the atmosphere thus increasing moisture content of the grain which favors fungal growth.

Due to lack of suitable grain storage structures and storage management technologies maize farmers are forced to sell their produce immediately after harvest, thus resulting in low market prices (Kimenju *et al.,* 2009). Postharvest insect pest losses are influenced by the storage time and population of insects involved in infestation. Farmers do traditional solar drying of maize in the open but still experience PHL since they use non-hermetic polypropylene bags for storage which do not prevent infestation with *P. truncatus*. This research aims at designing a technology that combines solar disinfestation and hermetic storage of maize to reduce *P. truncatus* populations and postharvest losses.

1.2. Justification

In a study carried out in Mozambique, *P. truncatus* infestation has been reported to cause weight losses of around 35% in stored maize after 3-6 months of storage (Cugala *et al*., 2007). Therefore, there is a need to develop a range of more radical options for the control of *P. truncatus* due to the dwindling availability of effective pesticides. *P. truncatus* can be controlled by use of pesticides however; traditional chemical insecticides are being eliminated due to regulatory, environmental, food safety and societal influences. Therefore, alternative management systems are needed for these pests. In addition, with increased resistance of *P. truncatus* to pesticides hence people resolve to the use of DDT which is of prime food safety concern as it has no acceptable residue level and is no longer allowed for use in stored grain pest control. Methyl bromide, an ozone depleting chemical, is a Restricted Use Pesticide (RUP) because of its high acute toxicity to applicators.

The possible phase-out of methyl bromide, likely limitations or withdrawal of some organophosphide and carbamate pesticides and the possibility of new restrictions on the use of phosphine has heightened the urgency of considering other options.

Heat disinfestation of *P. truncatus* followed by PICS hermetic bagging may be an appropriate control and containment measure to keep maize losses within acceptable bounds. In addition, heat disinfestation of grain combined with hermetic bagging has the potential for high market acceptance.

The aim of this study is to contribute towards alternative management options for control of *Prostephanus truncatus* in maize storage for reduced postharvest losses

1.3. Objectives

Overall objective

To determine the effect of solar disinfestation and Purdue Improved Crop Storage (PICS) in the control of the larger grain borer, *Prostephanus truncatus* Horn (*Coleoptera: bostrichidae*) and reduction of postharvest losses in maize (*Zea mays L.)*

Specific objectives

1. To determine the susceptibility of *P. truncatus* to heat during hot air oven heat treatment of infested maize.
2. To assess the effectiveness of solar disinfestation and hermetic PICS bagging of maize in controlling *P. truncatus* infestation and maize weight losses over six months of storage

CHAPTER TWO

2.0. GENERAL LITERATURE REVIEW

2.1. Stored-product Insect Pests

The majority of stored-product insects come from only two of the roughly 26 orders of the Class Insecta namely Coleoptera and Lepidoptera. On the other hand, predators and parasitoids of stored-product insects come from the orders Hemiptera, Hymenoptera and Diptera (Rees, 2004). Stored-product insects are often classified by whether they develop and feed inside or outside grain kernels, and thus are referred to as either internal developers or external developers.

As the most destructive of the stored-product insects, six species from two families of Coleoptera and one family of Lepidoptera are recognized as internal developers, sometimes referred to as primary invaders. These include the grain borers (Family: Bostrichidae), such as the lesser grain borer, *Rhyzopertha dominica* (Fabricius), which is one of the most damaging insects, and the larger grain borer, *Prostephanus truncatus* (Horn), a major tropical pest of maize; the grain weevils (Family: Curculionidae), such as the rice weevil, *Sitophilus oryzae* (L.), maize weevil, *S. zeamais* Motschulsky and the granary weevil, *S. granarius* (L.); and, finally, the angoumois grain moth (Family: Gelechiidae), *Sitotroga cerealella* (Olivier), which today is less destructive than the other internal developers because infestations commonly start in the field and can be minimized by modern methods of harvesting (e.g. combine harvesters) and storage (Tang *et al*, 2007).

2.2. *Prostephanus truncatus* description

P. truncatus is a bostrichid beetle with three distinct life forms, namely; the eggs which are white to yellow, with no surface features and have a broad ovoid (ellipsoidal) shape, the larva which is

white, fleshy and has a sparse covering of hairs, has a parallel-sided C-shaped (scarabaeiform) body, short legs and small head and finally the adult which is 3 - 4.5 mm long, dark brown with a body that looks like a flattened tube, the end of which appears to have been cut straight. The body surface is pitted and has many small wart-like outgrowths (tubercles). The head is curved under the thorax so that the back of the head cannot be seen from above. The antennae have 10 segments, made up of a 7-segment 'stem' and a 3-segment 'club'.

2.3. Geographical Distribution of *Prostephanus truncatus* in Africa

P. truncatus was accidentally introduced from Central America into Tanzania in the late 1970s, and spread to other countries in the African region. *P. truncatus* is a beetle that is a major destructive agent in farm-stored maize and dried cassava across Africa (Golob, 2002; Vowotor et al., 2005).

The larger grain borer is spread over longer distances almost entirely through the import and export of infested grain. Local dispersal is through the local movement of infested maize and dried cassava and by flight activity of the adult beetles.

2.4. Damage caused by *Prostephanus truncatus*

P. truncatus is a serious pest of stored maize and dried cassava roots, and will attack maize on the cob, both before and after harvest **(**Golob, 2002; Vowotor et al., 2005). The adults prefer grain on cobs to shelled grain, thus damage on unshelled maize is greater than on loose, shelled maize. When infesting stored maize cobs with husk intact, the adults frequently begin their attack by boring into the maize cob cores, and eventually gain access to the grain at the apex of the cob by crawling between the cob and husk. They may also bore directly through the husk. They cause considerable losses in stored maize; weight losses as high as 35 % have been observed

after only 3 to 6 months storage (Cugala, 2007). Losses in dry cassava can be very high too; the dried roots may be readily reduced to dust by boring adults. During a field survey in Tanzania, postharvest losses of up to 19 % after 3 months and up to 63 % were recorded after four to five months due to the infestation of *P. truncatus* on cassava (Hodges et al., 1995).

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Plate 2.1. Maize cob damaged by the larger grain borer

Plate 2.2 Aerial view of *P.truncatus*

Plate 2.3 Side view of *P.truncatus*

2.5. Developmental stages of *Prostephanus truncatus*

Eggs are laid in tunnels and chambers bored by the females in the food source. Larvae hatch from the eggs after 3 to 7 days. The larvae develop within the grain or in the flour that accumulates by the feeding action of the adults. The aerial and side views of adult *P.truncatus* are shown in Plates 2.2 and 2.3. They pupate inside the food source. The adult beetle is 3 to 4.5 mm long and dark brown in colour. It has a cylindrical body shape, when viewed from above the rear of the insect is square shaped. The thorax bears rows of teeth on its upper front edge and the head is turned down underneath the thorax so that it cannot be seen from above. The female lays 30 to 50 eggs into the produce (maize, cassava, etc). The lifecycle can be completed within 25 to 26 days at optimum conditions i.e. high temperature (about 30°C) and relatively high humidity (about 70 % RH and 13 % grain moisture content). Development takes longer under cooler or drier conditions. *P. truncatus* develops more rapidly on maize grain than on cassava (Cugala *et al*., 2007).

2.6. Management and Control of *Prostephanus truncatus*

2.6.1. Cultural Practices

2.6.1.1. Detection and Inspection Methods, Store Hygiene, Timely Harvesting

It is not possible to detect the pest by visual inspection except when populations are very high. The immature stages develop within the food source, and therefore are not normally seen. Traps baited with the chemical attractant (pheromone) produced by the male beetle are useful to detect and monitor adult beetles. This pheromone is synthesized in the laboratory and loaded into plastic capsules, which then release the pheromone slowly through the walls of the capsule. A pheromone capsule is then placed in a suitable trap.

Flight traps, such as funnel, delta or wing traps baited with the pheromone are considered the best for monitoring the larger grain borer. These traps are suspended about 1 to 2 m from the ground outside the store or the standing maize crop. They should be placed at least 100m from stores or from the field to avoid attracting the beetles to these food sources. The traps are useful for researchers and for plant protection authorities. Traps are an important tool for phytosanitary purposes and for warning farmers about impeding attack by the larger grain borer. Presently, the only means of assessing infestations in store is by manual sampling of the produce. A detailed leaflet giving recommendations on the use of pheromone traps to monitor the *P. truncatus* has been prepared (Hodges and Pike, 1995). Although the traps and pheromones are available commercially, they are expensive and not easy to get.

Good store hygiene is very important in limiting infestation. The stores should be cleaned thoroughly between harvests and infested residues burnt before the new stock is stored. Used sacks should be immersed in boiling water to eliminate residual infestations. Residual infestation in the wooden structure of the store can be eliminated by removing timber or by fumigating the whole store under a gas-tight sheet. When maize is ready for harvest, it should not be left for too long in the field; the larger grain borer or other storage pests could attack it. Studies in Benin have shown that maize harvested 3 weeks after physiological maturity gave better economic returns when stored for 8 months than maize harvested only 1 week after physiological maturity. Leaving the maize in the field for extended periods after physiological maturity resulted in severe grain losses after 8 months of storage, mainly due to damage by the larger grain borer. However, early harvested maize had a higher proportion of mouldy grain (Borgemeister et al., 1998).

2.6.1.2. Post harvest Handling and Storage Practices

In locations where *P. truncatus* is a problem, infested cobs should be shelled as soon as possible before storing and dried completely to a safe critical moisture content of 13 %; when the kernels are too hard to bite through with the teeth they are usually dry enough for bagging. The grain should then be treated with a botanical pesticide. Traditional varieties with good husk cover are much less likely to be attacked, thus when storing these varieties on the cob, reject any cobs with damaged or open sheathing leaves (Meikle *et al.,* 2002; Borgemeister*, et al.,* 2003). In the case of cassava, roots should be left in the ground for as long as possible to reduce the storage period in order to minimize losses. After harvest, the cassava should be sun dried and immediately transferred to sealed containers. Only clean produce should be stored. The store should be carefully inspected before the newly harvested maize or cassava is placed inside. The grain should be stored in a suitable container since *P. truncatus* easily attacks grains stored in gunny bags (jute) or gourds. The most suitable containers are those that can be sealed such as metallic containers, old oil drums or mudded cribs or baskets. They provide a very effective barrier to pest attack and can be used provided the stock is sufficiently dried so that ventilation is not required. Brick stones should be used to construct the granaries since wood and grass would encourage breeding and multiplication of the *P. truncatus*. Iron sheet roofing is better for the stores to avoid harboring the pest. If grass is used for thatching, it should be a thick layer and cone shaped; the roofing should be replaced after a certain interval period to minimize leaking. The maize can be sold within the first 3 months since the extent of *P. truncatus* infestation during the first 3 months of storage is generally low. Alternatively the maize harvest is split into two portions. One portion, destined for consumption by the families should not be kept longer than three months in the store. The other portion to be kept longer in the store should be treated with insecticide if *P. truncatus* was observed the previous year. If not, the stock should be

regularly inspected. If the pest is found subsequently then grain shelling and treatment either with a botanical or with an inert dust is required.

2.6.2. Biological control of *Prostephanus truncatus*

2.6.2.1. Natural enemies of Prostephanus truncatus

The beetle *Teretrius nigrescens*, which is a specific predator of the *P. truncatus* in Central America, was introduced into Africa. The adult and the immature stages of this predatory beetle feed on eggs and larvae of the *P. truncatus*. The predatory beetle has been released in Benin, Ghana, Guinea-Conakry, Kenya, Malawi, Tanzania, Togo and Zambia. It became well established and spread in most countries. However, despite the successful introductions, there are still regular outbreaks of the *P. truncatus* and farmers still suffer losses. Nevertheless this predator has a role to play in the management of the *P. truncatus*, as it is able to reduce the density of the pest.

2.6.2.2. The use of Neem Oil to Control *Prostephanus truncatus*

Several plant extracts have been reported to control *P. truncatus* as shown in the table below.

Table 2.1: Plants Used to Control *P. truncatus*

Plant	Plant part	Product/ concentration	Effect on damage
Castor beans	Seed	10% ethanolic extract	-
Neem	Seed	5-10% slurry	< 10% damage
Neem	Oil	1.5% (vol/vol)	< 16% damage
Pyrethrum	Flower	0.5% powder (w/w)	Highly effective
Velvet leaf	Leaf, root	2.5-10% slurry	< 10% damage

Source: G. Stoll, 2003

Using plant material in the form of slurry has given better results than plant powders. The slurry can be prepared by weighing out powder into 150 ml containers and adding sufficient water to give a 10% concentration (w/w), and stirring until a smooth paste is obtained. Then, the grain is poured into prepared slurries and stirred with a rod until all grains are coated (Stoll, 2003). Neem has shown considerable potential for controlling pests of stored products (Opareke *et al.*, 1998). Jute sacks are also treated with neem oil or neem extracts to prevent pests - particularly, weevils and flour beetles- from penetrating for several months. However, neem products are not as effective for protection of maize grain against the *P. truncatus* as against grain weevils but pyrethrum is much more effective (Loth *et al.,* 2010). Since these two pests are usually found together, a mixture of neem and pyrethrum known as ("Nimpyr") is a better option to protect

stored maize. Trials in Tanzania showed much lower grain damage in maize treated with "Nimpyr" (0.5 - 6% kernel infested) compared to untreated maize (17 % to over 90 %) 6 months after treatment. But there are some shortcomings to the use of this mixture, namely; a relatively large amount of the mixture is needed to protect grain (2 to 3 kg/ 100 kg grain), the labor input needed to produce "Nimpyr" is considerable. Other disadvantages include; the active principles of pyrethrum deteriorate relatively rapid on exposure to heat and/or light, pyrethrum has an unpleasant odour, whilst neem has a bitter taste (although this can be eliminated by soaking and washing the grains in water for a sufficient period) and the mixture is unlikely to give protection in maize stored on cobs, since the pests are protected under the husks.

2.6.2.3. The use of Ash/Chilli Mixture to Control *Prostephanus truncatus*

Ash/chilli mixture and a thick layer of paddy husk ash covering the stock is reported to be effective in preventing *P. truncatus* attack (Borgemeister *et al.,* 2003). To prepare an ash/chilly mixture to protect maize from *P.truncatus*; dry the chillies and pound them to a fine powder then sieve cold wood ash from the fireplace. Mix 2 kg of wood ash with 1 tablespoon of chilli powder and mix them properly. Mix 1 part ash/pepper mixture with 4 parts of dried maize grain and store (Borgemeister *et al.*, 2003)

2.6.2.4. The use of Diatomaceous Earth to Control *Prostephanus truncatus*

The use of diatomaceous earth for control of grain boring insects during storage has in many cases been successful (Golob, 1997).The diatomite powder is mixed with shelled maize grain or newly harvested dry cobs before storing in bags. 1 kg diatomite is used per bag of maize. Some confusion exists on the use of diatomaceous earth, as finer ground diatomite products commonly used for sifting beverages is not effective as insect control. However, unprocessed products such

as 'Kensil Lagging' work on the same principles as laterite mentioned below, by dehydrating the insects and by destroying the insects' articulations.

2.6.2.5. The use of Laterite to Control *Prostephanus truncatus*

The common red soil of the arid tropics, when finely crushed protects stored grains and beans. In family grain stores or in sealed clay pots, the dust deters insects from boring into or laying their eggs on the dusted grains. Laterite rubs off the waterproof waxy coating the insect bodies and they dehydrate and die. In sealed storage pots insects suffocate because enough dust is poured in with grain to exclude air and also trapped insects dehydrate and die as their outer coating is damaged by abrasion**.**

2.6.3. Chemical control

2.6.3.1. The use of Fumigants to Control *Prostephanus truncatus*

Fumigants generally enter the insect through the respiratory system, and are toxic to all life stages. They are gaseous chemicals at ambient temperature and pressure, and can produce gas from a solid or liquid. They diffuse through air, permeate products and have little or no residual insecticidal effect (Harein and Davis, 1992). They mainly include phosphine (PH_3) and Methyl bromide (CH_3Br). Other fumigants include hydrogen cyanide, carbon disulphide, and chloropicrin and ethyl formate. Modified atmospheres, high carbon dioxide and high nitrogen (or low oxygen) have been used to a limited extent to disinfest grain or structures as alternatives to fumigation. In leaky structures, carbon dioxide is better than nitrogen, as concentrations of 35 % are lethal to all life stages of stored-product insects. In contrast, nitrogen must reduce the level of oxygen to below 2 %, requiring airtight storage to be cost-effective. Thus, the technology is

hindered by the necessity for sealed storages and access to large amounts of cheap carbon dioxide or nitrogen.

2.6.3.1. The use of Contact Insecticides to Control *Prostephanus truncatus*

Contact insecticides, or protectants, generally enter the insect orally or across the cuticle. They are applied either to grain, floor–wall junctions, general surfaces or crevices in warehouses and food-processing facilities. Protectants are defined as insecticides that prevent infestations from becoming established in a commodity, but are less effective at managing a well-established infestation, and infested commodities and structures are often better treated by fumigation. The most commonly used contact insecticides are: (i) organophosphates (malathion, dichlorvos, fenitrothion, pirimiphos-methyl, chlorpyrifos-methyl, chlorpyrifos-methyl with deltamethrin);(ii) pyrethroids (bioresmethrin, permethrin and deltamethrin); and (iii) synergized pyrethrins (Snelson, 1987). Moreover, as with fumigants, insect resistance is a major issue, which further adds to the complexity of application. Other less commonly used contact insecticides include diatomaceous earth (Paula *et al*., 2002) and insect growth regulators such as methoprene and hydroprene (Edwards *et al*., 1987). Spinosad, an insecticide based on bacterial fermentation products (Spinosyns A and D), has been shown to be effective against stored-product insects in laboratory and field evaluations (Fang *et al*., 2002a, b; Flinn *et al*., 2004) and is registered in the USA as a grain protectant.

2.6.4. Physical disinfestation methods

Chemical methods of insect disinfestation face significant challenges. Methyl bromide is becoming more and more costly and is being phased out under the 1987 Montreal Protocol in both developed and developing countries. The use of phosphine is being increasingly regulated

because of safety issues and insect resistance. Resistance to insecticides is widespread and chemical residues– owing to increased dosages–are becoming increasingly unacceptable to grain buyers and consumers. Not only do contracts with buyers now stipulate acceptable insect contamination and damage, but also which insecticides are acceptable and what constitutes the upper residue limit. On the other hand, a physical method of grain protection and disinfestation would not carry these problems. One such method might be the use of mechanical methods such as impact or combinations of sieving, aspirating and blowing, which are used in food-processing facilities. One of the most widely researched physical methods, however, is the use of extreme temperatures for insect disinfestations in bulk-stored grain, associated structures and food-processing facilities. The use of elevated temperatures has the major advantage of giving complete disinfestation while being comparatively rapid. It is chemical-free, and insects are not as likely to develop resistance to it. Methods using heat have been developed that disinfest grain both at the on-farm and commercial storage levels, as well as storages, processing facilities and equipment. However, there are scientific, technical and economic issues still to overcome.

2.6.4.1: Heat Disinfestation in the Control of Stored Grain Pests

The first recorded use of high temperature to disinfest grain was in China, 1,500 years ago (Liu et al., 1983). Research work shows that death can be caused at high temperatures because lipids in the nerve membranes and waxy layers of the insect cuticle degrade (Strang, 1992 and Fields, 1992). There is also evidence that for some species certain enzymes important to the metabolism of insects are inactivated at temperatures above 55 to 60°C which leads to the death of the insect. At temperature above 45˚C most stored product insects die within 24 hrs. There are a number of factors that affect the mortality of insects when exposed to high temperature; duration of exposure, temperature, species, stage and acclimation.

2.6.4.1: Solar Disinfestation in the Control of Stored Grain Pests

Utilizing solar energy as a possibility for the thermal disinfestation of grain with a 12% wwb moisture content has been tested by collecting solar energy in a $1m^2$ solar collector which achieved necessary 60˚C grain temperature, which is a lethal temperature for all insects. This was done under normal field conditions in Germany (Ahmed and Lücke, 2005). In Africa, it is not rare to find farmers who have spread out their harvested grain on the edges of roads, on mats or on flat stones in the sun to dry. This is indigenous knowledge which if coupled with science can be used to come up with the idea of drying grain on black polythene so as to concentrate the sun's heat energy. Most work with solar heat treatments of commodities has targeted bruchid pests of seeds using a variety of solar heating methods including plastic bags, corrugated metal and wooden racks (Murdock and Shade, 1991). In the case of the cowpea bruchid this is 57 8C, with all life stages of the insect (egg, larvae, pupa and adult) killed when exposed to this temperature for 1 hr (Murdock and Shade, 1991). To achieve this temperature, and thus disinfest cowpeas, Murdock and Shade used plastic sheeting to enclose and heat the cowpea grain. Black plastic sheeting (woven wicker mats can serve nearly as well) is laid upon the ground, and then covered to a depth of 1–2 cm with infested cowpea grain. A second, translucent plastic sheet is used to cover the lower sheet and grain, and then the edges of the two plastic sheets are sealed by folding the upper sheet under the lower one and securing the envelope so formed with small stones laid around the edges. When exposed to the sunlight, the temperature within the envelope rises rapidly thanks to solar energy passing through the translucent upper sheet and being absorbed by the cowpea grain and the underlying black plastic sheet. Within 15–30 min the temperature within the cowpea grain typically rises to 60–70.8˚C, more than adequate to kill all stages of the cowpea weevil (Murdock and Shade, 1991). Solarisation is the use of solar

radiation to increase the temperature of (dark) materials covered by a transparent plastic film or glass. The method is based on the fact that solar radiation (280 to 2500 nm) passes through the transparent layer, reaching the dark surface where it converts into convective heat and radiant heat of lower wave- length. Because the transparent film is much less permeable to long wavelength thermal radiation, the heat is contained within the system, increasing the temperature of the enclosed air volume. Solarisation for disinfesting crops and stored products has been used occasionally in tropical countries. Other applications of solarisation are drying of stored products and timber.

Hermetic Storage and Storage structures and enclosures developed for Hermetic Storage

Hermetic storage is a type of modified atmosphere that has now been applied for the protection of stored agricultural commodities including cocoa beans as well as coffee, rice, maize, pulses and seeds (Navarro et al., 1984; 1993; Navarro, 2006). It is also called "sealed storage" or "airtight storage" or "sacrificial sealed storage" or "hermetic silo storage". This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the modified atmosphere by reducing the O_2 and increasing the CO_2 concentrations through respiratory metabolism. It has been shown that hermetic storage allows safe storage for periods ranging from weeks to many months, as well as during shipment across intercontinental distances with storage losses typically well below 1 %.

Modern hermetic storage systems use special low permeability flexible plastic enclosures. These hermetic storage containers have evolved to store a variety of dry commodities in the range of 60 kg to 20,000 tonnes. They became commercially available starting in the early 1990's, and today are in use in more than 38 countries in a variety of configurations. A few specialized applications require rapid disinfestation, such as in 3 days for dried figs (Ferizli and Emekci, 2000). In these,

oxygen levels are reduced rapidly, either by purging with CO^2 (Gas – Hermetic Fumigation "GHF"), or by applying a significantly high vacuum (Vacuum - Hermetic Fumigation "VHF"). In either case, the process can quickly reduce oxygen content to below 1 % to 2 % (Navarro *et al*., 2002; Villers et al., 2008). The most widely used form of hermetic storage is the Cocoon™ (Fig. 1A). It is manufactured in capacities ranging from 5 tonnes to 300 tonnes. Cocoons are made from specially formulated flexible 0.83 mm thick PVC with permeability to oxygen of 400 $cc/m^2/day$ and to water vapor of 8 $gm/m^2/day$. A newer type of Cocoon called the MegaCocoon™ has more recently been introduced for larger scale storage of up to 1050 tonnes, with initial installations in Sudan.

2.6.6. PICS bagging

PICS bagging is a technology developed by Purdue University in collaboration with African researchers, known as Purdue Improved Crop Storage (PICS). The PICS bag is a triple-layer bag system which utilizes 2 thin, transparent and low permeability co-extruded multi layer plastic papers, which are each 80 µ thick, as a liner to a conventional polypropylene bag (Navarro and Donahaye, 2005). PICS bags provide an airtight seal for long-term, pest-free storage. PICS works by sealing grains in an airtight environment. This kills all the adult insects and most of the larvae within days. At the same time the triple bags keep the remaining larvae dormant and unable to damage the seeds. Triple bagging of the cowpeas, proved to be a cost effective storage method for cowpeas without use of chemicals (Moussa *et al.,* 2009). PICS technology has been quickly adopted by small scale farmers and other organizations (Baributsa *et al*., 2010). A study carried out in Benin on maize stored pests control by PICS-Bags has shown the effectiveness of this technology in maize storage with respect to aflatoxin development (Hell *et al*., 2010).

CHAPTER THREE

3.0 Susceptibility of *Prostephanus truncatus* to heat during hot air oven heat treatment of infested maize.

Abstract

Maize infested with adult insects of *Prostephanus truncatus* was used to study the heat tolerance of *P. truncatus* so as to lay the basis for solar disinfestations of maize prior to storage. The experiments were carried out in a Memmert (B54 Scwabach, Western Germany) air oven for different time-temperature combinations. The time-mortality data was first subjected to analysis of covariance and variance (ANCOVA) to determine the effect of time, temperature and the interaction of time and temperature on the mortality of *P. truncatus.* Time had no significant effect ($p > 0.05$) and as a result one-way ANOVA was done for each exposure time separately. Complementary log-log transformation (logistic regression) analysis was done as well and the lethal dose values were calculated to determine differences in lethal temperature (LT) values among the five exposure times (30, 60, 90 and 120 min). The oven tests showed a critical temperature of 60˚C as essential to effect a near complete kill of 98.5% mortality for an exposure period of 90 min. Exposure to higher temperatures of 65 and 70˚C of 60min and 30 min to achieve 98 % and 100 % mortality respectively. The LT_{95} for an exposure time of 120 min was 55.3˚C while for 60 min it was 61.3˚C. Longer exposure times resulted in further significant reduction in lethal temperature values. Preliminary solar disinfestation experiments showed that it was possible to achieve up to 60˚C on a sunny day with temperatures of at least 26˚C between 11:30 am and 2:30 pm when the sun is hottest. Therefore, it was concluded that heat disinfestation is an effective low-cost alternative to maize grain fumigation to control *P.truncatus* in Sub-Saharan African.

3.1. Introduction

Maize (*Zea mays L.)* is the most important cereal crops grown in Sub-Saharan Africa and contributes significantly to food security for her people. However, recurrent adverse weather conditions coupled with field pests and diseases contribute negatively to yields (Mugo *et al*., 2002). Loss and deterioration of available maize resources in storage further add to the problem. Storage pests further reduce the amount of harvested grain that ultimately becomes available for consumption. If control measures are not applied, these losses significantly affect the availability of the staple food to people. Despite these heavy losses incurred in storage, very little attention has been given to research on stored product pests in general and maize in particular until recently. Technologies have been developed to reduce the impact of field pests and diseases (Langyintuo, 2004), but storage pests, such as *Prostephanus truncatus* remain a problem. *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is the most destructive storage insect pest on stored maize in many parts of Africa (Farrell *et al*., 1996). Post-harvest maize grain losses of up to 80% attributable to *P. truncatus* infestation have been reported on shelled maize after six months of storage (Singano *et al*., 2007). In very intense cases of infestation, the stored maize can practically be completely destroyed; resulting in total loss of this staple food (Singano and Nkhata, 2004).

Control strategies against this pest have solely relied on the application of synthetic insecticides and fumigants. Although synthetic pesticides are known to have undoubted benefits, their adoption rate and use for insect pest control has remained remarkably low in resource-poor environments. In addition, the use of pesticides to protect storage products is harmful to the health of farmers and the consumers. The use of heat to kill insects and control infestations in postharvest handling is well known to farmers in Sub-Saharan Africa. In many countries of

Africa, it is not rare to find farmers spreading out their harvested grain on the edges of roads, mats or flat stones in the sun for drying. The first recorded use of high temperature to disinfest grain was in China, 1,500 years ago (Liu *et al*., 1983). The supporting data shows that death occurs at high temperatures because lipids in the nerve membranes and waxy layers of the insect cuticle get degraded (Strang, 1992; Fields, 1992). There is also evidence that for some species certain enzymes important in the metabolism of insects are inactivated at temperatures above 55 to 60°C which leads to the death of the insect. At temperature above 45°C most stored product insects die within 24 hrs. There are a number of factors like temperature, duration of exposure, species, stage and acclimation which affect the mortality of insects when exposed to high temperature. The aim of this study was to obtain minimum time -temperature combination at which we could achieve at > 50 % mortality and finally determine time–temperature combination for practical application of solar disinfestations.

3.2. Materials and Methods

3.3.1 Rearing of Test Insects

Adult *P. truncatus* were reared in 1 L glass jars in the Insectariums at *icipe*'s, Duduville Headquarters, Nairobi. The insect culture was maintained on previously sterilized maize grain (13 % moisture content) and incubated at 26 ± 2°C, 40 ± 5% relative humidity and with alternating light and dark periods of 12 hr. The adult insects were separated after sieving out the maize. They were then placed in different glass tubes for later use.

3.2.2. Preliminary Heat Disinfestation Test

In order to come up with the range of temperatures to be studied, preliminary tests were carried out by subjecting the insects alone to heat over a wide range of temperature. The hot air oven (Memmert (B54 Scwabach, Western Germany) temperatures for the study were as follows: 45, 50, 55, 60, 65 and 70˚C. As the oven was heating, adult *P. truncatus* were separated carefully from colonies using feather tipped forceps and put into 20 glass tubes (75 mm by 25 mm). Fifty unsexed adults were placed in each glass tube. *P. truncatus* adults were then held at each temperature for varying lengths of time between 1 minute and 24 hr depending on the temperature. This was done to facilitate getting a series of treatments causing 0 - 100 % mortality at each temperature as shown in Table 3.1. After the heat treatment, the glass tubes were removed from the oven at the designated time and the *P. truncatus* adults kept for 3 days before taking final mortality counts.

Table 3.1: Preliminary heat disinfestation tests results

temp	time taken to achieve 100% mortality
40˚C	no death after 24hrs
45˚C	no death after 24hrs
50˚C	1hr
55˚C	15min
60˚C	10min
65˚C	4min
70˚C	2min
75˚C	1min

3.2.3. Actual Heat Disinfestation of Infested Maize Grain in the Oven

The preliminary heat experiments showed that a temperature range of 50 – 70˚C was most destructive. This is because at temperatures below 50˚C, *P. truncatus* could survive for up to 24 hrs and for temperatures above 70˚C; exposure time was less than a minute and hence impractical for solar disinfestation. The infestation of maize was achieved by putting 50 unsexed adult insects collected from reared *P. truncatus* colonies in the Insectariums at *icipe*'s Duduville campus into separate glass tubes. The 75 mm by 25 mm glass tubes were then filled with 20 g of clean and not chemically treated maize. The insects were allowed to infest the maize by incubating the samples for seven days. Four replicate lots of infested maize were prepared. Five separate hot air ovens (Memmert (B54 Scwabach, Western Germany) were preheated to 50, 55, 60, 65 and 70˚C. Once the desired temperatures were attained the samples of infested maize were put into the hot air oven. 3 days after heat disinfestation, each glass tube was analyzed for dead and surviving insects.

3.2.4. Statistical Analysis

The outcome of interest was defined as the proportion of dead insects at a given temperature for the given duration of exposure. Since each group of insects in a glass tube was only observed once, these observations were considered to be independent. In order to determine whether temperature, exposure time and/or interaction of time and temperature had a significant effect on the mortality of *P. truncatus,* an Analysis of Covariance and Variance (ANCOVA) model was fitted with temperature, time and the interaction of temperature and time as factors. The proportions of insects that died were first arcsine transformed to stabilize the variance before performing ANCOVA. Due to the inherent limitations of ANOVA in describing the response curves, a log-logistic regression model (three parameter) was further fitted:

$$y = d / (1 + \exp [b (\log x - \log e)])$$

Where the parameter

e - is the temperature giving 50% response (LT_{50}).

d - is the upper limit and corresponds to the mean response of the control.

b describes the slope of the curve around e.

N.B. The greater the value of *b*, the steeper the slope of the curve.

The log-logistic model relied heavily on the 'drc' package (Ritz and Streibig, 2005).

The analyses were performed using R version 2.11.1 (R Development Core Team, 2010) with test performed at 5% level of confidence.

3.4. Results

The ANCOVA results indicated a highly significant effect of temperature ($p<0.01$) and interaction of temperature and time ($p<0.01$). Time was not significant ($p>0.05$). Since time was not significant, one way ANOVA was done for each exposure time separately and the results presented in table 3.2 below.

Table 3.2: Percent Dead Insects at Different Time-Temperature Treatments

	% dead insect			
	30min	60min	90min	120min
50˚C	3.5 ± 1.915^{a}	10 ± 1.633^{a}	24.5 ± 5.745^{a}	90.5 ± 5^{a}
55˚C	19 ± 5.292^{b}	51.5 ± 7.550^{b}	95 ± 4.761^{b}	90.5 ± 5^{a}
60˚C	94 ± 4.320^{c}	94.5 ± 1.915^{c}	98.5 ± 1.915^{b}	99 ± 2^{a}
65˚C	94.5 ± 1.915^{c}	98 ± 1.633^{c}	98.5 ± 1.915^{b}	100 ± 0^{b}
70˚C	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{b}	100 ± 0^{b}

Means in the same column with the same subscript are not significantly different ($p\geq0.05$)

Table 3.2 shows the average percent mortality at each time and temperature combination. The results indicate that the average percent mortality for each exposure time increases with increase in temperature. For 30min and 60min exposure, the mortality at 60˚C and 65˚C were not significantly different. For 90min exposure, mortality at 55, 60 and 65˚C was not significantly different. For 120min exposure, mortality at 50, 55 and 60˚C was not significantly different and that at 65˚C and 70˚C was not significantly different either.

Indeed, the same observations were made from the log-logistic model results as well (Figure 3.1). The results in figure 3.1, shows that at shorter holding times (30min, 60min), the proportion mortality curves followed a sigmoid shape had an initial lag phase followed by a steep almost linear rise and a final leveling off of the curve. In the same figure 3.1, at longer holding times (90min and 120min), there was no initial lag phase. For 90min there was a steady rise in proportional mortality almost directly from the onset. For 120min the proportional mortality was almost constant for all the temperatures.

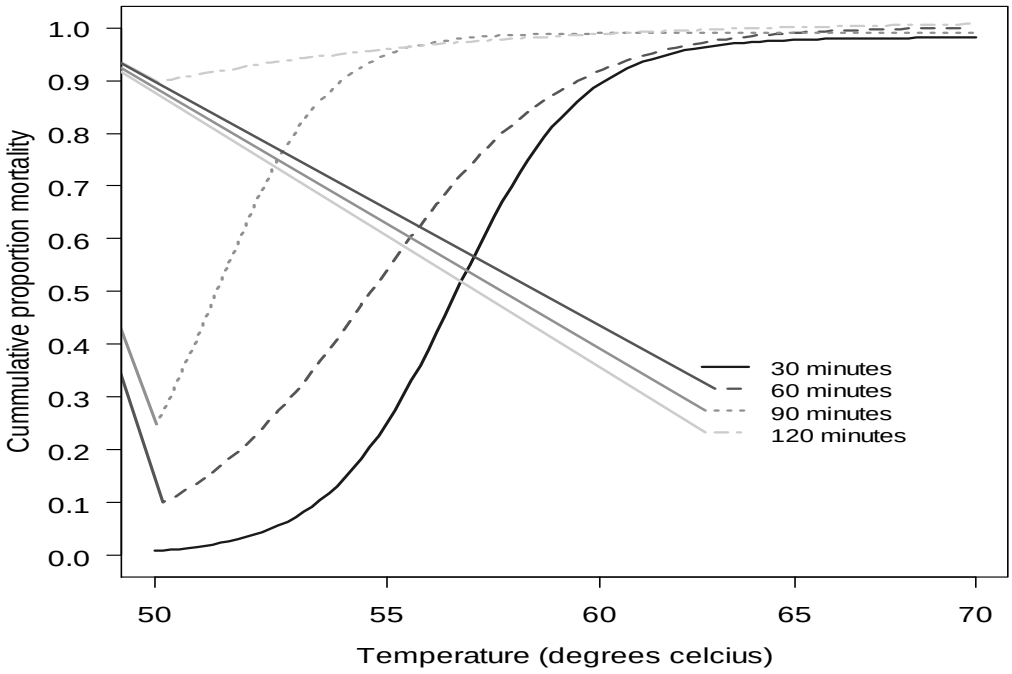


Figure 3.1: The log-logistic model estimated mortality response curves for *P.truncatus*

A brief exposure of the insects to 70˚C led to instant kill of the insects and within the first 30min all insects were dead. The estimated lethal temperatures (LTs) at 50^{th}, 90^{th} and 95^{th} percentiles together with the slope of the curves are presented in Table 3.2. It can be seen that generally, the

LTs decrease as time increases. Both Figure 3.1 and Table 3.2 (Column 3) show that the slope for 90 minutes was the steepest, followed by 30 minutes, 60minutes and 120 minutes.

Table 3.3 shows that the lethal temperature required to achieve a near complete kill (LT_{95}) of 200 *P. truncatus* predicted by the log-log model was in synchrony with the experimental results. Longer exposure times at lower temperatures and shorter exposure times at higher temperatures can achieve the same level of insect mortality. The longer the exposure time, the lower the temperature needed to effect complete kill of *P. truncatus.*

Table 3.3: Estimated parameters for the different exposure times from the log-logistic model

Time (min)	N*	*b***	LT_{50}	LT_{90}	LT_{95}
30	200	-38.76	56.5	59.8	61.0
60	200	-25.70	54.7	59.5	61.3
90	200	-44.47	51.3	53.9	54.8
120	200	-9.05	_	51.0	55.3

* total number of insects at each temperature and time combination
**the slopes.

Discussion

The observations made were because at any given temperature, mortality increased with an increase in exposure time and, at any given exposure time, mortality increased with an increase in temperature. This was similar to time-temperature relationships on mortality of insect pests reported by Mahroof et al., (2003b); and Boina and Subramanyam (2004).

High temperatures above 32°C have been reported to cause a number of adverse biochemical changes in insects namely; lower ion concentrations (e.g. pH), inactivation of major glycolysis enzymes, disruption of plasma membranes and denatured proteins, nucleic acids, lipids and carbohydrates (Hochachka and Somero, 1984; Neven, 2000).

Between temperatures of 45-55°C, the insect pest survives for several hours after which it undergoes severe water stress and dies eventually. Above 55° C they undergo rapid mortality and the entire population succumbs to death within minutes or seconds.

Probit analysis is a satisfactory means of determining estimates and confidence limits of mortality, giving the linearized probit transformation of sigmoid distributions (Claflin et al., 1986; Evans, 1987; Bruce et al., 2004). However in this study the complementary log–log transformation was used because it may at times fit the data better (Morgan, 1992), especially if mortality is being predicted as a function of temperature or time. Mahroof et al. (2003b) and Boina and Subramanyam (2004) preferred a complementary log–log transformation for estimating LT_{99} values for various life stages of *Tribolium castaneum* and *Tribolium confusum* exposed to several constant elevated temperatures.

According to these observations, LT_{95} for 30 min required a high temperature of 61.0˚C which is not possibly achievable with solar disinfestations. The highest solar radiation temperatures

achieved were 60˚C at *icipe* Kasarani and 58.8˚C in Kabete (Aberi, 2012). However, the LT_{95} for 120 min which required 55.3˚C is more practical and within achievable limits. Therefore, solar disinfestations done for a period of not less than 2 hrs at temperatures above 55°C can achieve total mortality of *P. truncatus*. According to our preliminary test results, these temperatures are achievable in the tropics at around midday from 12:30pm to 2:00 pm when the sun is hottest. Solar disinfestation of maize grain is economically feasible even for small scale farmers and serves as a possible phytosanitary measure against postharvest pests. With proper observation of the exposure times and temperatures, solar disinfestation can serve as an effective cheap alternative to fumigation and contact insecticides.

Longer exposure time is more desirable since heat can penetrate the maize grain and destroy *P. truncatus* that are lodged within the maize grain. The rate at which an insect dies depends on the extent to which it is cushioned from the heat. It takes less time to kill already isolated insects in a test tube placed in an oven than it would take to kill the same insects in a maize lot. Since it is not possible to achieve total mortality of *P. truncatus* at 55˚C for 120 min in heavily infested grains, the surviving population can be destroyed by storing the maize grain in hermetic bags. This formed the basis for the next experiment in our laboratory to establish the possibility of solar disinfestation of maize before hermetic storage of maize in triple bags (Purdue Improved Crop Storage (PICS) bags for six months.

CHAPTER FOUR

4.0. Effectiveness of solar disinfestation and hermetic PICS bagging of maize in controlling *P.truncatus* infestation, and grain quality in terms of viability (germination).

Abstract

The objective of this study was to compare the effectiveness of the integration of PICS bags and solar disinfestation compared to storage in woven PP (polypropylene) bags for the control of *Prostephanus truncatus* Horn. It has been established that hermetic post-harvest maize storage can effectively control maize weevil, *Sitophilus zeamais*, which can be responsible for up to 50% damage to stored maize grain. Its use eliminates the need for toxic and expensive chemicals. Therefore, laboratory experiments with *Prostephanus truncatus* Horn which causes more severe damage were carried out. Maize infested as well as non-infested with *P.truncatus*, was stored for 6 months under hermetic (PICS bags) and non-hermetic (woven polypropylene (PP) bags) conditions. Grain moisture content at the beginning of the experiment in January 2012 ranged from 12.30 to 13.31% and the weight loss damage was 0%. Under hermetic conditions, after six months' storage gas composition levels were at 6.82, 7.68 and 9.34% and CO_2 level had risen to 13.52, 12.75 and 9.88%. Insect counts were low in the PICS bags, 2±1 insects per 125g sample, and very high in the woven bags, 52.00±9.85 live *P.truncatus*, 68.67±9.07 *P.truncatus* larvae and 74.33±12.10 *S.zeamais* per 125g sample in the worst case. Germination capacity did not change much for the PICS bags but decreased greatly for woven bags up to 12% in the worst case. Losses were also significantly different for the maize stored in the PICS bags and the woven bags with losses as high as 47.66±4.59% recorded for the woven bags.

4.1. Introduction

In Sub- Saharan Africa, maize production is undertaken by resource-poor farmers with little or no control measures against *P. truncatus* during storage. Many smallholder farmers in Sub-Saharan Africa produce maize, but more often than not lack access to postharvest technology, including the most basic of storage structures or technical assistance to help store their maize. This situation forces small producers to sell their maize at harvest time, with the disadvantage of low market prices. Later, they buy it back for their own consumption at inevitably higher prices. Therefore, storage alternatives must be found to minimize the qualitative and quantitative grain maize losses, preferably without the use of pesticides. This goal can be achieved with simple and low cost technologies, including hermetic grain storage. Hermetic grain storage has been used since ancient times for grain preservation (Sigout, 1980; De Lima, 1990). Its basic principle is drastic elimination of oxygen in conjunction with an increase in carbon dioxide within the storage atmosphere which is achieved by the respiration of insects, fungi, and grain (Varnava *et al*., 1995; Moreno *et al*., 2000). The insects die when the air in the storage container is reduced to 3 % oxygen or less; and (Navarro *et al*., 1994). Fungal development also ceases when the oxygen level decreases to 1% (Moreno *et al*., 2000). Specific storage practices vary widely according to climate zone, cultural traditions, and production scale or socioeconomic condition of farmers. Storage recommendations are based on length of grain storage, *P. truncatus* presence, and hybrid vs. local maize varieties (Golob, 2009). Tropical heat, moisture and open-air storage promote rapid insect multiplication and mold formation in stored maize (FAO, 1994). Rapid insect development occurs when temperature is within 5 to10°C of optimal temperature, which for most storage insects, is in the range of 25 to 35°C (FAO, 1994). Hermetic storage isolates the storage ecosystem from the external environment while respiration within the storage ecosystem

causes oxygen reduction and carbon dioxide accumulation, leading to suffocation and dehydration of weevils Hermetic post-harvest maize storage has been shown to effectively control maize weevil, *Sitophilus zeamais*, which can be responsible for up to 50 % damage to stored maize grain (Yakubu *et al*., 2010). In rural areas, hermetic storage offers practically the only hope for an effective, cost-efficient, and chemical-free insect control thereby maintaining the quality of the grain, affording the farmer protection from seasonal fluctuations in maize prices and providing a safe grain maize supply for family consumption. With this knowledge of the possible success of hermetic storage came the idea of crop storage with hermetic PICS bags. Triple-layer hermetic Purdue Improved Crop Storage (PICS) bags were developed under the Bean/Cowpea Center for Research in Security Prices (CRSP) project in the late 1980s through funding from USAID, as a simple, low-cost, practical and effective way to enable low-resource farmers in West and Central Africa to preserve their cowpea grain after harvest with minimal losses to storage insects (Murdock *et al*., 2003). To bring systematic research to bear on the problem of storage of other crops in sub-Saharan Africa after the success of PICS bags with cowpeas in West Africa, the PICS team proposed to carry out systematic studies of the triple bagging technology for African crops other than cowpea. To date, PICS bags have displayed 50 % lower cassava chip storage losses compared to conventional polypropylene bags over a two month period (Ognakossan *et al*., 2010). It has also been displayed that PICS bags can provide extremely high rates of protection for maize grain, remaining under 0.5 % dry weight loss after a six month period (Hell *et al*., 2010). The objective of this research is to obtain information on the novel approach of using solar disinfestation and hermetic storage based solely on biogenerated CO_2 in PICS bags as a possible control of *P.truncatus*, and quality preservation method for stored maize. Since the introduction of *P. truncatus* in Kenya over 20 yrs ago, there is a need to

investigate the possibility of using the same technology of hermetic storage to control *P. truncatus*. Therefore, a study to test the combination of solar disinfestation and the PICS bagging in the control of *P. truncatus* in stored maize was carried out at *icipe* Nairobi, Kenya.

4.2. Materials and methods

4.2.1. Solar Disinfestation of Maize

The solar disinfestation of maize was carried out at the International Center for Insect Physiology and Ecology (*icipe*), Duduville Campus Kenya for six months (December, 2011 to July, 2012). Six bags of 90 kg of freshly harvested and dried clean shelled maize (variety H614D) that was not treated with any pesticide was purchased directly from a farmer in Uasin Gishu County, Rift valley, Kenya in order to ensure the uniformity of the commodity. The solar drying was done by spreading out a thin layer of maize on black Low Density Polyethylene (LDPE) on level ground in an open field. Then a transparent LDPE plastic paper was spread out over the maize and the edges were folded and stones placed on it to ensure no heat escapes and avoid aerial contamination.

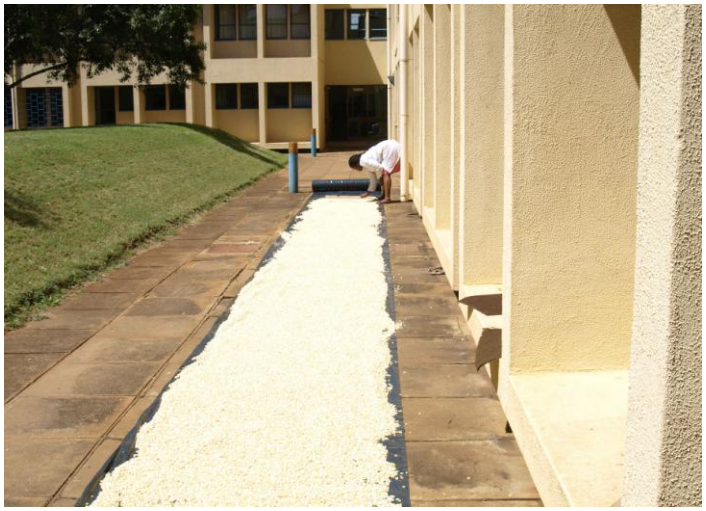


Plate 4.1. Spreading out the maize in the sun

The solar disinfestation was carried out on a sunny day at a time (between 11:00 a.m. and 2:30 p.m.) with ambient temperatures of 26˚C. The temperature inside the covered maize was

monitored using a Thermo-Hygrometer (deltatrak). As predetermined in our previous experiment on susceptibility of *P. truncatus* to heat in the hot air oven, when the temperatures of 55-60˚C were achieved, the maize was held for 2 hrs with constant monitoring of the temperature. After solar disinfestations, the maize was bagged in different types of bags for storage.

Plate 4.2: Temperature measurement with a thermo-hygrometer during solar disinfestation

4.2.2. Bagging of Solar Disinfested Maize

The experimental design was a complete randomized block design of 6 treatments with 3 replicates per block. The solar disinfested maize grain was analyzed for initial pest infestation, germination (viability) and moisture content in quadruplicate subsamples before use in this experiment. A total of 18 bags, comprising of 9 hermetic PICS bags and 9 ordinary polypropylene bags normally used by farmers for maize storage, were used in the study. Each bag was filled with 25 kg of maize grain. The PICS bags were prepared and filled with maize using the Fiche technique as illustrated in Appendix 1 for Cowpea storage. Of the 9 PICS bags a set of 3 bags were filled with clean solar disinfested maize (T1). A second set of 3 bags were filled with infested maize, that had been infested by 100 randomly selected unsexed adults of *P.*

truncatus into 3 lots and incubated for 7days for the insects to bore into the grain. After 7 days of incubation each lot was then subjected to solar disinfestation prior to bagging (T2). A third set of 3 PICS bags were filled with solar disinfested maize which was then infested with 100 randomly selected unsexed adults of *P. truncatus* (T3). The hermetic PICS bags were tightly sealed using the Fiche technique as shown in Appendix 1. Of these 9 ordinary polypropylene bags, a set of 3 were filled with clean solar disinfested maize (T4). Another set of 3 ordinary polypropylene bags were filled with solar disinfested maize and infested with 100 randomly selected unsexed adults of *P. truncatus* (T5). A third set of 3 ordinary polypropylene bags were filled with maize that was not solar disinfested and sealed. This served as a control that simulates what really happens at farm level (C). These ordinary polypropylene bags were tied tightly using a string. All the sealed bags were then placed on wooden planks above the ground with sufficient space between the bags in the experimental room. Temperature and relative humidity in the bags were monitored continuously during the storage period using data loggers (Easy log USB-502, LASCAR). The data logger was programmed to record data every 30minutes for the six months of storage.

Plate 4.3: Lascar EL-USB-502 data logger

4.2.3. Gas composition in PICS bag

Plate 4.4: MOCON PAC-CHECK for gas composition measurement

Oxygen (O_2) and carbon dioxide (CO_2) content in PICS bags was measured at the beginning of the trial and every month on each sampling day before opening the bags with an O_2/CO_2 portable analyzer (MOCON PAC CHECK Model 325). The inner plastic of PICS bags was perforated at 3 levels (top, middle and bottom) with the analyzer's needle and measurements taken from inside

the bag. After measurement, the tiny needle holes were closed with scotch and tape. The subsequent O_2/CO_2 measurements were done at the same spot by removing the tape and scotch.

4.2.4. Sample Analysis

The bags were also visually assessed for holes made by the insects. Each month all the bags were opened and 500 g quadruplicate samples were drawn using a hollow plastic tube (sampling spear) of 2 inches diameter inserted at different points in each bag up to a storage period of six months.. The tube was pushed all the way to the bottom of the bag so that the resultant maize grains would originate from all layers of the bag. The samples were analyzed for moisture content, the number of adult insects and larva, percentage insect damaged maize grains and seed quality (germination percentage).

4.2.4.1. Determination of Moisture Content of Maize Grains

Each of the 500 g quadruplicate samples from each bag were put into the MINI GAC® moisture meter (DICKEY-john CORPORATION) and moisture readings recorded. The maize moisture content was taken at the beginning of the experiment and thereafter at monthly intervals.

4.2.4.2. Assessment of Insect Damaged Maize Grains

Insect damaged grains were assessed using the count and weigh method (FAO, 1985). The 500 g quadruplicate samples from each bag were mixed and a 125 g sample drawn. The 125 g sample was then sorted into the following damage categories: insect damaged (*P. truncatus* damage), mould damage, broken pieces and undamaged grains. Because our interest was on the weight loss caused by storage insect damage, only the *P. truncatus* damaged grains were compared with undamaged lot.

The number and weight of insect damaged grains was then determined and used in the equation below:

$$\% \text{ Weight loss} = \frac{(U*Nd) - (D*Nu)}{U\,(Nu + Nd)} \times 100$$

Where:-

U = weight of undamaged grain,

D = weight of insect damaged grain,

Nu = number of undamaged grains,

Nd = number of insect damaged grains.

The insect damage was then expressed as percentage weight loss**.**

4.2.4.3. Assessment of the Number of Adult Insects and Larvae

The 500 g quadruplicate samples from each bag were mixed and a 125 g sample drawn. The 125 g sample was then put in refrigerator at 2˚C for 3 hours to immobilize the crawling insects. *Prostephanus truncatus* and *Sitophilus zeamais* adults were then removed from the sample counted and recorded. The damaged grains were broken open to remove *P. truncatus* adults and larvae lodged within the grain and their number recorded. The results were expressed as live adult *P. truncatus* per 125 g, live adult *S. zeamais* per 125 g and *P. truncatus* larvae per 125 g.

4.2.4.4. Assessment of Seed Quality

From 500 g sample, 40 maize grains were drawn and steeped in 250 ml of water for about 15 minutes to hydrate. The grains were then placed on moistened Whatman Filter Paper. The filter paper was folded over the seeds to keep them moist. The folded filter paper with the maize was

then placed into a quart Ziploc bag (1 litre capacity) and left for three days at 25°C place and germinated seeds were counted. The seed quality was then expressed as percent germination.

4.2.9. Statistical Analysis

All the obtained data was subjected to One Way ANOVA Linear Model analysis using Stata SE 9 and significant difference ($p < 0.05$) means were separated by using the Bonferroni test. The percentage weight loss and percent germination data were arcsine transformed prior to analysis to stabilize the variance.

4.3. Results and Discussion

4.3.1. Solar Disinfestation of Maize

The results for solar disinfestation temperatures are shown in Figure 4.1. The solar disinfestation temperature started from a low of 46.7˚C at 11.00 a.m. The temperatures rose steadily to 60.1˚C at 12.30 p.m. The highest temperature of 61.5˚C was realized at 1.20 p.m. Based on the results of our previous study on the susceptibility of *P. truncatus* to heat in the hot air oven (Chapter 3), the targeted temperatures of above 55˚C were achieved during the solar disinfestation. Even with fluctuations in temperature, we were able to hold the maize at temperatures above 55˚C for more than 2hrs between 12.00 noon and 2.30 p.m.

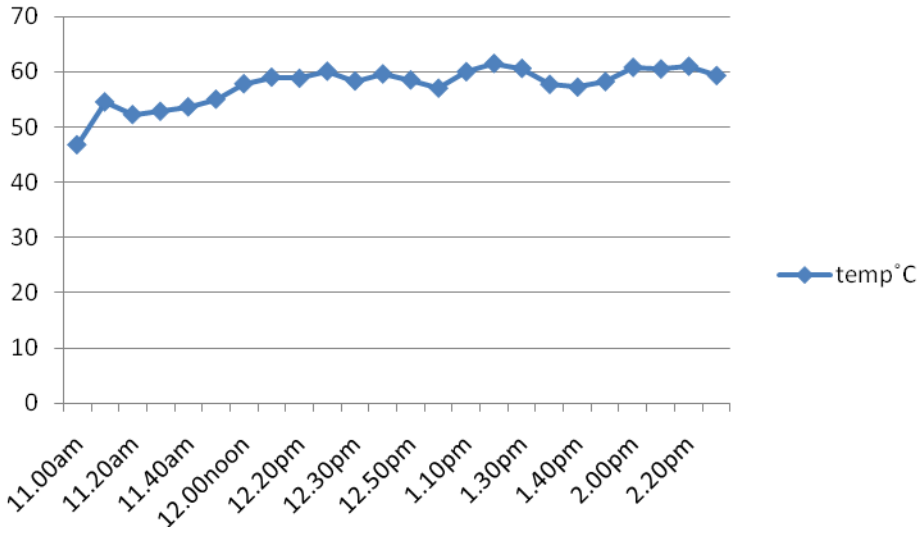


Figure 4.1: variation of solar disinfestation temperatures

4.3.2. Variation in Temperature and Relative Humidity during Storage

The results for variation in temperature and relative humidity during storage are shown in the figures 4.2 and 4.3 below. The temperature in maize stored in the hermetic PICS bags (T1, T2 and T3) dropped progressively, during the six months storage period, from initial of 25˚C in

January to final of 21˚C in July. However, the temperature in maize stored in polypropylene bags did not change over that period.

The temperatures in polypropylene bags containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5) and the maize that was neither solar disinfested nor artificially infested with *P .truncatus* (C) had higher temperature of 28˚C (in May) and 26˚C (in March), respectively. This was attributable to the prevailing dry conditions at the study site (*icipe*, Nairobi) and also due to insect activity in these treatments. However, the temperatures finally dropped to 25˚C by the end of the experiment.

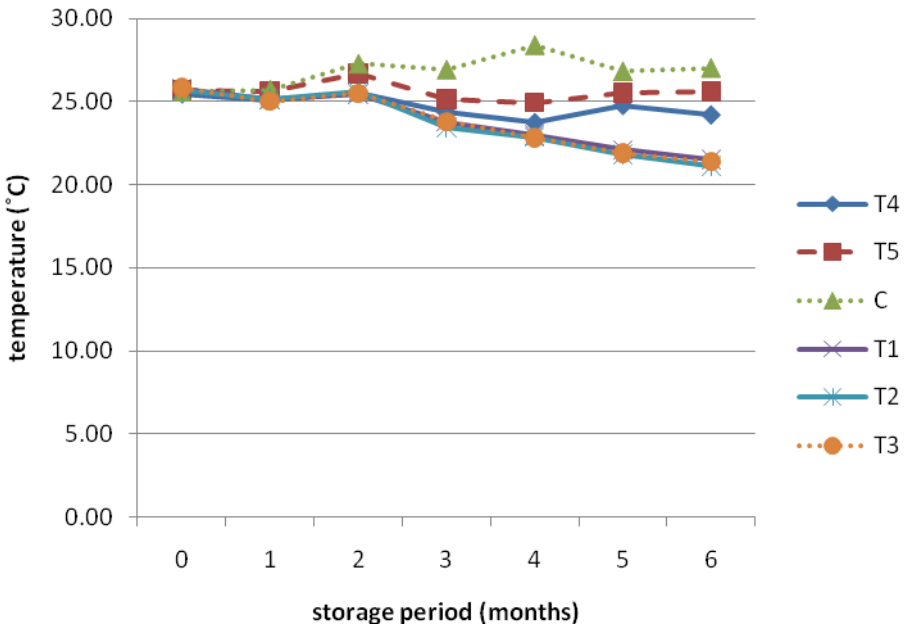
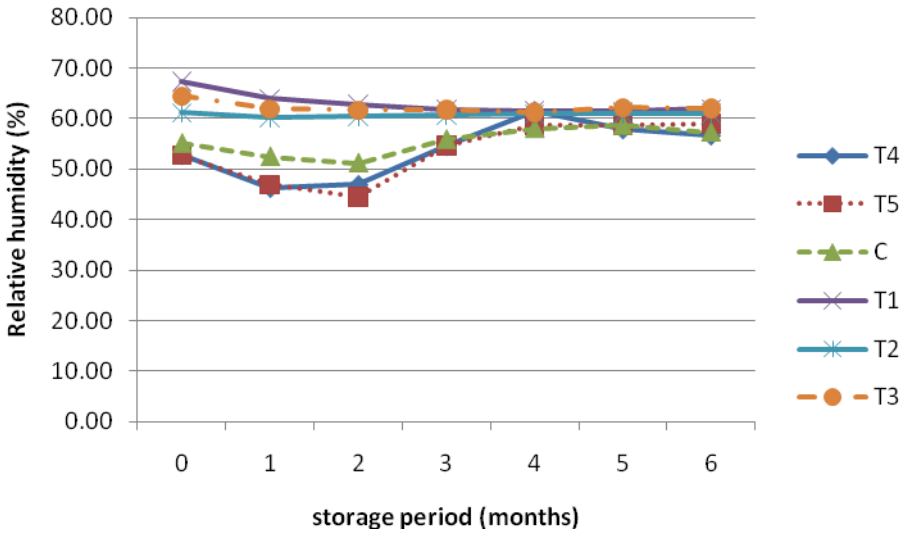


Figure 4.2: Variation of temperature in different storage bags over six months storage period



T1 – PICS bag with clean solar disinfested maize

T2 – PICS bag with maize that was artificially infested with *P.truncatus* then solar disinfested

T3 – PICS bag with maize that was solar disinfested then artificially infested with *P.truncatus* prior to bagging

T4 – PPbag with clean solar disinfested maize

T5 - PPbag with maize that was solar disinfested then artificially infested with *P.truncatus* prior to bagging

C – PPbag with maize that was neither solar disinfested nor artificially infested with *P.truncatus* (CONTROL)

Figure 4.3: Variation of relative humidity in different storage bags over six months storage period

At the beginning of the storage period, all the treatments (PICS and Polypropylene bags) had a relative humidity of between 55-65% RH with the PICS bags having a higher value than the polypropylene bags. The relative humidity in all the treatments in the PICS bags remained constant at 60% RH, while the relative humidity for treatments in the polypropylene bags decreased steadily from the onset of the experiment up to the third month of storage (March) where it dropped to a minimum value 45% RH then started rising steadily in the remaining months up to approximately 60% RH at the sixth month (July). The fall in RH during the first

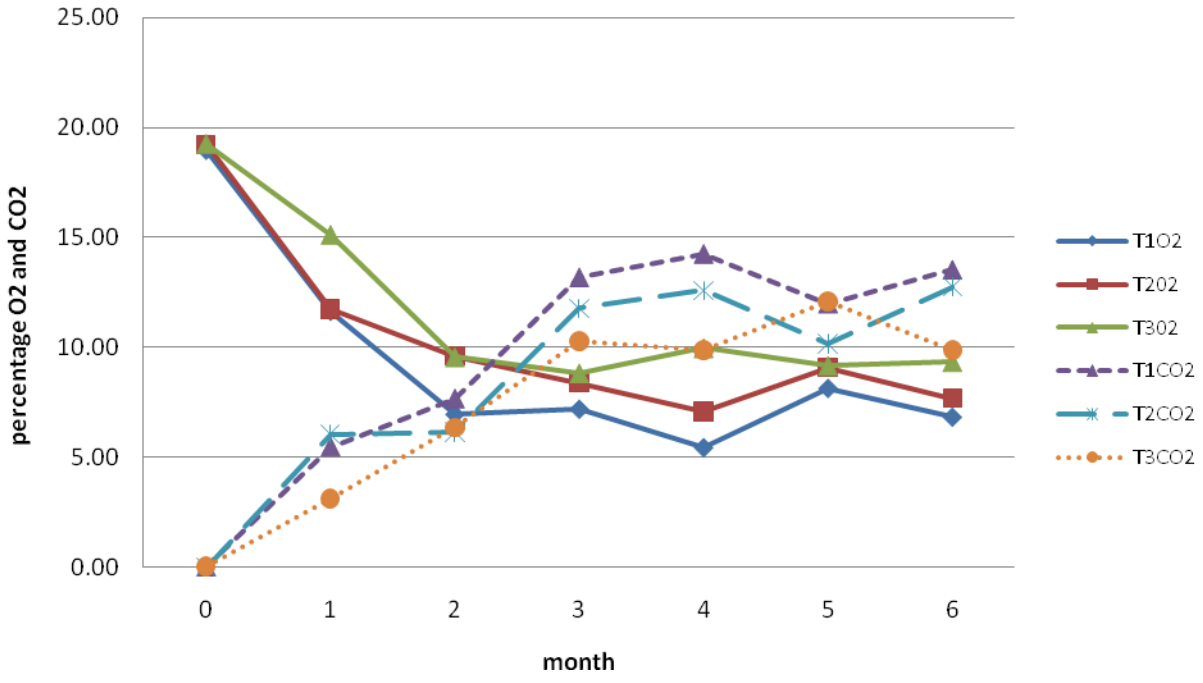
three months was attributed to prevailing dry conditions at the study site ((*icipe*, Nairobi)). The rise in the subsequent months was attributed to increased insect activity and the prevailing wet weather conditions. It has been reported that hermetic bags prevent entry of water vapor and protects the commodity from external humidity. The PICS bags also have been reported to create a microenvironment with an equilibrium relative humidity, which ensures that moisture content of maize stored in PICS bags does not increase to beyond the safe critical moisture level of 12-13 % (Villers *et al.*, 2008).

4.3.3. Gas composition in PICS bag

The results for variation of O_2 and CO_2in hermetic PICS bags over a six months storage period are shown in Figure 4.4. The initial concentration of O_2 in all the hermetic PICS bags were 18.98, 19.22 and 19.24% for PICS bags with clean solar disinfested maize (T1), PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3), respectively while CO_2 concentration was 0% for all the treatments.

By the third month of storage, the O_2 concentration dropped gradually to 6.94, 9.60 and 9.55%, while the CO_2 concentration increased gradually to 7.66, 6.16 and 6.35%, for PICS bags with clean solar disinfested maize (T1), PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3), respectively. Thereafter, the concentration of O_2 leveled at between 5-10% while CO_2 leveled at about 14%. This explains why there was approximately 2±1 adult *P. truncatus* surviving in PICS bag with maize that was

solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3) at the end of the six months storage period.



T1 – PICS bag with clean solar disinfested maize

T2 – PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested

T3 – PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging

Figure 4.4: Variation of CO_2 and O_2 in hermetic PICS bags over a six months storage period

The lowest concentration of O_2 reported for multiplication of insect pest is 2 % (Villers *et al.*, 2008). It has been well-established that the extent of oxygen depletion depends largely on the elements of the storage system, such as the insect population, moisture content, fungal inoculum, quality of the grain, and gas-tightness of the storage system (Moreno-Martinez *et al.*, 2000). Under sealed storage conditions in maize, insects and fungi deplete the oxygen supply, creating an unfavorable atmosphere for their own survival (Moreno-Martinez *et al.*, 2000). All these

parameters were well balanced in the hermetic PICS bags and the bags were therefore able to limit insect activity and multiplication hence preserving the maize for six months.

4.3.4. Sample Analysis

4.3.4.1. Determination of Moisture Content of Maize Grains

The results for moisture content of solar disinfested maize bagged in different types of bags (hermetic PICS and polypropylene) under different treatments are shown in table 4.1. The initial moisture content for maize stored in hermetic PICS bags ranged from 12.30 to 12.39%, while moisture content for maize stored in polypropylene bags ranged from 12.48 to 13.31%. During the 6 months of storage, there was no significant differences ($p > 0.05$) in moisture content in PICS bags with clean solar disinfested maize (T1), PICS bags with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bags with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3). There was no significant difference ($p > 0.05$) in moisture content in maize bagged in all the treatments in PICS bags compared to polypropylene bags with clean solar disinfested maize (T4) until after the 4^{th} month when significant differences ($p < 0.05$) in moisture content was noted. It is only from the 5^{th} and 6^{th} months of storage that differences in moisture content in PICS bags treatments (T1, T2 and T3) and polypropylene bags with clean solar disinfested maize (T4) were noted. This was because of the high number of insect counts in this PP bags treatment as shown in table 4.2. For the first 2 months of storage, there was no significant difference ($p > 0.05$) in moisture content in PICS bags (T3) and PP bags (T5) both containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging and the control (C) containing maize that was neither solar disinfested nor artificially infested with *P. truncatus.*

Table 4.1: Variation of moisture content (%)in different storage bags over six months' storage period

	Storage Period (Months)					
Treatments*	**1**	**2**	**3**	**4**	**5**	**6**
T1	12.03±0.42b	11.97±0.12a	12.17±0.51b	12.92±0.38a	12.93±0.25ab	12.88±0.30a
T2	12.31±0.34b	12.23±0.40a	12.59±0.24b	12.99±0.25a	12.74±0.27a	12.83±0.27a
T3	12.46±0.43b	12.25±0.29a	12.49±0.38b	12.95±0.24a	13.02±0.26b	13.34±0.26bc
T4	11.75±0.32a	11.98±0.54a	11.91±0.44b	12.88±0.49a	12.58±0.31a	13.50±0.18c
T5	12.07±0.49ab	12.12±0.52a	11.4±0.69a	13.34±0.26b	13.13±0.33b	13.55±0.19c
C	12.09±0.27ab	12.16±0.34a	11.4±0.52a	13.09±0.34b	13.42±0.26c	13.18±0.24b

Mean with same letter in the same column are not significantly different (p>0.05)

*T1 – PICS bag with clean solar disinfested maize

T2 – PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested

T3 – PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging

T4 – PP bag with clean solar disinfested maize

T5 – PP bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging

C – PP bag with maize that was neither solar disinfested nor artificially infested with *P. truncatus* (CONTROL)

However, after the 2nd month of storage significant differences in moisture content between those treatments were noted. Overall, after six months of storage, the PICS bags treatments with no artificial infestation were able to maintain the moisture content at the recommended storage level for maize of 12 - 13% than all treatments in polypropylene bags, where the moisture content was above 13%. The increase in moisture content in the polypropylene bags was due to the high insect growth and grain damage resulting in production of a lot of dust and frass which are hygroscopic and imbibes water from the environment.

4.2.4.3. Assessment of the Number of Adult Insects and Larvae

The results for insect counts for surviving adult *P. truncatus,* and adult *S. zeamais* and *P. truncatus* larvae are shown in Table 4.2. There was no significant difference ($p > 0.05$) in the number of live adult *P. truncatus, P. truncatus* larvae and live adult *S. zeamais* in the hermetic PICS bags throughout the storage period. There were no *P. truncatus* in PICS bag with clean solar disinfested maize (T1) and PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2). This was due to lack of sufficient oxygen and also because the bags did not allow re-infestation from the environment in which the maize was stored. Hermetic conditions with less than 1% oxygen have been reported to be suboptimal conditions for the insects' multiplication and survival (Annis 1986; Fleurat-Lessard, 1987). There are several studies wherein insect pests had been successfully and economically controlled without using any chemical toxicant (Zuxun *et al.,* 1999). More research has confirmed that stored grain insect pests can be controlled by decreasing oxygen concentration, increasing CO_2 levels as well as by increasing temperature (Mueller, 1994b). As reported earlier, the concentration of oxygen in the PICS bags did not drop to below 5%, hence the PICS bags with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3) had a few surviving

insects, (2 insects per 125 g sample) at the end of the storage period. These findings agree with (Villers *et al.,* 2008) who reported that the critical concentration of oxygen that can result in death of all insects in hermetic storage is below 2%. However, the surviving insects and larvae exhibited little activity and the weight loss/insect grain damage was only 2 ± 0.25 % at the end of the storage period (table 4.3). At the end of the six months storage period, there was no significant difference ($p > 0.05$) in the counts of *P .truncatus* adult and larva in the PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3). This study shows that it is not mandatory to carry out solar disinfestation in order to ensure that the maize in PICS bags does not get damaged by *P. truncatus.* This is an important observation implying that, maize infested with *P. truncatus* can be preserved for six months under hermetic conditions of PICS bags without having to solar disinfest the maize prior to bagging.

The treatments in PICS bags had no insect activity, however, the treatments in polypropylene bags, the counts for adult *P. truncatus,* and *S. zeamais* and *P. truncatus* larva increased gradually over the storage period. This is attributable to non-hermetic conditions in the polypropylene bags compared to PICS bags. The maize stored in polypropylene bags were infested by *S. zeamais* by the second month of storage. By the end of the storage period, the polypropylene bags with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5) had the highest counts of live *P. truncatus* (52.00 ± 9.85), *P. truncatus* larvae (68.67 ± 9.07) and *S. zeamais* (74.33 ± 12.10) per 125 g sample. This treatment (T5) shows a significant difference in the counts of adult *P. truncatus*, *P. truncatus* larvae and adult *S. zeamais* compared to T4 and the control.

Table 4.2: Insect counts in the different treatments over a five months' storage period

Treatment *	Live *P. truncatus* per 125g sample	*P. truncatus* larvae per 125g sample	Live *S. zeamais* per 125g sample
March 2012			
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.00±0.00a	0.00±0.00a
T3	1.00±1.00a	0.00±0.00a	0.00±0.00a
T4	2.00±1.00a	0.00±0.00a	28.00±2.00b
T5	11.33±3.1b	17.67±2.52b	20.00±2.00c
C	1.67±0.58a	0.00±0.00a	26.00±2.65b
April 2012			
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.00±0.00a	0.00±0.00a
T3	1.67±1.15a	0.00±0.00a	0.00±0.00a
T4	0.67±0.58a	0.00±0.00a	7.67±2.52b
T5	23.33±1.53b	35.00±5.00b	18.33±3.06c
C	1.67±2.08a	0.00±0.00a	10.33±2.08b
May 2012			
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.00±0.00a	0.00±0.00a
T3	3.67±0.58a	1.67±0.58a	0.00±0.00a
T4	2.67±2.31a	1.33±1.15a	21.00±2.65b
T5	26.67±1.53b	45.00±5.00b	40.33±8.39c
C	4.33±3.21a	1.00±1.00a	37.33±6.43c
June 2012			
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.00±0.00a	0.00±0.00a
T3	3.67±1.53a	2.33±1.53a	0.00±0.00a
T4	5.67±2.08a	3.00±1.00a	33.00±7.00b
T5	55.67±6.03b	43.00±2.65b	48.33±5.51b
C	6.67±1.15a	4.00±2.00a	58.67±3.21c
July 2012			
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.00±0.00a	0.00±0.00a
T3	2.00±1.00a	0.00±0.00a	0.00±0.00a
T4	11.00±1.00a	6.00±2.65a	55.67±8.14b
T5	52.00±9.85b	68.67±9.07b	74.33±12.10bc
C	9.67±2.08a	8.67±3.06a	85.67±8.15c

Column means followed by the same letter are not significantly different ($P > 0.05$)

*T1 – PICS bag with clean solar disinfested maize
T2 – PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested
T3 – PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging
T4 – PP bag with clean solar disinfested maize
T5 – PP bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging
C – PP bag with maize that was neither solar disinfested nor artificially infested with *P. truncatus* (CONTROL)

At the end of the six months storage period, the polypropylene bags containing clean solar disinfested maize (T4) had 11 ± 1 adult *P. truncatus*, 6 ± 3 *P. truncatus* larvae and 56 ± 8 *S. zeamais* per 125 g sample and a concomitant weight loss of 36.33 ± 2.71 %. On the other hand, polypropylene bags containing maize that was neither solar disinfested nor artificially infested with *P. truncatus* (C) had 10±2 adult *P. truncatus*, 9 ± 3 *P. truncatus* larvae and 86 ± 8 *S. zeamais* per 125 g sample and a concomitant weight loss of 30.50 ± 6.26 %.

The high weight loss of 36.33 ± 2.71 % in polypropylene bags containing clean solar disinfested maize (T4) could be attributable to infestation from storage environment and solar disinfestation of the maize which eliminated other contaminants but favored growth and multiplication of *P. truncatus.* This storage study in *icipe* could very well simulate what happens at farm level especially due to the mass invasion of the polypropylene bags by *S. zeamais* (Meikle, 2002).

4.2.4.3. Assessment of Insect Damaged Maize Grains

The results for the extent of insect damaged maize grains expressed as weight losses are shown in Table 4.1. The clean solar disinfected maize stored in PICS bags (T1) showed no loss in weight during the six months of storage while the clean solar disinfected maize stored in polypropylene bags (T4) started to show an increase in weight loss (from 0.65 to 36.33 %) after 6 months of storage.

There was a significant difference ($p < 0.05$) in grain weight loss between PICS bags containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3) and polypropylene bags containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5). There was higher increase in weight loss in the latter treatment (up to 47.66%) than in former treatment (up to 2%) after 6 months storage period.

Table 4.3: Percentage weight loss of maize in different storage bags over a six months' storage period

	Storage period (Months)						
Treatment *	**0**	**1**	**2**	**3**	**4**	**5**	**6**
T1	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
T2	0.00±0.00a	0.73±0.07d	0.83±0.08b	1.15±0.16bc	1.38±0.20b	0.81±0.25b	1.07±0.19b
T3	0.00±0.00a	0.47±0.10c	0.89±0.06b	0.94±0.16b	1.39±0.16b	1.73±0.39c	2.00±0.25c
T4	0.00±0.00a	0.00±0.00a	0.65±0.03a	1.21±0.25c	1.69±0.66b	3.58±0.41d	36.33±2.71e
T5	0.00±0.00a	1.81±0.05e	3.72±0.19c	6.10±0.38d	31.95±5.05d	41.24±2.86f	47.66±4.59f
C	0.00±0.00a	0.09±0.03b	0.87±0.21b	2.68±0.44c	7.27±2.39c	11.12±2.98e	30.50±6.26d

Column means followed by the same letter are not significantly different ($P > 0.05$)

*T1 – PICS bag with clean solar disinfested maize

T2 – PICS bag with maize that was artificially infested with *P.truncatus* then solar disinfested

T3 – PICS bag with maize that was solar disinfested then artificially infested with *P.truncatus* prior to bagging

T4 – PPbag with clean solar disinfested maize

T5 - PPbag with maize that was solar disinfested then artificially infested with *P.truncatus* prior to bagging

C – PPbag with maize that was neither solar disinfested nor artificially infested with *P.truncatus* (CONTROL)

These findings showed that solar disinfestation alone does not protect the maize from infestation with storage pests. Solar disinfestation therefore needs to be combined with hermetic storage in order to ensure there is no infestation of stored maize. There was a significant difference ($p < 0.05$) in grain weight loss between polypropylene bags containing clean solar disinfested maize (T4) and polypropylene bags containing maize that was neither solar disinfested nor artificially infested with *P. truncatus* (C).

The increase in weight loss was gradually slow for the first 3 months of storage; however the weight loss increased at a faster rate during the last 3 months of storage up to 36.3 and 30.5 %, respectively. The higher increase in weight loss for polypropylene bags containing clean solar disinfested maize (T4) compared to polypropylene bags containing maize that was neither solar disinfested nor artificially infested with *P. truncatus* (C) was due to the high counts of 11 ± 1for adult *P. truncatus*, 6 ± 3 for *P. truncatus* larvae and 56 ± 8 for *S. zeamais* per 125 g sample as shown in table 4.2. The polypropylene bags containing maize that was neither solar disinfested nor artificially infested with *P. truncatus* (C) simulated the situation in a farm-store where a farmer stored clean maize in a woven bag next to infested bags and the maize got infested and damaged after six months of storage.

By the third month of storage, all the treatments with the exception of the polypropylene bags containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5) had less than 3 % grain weight loss. By the fourth month of storage, the percentage grain weight loss for these polypropylene bags containing maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5) had increased to 31.9 ± 5.05 % weight loss and by the end of the six months storage period this treatment's percentage weight

loss was significantly higher (47.66 ± 4.59 %) than that of the other treatments ($p < 0.05$). This was attributable to the synergistic effect of the damage by both *S. zeamais* and *P. truncatus.* These insects did not infest maize in any of the hermetic PICS bags treatments because the bag was in three layers (triple layer bag). The three layers create a physical barrier to the *P. trun*catus and *S. zeamais* that may be present in the environment in which maize is stored (Vachanth *et al.*, 2010).

4.2.4.4. Assessment of Seed Quality

The results for assessment of seed quality expressed as percentage germination are as shown in Table 4.4. During the first five months of storage, there were no significant differences ($p > 0.05$) in the seed quality of maize stored in the hermetic PICS bags. The germination percent for these treatments was maintained above 80% for these first five months. However, during the sixth month of storage, the germination capacity dropped to 78.13, 69.17 and 71.04% for the PICS bag with clean solar disinfested maize (T1), PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested (T2) and PICS bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T3), respectively. This was probably due to physiological change in the seed coat of the maize grain such that they did not absorb enough water during the 30 minute soaking period before putting them on the Whatman filter paper.

During the first two months of storage, there were no significant differences ($p > 0.05$) between the germination capacities of the maize stored in PICS bags and maize stored in polypropylene bags. After the third month, significant differences ($p < 0.05$) were observed as the germination capacity of maize stored in polypropylene bags started dropping.

Table 4.4: Percentage germination (seed quality) of maize in different storage bags over a six months' storage period

	Storage Period (months)					
Treatment*	**1**	**2**	**3**	**4**	**5**	**6**
T1	83.75±9.74a	92.29±3.28bc	82.71±6.61b	81.46±4.45c	84.58±5.92c	78.13±5.75d
T2	84.79±5.98a	85.42±3.17a	79.58±4.87b	82.50±8.12c	82.08±3.17c	69.17±3.74c
T3	81.67±5.47a	90.63±3.22bc	82.71±3.91b	76.88±4.28c	83.41±3.92c	71.04±3.91c
T4	83.13±7.99a	93.13±3.71c	72.08±3.82a	63.54±8.82b	67.71±5.98b	36.96±7.65b
T5	78.33±8.62a	88.33±4.04ab	66.67±3.74a	47.08±10.97a	47.71±8.49a	12.71±5.48a
C	83.54±7.94a	88.33±3.26ab	78.96±3.28b	46.67±9.67a	54.38±8.33a	36.88±3.56b

Column means followed by the same letter are not significantly different ($P > 0.05$)

*T1 – PICS bag with clean solar disinfested maize

T2 – PICS bag with maize that was artificially infested with *P. truncatus* then solar disinfested

T3 – PICS bag with maize that was solar disinfested then artificially infested with *P .truncatus* prior to bagging

T4 – PP bag with clean solar disinfested maize

T5 – PP bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging

C – PP bag with maize that was neither solar disinfested nor artificially infested with *P. truncatus* (CONTROL)

After six months of storage, the germination capacity had dropped to 36.96±7.65, 12.71±5.48 and 36.88±3.56 %, for the polypropylene bag with clean solar disinfested maize (T4), the polypropylene bag with maize that was solar disinfested then artificially infested with *P. truncatus* prior to bagging (T5) and the polypropylene bag with maize that was neither solar disinfested nor artificially infested with *P. truncatus* (C). This was due to the high level of insect damage by both *P. truncatus* and *S. zeamais* as well as the observed high moisture content. Many studies in various countries have shown that triple-bagging maintains germination of 85% or more for periods up to 9 months, while conventional storage in jute bags reduces germination down by 14 % to 76 % within 3 months (Omondi *et al*., 2011) This has led to the adoption of hermetic storage by some leading seed producers (Anankware *et al*., 2012).

CHAPTER FIVE
5.0. GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The results of thermal disinfestation experiments showed that use of high temperatures is a promising method to provide a rapid, non-chemical alternative to fumigation and other methods of chemical control of *P. truncatus* in maize. This is especially good during these times when the demand for safer residue free maize is high. The use of polythene (black and transparent LDPE) for solar disinfestations of maize is both cheap and sustainable in Sub-Saharan Africa and it has potential for commercial application. Though the use of heat is constrained by its high cost compared to other chemical alternatives, this simple technology can undoubtedly achieve lethal temperatures of between 55-60˚C required to achieve between 95-99 % mortality of *P. truncatus* populations in infested maize grain.

The hermetic storage studies show that PICS bags are effective for the control of *P. truncatus* damage, preservation maize grain viability and the moisture content at below 14 % in stored maize. This means the grain can be used for planting in the next season by farmers.PICS bags would be a good option for storage where the store has not been completely disinfested or where there is fear of an outbreak of *P. truncatus* or *S. zeamais*. The maize stored in the polypropylene bags was not suitable for human consumption as it was highly damaged. If such a scenario were to present itself, it would seriously threaten food security, lead to loss of livelihoods and also pose a risk to food safety as such highly damaged grain could have aflatoxins. The losses recorded were based on weight loss but in real sense more was lost in terms of quality because of

the debris which makes the grain dirty, nutritional loss and lack of aesthetic appeal. Damaged kernels are of lighter weight and result in discounts when marketed.

In addition to these findings, this work has shown and cleared doubts and fears that the PICS bags could not protect against *P.truncatus.* There were concerns that the insect can make holes in the bags, but by the end of the six months storage, no holes were observed on these bags.

In terms of effectiveness in storing maize without damage, after six months of storage, PICS bags appeared to be better compared to polypropylene bags and PICS bags do not need any integration with solar disinfestation before storage. More studies are needed to confirm the results of the present study.

5.2. Recommendations

There is need to come up with a technology which can be used to carry out solar dinfestation in highland regions which experience day temperatures of less than 26˚C because they may not be able to achieve 60˚C which is needed to kill *P. truncatus.*

Since this work has been carried out successfully in the laboratory, more tests need to be done in the field to see if the results will be the same.

There is need for cost-benefit analysis to see how easy it is to pass on the new technology to the farmers.

Since the PICS bags have performed well with maize, further research with other high value cereals and legumes should be carried out here in Kenya to see if it will perform equally well.

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APPENDICES

APPENDIX 1: Fiche technique

Cowpea storage without chemical using PICS Bags

The Purdue Improved Cowpea Storage (PICS) Project is a regional project for West and Central Africa. By 2012 it aims to achieve storage of 50 percent of the cowpea in the region using the nonchemical, hermetic storage PICS bags.

Cowpea suffers heavily from insect attack, both in the field and in storage. *Callosobruchus maculatus*, also known as the cowpea weevil or cowpea bruchid, is the major storage pest of cowpea in West Africa. Infestations start in the field on pods and the population grows rapidly when eggs are laid directly on the seeds.

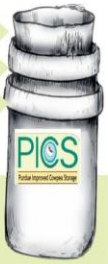
The adult female lives 5-10 days and lays 40-60 eggs, which she glues to the cowpea seeds. Bruchid larvae feed and develop inside the seeds and emerge as adults after about 3-4 weeks. The adults mate and produce another generation, and the cycle begins again. Since each female lays so many eggs and there are multiple generations, even a small infestation at harvest can lead to almost a total loss of stored cowpea after a few months.

The initial infestation of the cowpea by bruchids takes place in the field before harvest. At harvest the seeds look healthy but already contain larvae and eggs of the bruchids. An initial rate of infestation of 3 to 5% is often reported before storage.

Storage in triple layer bag

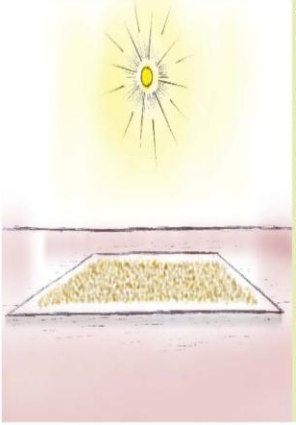
The triple layer bag consists of two polyethylene plastic bags that are 80 microns thick (inner bags) and a third sack (outer bag) made of woven polypropylene.

Inside bag: HDPE 80 microns
Middle bag: HDPE 80 microns
Exterior bag: woven polypropylene



Buy PICS bags from approved merchants.

Procedure

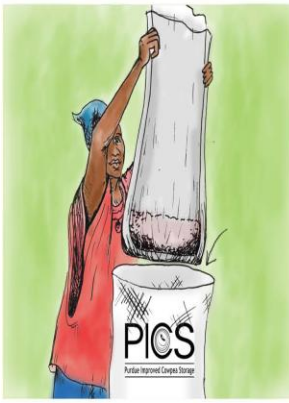


1
Ensure that your cowpea is completely dry and clean. Remove all the debris from the cowpea grain. Drying before storage may help to reduce the initial rate of infestation.

2
Take the three PICS bags apart and check the two inner bags for holes and tears. Do not use a bag that has holes or tears.

3
Pour a small amount of cowpea into the inner bag, starting gently. This will help to easily insert the first bag into the second.

4
Insert the first polyethylene bag into the second polyethylene bag. Make sure there are no air pockets at the bottom.



5
Insert the two Polyethylene bags into the woven polypropylene bag.

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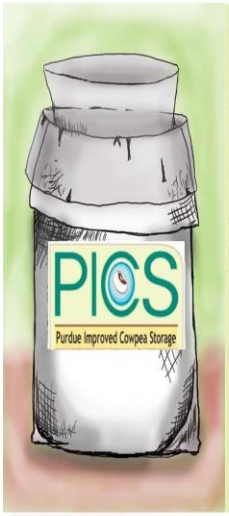
PICS Purdue Improved Cowpea Storage



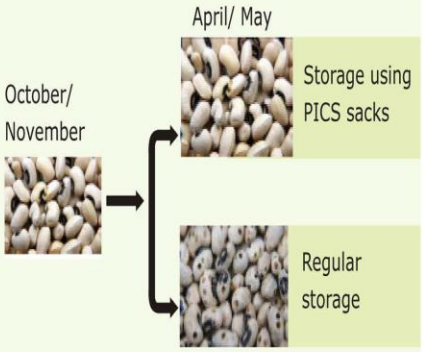
6

Fold over the top of the woven polypropylene bag. Do the same for the second polyethylene bag.

10

Pull the middle bag up over the first one so that it completely surrounds it. Twist the lip shut, fold over and tie, as before. Follow the same steps for the outer bag.

Economics of PICS technology

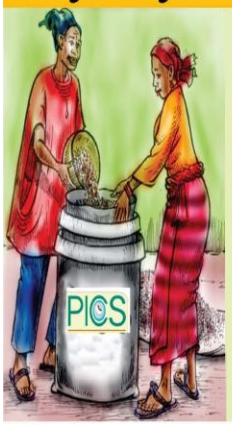


Savings for a farmer selling 100 kg

Option 1: Sell at harvest
Cowpeas sold at harvest time at 50 Naira/kg
50 Naira/ kg * 100 kg = 5000 Naira

Option 2: Sell later
Cowpeas sold 4 to 6 months after harvest can be sold for 100 Naira/kg
100 Naira / kg * 100 kg = 10000 Naira
Buy a bag at 300 Naira / bag = 300 Naira
Net: Naira 10000 - 300 = 9700 Naira

Filling the Bag



7

Fill the inner bag with more cowpea. Shake gently from time to time to reduce the pockets of air. Make sure no grain gets between the bags.

Tying steps

Step 1	Step 2	Step 3
Inner bag first	Middle bag next	Outer bag last

8

Fill the bag far enough so that a lip remains for tying. Pack the grain tightly to remove air.

9

Twist the lip of the first bag tightly shut. Fold it over and tie firmly with heavy string at the base of the twist and over the folded twist.

Advantage

Cowpeas stored in PICS bags for a few months sell 5-10% higher than cowpeas stored with chemicals.

Precaution

The triple layer bag is recommended for storing cowpea for a long period (at least 2 months). It is recommended that the bag remain sealed at all times for the duration of the storage. Keep the stored cowpea in a safe and dry place, and out of reach of rodents. Rodents may make holes in the plastic bags. The hermetic triple layer plastic bag technology is easy, effective, and safe.

Contacts

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APPENDIX 2: stata anova output

COUNT AND WEIGH

-> month = apr

Summary of percentage loss			
treatment	Mean	Std. Dev.	Freq.
C 1	1.21	.24932637	12
C 2	6.0958333	.38469498	12
C 3	2.675	.4408772	12
T 1	0	0	12
T 2	1.1466667	.15674723	12
T 3	.94416668	.16384353	12
Total	2.0119444	2.018881	72

Analysis of Variance

-> month = feb

Summary of percentage loss			
treatment	Mean	Std. Dev.	Freq.
C 1	0	0	12
C 2	1.8108333	.04621262	12
C 3	.0925	.03441062	12
T 1	0	0	12
T 2	.72666666	.070367	12
T 3	.46583333	.09548521	12
Total	.51597222	.64366393	72

-> month = jan

Summary of percentage loss			
treatment	Mean	Std. Dev.	Freq.
C 1	0	0	12
C 2	0	0	12
C 3	0	0	12
T 1	0	0	12
T 2	0	0	12
T 3	0	0	12
Total	0	0	72

-> month = july

Summary of percentage loss			
treatment	Mean	Std. Dev.	Freq.
C 1	36.3275	2.7118937	12
C 2	47.6625	4.5885808	12
C 3	30.5	6.2638948	12
T 1	0	0	12
T 2	1.0658333	.19322424	12
T 3	1.9975	.25125595	12
Total	19.592222	19.655112	72

-> month = june

Summary of percentage loss			
treatment	Mean	Std. Dev.	Freq.

```
     C 1 |   3.5833333   .40861929        12
     C 2 |    41.2375   2.8648501        12
     C 3 |   11.124167    2.981755        12
     T 1 |         0         0         12
     T 2 |   .80916666   .25039816        12
     T 3 |   1.7258333    .3864867        12
-----------+------------------------------------
   Total |   9.7466667   14.747382        72
-> month = mar

         |    Summary of percentage loss
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |   .65416667    .0274552        12
     C 2 |   3.7166666   .19462475        12
     C 3 |       .865   .21466676        12
     T 1 |         0         0         12
     T 2 |   .82583333    .0752521        12
     T 3 |       .885   .05760366        12
-----------+------------------------------------
   Total |   1.1577778   1.1984629        72


-> month = may

         |    Summary of percentage loss
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |   1.6933333   .66397336        12
     C 2 |   31.950833   5.0547069        12
     C 3 |   7.2733334   2.3901934        12
     T 1 |         0         0         12
     T 2 |   1.3791667   .19865266        12
     T 3 |     1.3925   .15615406        12
-----------+------------------------------------
   Total |   7.2815278   11.565823        72
```

GERMINATION

by month, sort : oneway percentgerminated treatment, bonferroni tabulate

```
-----------------------------------------------------------------------------------------------------------------------------------------------------
-> month = apr

         |    Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |   72.083333   3.8188131        12
     C 2 |   66.666667   3.7436815        12
     C 3 |   78.958333   3.2784304        12
     T 1 |   82.708333   6.6107981        12
     T 2 |   79.583333   4.8656184        12
     T 3 |   82.708333   3.9106982        12
-----------+------------------------------------
   Total |   77.118056    7.335757        72

-> month = feb

         |    Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |     83.125   7.9861386        12
     C 2 |   78.333333   8.6164044        12
     C 3 |   83.541667   7.9385662        12
```

```
     T 1 |      83.75   9.7409632        12
     T 2 |   84.791667    5.978972        12
     T 3 |   81.666667   5.4703055        12
-----------+------------------------------------
   Total |   82.534722   7.7765114        72

-> month = jan

         | Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |      73.75   7.0307765        12
     C 2 |   77.708333   7.0274085        12
     C 3 |   82.291667   11.101716        12
     T 1 |   73.958333   8.0098188        12
     T 2 |       77.5   8.0481505        12
     T 3 |   72.083333   5.5219946        12
-----------+------------------------------------
   Total |   76.215278   8.4126943        72
-> month = july

         | Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |   36.458333   7.6469196        12
     C 2 |   12.708333   5.4832735        12
     C 3 |     36.875   3.5555654        12
     T 1 |     78.125   5.7529637        12
     T 2 |   69.166667   3.7436815        12
     T 3 |   71.041667   3.9106982        12
-----------+------------------------------------
   Total |   50.729167   24.301646        72


-> month = june

         | Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |   67.708333    5.978972        12
     C 2 |   47.708333   8.4918633        12
     C 3 |     54.375   8.3342802        12
     T 1 |   84.583333   5.9192803        12
     T 2 |   82.083333   3.1682612        12
     T 3 |   83.409091   3.9167473        11
-----------+------------------------------------
   Total |   69.788732   15.961709        71

-> month = mar

         | Summary of percent germinated
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
     C 1 |     93.125   3.7119279        12
     C 2 |   88.333333   4.0358244        12
     C 3 |   88.333333   3.2566947        12
     T 1 |   92.291667   3.2784304        12
     T 2 |   85.416667   3.1682612        12
     T 3 |     90.625   3.2201426        12
-----------+------------------------------------
   Total |    89.6875   4.2570881        72

-> month = may
```

| Summary of percent germinated
treatment	Mean Std. Dev. Freq.
C 1	63.541667 8.8200658 12
C 2	47.083333 10.966547 12
C 3	46.666667 9.6726732 12
T 1	81.458333 4.4541009 12
T 2	82.5 8.1184414 12
T 3	76.875 4.2806382 12
Total	66.354167 17.095102 72

. INSECT COUNTS
LIVE PROSTEPHANUS
-> month = april

| Summary of live prostephanus
treatment	Mean Std. Dev. Freq.
C1	.66666667 .57735027 3
C2	23.333333 1.5275252 3
C3	1.6666667 2.081666 3
T1	0 0 3
T2	0 0 3
T3	1.6666667 1.1547005 3
Total	4.5555556 8.726041 18

-> month = july

| Summary of live prostephanus
treatment	Mean Std. Dev. Freq.
C1	11 1 3
C2	52 9.8488578 3
C3	9.6666667 2.081666 3
T1	0 0 3
T2	0 0 3
T3	2 1 3
Total	12.444444 19.076386 18

-> month = june

| Summary of live prostephanus
treatment	Mean Std. Dev. Freq.
C1	5.6666667 2.081666 3
C2	55.666667 6.0277138 3
C3	6.6666667 1.1547005 3
T1	0 0 3
T2	0 0 3
T3	3.6666667 1.5275252 3
Total	11.944444 20.417617 18

-> month = march

| Summary of live prostephanus
treatment	Mean Std. Dev. Freq.
C1	2 1 3
C2	11.333333 3.0550505 3
C3	1.6666667 .57735027 3

```
      T1 |         0        0        3
      T2 |         0        0        3
      T3 |         1        1        3
-----------+------------------------------------
   Total |   2.6666667   4.2287531       18

-> month = may

         |   Summary of live prostephanus
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |   2.6666667   2.3094011        3
      C2 |   26.666667   1.5275252        3
      C3 |   4.3333333   3.2145503        3
      T1 |         0        0        3
      T2 |         0        0        3
      T3 |   3.6666667   .57735027        3
-----------+------------------------------------
   Total |   6.2222222   9.6744391       18
PROSTEPHANUS LARVAE
-> month = april

         |   Summary of prostephanus larvae
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |         0        0        3
      C2 |        35        5        3
      C3 |         0        0        3
      T1 |         0        0        3
      T2 |         0        0        3
      T3 |         0        0        3
-----------+------------------------------------
   Total |   5.8333333    13.53101       18

-> month = july

         |   Summary of prostephanus larvae
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |   8.6666667   3.0550505        3
      C2 |   68.666667   9.0737717        3
      C3 |         6   2.6457513        3
      T1 |         0        0        3
      T2 |         0        0        3
      T3 |         0        0        3
-----------+------------------------------------
   Total |   13.888889   25.671886       18

-> month = june

         |   Summary of prostephanus larvae
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |         3        1        3
      C2 |        43   2.6457513        3
      C3 |         4        2        3
      T1 |         0        0        3
      T2 |         0        0        3
      T3 |   2.3333333   1.5275252        3
-----------+------------------------------------
   Total |   8.7222222   15.899706       18

-> month = march
```

| Summary of prostephanus larvae

treatment	Mean	Std. Dev.	Freq.
C1	0	0	3
C2	17.666667	2.5166115	3
C3	0	0	3
T1	0	0	3
T2	0	0	3
T3	0	0	3
Total	2.9444444	6.829626	18

-> month = may

| Summary of prostephanus larvae

treatment	Mean	Std. Dev.	Freq.
C1	1.3333333	1.1547005	3
C2	45	5	3
C3	1	1	3
T1	0	0	3
T2	0	0	3
T3	1.6666667	.57735027	3
Total	8.1666667	17.05786	18

LIVE SITOPHILUS

-> month = april

| Summary of live sitophilus

treatment	Mean	Std. Dev.	Freq.
C1	7.6666667	2.5166115	3
C2	18.333333	3.0550505	3
C3	10.333333	2.081666	3
T1	0	0	3
T2	0	0	3
T3	0	0	3
Total	6.0555556	7.2149536	18

-> month = july

| Summary of live sitophilus

treatment	Mean	Std. Dev.	Freq.
C1	55.666667	8.1445278	3
C2	74.333333	12.096832	3
C3	85.666667	8.1445278	3
T1	0	0	3
T2	0	0	3
T3	0	0	3
Total	35.944444	38.494419	18

-> month = june

| Summary of live sitophilus

treatment	Mean	Std. Dev.	Freq.
C1	33	7	3
C2	48.333333	5.5075705	3
C3	58.666667	3.2145503	3
T1	0	0	3

```
      T2 |         0         0         3
      T3 |         0         0         3
-----------+------------------------------------
   Total |  23.333333  25.414216        18
```

-> month = march

```
         |  Summary of live sitophilus
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |        28         2         3
      C2 |        20         2         3
      C3 |        26  2.6457513         3
      T1 |         0         0         3
      T2 |         0         0         3
      T3 |         0         0         3
-----------+------------------------------------
   Total |  12.333333  12.997737        18
```

```
                Analysis of Variance
   Source          SS       df      MS         F     Prob > F
------------------------------------------------------------------------------
Between groups          2842      5       568.4    227.36     0.0000
 Within groups          30     12        2.5
------------------------------------------------------------------------------
   Total             2872     17   168.941176
```

Bartlett's test for equal variances: chi2(2) =   0.1797 Prob>chi2 = 0.914

-> month = may

```
         |  Summary of live sitophilus
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |        21  2.6457513         3
      C2 |  40.333333  8.3864971         3
      C3 |  37.333333  6.4291005         3
      T1 |         0         0         3
      T2 |         0         0         3
      T3 |         0         0         3
-----------+------------------------------------
   Total |  16.444444  18.398281        18
```

MOISTURE

-> month = april

```
         |      Summary of moisture%
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
      C1 |  11.908333   .4420169        12
      C2 |      11.4   .68622814        12
      C3 |     11.15   .52483767        12
      T1 |  12.166667   .51227353        12
      T2 |  12.591667   .23915891        12
      T3 |  12.491667   .38484562        12
-----------+------------------------------------
   Total |  11.951389   .71068167        72
```

-> month = february

```
         |      Summary of moisture%
 treatment |       Mean   Std. Dev.      Freq.
-----------+------------------------------------
```

```
      C1 |      11.75   .32051099        12
      C2 |   12.066667   .48865932        12
      C3 |   12.091667   .27122055        12
      T1 |   12.033333   .42497761        12
      T2 |   12.308333   .33698765        12
      T3 |   12.458333   .42524516        12
---------+--------------------------------------
   Total |   12.118056   .43389053        72

-> month = january

         |       Summary of moisture%
 treatment |       Mean   Std. Dev.      Freq.
---------+--------------------------------------
      C1 |   12.683333   .54744582        12
      C2 |   13.308333   .52649494        12
      C3 |   12.483333   .44278742        12
      T1 |   12.391667   .60671747        12
      T2 |       12.3   .49726528        12
      T3 |   12.391667   .28431213        12
---------+--------------------------------------
   Total |   12.593056   .58798448        72

                Analysis of Variance
  Source           SS       df      MS         F     Prob > F
------------------------------------------------------------------------------
Between groups     8.38569368     5   1.67713874     6.85     0.0000
 Within groups     16.1608345     66   .244861128
------------------------------------------------------------------------------
   Total         24.5465281     71   .345725748

Bartlett's test for equal variances:  chi2(5) =   6.2362  Prob>chi2 = 0.284


-> month = july

         |       Summary of moisture%
 treatment |       Mean   Std. Dev.      Freq.
---------+--------------------------------------
      C1 |       13.5   .17580984        12
      C2 |      13.55   .19306155        12
      C3 |   13.183333   .24058017        12
      T1 |     12.875   .30188799        12
      T2 |   12.833333   .27080131        12
      T3 |   13.341667   .26097147        12
---------+--------------------------------------
   Total |   13.213889   .36782789        72

-> month = june

         |       Summary of moisture%
 treatment |       Mean   Std. Dev.      Freq.
---------+--------------------------------------
      C1 |   12.583333   .30993636        12
      C2 |     13.125   .33337119        12
      C3 |   13.416667   .26227445        12
      T1 |   12.933333   .25346097        12
      T2 |   12.741667   .27122055        12
      T3 |     13.025   .26328343        12
---------+--------------------------------------
   Total |   12.970833   .38398852        72


-> month = march
```

| Summary of moisture%
treatment	Mean Std. Dev. Freq.
C1	11.983333 .54244129 12
C2	12.116667 .51669118 12
C3	12.158333 .33698761 12
T1	11.966667 .12309156 12
T2	12.225 .39800638 12
T3	12.25 .28762352 12
Total	12.116667 .3953961 72

-> month = may

| Summary of moisture%
treatment	Mean Std. Dev. Freq.
C1	12.883333 .49144188 12
C2	13.341667 .26097138 12
C3	13.091667 .33698753 12
T1	12.916667 .37859376 12
T2	12.991667 .2466441 12
T3	12.95 .23931731 12
Total	13.029167 .36092113 72

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