Pesticide free methods of maize weevil control in stored maize for developing countries

by

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DEDICATION

This thesis is dedicated to my mom, Ms. Jane Nantongo, and to my siblings, Miriam Nakabiito and Ronald Sserunkuma.

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NOMENCLATURE

Avg	Average
BCFM	Broken corn and foreign material
C	Control
HS	Hermetic storage
MA	Maize-amaranth
MC	Moisture content
MD	Mechanical damage
NH	Non-hermetic storage
Std	Standard deviation
TW	Test weight

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ABSTRACT

Maize, being an important agricultural crop and among the three most widely grown in the world, plays an important role in the livelihood of smallholder farmers contributing 34-36% daily calorific intake in East Africa and 10% in West Africa. In developing countries, population growth is expected to occur with increasing food demand. Conversely, maize experiences post-harvest losses (PHLS) especially from the maize weevil (*Sitophilus zeamais*), which if minimized, could help to reduce the number of hungry people in the world that is about 870 million people and majority (850 million people) being in developing countries. For the economies of smallholder farmers, better storage leads to fewer losses, more income, more grain available to seed, greater family stability, lower risks of family/country conflicts, improvement in political stability and quality of life. The different approaches which were studied to control the maize weevils included: evaluation of hermetic maize storage for smallholder farmers, effect of blending maize kernels with amaranth during storage on maize weevil mortality, and effect of storage containers physical disturbance on maize weevil mortality.

The first study found 100% maize weevil mortality for hermetically sealed containers and the oxygen levels inside them declined from 21% to between 3 and 10%. From the maize, maize-amaranth experiment, it was concluded that blending maize with amaranth during storage reduced maize weevil population growth by 46% compared to storing maize alone. From the last experiment, we found out that physical disturbance resulted in 81% weevil mortality. The overall conclusion is that there are effective low-cost ways to control maize weevils by hermetic storage, physical disturbance and blending maize with amaranth during storage. Hermetic storage is the best among the researched methods to effectively

control the maize weevils, followed by physical disturbance and then maize-amaranth mixture.

Possible future research can be to:

- Investigate the possible causes and how to eliminate and/or minimize maize spoilage on barrel walls. This experiment will seek to minimize and/or eliminate molds that were observed and the aflatoxin detected from samples picked from barrel walls.
- Hermetic storage should be investigated without letting the weevils to first go first through lifecycles to increase in population. This experiment will investigate if kernel spoilage occurs on barrel walls if hermetic sealing in done from the first day of storage.
- Investigate how long it takes for a female maize weevil to bore through a kernel. This test will establish how frequently it is necessary to disturb the weevils.
- Setting up the same maize-amaranth experiment (50:50 by volume) but having an extra layer of amaranth on top to investigate if this can help completely control the maize weevil. This extra layer will reduce and/or eliminate the maize kernels that were available during our experiment.
- Due to observation of no spoilt kernels for maize blended with grain amaranth on barrel walls, more research should be done to quantify the observations. This experiment may lead to hermetic and maize-amaranth mixture methods being used together by smallholder farmers to eliminate and/or mimimize weevils while experiencing no mold maize in metallic storage containers.

- Investigate the effect of physical disturbance using larger storage containers. Since farmers use larger storage containers compared to what we investigated in laboratory setting, it is necessary to find out what will happen in real life.
- Implement and test the researched methods in a developing country. These tests will help determine if the proposed methods are feasible.

CHAPTER 1: INTRODUCTION

Thesis Organization

The information presented in this thesis is organized into five chapters. The first chapter is introduction, with sections on the thesis organization, objectives, and literature review. The second chapter contains a paper entitled "Evaluation of hermetic maize storage steel barrels for smallholder farmers," the third chapter contains a paper entitled "Effect of blending maize kernels with amaranth on maize weevil mortality," the fourth chapter contains a paper entitled "Effect of storage container physical disturbance on maize weevil mortality". The fifth is the "General conclusions" chapter, based on the information contained in previous chapters, and answering objectives from chapter 1. Chapters two, three, and four, were prepared for publication in journals and are formatted in accordance with the guidelines of papers submitted to those journals.

Literature Review

Maize is a major staple crop for many smallholder farmers over the world. However it experiences post-harvest losses due to the maize weevil (*Sitophilus zeamais*). This section covers literature review about maize, maize weevil, factors affecting maize storage, storage methods, hermetic storage, amaranth as a grain that is postulated to reduce maize weevil movement if blended with maize kernels during storage and physical disturbance of storage containers as method to control maize weevils.

Maize

The words maize and corn are used interchangeably to refer to *Zea mays* that belong to the grass family (*Gramineae*) which is a tall plant with an extensive fibrous root system and it’s a cross pollinated species, with the ear (female) and tassel (male) flowers in separate positions along the plant (Encyclopedia, 2014; FAO, 1992). Corn meaning depends on the country where you are: for example, in U.S. corn means maize or Indian corn, in England corn means wheat and in Scotland and Ireland it means oats (Lance and Garren, 2002). According to Lance and Garren (2002), corn mentioned in the Bible is assumed to mean wheat or barley. United States of America continues to be the largest producer of maize with a projected production of 355.6 million Mg (14.5 billion bushels) for 2014 (USDA, 2014, 2013a). In 2013, USA produced 353.06 million Mg (13.9 billion bushels) of maize (FAOSTAT, 2014). Other large scale producer countries include: China, Brazil, Mexico, Indonesia, India, France, Argentina, South Africa and Ukraine. By 2025, maize will be become the mostly highly produced crop in developing countries (CIMMYT and IITA, 2011). In Eastern Africa, Ethiopia and the United Republic of Tanzania are the largest producers of maize (Table 1.1).

Table 1.1. Maize production, yield and area harvest of 2013 for East African countries (FAOSTAT, 2014)

Country	**Element name**	**Unit**	**Value**	**Remarks**
Burundi	Area harvested	Ha	122,871	Official data
	Yield	Hg/Ha	13,219	Calculated data
	Production	tonnes	162,417	Official data
Comoros	Area harvested	Ha	3,000	FAO estimate
	Yield	Hg/Ha	26,000	Calculated data
	Production	tonnes	7,800	FAO estimate
Djibouti	Area harvested	Ha	8	FAO estimate
	Yield	Hg/Ha	20,000	Calculated data
	Production	tonnes	16	FAO estimate
Kenya	Area harvested	Ha	2,100,000	FAO estimate
	Yield	Hg/Ha	16,147	Calculated data
	Production	tonnes	3,390,941	Official data
Madagascar	Area harvested	Ha	300,000	FAO estimate

Table 1.1 (Continued)

	Yield	Hg/Ha	15,167	Calculated data
	Production	tonnes	455,000	Unofficial figure
Malawi	Area harvested	Ha	1,676,758	Official data
	Yield	Hg/Ha	21,708	Calculated data
	Production	tonnes	3,639,866	Official data
Mauritius	Area harvested	Ha	93	Official data
	Yield	Hg/Ha	68,172	Calculated data
	Production	tonnes	634	Official data
Mozambique	Area harvested	Ha	1,700,000	FAO estimate
	Yield	Hg/Ha	9,594	Calculated data
	Production	tonnes	1,631,000	Unofficial figure
Eritrea	Area harvested	Ha	20,000	Unofficial figure
	Yield	Hg/Ha	10,000	Calculated data
	Production	tonnes	20,000	Unofficial figure
Zimbabwe	Area harvested	Ha	900,000	FAO estimate
	Yield	Hg/Ha	8,878	Calculated data
	Production	tonnes	799,000	Unofficial figure
Réunion	Area harvested	Ha	1,700	FAO estimate
	Yield	Hg/Ha	95,294	Calculated data
	Production	tonnes	16,200	FAO estimate
Rwanda	Area harvested	Ha	292,326	Official data
	Yield	Hg/Ha	22,846	Calculated data
	Production	tonnes	667,833	Official data
Somalia	Area harvested	Ha	123,556	Unofficial figure
	Yield	Hg/Ha	12,100	Calculated data
	Production	tonnes	149,497	Unofficial figure
Tanzania	Area harvested	Ha	4,120,269	Official data
	Yield	Hg/Ha	13,000	Calculated data
	Production	tonnes	5,356,350	Official data
Uganda	Area harvested	Ha	1,000,000	FAO estimate
	Yield	Hg/Ha	27,480	Calculated data
	Production	tonnes	2,748,000	Official data
Ethiopia	Area harvested	Ha	2,069,267	Official data
	Yield	Hg/Ha	32,253	Calculated data
	Production	tonnes	6,674,048	Official data
Zambia	Area harvested	Ha	997,880	Official data
	Yield	Hg/Ha	25,382	Calculated data
	Production	tonnes	2,532,800	Official data

Human maize consumption has an increasing trend due to global population growth and its alternative uses that include; animal feed, fiber, and ethanol production. In the U.S. alone, during the “green revolution” (1961-1990), there were enough food yields in relation to the population (Rattray, 2012) due to new and improved farming techniques that were introduced by that time which allowed seeds to utilize more water and fertilizers than before and the massive land that was available for new crop farming with cheap water. Unfortunately, during 1990-2007 the population growth surpassed agricultural production reducing land available for farming because more people settled on land that would have been available for agricultural production. Also this was due to scarcity of water in developed countries and some fertilizers plus pesticides were banned for use (Rattray, 2012) leading to decline in agricultural production. Maize consumption is expected to increase by 2.3% p.a in the 2014/15-2017/18 period compared to 1.5% for wheat and 1.7% for barley (IGC, 2013) thus making maize very important to humans. According to Kearney (2010), maize consumption has been increasing and it will reach its peak by 2050 due to industrial countries particularly in North America that use maize as a sweetener: and as feedstock for biofuels. Maize, being cheaper than other cereals such as rice and wheat, is used as a commodity for food aid in developing countries.

Maize is considered a staple food source particularly in Latin America and Africa (UNDP, 2010). In Africa, it’s among the most crucial and strategic crops being grown in different parts under different climatic and ecological conditions (FARA, 2009). In developed countries, because of being cheap to produce, it’s used for animal feed and as a raw material for other industrial processes. Maize was identified along with other commodities such as cotton, oil palm, beef, diary, poultry and fish by the African heads of state and government as a strategic commodity for achieving food security and poverty reduction during the 2006 Abuja summit on food security in Africa. Maize production is to be promoted by African countries to achieve self-sufficiency by 2015 (AUC, 2006). The major components of maize are: bran (seed coat), endosperm, and germ (Fig 1.1).

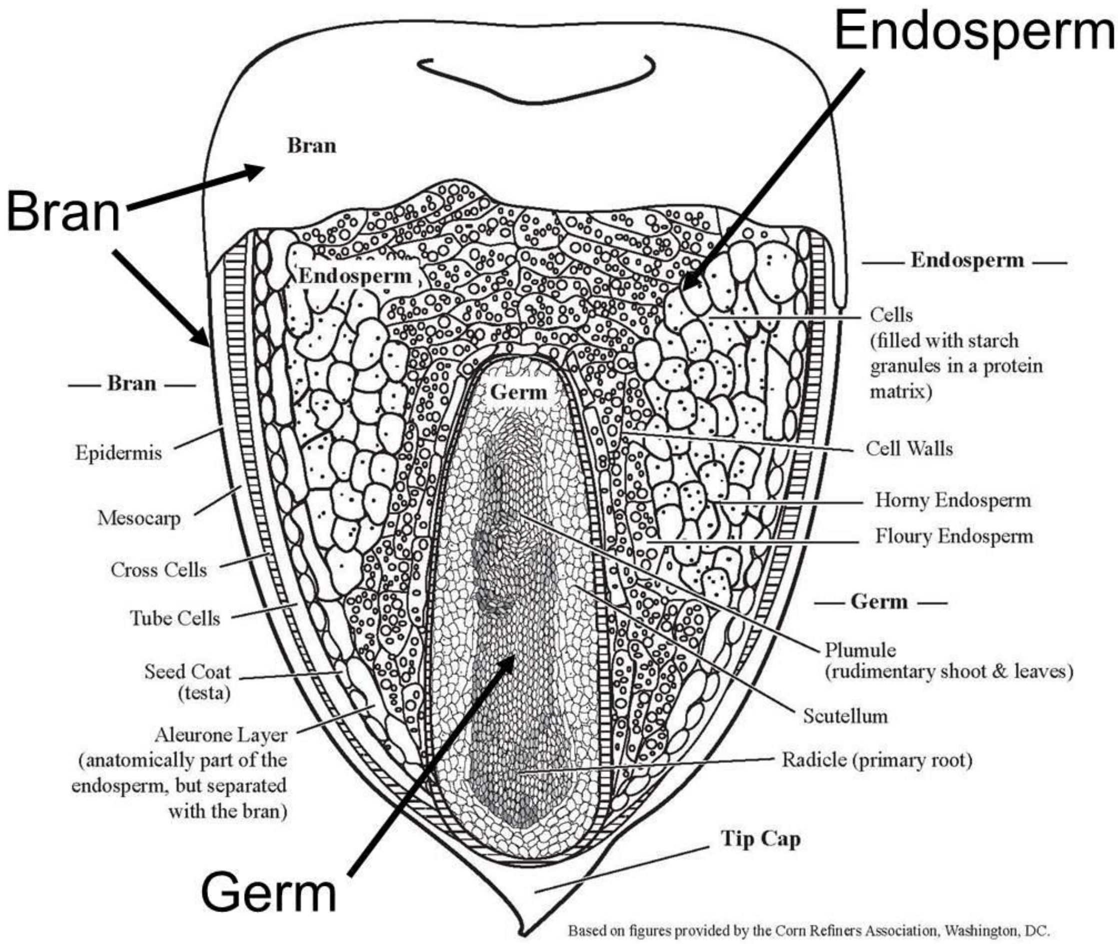

Figure 1.1. Maize kernel parts

(Center for Crops Utilization Research, 2013)

Maize weevil

The maize weevil, *Sitophilus zeamais,* is the most destructive pest of stored maize. It belongs to Coleoptera order and family *Curculionidae*. It has 2-to 4-mm body length with its head protruded into a snout. At the end of the snout, there is a pair of mandibles. It is generally reddish brown in color. It has a long snout with clubbed segmented elbowed antennae and four light reddish brown oval spots on the elytra (Khare, 1994). The lifecycle is on average 36 days at 27 ± 1^{0}C, and 69±3% relative humidity (RH) (Sharifi and Mills, 1971). Maize weevils can be extremely economically destructive to maize under good conditions of tropic and sub-tropic temperatures and maize moisture content ranges of 10 to 14%. Adult weevils damage grain by feeding on the endosperm of the grain kernel. The female deposits an egg a kernel, eggs hatch into larva (tiny grubs) which feed on the endosperm inside the kernel (Hill, 1983). This impacts the quality of the grain in terms of bulk density, moisture content and endosperm value while also producing significant grain dust (maize components in powder form). When not controlled, weevils will completely consume all components inside the maize kernel during storage. Exposure due to damage inflicted on the kernels also facilitates disease and fungal growth in the grain (CGC, 2013a). Factor variety difference within grains have been reported to affect the development time and reproductive capacity of maize weevils (Adams, 1976; Dobie and Kilminster, 1978; Gomez et al., 1983).

Maize storage

Storage is a post-harvest stage in which the products/harvests are kept in such a way as to guarantee food security and/or seeds for the following growing season. For storage to be effective, measures to preserve the quality and quantity have to be adopted. Bern et al. (2013) states that "preservation cannot improve upon the grain as it was at harvest and it cannot even stop deterioration completely but it slows deterioration to an acceptable rate."

Different methods of preservation that include drying, refrigeration, ionizing radiation, mechanical isolation and chemical treatment are explained in detail by Bern et al. (2013).

Degradation of products during storage is influenced by a combination of moisture, temperature and oxygen factors. Moisture and temperature are determining factors in accelerating or delaying the biochemical oxidation of grain and other living organism. Also, they influence the rate at which insects and microorganisms such as molds, fungi, yeasts and bacteria grow, and the premature germination of maize.

Storage methods for smallholder farmers have been well documented and categorized by FAO (2009, 1994) into: a) traditional farm/village storage methods which include: temporary storage methods (aerial storage, ground or drying floor storage, open timber platforms), long-term storage (basket storage, calabashes, gourds, earthenware pots, jars, solid wall bins, underground storage), b) 'improved' farm/village storage methods: these are meant to address weaknesses of traditional farm/village storage methods and c) alternative storage technology (other than traditional and improved traditional systems) at farm/village level such as: metal or plastic drums, alternative solid wall bins e.g. "USAID" bin, concrete silo, "Pusa" bin, metal silos and synthetic silos. Moussa et al. (2011) reports that storage level technology acceptance is highly significant in countries that receive specific training compared to those that do not though in non-trained countries, other approaches such as general extension services can be used.

In developed countries and in particular the U.S., most cereals are stored in commercial grain elevators. Monitoring of grain temperature and calendar-based fumigations using phosphine are among the control measures taken for insects in grain elevators (Hagstrum et al., 1999). Bins can be built from steel or concrete. In comparison, steel bins are built with aeration fans whereas large upright concrete bins are frequently not built with them (Flinn et al., 2007). Insects in upright concrete bins are controlled through fumigation and turning of grain to add aluminum phosphide pellets. U.S. cereals like wheat are not infested in the field (Cotton and Winburn, 1941).

Hermetic storage

Hermetic storage is a storage system that aims at depleting oxygen and enriching carbon-dioxide inside a storage system due to respiration of stored products and living organisms through sealing which prevents interaction of stored products and organisms with the outside environment. Hermetic storage works to reduce oxygen levels in the storage structure to less than 10%, at which insect activity ceases (IRRI, 2013).

Different forms of hermetic storage are used depending on the financial status of the user. They include: a) organic-hermetic storage which relies on the metabolic activity and respiration of insects, micro-flora and the commodity itself to generate a modified depleted oxygen and enriched carbon-dioxide atmosphere which is non-life sustaining (GrainPro, 2014); b) Vacuum-Hermetic Fumigation (V-HF) which uses the principle of lowering pressure inside the storage structure using a vacuum pump for accelerated disinfestation of non-crushable commodities through asphyxiation (GrainPro, 2014); and c) Gas-Hermetic Fumigation (G-HF) which uses an external gas source e.g. CO_2 to enrich the environment for crushable commodities such as fruits prior to shipment (GrainPro, 2014).

Hermetic storage types include: hermetic plastic and bag storage systems (e.g. triple and double bagging, plastic bags), hermetic bulk storage systems, and locally available hermetic storage system such steel barrels. These are described in detail by Yakubu, 2012, 2009.

Yakubu et al., 2011 studied the effects of temperature, time, maize moisture, and oxygen volume on maize weevil mortality. Weevil infested commercial hybrid maize grain samples in 476-mL (1-pint) jars were held under hermetic conditions at maize moisture levels of 6.3% and 16% w.b., and at two levels of temperature, 10^0C and 27^0C. The hermetic conditions were effective in killing weevils. There were significant effects due to temperature and moisture content. Equations were developed to predict the time to 100% adult weevil mortality as a function of temperature, maize moisture content and initial oxygen volume. The equation used to predict 100% maize weevil mortality is in appendix A. Yakubu's experiment was the baseline to investigate hermetic storage in 208-L (55-gal) steel barrels.

The Sukup food storage assembly has been suggested for use to hermetically control the maize weevil thus there is need to investigate its effectiveness. It consists of a 208-L (55-gal) steel drum, a lid that provides a hermetic seal, and a cradle to support the drum (Fig 1.2).

Amaranth

Amaranth belongs to the order *Caryophyllales*, family *Amaranthaceae*, sub family *Amaranthoideae*, genus *Amaranthus* (J. D. Sauer, 1967). *Genus amaranthus* is estimated to be made up of 60 species most of which are cosmopolitan weeds (*A. retroflexus L., A. hybridus., A. powelli S. Watt., A. spinosus L.*). Amaranth is used as a vegetable, food, forage and some as ornamental (Brenner et al., 2000a) while others are weeds (Kauffman and Weber, 1990a). Amaranth can grow in diverse conditions and it's pollinated by wind and/or insects.

Figure 1.2. Sukup food storage assembly

For our interest, amaranth is postulated to fill kernel inter-granular spaces which by doing so restrict maize weevil movement to access kernels thus contributing to weevil mortality and quality control. Laswai et al. (2013), studied the effectiveness of finger millet, sorghum, rice husk, and sunn-hemp in Tanzania as a control measure for post-harvest maize losses to larger grain borer (*Prostephanus truncates)* and maize weevils (*Sitophilus zeamais)* and concluded that maize weevils were less destructive in terms of damage compared to *P. truncates.* The effectiveness was as: actellic super dust (synthetic insecticide) > sunnhemp seeds > rice husks > finger millet > sorghum. Other natural control measures involve use of plant products (Asawalam, 2012; Nukenine et al., 2013; Ogendo et al., 2004; Rajendran and Sriranjini, 2008).

Amaranth has higher protein content compared to other cereals thus making it a good grain for human consumption and to help remedy world hunger and malnutrition (Tagwira et al., 2006a). Furthermore, Kauffman and Weber (1990), reported that amaranth produces large amounts of biomass in a short period of time. Amaranth for human consumption is classified into vegetable amaranth that have edible leaves and grain amaranth. Grain amaranth belongs to a group of cereal-like crop or pseudocereals and it’s an annual herbaceous plant. Amaranth is nutritious with high amounts of vitamin C, iron, beta carotene, folic acid and protein (Table 1.2). The amaranth protein is high in the amino acid lysine that is a limiting factor in cereals like maize, rice and wheat.

Table 1.2. Estimated composition of grain and uncooked leaves of amaranth (100 g portions)

Component	**Vegetable**	**Grain**
Protein	3.5 g	15.0 g
Fat	0.5 g	7.0 g
Total carbohydrates	6.5 g	63.0 g
Fiber	1.3 g	2.9 g
Calories	36	391
Phosphorus	67 mg	477 mg
Iron	3.9 mg	
Potassium	411 mg	
Vitamin A (beta carotene)	6100 IU	0
Riboflavin	0.16 mg	0.32 mg
Niacin	1.4 mg	1.0 mg
Ascorbic acid ©	80 mg	3.0 mg
Thiamin (B1)	0.08 mg	0.14 mg
Ash	2.6 g	2.6 g
Calcium	267 mg	490 mg

Source: (Cole, 1979).

Amaranth leaves and stems may be eaten in either raw or in cooked form as any vegetables whereas its seed can be milled into flour or popped. Amaranth helps in the general improvement of human health plus prevention and improvement of specific ailments and symptoms. It's reported to be of benefit for people suffering from HIV/AIDS (Larry, 2008; Muyonga et al., 2008) and for those using anti-retroviral drugs. Amaranth oil effects of lowering total serum triglycerides and density lipoproteins (LDL) have been reported by Escudero et al. (2006) in animal studies and Martirosyan et al. (2007) in human studies. High levels of serum LDL are associated with coronary heart disease. Unsaturated forms of vitamin E and squalene in amaranth oil are the ones considered responsible for the serum LDL lowering effect. Cooked or autoclaved amaranth grain has been recently researched as a good feed for chickens giving comparably good results to a corn-soybean ration (Acar et al., 1987). Ravindran et al. (1996), reported that *A. hypochondriacus* processed grain is a potential source of energy supplement for broiler diet.

Physical disturbance as a control measure to insects

The simple physical disturbance interferes with insect damage to stored products since the female insect needs time to bore and/or lay eggs inside maize kernels. By subjecting them to disturbance, the maize weevils die before laying eggs. Quentin et al. (1991), investigated the effect of bean tumbling to control the bean bruchids during storage. It was assumed that when beans are physically disturbed numerous times, the larva not damaged by disturbance would capitulate due to exhaustion of energy reserves before gaining access to the cotyledon. The experiment consisted of: tumbling and stationary treatments: 0.8-L glass jars, 16-L plastic buckets, and 45-kg sacks. Each of the storage container types was either loaded half-way with 100% intact or 50% damaged beans. Glass jars, plastic buckets, and sacks were also loaded with 30, 480, and 1000 bean weevils respectively. For jars, and plastic buckets: two plastic tubes were fixed into the inside walls to act as baffles so as to avoid just sliding of beans during tumbling and physical disturbance was done after every eight hours by rotating them through 360^0C back to their original positions. Sacks were turned end-over-end two to three times a day. There was reduction in the number of bean bruchids in physically disturbed compared to stationary treatment as follows: 98% (for 100% intact beans) in glass jars, 95% (for 50% intact beans) in glass jars, 98% (for >98% intact beans) in plastic buckets, 97% (for >98% intact beans) in sacks. A 97% overall mean reduction in bean bruchid population was noticed due to storage container physical disturbance. Quentin et al. (1991), got good results for the bean weevils, and this prompted us to investigate the same treatment effects on the maize weevil.

Synopsis

This research addresses the need of investigating pesticide-free and economically feasible solutions to post-harvest losses of maize due to maize weevils. Smallholder farmers need pesticide-free, cheap, affordable, and easy to use technologies that minimize and/or eliminate post-harvest losses of maize during storage. Research targeting pesticide approaches to control stored products insects in developing countries will have limited applications. Minimizing post-harvest losses will reduce the number of hungry people in the world that is approximated to be 870 million people and majority (850 million) are in developing countries thus by controlling PHLs, we can make more food available to people without increase in field production. For the economies of smallholder farmers, better storage leads to fewer losses, more income, and more grain available to seed, greater family stability, lower risks of family/country conflicts, improvement in political stability and quality of life.

General Objectives

The research objectives were to:

- Determine the effectiveness of the Sukup Food Storage Assembly as hermetic storage for maize weevil control.
- Determine the effectiveness of amaranth-maize blending during storage for maize weevil control.
- Determine the effectiveness of physical disturbance during storage on maize weevil control.

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CHAPTER 2: EVALUATION OF HERMETIC MAIZE STORAGE STEEL BARRELS FOR SMALLHOLDER FARMERS

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Abstract

Maize is an important crop for many smallholder farmers in the world. Maize weevils (*Sitophilus zeamais*) cause a significant loss in quality and quantity during maize storage especially in tropical regions. Hermetic storage of maize has been shown to be effective in controlling maize weevils in laboratory settings. The objective of this research was to test the effectiveness of hermetic storage containers that could be used by smallholder farmers. Six 208-L (55-gallon) steel barrels were each loaded with 170 kg (375 lb) of maize with initial weevil populations of 25 live weevils/kg of maize (11 live weevils/lb). The barrels were placed in a room at 27^0C (81^0F) under non-hermetic conditions for about three weevil lifecycles. After 120 days, the weevil population increased to an average of 99 live weevils/kg (45 live weevils/lb). Three of the six barrels were then hermetically sealed. After 21 days, the weevil population was zero live weevils/kg in the hermetically sealed barrels (100% mortality) and an average of 214 live weevils/kg (98 live weevils/lb) in the non-hermetic barrels. Means of barrel oxygen content, ending number of live weevils per kg of maize, test weight (TW), moisture content (MC), temperature and humidity were

significantly different between the hermetic and non-hermetic storage treatments. Broken corn and foreign material (BCFM) and mechanical damage (MD) were not significantly different. Hermetically sealed steel barrels for maize storage control maize weevils and they may be an effective storage option for smallholder farmers.

Keywords: Maize weevil, tropical regions, maize storage, test weight, BCFM, mechanical damage, moisture content, hermetic storage

Introduction

Maize (*Zea mays)* is a major staple crop for smallholder farmers with over 300 million consumers in Africa (Daily Guide, 2010). In 2011, maize was harvested on 170 million ha worldwide resulting in 883 million Mg of production (FAOSTAT, 2014). By 2025, maize will be the most highly produced crop globally (CIMMYT and IITA, 2011; Rosegrant et al., 2008).

The maize weevil (*Sitophilus zeamais*) can be extremely destructive to stored maize. The female weevil bores through the pericarp of undamaged kernels and deposits eggs into the intact inner portion of the kernel which is then sealed off by mucus like substance. The pupa consumes the inner portion of the kernel. After emergence, adult weevils damage grain by feeding on the endosperm of the kernel plus chewing a 1.5-mm hole in the pericarp (Kranz et al., 1997). Up to 50% or more loss of maize can occur due to weevils during storage (Boxall, 2001). Damage inflicted on the kernels also provides potential openings for disease and fungal growth in the grain (CGC, 2013b).

Hermetic storage (HS) of maize depletes oxygen and increases carbon dioxide inside a storage system due to respiration of stored products and other living organisms (i.e., maize weevils) through sealing which prevents interaction with the outside environment. When oxygen levels fall below about 5%, insect activity ceases and insects die (Gummert et al., 2004).

Previous research studied the effects of temperature, time, maize moisture and oxygen levels on maize weevil mortality (Yakubu et al., 2011). Weevil infested commercial hybrid maize samples in 476-mL (1-pint) jars were held under hermetic conditions at maize moisture levels of 6.3% and 16% w.b., and at 10 and 27^{0}C. The hermetic conditions were effective in killing weevils. There were significant effects due to temperature and moisture content. Equations were developed to predict the time to 100% adult weevil mortality as a function of temperature, maize moisture content and initial oxygen volume.

Double and triple plastic bags are being used by smallholder farmers to store cowpeas in West Africa (Baoua et al., 2013; Murdock and Baoua, 2014; Murdock et al., 2012). Experience may be necessary to achieve good results and hermetic conditions may be interfered with by rodents. It is difficult to maintain a sealed system for a long period of time, and molding of grain can occur where moisture has accumulated in the storage bags (Caddick, 2007). While bagging has proven effective in killing insects, it doesn't provide mechanical protection against rodents and the bag usually has no more than two years of useful life (Brooks and Lavoie, 1990; Jayas et al., 1994). Steel containers provide mechanical protection and are of a size useful to many smallholder farmers. They can be fabricated from locally available materials like galvanized steel sheets and their construction by local artisans creates jobs for example, the postcosecha steel silo is built from 26-gauge (0.7-mm) galvanized steel sheet and lead based solder. A 5-mm fold is formed to make the joints, and seams are crimpled and soldered (Tefera et al., 2011). It is very difficult to achieve hermetic seal for postcosecha steel silos.

Drums or barrels can also be used for hermetic maize storage. However, we have not found reports of studies which tested the hermetic efficacies of drums or barrels. Metal drum technology can be well adopted by use of general extension services (Moussa et al., 2011). The objective of this research was to evaluate the effectiveness of 208-L (55-gallon) steel barrels for hermetic maize storage. The specific objectives were to: determine weevil mortality for hermetically sealed grain, determine quality of hermetically sealed grain, and determine whether hermetically sealed grain becomes re-infested when unsealed.

Material and Methods

Containers

Six 208-L (55-gallon) open head, unlined, steel barrels (Sioux Chief Mfg Co. Model 882-35, 24110 S Peculiar Dr, Peculiar, MO 64078) were used as storage containers. The barrels could be covered either with: (1) screens to retain weevils but yet allow for air passage (long ultra-sun block solar screens, New York Wire, Mt. Wolf, PA); (2) or hermetically sealable lids from the Sukup Food Storage System (Sukup Manufacturing Co. Sheffield, IA). Before cleansing, all barrels with lids installed were filled with warm water and turned upside down to check for water leaks. They were then cleansed with Ajax triple action liquid soap, a large cotton mop and a medium handle brush with warm water. After thorough rinsing, the barrels were left to dry.

Weevils

Weevil-infested commercially comingled maize was used as the source of maize weevils. Weevils were separated from the maize by passing the infested maize through a

Carter Day Dockage tester (CEA, Minneapolis, MN 55432) with 4.76-mm (12/64-in) screen to retain the maize and a 0.99-mm (2.5/64-in) screen to retain the weevils plus some small broken maize kernels. Three representative samples of weevils were used to determine an average weight of 36.72 g per 1,000 weevils. Weevil quantities for seeding the barrels were determined by weight rather than counting.

Experimental procedure

The six barrels were each loaded with 170 kg (375 lb) of weevil-free commercial comingled bulk maize from the 2012 harvest in central Iowa. The maize had an average a moisture content of 13.4%. Each barrel was seeded with 25 live weevils/kg of maize (time T = 0 days) and covered with a screen to prevent weevil escape. The loaded barrels were held in a room maintained at 27 ± 2^0C and with fluorescent lights on.

Measurements

Representative samples of the maize were drawn at different times using a Seedburo brass sampling probe (2293 S. Mt Prospect Road Des Plaines, IL 60018) inserted three times into each barrel at a diagonal angle. Weevil mortality in the samples was determined (Gullan and Cranston, 2010; Yakubu et al., 2010). Samples were analyzed for broken corn and foreign material (BCFM) (USDA, 2013b), moisture content (ASABE, 2006), test weight (TW) (USDA, 1996) and mechanical damage (MD) (Steele, 1967). Oxygen level inside the hermetically sealed barrels was measured using oxygen sensors (Model 65, AMI, Huntington Beach, CA) mounted in the center of the sealable lids and connected to a computer via a PMD 1408FS DAC system. Aflatoxin analysis was performed at the end of the experiment using a Charm ROSA-M reader (Charm Science, Inc 659 Andover Street Lawrence, MA 01843-1032 USA). It detects the sum of aflatoxins B1, B2, G1, and G2 (Appendix A).

Temperature and relative humidity inside barrels was measured using haxo-8 temperature and humidity loggers (879 Maple Street Contoocook, NH 03229). Data were analyzed using JMP Pro 10 and Microsoft Excel.

Results and Discussion

Weevil mortality

From time, T = 0 to T= 120 days, the weevils were left to go through several lifecycles so as to increase in population density. The target was to have 100 weevils/kg of maize. The initial population density was 25 weevils/kg of maize; at 120 days, it was 99 weevils/kg on average and three randomly selected barrels (HS1, HS2, and HS3) were sealed (Table 2.1). These three hermetic barrels were then unsealed due to suspected malfunctioning of the oxygen sensors. Upon unsealing, apparently dead weevils were seen to have accumulated mostly on top of the maize, below the oxygen sensors and on the sides under the lids. After 24 h (T=122 days) of exposure to oxygen with screens on top, all barrels were sampled. Live weevils dropped from 99 weevils/kg to 17 weevils/kg on average (Fig 2.1). This meant that though weevils seemed to be dead by visual observation, some were just dormant and, after exposure to oxygen became active again. This could have been a narcotic effect of carbon dioxide leading to immobilization and/or knock-down of weevils (Aliniazee, 1971; Edwards and Rollas, 1973; Navarro, 2006). The calculated expected mortality days for 99 weevils/kg was 8 days (Yakubu et al., 2011) (Appendix A). The three hermetic barrels (HS1, HS2 and HS3) were left unsealed for 7 days (from T =122 to T= 129) but with a screen on top to prevent escape of live weevils. At T= 129 days, barrels HS1, HS2 and HS3 were sealed again. The calculated time to mortality for 17 weevils/kg was 20 days. After 20 days (T=149 days), the same observations were seen as those observed after one day of sealing. Weevils were exposed to oxygen for 24 h (T = 150 days). After 24 hours, all barrels were sampled and the population density was 0 (zero) live weevils/kg in HS1, HS2, and HS3. From T= 150 to T= 190 days, all six barrels were left with screens on top to prevent escape of weevils. The purpose for this time period was to determine if the hermetic storage had an effect on other life stages of maize weevils, that is to say eggs, larva and pupa.

Fig 2.1 shows the average number of weevils (live weevils/kg maize) as a function of time for the hermetically and non-hermetic barrels. At the time of hermetic sealing (T=120 days), the six barrels averaged 99 live weevils/kg. The number of live weevils was not significantly different among barrels for the first 120 days. There was an increase of weevils in the non-hermetic barrels up to an average of 214 weevils/kg while the hermetically sealed weevil population declined to 0 (zero) weevils/kg at T=190 days (Fig 2.1). The number of weevils in hermetically sealed barrels was significantly lower than in the non-hermetic barrels after one day of sealing (p = 0.0060, R^2=87.61%), after complete sealing, T =150 days (p = 0.0011, R^2=94.68%) and at T= 190 days (p = 0.0002, R^2=97.60%) (Table 2.1). Analysis within each treatment was also done for the different time periods (Table 2.1). A 95% confidence interval was considered. Some of the data analyzed is shown in Appendix A. The decline (T =40 days) was attributed to the weevils not yet being adapted to the new environment and probably some were ending their lifecycle. The population increase in the non-hermetic barrels was because of the favorable maize moisture and temperature (Sone, 2000) and the complete mortality in hermetically sealed barrels was because of oxygen depletion and CO_2 enrichment (Anankware et al., 2013; Anankware and Bonu-Ire, 2013; Fleurat, 1990; Foster et al., 1955; Navarro, 2006; Navarro et al., 1990; Oxley and

Wickenden, 1963; Villers et al., 2010; Yakubu et al., 2011, 2010). At the end of the experiment (T=190 days), zero live weevils in all hermetic barrels was due to the hermetic storage effect on other life stages of maize weevils (egg, larva and pupa) and the 214 weevils/kg on average in non-hermetic barrels was due to favorable temperature, maize moisture content and availability of kernels.

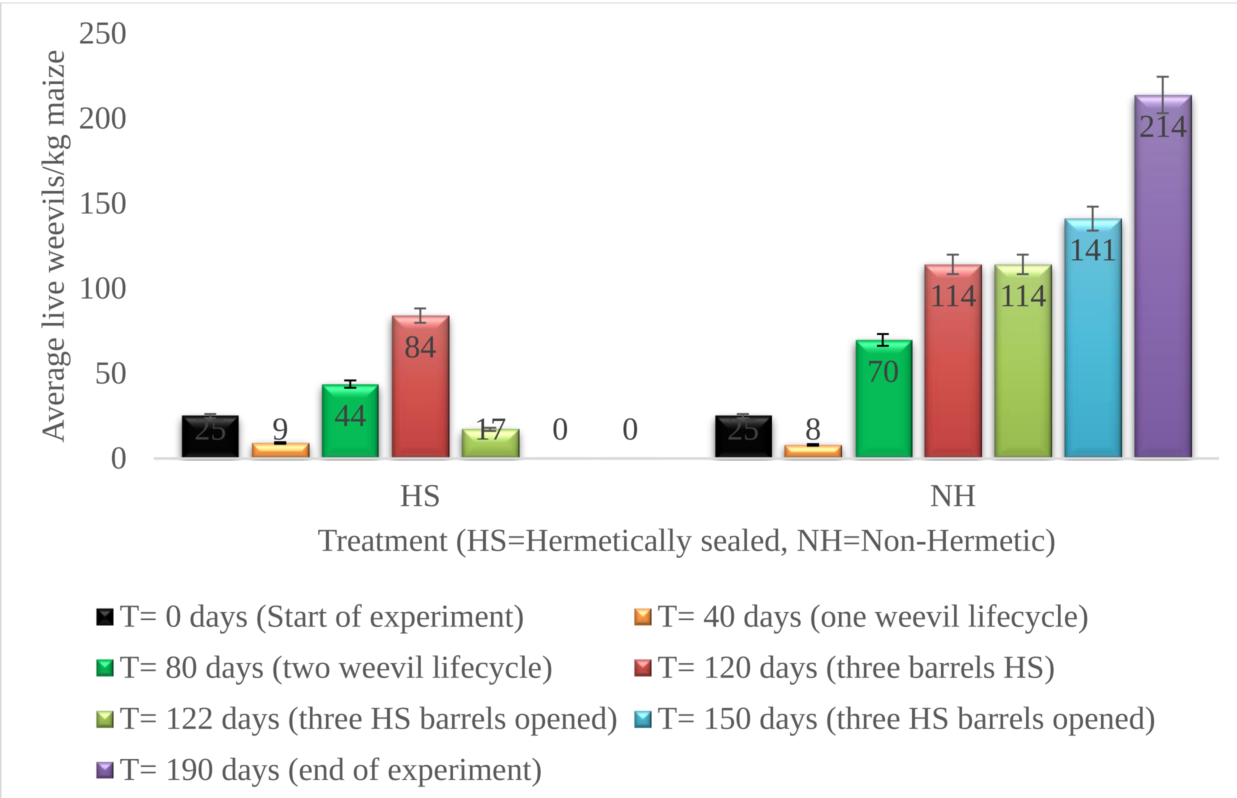

Figure 2.1. Mean live weevils/kg of maize at different time periods for HS vs. NH treatments

Table 2.1. Tukey's means comparison of live weevils and maize quality values for hermetic versus non-hermetic storage experiment

Item	Treatment	T=0	T=40	T=80	T=120	T=122	T=150	T=190
Number of live weevils/kg	HS	25±0$^{\text{Abc}}$	9±2$^{\text{Acd}}$	44±12$^{\text{Ab}}$	84±13$^{\text{Aa}}$	17±11$^{\text{Acd}}$	0±0$^{\text{Ad}}$	0±0$^{\text{Ad}}$
	NH	25±0$^{\text{Ad}}$	8±5$^{\text{Ad}}$	70±17$^{\text{Acd}}$	114±30$^{\text{Abc}}$	114±30$^{\text{Bbc}}$	141±29$^{\text{Bb}}$	214±29$^{\text{Ba}}$
Temp (^{0}F)	HS	N/A	87.0±6.0$^{\text{Uc}}$	88.4±1.5$^{\text{Ua}}$	87.6±1.9$^{\text{Ub}}$	87.2±1.4$^{\text{Uabc}}$	82.5±1.4$^{\text{Ud}}$	86.9±2.3$^{\text{Uc}}$
	NH	N/A	87.0±6.3$^{\text{Uc}}$	88.9±1.6$^{\text{Ta}}$	88.2±1.5$^{\text{Tb}}$	86.9±1.2$^{\text{Ubc}}$	88.4±1.3$^{\text{Tab}}$	88.8±1.7$^{\text{Ta}}$
RH (%)	HS	N/A	65.2±1.3$^{\text{Mf}}$	66.9±1.0$^{\text{Me}}$	69.9±0.7$^{\text{Md}}$	71.5±0.4$^{\text{Mb}}$	70.4±0.7$^{\text{Mc}}$	74.5±1.1$^{\text{Ma}}$
	NH	N/A	64.8±1.2$^{\text{Nf}}$	66.6±1.0$^{\text{Ne}}$	69.4±1.4$^{\text{Nd}}$	70.9±1.8$^{\text{Mc}}$	72.6±2.0$^{\text{Nb}}$	74.7±2.7$^{\text{Ma}}$
MC (%)	HS	13.0±0.6$^{\text{Dab}}$	13.3±0.0$^{\text{Da}}$	13.2±0.1$^{\text{Dab}}$	12.8±0.1$^{\text{Dab}}$	12.8±0.1$^{\text{Dab}}$	13.3±0.1$^{\text{Dab}}$	12.8±0.2$^{\text{Db}}$
	NH	13.2±0.1$^{\text{Dab}}$	13.4±0.2$^{\text{Da}}$	13.3±0.1$^{\text{Dab}}$	12.8±0.1$^{\text{Dc}}$	12.8±0.1$^{\text{Dc}}$	13.1±0.1$^{\text{Ebc}}$	12.6±0.1$^{\text{Dc}}$
MD (%)	HS	4.6±1.8$^{\text{Xbc}}$	3.8±0.5$^{\text{Xc}}$	4.5±0.4$^{\text{Xbc}}$	6.4±1.0$^{\text{Xab}}$	6.9±0.9$^{\text{Xab}}$	6.0±0.4$^{\text{Xabc}}$	7.4±0.4$^{\text{Xa}}$
	NH	4.1±0.9$^{\text{Xd}}$	3.9±0.3$^{\text{Xd}}$	4.4±0.5$^{\text{Xcd}}$	6.1±0.6$^{\text{Xbc}}$	6.1±0.6$^{\text{Xbc}}$	7.4±0.9$^{\text{Xb}}$	9.4±1.1$^{\text{Za}}$
BCFM (%)	HS	1.5±0.1$^{\text{Kc}}$	1.9±0.1$^{\text{Kbc}}$	2.3±0.1$^{\text{Kabc}}$	3.2±0.5$^{\text{Kab}}$	3.2±0.5$^{\text{Ka}}$	3.3±0.5$^{\text{Ka}}$	2.3±0.5$^{\text{Kabc}}$
	NH	1.7±0.5$^{\text{Kc}}$	1.9±0.1$^{\text{Kbc}}$	2.3±0.3$^{\text{Kbc}}$	2.8±0.3$^{\text{Kab}}$	2.8±0.3$^{\text{Kab}}$	3.3±0.4$^{\text{Ka}}$	2.3±0.3$^{\text{Kbc}}$
TW (Ib/bu)	HS	57.4±0.2$^{\text{Ra}}$	57.1±0.1$^{\text{Rab}}$	56.7±0.2$^{\text{Rbc}}$	56.2±0.1$^{\text{Rcd}}$	56.1±0.2$^{\text{Rde}}$	55.5±0.1$^{\text{Re}}$	55.2±0.2$^{\text{Rf}}$
	NH	57.4±0.2$^{\text{Ra}}$	57.1±0.1$^{\text{Rab}}$	56.7±0.2$^{\text{Rbc}}$	56.0±0.1$^{\text{Rc}}$	56.0±0.1$^{\text{Rc}}$	55.2±0.1$^{\text{Sd}}$	54.4±0.2$^{\text{Sd}}$

Values not followed by same upper case letter at each time for each item, and levels not connected by lower case letter in each treatment are significantly different at 0.05 level

Temperature

There was a range of 21.6 to 34.7^{0}C (70.9 to 94.5^{0}F) in temperature inside the barrels with 30.7^{0}C (87.2^{0}F) being the average. At T=120 days, temperatures inside NH1, NH2, NH3, HS2, and HS3 were not significantly different from each other while that of HS1 was significantly higher than the rest of the barrels. After one day of sealing (T=122 days), the temperatures inside hermetic and non-hermetic barrels were not significantly different. At the end after barrels were resealed (T=150 days), temperatures inside hermetic barrels were significantly lower ($P<0.0001$) than those of non-hermetic barrels. At T=190 days, the previously hermetically sealed barrels' temperature were significantly lower ($p<0.0001$) from those of non-hermetic barrels. Also analysis within each treatment was done for the different time periods (Table 2.1). The temperature values were attributed to respiratory and/or metabolic processes of maize and the weevils (Bern et al., 2013). Temperatures recorded inside the barrels were slightly higher than those recorded by Foster et al., 1955 (21.1 – 26.7^{0}C). This could have been because of having a controlled room temperature in comparison to their experiment which was exposed to winter, summer and spring weather.

Relative humidity

The measured relative humidity range was 59 to 83% inside the barrels with 70% being the average, and it showed an increasing trend with time (Table 2.1). At T=120 days, relative humidities inside HS1, HS2 NH1, and NH2 were not significantly different from each other whereas those of HS3 and NH3 were significantly higher. After one day of sealing (T=122 days), relative humidities inside hermetic and non-hermetic barrels were not significantly different. At the end, after the barrels were resealed (T=150 days), relative humidity inside hermetic barrels were significantly lower than those of non-hermetic barrels

(P<0.0001). At T=190 days, humidities in previously hermetically sealed barrels were significantly lower ($p<0.0001$) than those in non-hermetic barrels (Table 2.1).

Maize moisture content (MC)

Maize moisture ranged from 13.7% to 12.5% during the 190 days of the experiment with a general increase for the first 40 days and a decline after that. Maize moisture in all barrels during population increase (T = 0 to T=120) were not significantly different. Moisture differences between hermetically sealed and non-hermetic barrels were not significant after one day of sealing but MC was significantly higher for hermetically sealed after resealing, T =150 days ($p=0.0488$, R^2=66.2%) (Table 2.1). At T=190 days, moisture was not significantly different between barrels. Also analysis within each treatment was done for the different time periods (Table 2.1). The increase in moisture during the first 40 days was probably due to the respiration of maize and weevils. The decline would have perhaps been due to maize establishing equilibrium moisture. Under this process moisture is assumed to have moved to the sides of the barrels to where the probe could not get samples. This assumption is supported with the observation of mold and/or deteriorated maize which was on barrel walls. Metal silos have a disadvantage of moisture migration and condensation in hot climates and this may limit hermetic storage (Navarro, 2006).

Because of the slightly higher temperature in the barrels, moisture transfer and accumulation near the sides of barrels may have led to deterioration of and/or moldy maize as observed when the barrels were emptied. Foster et al. (1955), observed a similar scenario. Navarro (2006); and Navarro et al. (1994) reported that both mold and insects release heat which can lead to temperature gradients within the stored grain thus creating convection currents within the stored grain product, encouraging warm moist air movement from the heating section to cooler sections where moisture is dropped as air cools. The deterioration may have an effect on farmers' acceptance of the technology (Navarro et al., 1994).

Mechanical Damage (MD)

MD is the percentage by weight of kernels with a missing portion or any visible crack or rupture of the seed coat (Steele, 1967). There was a general increasing trend from 4.34% to 8.43% on average in all treatments. MD was not significantly different between all barrels during weevil population increase (T= 0 upto 120 days). They were also not significant between hermetically sealed and non-hermetic barrels after one day of sealing i.e. T =122 days and after complete resealing T=150 days. However, MD was significantly different between previously hermetically sealed and non-hermetic barrels at T= 190 days ($p = 0.0349$) (Table 2.1). Also analysis within each treatment was done for the different time periods (Table 2.1). The increase in MD was attributed to the increasing number of weevils in the barrels. The significant difference at the end of the experiment was due perhaps to no weevils in the previously hermetically sealed barrels compared to non-hermetic barrels which had an increasing number of weevils. The results were in line as those observed by Foster et al. (1955) in which damaged kernel numbers varied considerably throughout the experiment.

Broken corn and foreign material (BCFM)

There was a general increase in the BCFM in all the six barrels from 1.6% to 3.14% on average from time T = 0 to 150 days and then there was a slight decline to 2.33% from T = 150 to T = 190 days. BCFM values were not significantly different between hermetically sealed and non-hermetic barrels at any time (Table 2.1). Also analysis within each treatment was done for the different time periods (Table 2.1). The increase in BCFM over time was attributed to the increased number of maize weevils while the decline between T=150 and

T=190 days was attributed to possible sampling error. BCFM absorbs moisture more rapidly than grain (Navarro, 2006). This favors mold development, a condition which was observed on maize and fines close to barrel sidewalls.

Test weight (TW)

There was a decline in TW from 739 to 705 kg/m^3 (57.4 to 54.8 Ib/bu) on average during the experiment. During the first 120 days, TW difference was not significant between treatments. After one day of sealing, the TW difference was not significant between hermetic and non-hermetic barrels. TW was significantly higher for hermetically stored maize at T=150 days ($p=0.0194$, $R^2=78.12\%$) and T=190 days, ($p=0.0048$ and $R^2=88.89\%$) (Table 2.1). Also analysis within each treatment was done for the different time periods (Table 2.1). As the maize was losing moisture, there was an expected increase in TW (Bern and Brumm, 2009) but the declining TW can be attributed to immature corn and/or a change in the dry material quantity (Bern and Brumm, 2009) or deterioration due to infestation of the maize by weevils.

Aflatoxins

At the end of the experiment there were regions of visible fungal growth on fine material and kernels on barrel walls in all replications of both hermetic and non-hermetic treatments. The aflatoxins are produced by *Aspergillus flavus*. Pearson et al. (2001), reports that most aflatoxins appear in maize if it experiences some sort of stress and presence of molds in kernels allow them to increase. Aflatoxins were detected (Appendix A) and their concentrations were less than 20 ppb on average. The samples analyzed were not representative of all the maize in the barrels. We seek further investigation to understand the specific cause of fungal growth as it was unexpected in view of the low beginning moisture content (<14%). We believe mold and aflatoxins would be unlikely if barrels are sealed as soon as weevil-infested maize is loaded.

Oxygen sensor readings for HS1, HS2 and HS3 (T=120-122 days)

There was a general decline in oxygen from 23 to 3% in the three sealed barrels on average by 120.2 days. Then the oxygen values increased to 6.7% from the 120.2-120.4 days and finally there was a decline to a constant value of 5.5% on average up to 120.8 days (Fig 2.2). Oxygen levels inside the sealed barrels were significantly lower than the atmospheric oxygen level ($p = 0.0027$).

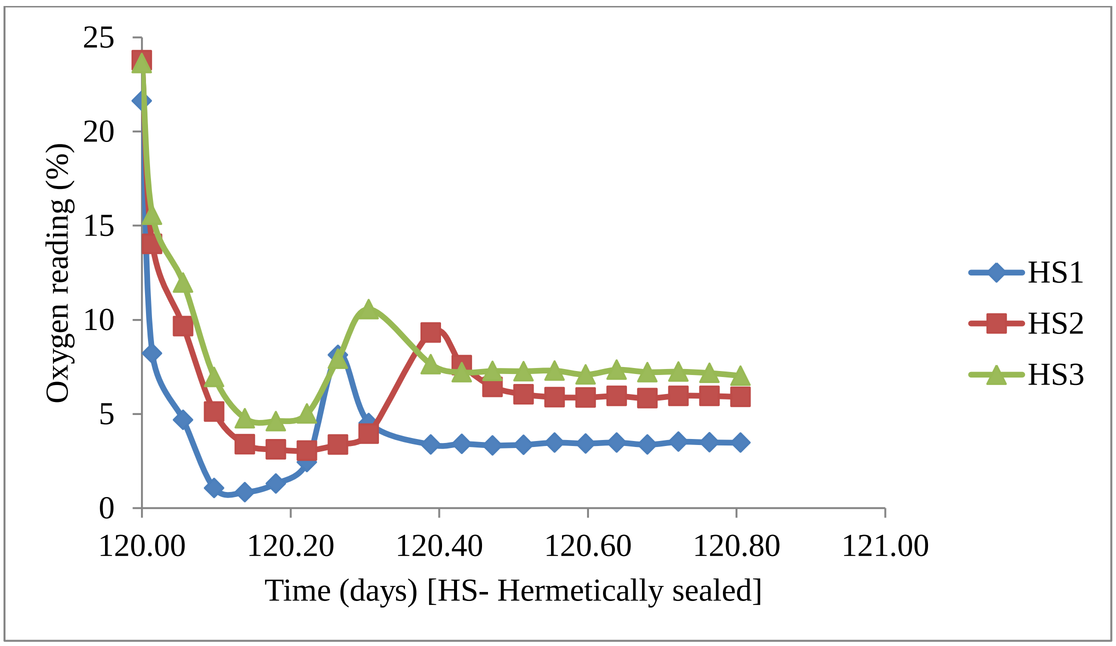

Figure 2.2. Oxygen content inside hermetically sealed barrels (T=120-122 days)

Oxygen sensor readings for HS1, HS2 and HS3 (T=129-150 days)

After resealing the barrels at T=129 days, the curve followed almost the same trend as the curve obtained after T=120-122 days of sealing (Fig 2.2) with a decline of below 5% at T=129.2 days. There was a rise of between 5% and 10% followed by a decline to a constant value (Fig 2.3). Oxygen readings remained constant in all three hermetic barrels HS1, HS2 and HS3 from T=130.4 days upto the end of the experiment (T=149 days). This whole portion is not shown on Fig 2.3. Oxygen level inside the sealed barrels were significantly lower than atmospheric oxygen level ($p < 0.0001$). The decline in oxygen with time was expected; however the slight increase between 129.25 and 129.5 days and a quick decline in oxygen before the expected calculated oxygen consumption days were unexpected. The trend was similar to that observed by Villers et al. (2010), while studying hermetic storage of cocoa beans. Generally, to some extent the results do not agree with the oxygen levels of <3% recommended for complete mortality by Banks and Annis (1990); Fleurat (1990); and Navarro (1978) for effective control. However 100% mortality was achieved. Bailey (1955, 1956, 1957, 1965), suppressed storage insects at about 5% oxygen with longer exposure time, which is almost the same concentration observed for our results but at a lower exposure time. Navarro (2006), stated that pure CO_2 environments in laboratory settings can kill product-stored insects within 10 and 48 h, which could have been created by weevils and maize. Mortality can be attributed to its correlation with a hypoxia condition that causes body water loss (Navarro, 1978) thus leading to death. Also temperature within the barrels might have favored intensive oxygen intake by the weevils thus leading to increased mortality (Navarro, 2006; Navarro et al., 1994). The fluctuations seen in Figs 2.2 and 2.3 were similarly observed by Hyde et al. (1973); Navarro et al. (1994, 1990); and Oxley and Wickenden (1963) for both laboratory and field experiments, and it was attributed to a residual insect population that may remain behind after an extended period of time before a steady-state is attained. The steady-state conditions for our experiment were not only supported by the constant oxygen reading after some time but also by the zero number of weevils counted after unsealing of barrels, as opposed to Navarro et al. (1994), who reported that a residue population may be observed after the grain is re-exposed to oxygen.

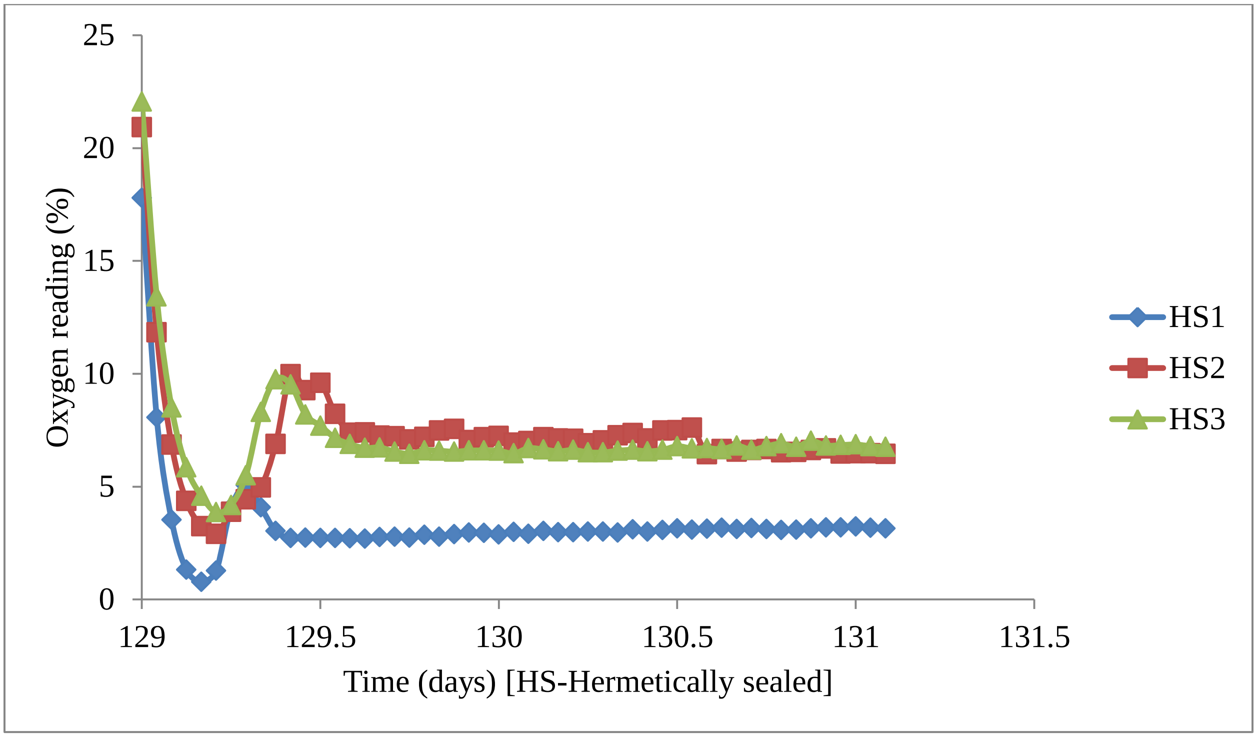

Figure 2.3. Oxygen content inside hermetically sealed barrels (T=129-150 days)

Conclusions

- Hermetic sealing barrels resulted in 100% adult weevil mortality.
- Eggs, larvae, and pupa were killed by hermetic sealing.
- Oxygen levels in hermetically sealed barrels declined from 21% to between 3 and 10%.
- HS vs. NH: BCFM was not significantly different at any time, MC was significantly higher only at T = 150 days, TW was significantly higher at T = 150 and 190 days, MD was significantly higher in NH at T = 190 days.
- Further investigation is necessary to understand the reasons for moldy maize on the walls of the barrels.

Implications

- Hermetic storage in a 55-gallon barrel is an effective non-pesticide approach to controlling weevils in maize.
- No high level of expertise is needed to implement this technology.

Acknowledgements

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CHAPTER 3: EFFECT OF BLENDING MAIZE KERNELS WITH AMARANTH DURING STORAGE ON MAIZE WEEVIL MORTALITY

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Abstract

Amaranth (*Amaranthus spp*.) is used as a vegetable, food, forage and sometimes an ornamental. Grain amaranth has higher protein content compared to other cereals thus making it a good choice for human consumption. Due to the small size of amaranth seeds, it has been postulated that amaranth can be blended with maize during storage to fill the inter-granular spaces between kernels thus minimizing maize weevil movements to access kernels and thus controlling maize weevil population. Maize is among the three most widely grown grains in the world, but it can experience large post-harvest losses during storage. The objective of this research was to investigate the effects of blending maize with grain amaranth during storage on maize weevil mortality versus maize stored alone. Three 208-L (55-gallon) steel barrels were loaded with 160 kg (353 lb) of maize and three were loaded with maize-amaranth (50:50 volume) all with initial weevil populations of 25 live weevils/kg of maize (11 live weevils/lb). Blending maize with amaranth during storage reduced maize weevil population growth after 160 days by 46% compared to storing maize alone.

Keywords: Amaranth, maize weevil, cereals, maize, post-harvest losses, test weight

Introduction

Amaranth belongs to the order *Caryophyllales*, family *Amaranthaceae,* sub-family *Amaranthoideae*, genus *Amaranthus* (Jonathan D Sauer, 1967). Genus *amaranthus* is estimated to be made up of 60 species most of which are cosmopolitan weeds (*A. retroflexus L., A. hybridus., A. powelli S. Watt., A. spinosus L.*). Amaranth is used as a vegetable, food, forage and sometimes as ornamentals (Brenner et al., 2000b) while others are weeds (Kauffman and Weber, 1990b). Amaranth can grow in diverse conditions and it's pollinated by wind and insects. Amaranth has higher protein content compared to several other cereals thus making it a good choice for human consumption and to help remedy world hunger and malnutrition (Tagwira et al., 2006b). Furthermore, Kauffman and Weber (1990), reported that amaranth produces large amounts of biomass in a short period of time. Amaranth for human consumption is classified into vegetable amaranth that has edible leaves and grain amaranth. Grain amaranth belongs to a group of cereal-like crops or pseudo-cereals and it's an annual herbaceous plant.

Maize is a very important agricultural crop and among the three most widely grown in the world (CIMMYT and IITA, 2011). By 2011, maize was produced on more than 170M ha in the world with about 35M ha in Africa (FAOSTAT, 2014). In least developed countries where maize plays an important role in the livelihood of smallholder farmers, production on about 23M ha counted for about 43M Mg of maize in 2013 (FAOSTAT, 2014). The maize crop contributes 34-36% of daily caloric intake in countries such as Kenya and Tanzania (WorldBank et al., 2011) whereas it contributes about 10% caloric intakes in West Africa (Byerlee and Heisey, 1996). Maize plays a significant role in the lives of smallholder farmers, but it experiences post-harvest losses (PHLs) which, if minimized, could help to reduce the number of hungry in the world. This number is about 870M people and the majority (850M people) are in developing countries (FAO et al., 2012). PHLs occur between harvest and consumption. In a grain chain, PHLs occur during harvesting, threshing, winnowing, assembling, drying, milling, storage, packaging and transportation. PHLs reduction is reorganized as part of the integrated approach to ensuring the full realization of agricultural potential to meet the world's increasing food and energy needs. In sub-Saharan Africa (SSA), grain PHL reduction is a critical objective because those losses are not only a waste of valuable food and other resources such as labor, seeds, land but are also symptoms of poorly performing value chains. Most PHLs occur during storage and the maize weevil (*Sitophillus zeamais*) is the critical PHL insect of stored maize in the tropics (Jacobs and Calvin, 2001; Longstaff, 1986, 1981).

Control of maize weevil by increasing its mortality rate in stored maize is desirable. One of the methods proposed is by storing maize mixed with amaranth which is postulated to reduce interstitial spaces between kernels. This leads to restricted movement of the weevils which in turn denies access of maize weevils to kernels thus leading to reduction in weevil population. In doing so, less infestation occurs, weevils are unable to reproduce, and eventually they die since they have limited access to maize kernels. Laswai et al. (2013), observed varying degrees of control of maize weevils when they blended maize with crotalatia seeds, finger millet, or sorghum. The effectiveness was: actellic super dust (synthetic insecticide) > sunnhemp seeds > rice husks > finger millet > sorghum The objective of this research was to investigate the effect of blending maize with amaranth during storage on maize weevil mortality compared to maize stored alone.

Methods and Materials

Containers

Six 208-L (55-gallon) open head, unlined, steel barrels (Sioux Chief Mfg Co. Model 882-35, 24110 S Peculiar Dr, Peculiar, MO 64078) were used as storage containers. The barrels can be covered with sun screens (New York Wire, Mt. Wolf, PA) to retain weevils but yet allow for air passage. Barrels were cleansed with Ajax triple action liquid soap, a large cotton mop and a medium handle brush with warm water. After thorough rinsing, the barrels were left to dry.

Maize weevils

Maize weevils from infested commercially comingled maize were used as the source of infestation. Weevils were separated from the maize by passing infested maize through a Carter Day Dockage tester (CEA, Minneapolis, Minnesota 55432 USA) with a screen of 4.76-mm (12/64-in) to retain the maize and a 0.99-mm (2.5/64-in) screen to retain the weevils. Weevil quantities for seeding the barrels used was by weight rather than counting.

Experimental maize and amaranth

Commercial comingled bulk maize used in this experiment was purchased from a local grain elevator in central Iowa with an initial average moisture content of 13%. Amaranth used in the experiment was organic whole grain amaranth of variety *Amaranthus Hypochondriacus* with an initial average moisture content of 11.7% grown and donated to Iowa State University by Mark & Marcie Jones (4498 Rd. 167 Oshkosh, NE 69154).

Three of the six barrels were each loaded with 160 kg (353 lb) of un-infested commercial comingled bulk maize whereas the other three were loaded with maize blended with amaranth (50:50 by volume). After each 21 kg of maize was loaded, 24 kg of amaranth was added and stirred by hand to make amaranth fill the voids between maize kernels. Each barrel was seeded with 25 live weevils/kg of maize. The six loaded barrels were covered with long ultra-sun block charcoal solar screening (New York Wire, P.O. Box 866, Mt. Wolf, PA 17347, USA) to allow air circulation while preventing weevil escape.

Measurements and statistical analysis

Representative samples were drawn after every 40 days up to 160 days using a Seedburo brass sampling probe (2293 S. Mt Prospect Road Des Plaines, IL 60018) inserted three times in each barrel at a diagonal angle. Weevil mortality was determined as described by Yakubu et al. (2010), and Gullan and Cranston (2010). Samples were analyzed for broken corn and foreign material (BCFM) (USDA, 2013b), moisture content (ASABE, 2006), test Weight (TW) (USDA, 1996) and mechanical damage (MD) (Steele, 1967). Temperature and relative humidity inside barrels was measured using haxo-8 temperature and humidity loggers (879 Maple Street Contoocook, NH 03229). Tukey's means comparison was used to compare the differences in treatments at α=0.05 using JMP Pro 10 and MS excel for descriptive analysis of data.

Results and Discussion

Live weevils

Fig 3.1 and Table 3.1 show the mean number of live maize weevils over time for the two treatments. For the first 80 d, the increase in the number of weevils in the two treatments was almost the same for all six barrels. This was probably due to the maize-amaranth treatment having some maize exposed to the weevils on top of the grain in the barrels that were used in population increase. The increase in the number of live weevils was higher at 120 d and 160 d for the maize stored alone compared to maize blended with amaranth. This was attributed to the availability of maize, favorable temperature and moisture content for the maize stored alone whereas the slight increase for the maize-amaranth mixture was probably because of the declining number of maize kernels available on top for weevil egg deposit. The number of live weevils between the two treatments was not significantly different for 40, 80 and 120 d but it was significantly higher for maize stored alone at 160 d ($p = 0.0415$) (Table 3.1). When the numbers of live weevils for maize-amaranth mixture results were tested for 0 vs. 40 vs. 80 vs. 120 vs. 160 days, the number of weevils at 80, 120, and 160 days were significantly higher than at 0 and 40 days. Raw data is shown in Appendix B. On average, the maize-amaranth mixture reduced population increase by 46%. For future research, we recommend a layer of amaranth on top to completely cover the maize kernels.

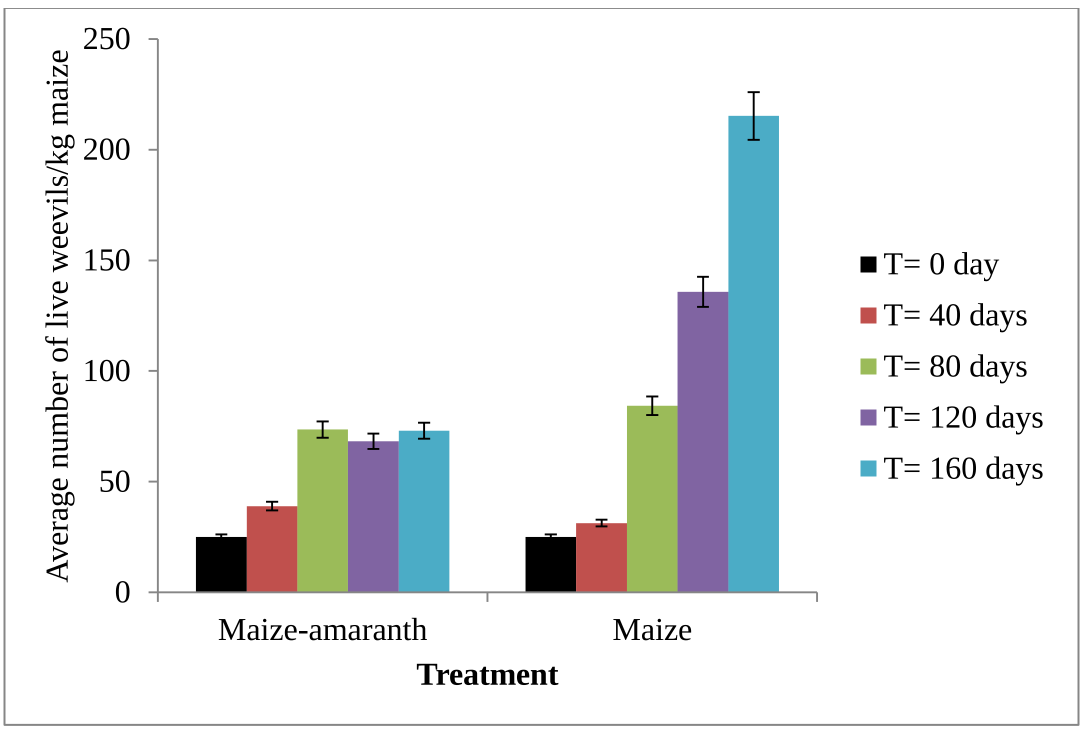

Figure 3.1. Mean live weevils/kg at different time periods for maize-amaranth mixture and maize stored alone experiment

Table 3.1. Tukey's means comparison of live weevils and maize quality values for maize-amaranth mixture versus maize stored alone

Item	Treatment	T=0	T=40	T=80	T=120	T=160
Number of live weevils/kg	Maize-amaranth	25±0^{Ab}	39±4^{Ab}	74±11^{Aa}	68±10^{Aa}	73±14^{Aa}
	Maize	25±0^{Ab}	31±6^{Ab}	84±34^{Aab}	136±64^{Aab}	215±82^{Ba}
Temp (^{0}F)	Maize-amaranth	-	78.7±4.9^{Ga}	83.2±3.4^{Gc}	80.0±1.7^{Gb}	82.9±1.1^{Ga}
	Maize	-	81.0±8.7^{Hc}	86.0±5.0^{Hab}	84.5±5.7^{Hab}	87.0±7.0^{Ha}
RH (%)	Maize-amaranth	-	65.6±4.5^{Aa}	59.5±9.9^{Abc}	57.9±11.6^{Ac}	60.9±9.3^{Ab}
	Maize	-	67.4±5.5^{Bd}	68.4±2.7^{Bc}	69.6±1.4^{Bb}	71.6±1.6^{Ba}
MC (%)	Maize-amaranth	13.0±1.9^{Ma}	12.1±2.6^{Mc}	12.2±1.9^{Mc}	12.2±1.7^{Mc}	12.6±1.6^{Mb}
	Maize	13.1±1.8^{Ma}	12.8±1.0^{Ma}	12.6±0.6^{Na}	12.7±0.8^{Na}	12.7±1.7^{Na}
MD (%)	Maize-amaranth	16.1±^{Pa}	15.7±^{Pa}	16.3±^{Pa}	16.7±^{Pa}	16.9±^{Pa}
	Maize	14.9±^{Pb}	16.2±^{Pb}	24.3±^{Qa}	25.9±^{Qa}	26.9±^{Qa}
BCFM (%)	Maize-amaranth	5.1±0.4^{Ka}	5.1±0.3^{Lab}	4.8±0.1^{Lab}	4.7±0.1^{Lab}	4.1±0.2^{Lb}
	Maize	4.8±1.0^{Kb}	7.1±0.6^{Ka}	7.4±0.5^{Ka}	7.5±0.5^{Ka}	7.8±0.5^{Ka}
TW (Ib/bu)	Maize-amaranth	56.6±0.2^{Ra}	56.1±0.0^{Rb}	56.0±0.0^{Rb}	55.5±0.3^{Rc}	54.5±0.1Rd
	Maize	56.6±0.5^{Ra}	56.0±0.2^{Rab}	55.8±0.1^{Sb}	54.9±0.2^{Rc}	53.8±0.3^{Sd}

Values not followed by same upper case letter at each time for each item, and levels not connected by same lower case letter in each treatment are significantly different at 0.05 level

Temperature and relative humidity

Relative humidity (RH) is the ratio of absolute humidity of air to the maximum possible absolute humidity of that air. The humidity for maize amaranth mixture ranged between 57.9 to 65.6 % on average whereas that of maize stored alone ranged from 67.4% to 71.6% on average. The trend of RH for maize-amaranth mixture was decreasing from T=40 to T= 120 d and they was a slight increase at T=160 d. This could have been due to the presence of amaranth. On the other hand, RH trend for maize stored alone was increasing with time. Relative humidity for maize stored alone was significantly higher at all time (Table 3.1). RH within maize stored along was significantly different at all times whereas that of maize-amaranth mixture was not significantly different at T=80, 120, and 160 days (Table 3.1).

Temperature had a general increasing trend in the two treatments though it was higher in maize stored alone at all times. The increasing trend was attributed to the respiration the stored products and maize weevils (Bern et al., 2013), however the low level increase for maize-amaranth mixture was attributed to the presence of amaranth. Temperature for maize stored alone was significantly higher at all times (Table 3.1). By feel of the hand when emptying the barrels, maize stored alone was warm and mold kernels were on the sides of the barrels whereas for the maize-amaranth mixture, it wasn't that all warm and no mold kernels were observed. The maize-amaranth mixture storage could be the future to eliminate and/or minimize spoilage of maize in metallic barrels and this would address Navarro (2006), opinion that metallic silos have limited use due to moisture migration that leads to mold maize.

Maize moisture

There was a decrease in moisture content of both treatments for the first 40 days probably due to moisture equilibrium establishment of the stored products with the surrounding environment (Bern et al., 2013) and moisture exchange between amaranth and maize (Table 3.1). The moisture changes for grain amaranth were not monitored. After 40 d, there was an increase in the average moisture content probably due to respiration of maize weevils (Bern et al., 2013) as modeled by the combustion of carbohydrate equation. Moisture content among the two treatments was not significantly different at 0 and 40 d, whereas it was significantly higher for maize stored alone (control) at 80 ($p = 0.0005$), 120 ($p = 0.0036$), and 160 days ($p = 0.0026$) (Table 3.1). Also analysis of moisture within each treatment was done over time (Table 3.1).

Visible mechanical damage

Results of visible mechanical damage for the maize-amaranth storage were almost constant throughout the experimental period whereas that of maize alone (control) was increasing with time (Table 3.1). The almost constant results were probably due to the observed weevils which were mostly on top of the barrel since when weevils crawled onto the top, they couldn’t penetrate again through the amaranth into the lower part of the barrel thus damage was concentrated in a specific area. The increasing percentage of mechanical damage was due to weevil population increase. Mechanical damage between treatments was not significantly different at 0 and 40 days while it was significantly higher for the control at 80 ($p = 0.002$), 120 ($p = 0.001$), and 160 days ($p = 0.0017$). Laswai et al., 2013 observed an increasing trend of mechanical damage with small grains of crotalatia seeds, finger millet, and sorghum used as physical control measure of post-harvest.

Broken corn and foreign material

Results of broken corn and foreign material for the maize-amaranth storage were almost constant throughout the experimental period whereas that of maize stored alone were increasing with time (Table 3.1). The almost constant results were probably due to the observed weevils which were mostly in the top end of the barrel since, when weevils crawled to the top; they couldn’t penetrate again through the amaranth into the lower part of the barrel. Amaranth filled the inter-granular spaces between the kernels. The increasing BCFM was due to weevil population increase. The BCFM for the control was significantly higher at

40 (p = 0.0074), 80 (p = 0.0011), 120 (p = 0.0008), and 160 days (p = 0.0005). Also analysis of BCFM within each treatment was done over time (Table 3.1).

Test weight

Test weight declined with time in both treatments but there was a greater decline at 120 and 160 days for stored maize in comparison to the maize-amaranth probably because more dry matter and/or endosperm loss caused by the increasing number of weevils was more. From observation, since weevils were on top for maize-amaranth storage, top kernels had almost nothing left inside them. Tukey's mean comparison of the two treatments was not significantly different at 0, 40, and 120 d whereas it was significantly higher for maize-amaranth mixture at 80 (p = 0.0249), and 160 days (p = 0.0161). Also analysis within each treatment was done over time (Table 3.1). As the moisture content was increasing, there was an expected decrease in test weight (Bern and Brumm, 2009) however damage and/or deterioration of kernels due to maize weevils may have contributed to test weight decline. Raw data is shown in Appendix B.

Conclusions

Based on this research

- Blending maize with amaranth (50/50 by volume) during 160 d of storage reduced maize weevil population by 46% compared to maize stored alone.
- The number of weevils between the two treatments was significantly higher for maize stored alone at 160 days.
- BCFM for maize stored alone was significantly higher at 40, 80, 120, and 160 days.
- Moisture content was significantly higher for maize stored alone at 80, 120, and 160 days.

- Test weight was significantly higher for maize-amaranth mixture at 80, and 160 days.
- Relative humidity and temperature for maize store alone was significantly higher at-all-times compared to maize-amaranth mixture.
- The same experimental set-up should be done with an extra layer of amaranth on top to completely cover the maize kernels that were exposed to the maize weevils.

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CHAPTER 4: EFFECT OF STORAGE CONTAINER PHYSICAL DISTURBANCE ON MAIZE WEEVIL MORTALITY

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Abstract

Maize is an important agricultural crop and among the most widely grown in the world. Maize, wheat, and rice supply at least 30% of the food calories in developing countries. Before the world thinks of increasing maize production due to increasing demand, there is also critical need to minimize post-harvest losses especially for smallholder farmers. Cheap, effective, affordable and easy to implement technologies will be of significant contribution to reduce post-harvest losses of grain during storage. Maize weevils cause post-harvest losses of maize thus the objective of this research was to investigate the effect of storage container physical disturbance on maize weevil mortality. The experiment consisted of two treatments: control and disturbed containers, three replications, four times of data collection (40, 80, 120, and 160 days). Clean plastic ground coffee containers were used for this experiment loaded with 1 kg of maize and 25 live weevils/kg. Every twelve hours, the containers to be disturbed were manually rolled one circumference up-to the original position. Number of live weevils and mechanical damage (MD) results were significantly higher for control treatment at 80, 120, and 160 days. Broken maize and foreign material (BCFM) was significantly higher for the control at 160 days. Test weight (TW) was significantly higher for the physically disturbed treatment at 160 days. Physical disturbance of storage containers reduced maize weevil population by 81% compared to the control.

Keywords: Maize, post-harvest losses, maize weevil, test weight

Introduction

Maize is an important agricultural crop and among the most widely grown in the world (CIMMYT and IITA, 2011). Maize in combination with wheat and rice supply 30% of the food calories to more than 4.5 billion people in 94 developing countries (von Braun et al., 2010). Maize plays a big role in the livelihoods of millions of smallholder farmers as an affordable food in both low and middle income countries in which it's used for food, feed and income.

Before the world can think of increasing maize production with regard to increasing demand, changes in natural resource depletion, food crisis, global financial crisis, increasing poverty and emerging climate changes, there is a need to minimize post-harvest losses (PHLs) to reduce the number of hungry and chronically undernourished people in the world that is about 870 million people and the majority of them (850 million) are in developing countries (FAO et al., 2012). PHL reduction is increasingly recognized as part of the integrated approach to ensuring the full realization of agricultural potential to meet the world's increasing food and energy needs (FAO, 2014; WorldBank et al., 2011). PHL reduction is a critical objective because those losses are not only a waste of valuable food but also resources such as labor, seeds, and land. Most PHLs occurs during storage and maize weevil (*Sitophillus zeamais*) is the critical PHLs insect of stored maize in the tropics (Jacobs and Calvin, 2001; Longstaff, 1986, 1981).

Quentin et al. (1991) investigated the effect of bean tumbling to control the bean bruchids during storage. It was assumed that when beans are physically disturbed numerous times, the larva not damaged by disturbance would capitulate due to exhaustion of energy reserves before gaining access to the cotyledon. The experiment consisted of tumbling and stationary treatments: 0.8-L glass jars, 16-L plastic buckets, and 45-kg sacks. Each of the storage container types was either loaded half-way with 100% intact or 50% damaged beans. Glass jars, plastic buckets, and sacks were also loaded with 30, 480, and 1000 bean bruchids respectively. For jars, and plastic buckets: two plastic tubes were fixed into the inside walls to act as baffles so as to avoid just sliding of beans during tumbling and physical disturbance was done after every eight hours by rotating them through 360^0C back to their original positions. Sacks were turned end-over-end two to three times a day. There was reduction in the number of bean bruchids in physically disturbed compared to stationary treatment as follows: 98% (for 100% intact beans) in glass jars, 95% (for 50% intact beans) in glass jars, 98% (for >98% intact beans) in plastic buckets, 97% (for >98% intact beans) in sacks. A 97% overall mean reduction in bean bruchid population was noticed due to storage container physical disturbance. Quentin et al. (1991), got good results for the bean bruchids, and this prompted us to investigate the same treatment effects on the maize weevil. Furthermore, turning stored wheat effectively controlled insects and mites (Muir et al., 1977). Bailey (1969), observed the same situation for the immature stages of grain weevil *Sitophilus granarius* in wheat. From their research, Cotton & Gray 1948 concluded that the beneficial disturbance of turning stored grain is a decline in temperature and to a lower extent moisture content though Gay 1941b and Gay 1941a reported that there is no significant mean temperature difference during turning and this was confirmed by Watters 1963 who concluded that there is minute heat lost to the external environment even when the ambient temperature is low. Heslop & Ray 1959 reported that there was increased oxygen consumption due to applied physical stress to cockroach *Periplaneta Americana*.

Effective, affordable and easy to implement technologies will be of significant contribution to reducing post-harvest losses of grain during storage. The objective of this research was to investigate the effect of storage container physical disturbance on maize weevil mortality.

Methods and Materials

Containers

Clean plastic ground coffee containers (net 788 g) were used for this experiment (Fig 4.1). Since the containers had two internal baffles, a third 1.5 x 1.5 x 10 cm wooden baffle was fixed in by means of screws for the containers that were to be physically disturbed. Holes of 10 cm in diameter were cut through the lids using a hole cutter and then ultra-sun block solar screens (New York wire, P.O. Box 866, Mt. Wolf, PA 17347, USA) were glued with silicon glue. The lids with screens were held on the containers by use of two rubber bands per container.

Figure 4.1. Plastic ground coffee container

Maize

Commercial comingled bulk maize used in the experiment was purchased from West Central Coop Elevator (1095 T Ave, Boone, IA 50036). Each plastic container was loaded with 1kg of maize which had on average a moisture content of 13.6%.

Weevils

The maize weevils for this experiment *(S. zeamais)* were obtained from an infested container of maize in the biomaterials laboratory at Iowa State University. The weevils were separated and retrieved from the infected maize through sieving method. Screen of 0.99-mm (2.5/64 in) diameter hole diameter retained the weevils. Twenty-five live maize weevils were loaded into each container.

Experimental design

The experiment consisted of two treatments: control and disturbed containers (Fig 4.2), three replications, different storage times (40, 80, 120 and 160 days) totaling 2x3x4=24 containers. Twenty five weevils/kg of maize were loaded into each of the containers which were then laid longitudinally into a chamber maintained at 27^0C. Every twelve hours, the containers to be disturbed were manually rolled one circumference.

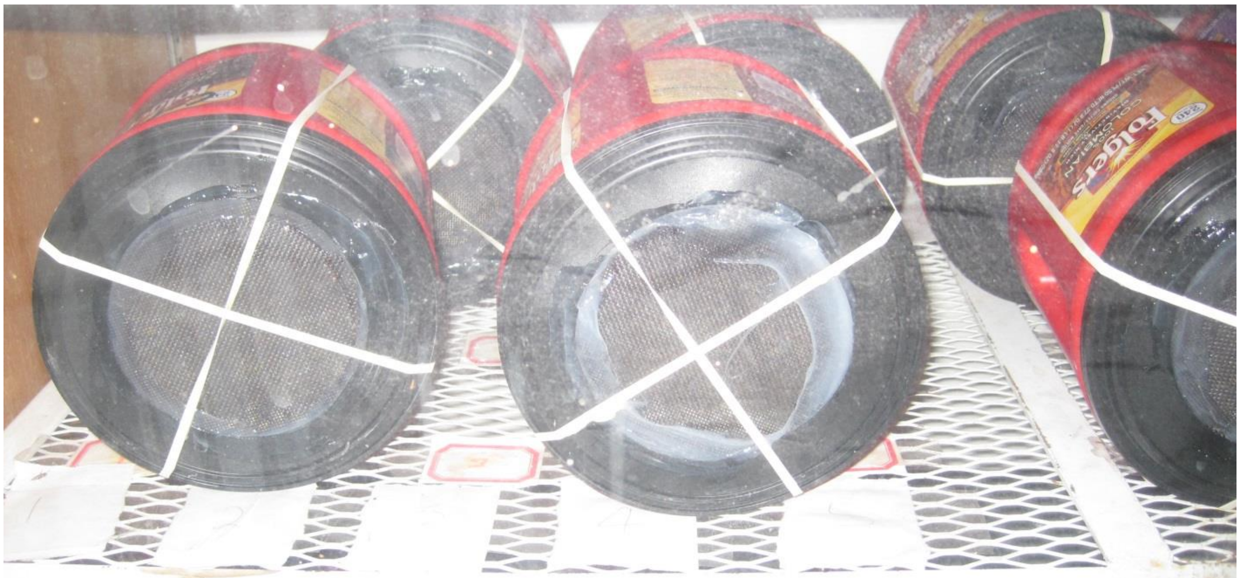

Figure 4.2. Experimental set-up of physically disturbed vs. control treatments

Measurements

After every 40 d up to 160 d, six containers (three for each treatment) were picked randomly from the experimental chamber for data collection. Weevil mortality was determined (Gullan and Cranston, 2010; Yakubu et al., 2010). Container contents were analyzed for broken maize and foreign material (BCFM) (USDA, 2013b), moisture content (ASABE, 2006), test weight (TW) (USDA, 1996) and mechanical damage (MD) (Steele, 1967).

Statistical analysis

Data analysis was done using JMP Pro 10 (Tukey's mean comparison at 5% level) and MS Excel descriptive analysis.

Results and Discussion

Live weevils

Live maize weevils declined from 25 to a range between 13 and 3 live weevils/kg of maize for the first 40 d in all containers. This was probably because some weevils were ending their life span. For the rest of the time periods, control containers showed an increase in the number of live weevils because they didn't experience any disturbance whereas disturbed containers continued to show a decline and at certain times there was zero number of live maize weevils thus physical disturbance lowered the maize weevil population relative to the control treatment (Table 4.1). Live weevil results were not significantly different at 0 and 40 d between treatments but there were significantly higher for control treatment at 80 ($p=0.0016$, $R^2=93.6\%$),), 120 ($p=0.0030$, $R^2=91.2\%$), and 160 d ($p=0.0006$, $R^2=96.1\%$). Analysis of the results with time was also done for each treatment (Table 4.1). Raw data is shown in Appendix C. On average the disturbance reduced the number of live weevils by 81% compared to the control treatment.

Table 4.1. Tukey's mean comparison of live weevils and maize quality values for physical disturbance versus control experiment

Item	Treatment	T=0 d	T=40 d	T=80 d	T=120 d	T=160 d
Number of live weevils/kg	Control	$25±0^{Ab}$	$11±1^{Ab}$	$15±2^{Ab}$	$40±8^{Aa}$	$44±5^{Aa}$
	Disturbed	$25±0^{Aa}$	$6±3^{Ab}$	$1±2^{Bb}$	$1±2^{Bb}$	$3±2^{Bb}$
MC (%)	Control	$13.7±0.2^{Ma}$	$13.5±0.2^{Ma}$	$13.3±0.1^{Ma}$	$13.4±0.2^{Ma}$	$13.2±0.2^{Ma}$
	Disturbed	$13.7±0.1^{Ma}$	$13.5±0.2^{Mab}$	$13.3±0.2^{Mabc}$	$13.2±0.1^{Mbc}$	$13.0±0.0^{Mc}$
MD (%)	Control	$14.4±0.5^{Gc}$	$16.1±1.3^{Gc}$	$16.8±0.7^{Gbc}$	$20.1±1.8^{Gab}$	$22.0±0.4^{Ga}$
	Disturbed	$14.7±0.3^{Ga}$	$15.3±0.5^{Ga}$	$15.1±0.1^{Ha}$	$15.1±0.2^{Ha}$	$14.1±0.1^{Ha}$
BCFM (%)	Control	$0.0±0.0^{Kb}$	$0.57±0.1^{Ka}$	$0.60±0.1^{Ka}$	$0.82±0.2^{Ka}$	$0.96±0.2^{Ka}$
	Disturbed	$0.0±0.0^{Kb}$	$0.59±0.1^{Ka}$	$0.68±0.0^{Ka}$	$0.65±0.2^{Ka}$	$0.45±0.0^{La}$
TW (Ib/bu)	Control	$56.7±0.0^{Ra}$	$56.6±0.0^{Rab}$	$56.5±0.0^{Rab}$	$56.3±0.2^{Rbc}$	$56.1±0.1^{Rc}$
	Disturbed	$56.7±0.1^{Ra}$	$56.6±0.1^{Ra}$	$56.5±0.1^{Ra}$	$56.5±0.1^{Ra}$	$56.5±0.1^{Sa}$

Values not followed by same upper case letter at each time for each item, and levels not connected by same lower case letter in each treatment are significantly different at 0.05 level

Moisture content

Moisture content during the experiment ranged between 13.9% and 12.9% w.b (Table 4.1). It declined with time as expected probably due to establishing an equilibrium moisture (Carl. J. Bern et al., 2013b). Our results were not in line with Cotton & Gray 1948 conclusion that disturbance by turning of stored grain leads to a lower decline in moisture content since for our experiment decline was observed in both control and physically disturbed containers. Moisture content was not significantly different between treatments throughout the whole period of the experiment (Table 4.1). Raw data is shown in Appendix C.

Mechanical damage

At the start of the experiment (T = 0 d), approximately 14% of the maize kernels were mechanically damaged. The control containers showed an increasing trend with time to approximately 23% mechanical damage as the highest whereas physically disturbed storage containers showed almost constant mechanical damage values of about 15% which is almost the initial recorded value. The low number of weevils in disturbed containers couldn't contribute much in mechanical damage. The increasing trend for controls was attributed to the increasing number of maize weevils that cause more damage to kernels whereas physically disturbed results might have been due to the declining number of weevils as expected. Mechanical damage between treatments was not significantly different at 0 and 40 d but they were significantly higher for control treatment at 80 ($p=0.0297$, $R^2=73.2\%$),), 120 ($p=0.0176$, $R^2=79.1\%$), and 160 d ($p<0.0001$, $R^2=99.5\%$) (Table 4.1). When Cotton and Gray, 1948 and Joffe, 1963 periodically transferred large stores of grain from one bin to another, they noticed reduced pest damage which may have been due to physical disturbance. The major effect of grain transfer and/or disturbance is to reduce damage impacts by insects (Bailey, 1969; Joffe and Clarke, 1963; Loschiavo, 1978).

Broken maize and foreign material

Broken maize and foreign material (BCFM) results throughout the experiment period ranged between 0.40% and 1.11%. Physically disturbed containers showed little increase in BCFM and this again was attributed to maize weevil population density decline that couldn't lead to increase in BCFM. On the other hand, control treatment showed an increasing trend as expected probably due to increasing number of live weevils. Broken maize and foreign material between treatments was not significantly different at 0, 40, 80, and 120 d but it was significantly higher for control treatment at 160 ($p=0.0162$, $R^2=79.9\%$), (Table 4.1).

Test weight

Test weight results ranged between 56.7 to 56.0 Ib/bu throughout the 160 d of the experiment. Physically disturbed container test weight results declined slightly almost to a constant value whereas the controls showed higher decline compared to physically disturbed containers probably because of the increasing number of live weevils. Bern and Brumm (2009), noted that as maize is losing moisture, there is a predictable increase in test weight though it wasn't the case for our experiment probably due to changes in the dry matter or deterioration that could have occurred due to infestation caused by maize weevils. Test weight between treatments was not significantly different at 0, 40, 80, and 120 d but it was significantly higher for control treatment at 160 d (p=0.0257, R^2=75.0%) (Table 4.1). Analysis of the results with time was also done for each treatment (Table 4.1).

Conclusions

The experiment was successfully carried out and we conclude that;

- Physical disturbance of storage containers reduced maize weevil population by 81% after 160 d compared to the control treatment.
- Number of live weevils and mechanical damage results were significantly higher for control treatment at 80, 120, and 160 days.
- Broken maize and foreign material between treatments was significantly higher for control treatment at 160 days.
- Test weight between treatments was significantly higher for control treatment at 160 days.

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CHAPTER 5: GENERAL CONCLUSIONS

Maize storage for smallholder farmers still remains a challenge to many in the world and that is why this research was based on finding out pesticide free methods that can be used by these farmers. The objectives of this research were: a) to evaluate the effectiveness of 208-L (55-gallon) steel barrels for hermetic maize storage and specifically to: determine weevil mortality for hermetically sealed grain, determine quality of hermetically sealed grain, and determine whether hermetically sealed grain becomes re-infested when unsealed, b) to investigate the effect of amaranth-maize blending during storage on maize weevils mortality versus maize stored alone, and c) to investigate the effect of storage container physical disturbance on maize weevil mortality.

The hermetic versus non-hermetic storage experiment resulted in effective control of maize weevils by hermetic storage without use of insecticides. The time period to achieve oxygen levels at which maize weevil life ceases was reached early than calculated. This can be thought of as an advantage of having more maize weevil population and maize itself that might create an environment having more CO_2 due to respiration leading to their death faster.

The maize-amaranth blending experiment results are promising since it’s the first of the kind experiment to be done with quite a large storage container of 208-L (55-gal) with small grains such as amaranth. The amaranth seems to fill the inter-spaces between kernels that restrict movement of the maize weevils. When weevils crawl through the amaranth from the lower sections of the storage container to the top, they cannot crawl and/or penetrate again to infest the lower section kernels thus they end-up being destructive to the top kernels. Also, there was no moldy corn on maize-amaranth barrel wall observed. The maize-amaranth mixture reduced maize population increase by 46% after 160 days of storage.

Physical disturbance is a simple approach that controls maize weevil. The number of weevils showed a declining trend with disturbed containers whereas control containers showed an increasing weevil trend. As it takes time for female weevils to bore through a kernel and to deposit an egg, when subjected to physical disturbance, they have to try again boring which in end stress, dying before laying eggs. Physical disturbance reduced maize population increase by 81% after 160 days of storage.

The overall conclusion is that there are effective low-cost ways to control maize weevils by hermetic storage, physical disturbance and blending maize with amaranth during storage. Hermetic storage is the best among the researched methods to effectively control the maize weevils, followed by physical disturbance and then maize-amaranth mixture.

Future research recommendations

Based on this research, possible future research can be:

1) Investigate the possible causes and how to eliminate and/or minimize maize spoilage on barrel walls. This experiment will seek to minimize and/or eliminate molds that were observed and the aflatoxin detected from samples picked from barrel walls.
2) Hermetic storage should be investigated without letting the weevils first go first through lifecycles to increase in population. This experiment will investigate if kernel spoilage occurs on barrel walls if hermetic sealing in done from the first day of storage.
3) Investigate how long it takes for a female maize weevil to bore through a kernel. This test will establish how frequently it is necessary to disturb the weevils.
4) Setting up the same maize-amaranth experiment (50:50 by volume) but having an extra layer of amaranth on top to investigate if this can help completely control the

maize weevil. This extra layer will reduce and/or eliminate the maize kernels that were available during our experiment.

5) Due to observation of no spoilt kernels for maize blended with grain amaranth on barrel walls, more research should be done to quantify the observations. This experiment may lead to hermetic and maize-amaranth mixture methods being used together by smallholder farmers to eliminate weevils while experiencing no moldy maize in metallic storage containers.
6) Investigate the effect of physical disturbance using larger storage containers. Since farmers use larger storage containers compared to what we investigated in laboratory setting, it is necessary to find out what will happen in real life.
7) Implement and test the researched methods in a developing country. These tests will help determine if the investigated methods are feasible.

APPENDIX A: HERMETIC VERSES NON-HERMETIC DATA

Estimated time to 100% mortality

Calculations below are the estimated mortality time at different population densities. Interpolation and extrapolation was applied for the weevil population densities of our experiment.

$$Mortality\,days = \frac{0.2099(((\frac{\%voids}{100})*(\frac{\%fill}{100})*(barrelvol))+((\frac{\%headspace}{100})*(barrelvol)))}{(\#weevils*(Oxy\,cm^3\,weevil^{-1}\,day^{-1})}$$

Source : (Yakubu, 2009)

	Barrel computations		
	Dia(in)	22.5	
	Height(in)	34.5	
	1 bu maize	1.245	ft^3
	56 lb	25.4	kg
	Maize bulk density(lb/ft^3)	45	
	Barrel vol (in^3)	13,717.47	
	Barrel vol (cm^3)	224,789.06	
	Barrel vol (ft^3)	7.94	
	Maize mass (bu)	6.38	
	Maize mass (kg)	161.96	
	Total maize mass for 9 barrels (bu)	47.82	
	Total maize mass for 9 barrels + 8% Misc (bu)	51.65	
	Total maize mass for 6 barrels + 8% Misc (bu)	34.43	
	Assuming different weevils/kg of corn		
	Weevils/kg	Total # weevils	
	50	8098	
	100	16196	
	150	24293	
	200	32391	
	Mortality calculations		
	Maize particle density (g/cm^3)	1.2601	

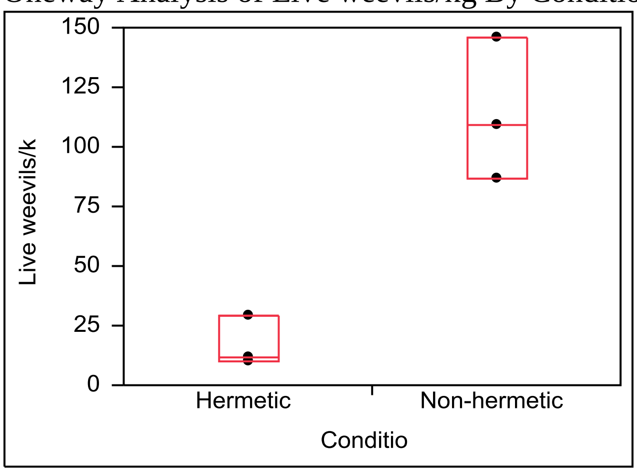
Maize bulk density (g/cm^3)	0.722	
Voids (%)	42.70	
Fill (%) (assumed)	95	
Headspace (%) (assumed)	5	
Oxy cm^3 $weevil^{-1}day^{-1}$ at 27^0C	0.18	At 27^0C and 13%

Mortality days at @ Weevils/kg	Days
50	15
100	8
150	5
200	4

Live maize weevils' analysis at different time periods for Hermetic-Non-hermetic experiment

T= 122 days

Oneway Analysis of Live weevils/kg By Condition



Oneway Anova

Summary of Fit

Rsquare	0.876098
Adj Rsquare	0.845122
Root Mean Square Error	22.33831
Mean of Response	65.5
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Condition	1	14113.500	14113.5	28.2836	0.0060*
Error	4	1996.000	499.0		
C. Total	5	16109.500			

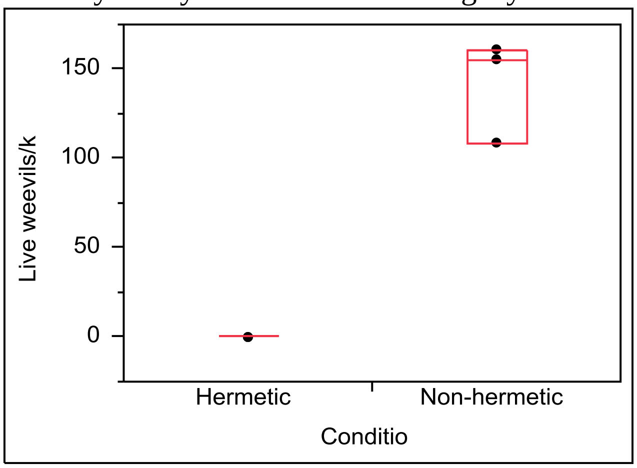
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level			**Mean**
Non-hermetic	A		114.00000
Hermetic		B	17.00000

Levels not connected by same letter are significantly different.

T=150 days

Oneway Analysis of Live weevils/kg By Condition



Oneway Anova
Summary of Fit

Rsquare	0.946768
Adj Rsquare	0.933459
Root Mean Square Error	20.52235
Mean of Response	70.66667
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Condition	1	29962.667	29962.7	71.1421	0.0011*
Error	4	1684.667	421.2		
C. Total	5	31647.333			

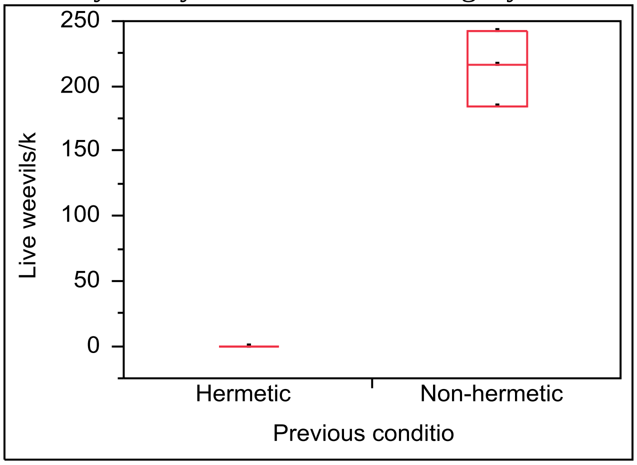
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level			**Mean**
Non-hermetic	A		141.33333
Hermetic		B	0.00000

Levels not connected by same letter are significantly different.

T=190 days

Oneway Analysis of Live weevils/kg By Previous condition



Oneway Anova

Summary of Fit

Rsquare	0.976017
Adj Rsquare	0.970021
Root Mean Square Error	20.54264
Mean of Response	107
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Previous condition	1	68694.000	68694.0	162.7820	0.0002*
Error	4	1688.000	422.0		
C. Total	5	70382.000			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Connecting Letters Report

Level			**Mean**
Non-hermetic	A		214.00000
Hermetic		B	0.00000

Levels not connected by same letter are significantly different.

Aflatoxins

The samples in table below are for the observed moldy maize on the sides of steel barrels. Small portions were picked from the barrel wall sides for analysis. The results are not representative of all the maize in the barrels. Aflatoxin analysis was performed using a Charm ROSA-M reader (Charm Science, Inc 659 Andover Street Lawrence, MA 01843-1032 USA). It detects the sum of aflatoxins B1, B2, G1, and G2.

Sample name	**Ppb total aflatoxins**
Non-Hermetic barrel 1	2
Non-Hermetic barrel 2	1
Non-Hermetic barrel 3	2
Hermetic barrel 1	30
Hermetic barrel 2	3
Hermetic barrel 3	0

Raw data for the hermetic vs. non hermetic storage experiment

Average broken corn and foreign material (%) at different time periods of hermetic vs. non-hermetic treatments

Treatment	T= 0 days (Start of experiment)	T= 40 days (one weevil lifecycle)	T= 80 days (two weevil lifecycle)	T= 120 days (three barrels HS)	T= 122 days (three HS barrels opened)	T= 150 days (three HS barrels opened)	T= 190 days (end of experiment)
HS1	1.65	1.84	2.41	2.50	2.78	3.39	2.33
HS2	1.48	1.77	2.16	3.33	3.76	3.20	2.78
HS3	1.48	1.70	2.36	2.61	3.14	2.50	1.76
NH1	2.12	2.01	2.36	2.56	2.56	2.75	2.00
NH2	1.65	1.90	2.55	3.17	3.17	3.62	2.51
NH3	1.17	1.82	1.94	2.73	2.73	3.38	2.63
Avg_all	1.60	1.84	2.30	2.82	3.02	3.14	2.33
Avg_Sealed	1.54	1.77	2.31	2.82	3.22	3.03	2.29
Avg_unsealed	1.65	1.91	2.28	2.82	2.82	3.25	2.38
Std (HS)	0.1	0.1	0.1	0.5	0.5	0.5	0.5
Std (NH)	0.5	0.1	0.3	0.3	0.3	0.4	0.3

Average test weight (Ib/bu) at different time periods of hermetic vs. non-hermetic treatments

Treatment	T= 0 days (Start of experiment)	T= 40 days (one weevil lifecycle)	T= 80 days (two weevil lifecycle)	T= 120 days (three barrels HS)	T= 122 days (three HS barrels opened)	T= 150 days (three HS barrels opened)	T= 190 days (end of experiment)
HS1	57.3	57.1	56.7	56.3	56.0	55.6	55.4
HS2	57.3	57.1	56.6	56.1	56.3	55.6	55.1
HS3	57.7	57.0	56.9	56.2	55.9	55.4	55.1
NH1	57.3	57.1	56.7	56.1	56.1	55.1	54.3
NH2	57.7	57.1	56.5	56.1	56.1	55.3	54.6
NH3	57.3	57.2	56.9	55.9	55.9	55.2	54.3
Avg_all	57.4	57.1	56.7	56.1	56.0	55.4	54.8
Avg_Sealed	57.4	57.1	56.7	56.2	56.1	55.5	55.2
Avg_unsealed	57.4	57.1	56.7	56.0	56.0	55.2	54.4
Std (HS)	0.2	0.1	0.2	0.1	0.2	0.1	0.2
Std (NH)	0.2	0.1	0.2	0.1	0.1	0.1	0.2

Average mechanical damage (%) at different time periods of hermetic vs. non-hermetic treatments

Treatment	T= 0 days (Start of experiment)	T= 40 days (one weevil lifecycle)	T= 80 days (two weevil lifecycle)	T= 120 days (three barrels HS)	T= 122 days (three HS barrels opened)	T= 150 days (three HS barrels opened)	T= 190 days (end of experiment)
HS1	6.62	4.40	4.84	7.55	7.87	6.45	7.77
HS2	3.75	3.48	4.06	5.94	6.04	5.63	6.91
HS3	3.43	3.52	4.58	5.72	6.87	5.95	7.50
NH1	3.43	4.16	4.82	6.73	6.73	6.43	8.35
NH2	5.04	3.97	4.60	6.13	6.13	8.04	9.62
NH3	3.75	3.58	3.89	5.54	5.54	7.73	10.46
Avg_all	4.34	3.85	4.47	6.27	6.53	6.71	8.43
Avg_Sealed	4.60	3.80	4.49	6.41	6.93	6.01	7.39
Avg_unsealed	4.07	3.91	4.44	6.13	6.13	7.40	9.48
Std (HS)	1.8	0.5	0.4	1.0	0.9	0.4	0.4
Std (NH)	0.9	0.3	0.5	0.6	0.6	0.9	1.1

Average moisture content (%) at different time periods of hermetic vs. non-hermetic treatments

Treatment	T= 0 days (Start of experiment)	T= 40 days (one weevil lifecycle)	T= 80 days (two weevil lifecycle)	T= 120 days (three barrels HS)	T= 122 days (three HS barrels opened)	T= 150 days (three HS barrels opened)	T= 190 days (end of experiment)
HS1	12.65	13.34	13.08	12.89	12.80	13.41	12.87
HS2	13.63	13.29	13.28	12.89	12.85	13.19	12.54
HS3	12.65	13.33	13.23	12.67	12.67	13.27	12.83
NH1	13.12	13.26	13.31	12.87	12.87	13.15	12.61
NH2	13.30	13.32	13.12	12.83	12.83	13.05	12.68
NH3	13.30	13.68	13.36	12.74	12.74	12.96	12.57
Avg_all	13.11	13.37	13.23	12.81	12.80	13.17	12.68
Avg_Sealed	12.98	13.32	13.20	12.82	12.78	13.29	12.75
Avg_unsealed	13.24	13.42	13.27	12.81	12.81	13.05	12.62
Std (HS)	0.6	0.0	0.1	0.1	0.1	0.1	0.2
Std (NH)	0.1	0.2	0.1	0.1	0.1	0.1	0.1

Average number of live Weevils/kg at different time periods of hermetic vs. non-hermetic treatments

Treatment	T= 0 days (Start of experiment)	T= 40 days (one weevil lifecycle)	T= 80 days (two weevil lifecycle)	T= 120 days (three barrels HS)	T= 122 days (three HS barrels opened)	T= 150 days (three HS barrels opened)	T= 190 days (end of experiment)
HS1	25	11	32	90	10	0	0
HS2	25	8	56	93	12	0	0
HS3	25	8	43	69	29	0	0
NH1	25	13	58	146	146	108	184
NH2	25	3	89	109	109	161	242
NH3	25	8	62	87	87	155	216
Avg_all	25	8	70	99	66	71	107
Avg_Sealed	25	9	44	84	17	0	0
Avg_unsealed	25	8	70	114	114	141	214
Std (HS)	0	2	12	13	11	0	0
Std (NH)	0	5	17	30	30	29	29

APPENDIX B: MAIZE KERNELS BLENDED WITH AMARANTH VERSUS MAIZE STORED ALONE EXPERIMENT RAW DATA

Maize and amaranth required for the experiment

Assumptions, 50% Amaranth : 50% Corn			
Barrel computations			
Dia(in)	22.5		
Height(in)	34.5		
1 bu maize	1.245	ft^3	
56 Ib	25.4	kg	
Maize bulk density(Ib/ft^3)	45		
	Volume (in^3)		
	Barrel		
Barrel volume (in^3)	13,717.47		
Corn (50% of Barrel vol)	6,858.73	in^3	
Corn (50% of Barrel vol)	3.97	ft^3	
Corn (50% of Barrel vol)	3.19	bu	
Corn Mass @barrel (50% of Barrel vol)	80.98	kg	
Amaranth (50% of Barrel vol)	6,858.73	in^3	
Amaranth (50% of Barrel vol)	3.97	ft^3	
Amaranth bulk density	843	kg/m^3	Dokok et al., 1994.
1 in =	0.03	m	
Amaranth Mass required@barrel (50% of Barrel vol)	94.75	kg	
Total Amaranth needed for the 3 barrels	284.25	kg	
Total Amaranth + Miscellenous ~	306.99	kg	
Total Amaranth + Miscellenous ~	676.90	Ib	
Experiment set-up			
NOTES			
25 weevils/kg of maize			
After every 20.24~21 kg of maize + 23.69~24 kg of amaranth we would stir with hands to fill the interspaces between kernels			

Source: Dokok, L. A. A. Modhir, G. Halasova, I. Polacek and B. Hozova. 1994. Importance and utilization of amaranth in food industry Part I. John Wiley & Sons, Ltd. Vol. 38, Issue 4, pages 378-381

Average BCFM (%)

Treatment	T= 0 day	T= 40 days	T= 80 days	T= 120 days	T= 160 days
MA1	5.03	5.31	4.71	4.62	4.26
MA2	4.95	5.30	4.86	4.61	4.66
MA3	5.65	4.72	4.74	4.71	4.60
C1	5.66	7.76	7.89	8.05	8.31
C2	3.69	7.14	7.48	7.58	7.75
C3	5.02	6.53	6.82	6.97	7.31
Avg_all	5.00	6.12	6.08	6.09	6.15
Avg_ Maize-Amaranthr (MA)	5.21	5.11	4.77	4.65	4.51
Avg_Control (C)	4.79	7.14	7.40	7.53	7.79
STD (MA)	0.4	0.3	0.1	0.1	0.2
STD (C)	1.0	0.6	0.5	0.5	0.5

Average Test weght (Ib/bu)

Treatment	T= 0 day	T= 40 days	T= 80 days	T= 120 days	T= 160 days
MA1	56.7	56.1	56.0	55.6	54.6
MA2	56.4	56.1	56.0	55.8	54.4
MA3	56.6	56.1	56.1	55.2	54.4
C1	56.2	55.8	55.8	54.9	53.9
C2	56.6	55.9	55.7	54.7	54.0
C3	57.1	56.3	55.9	55.2	53.5
Avg_all	56.6	56.1	55.9	55.2	54.1
Avg_ Maize-Amaranthr (MA)	56.6	56.1	56.0	55.6	54.5
Avg_Control (C)	56.6	56.0	55.8	54.9	53.8
STD (MA)	0.2	0.0	0.0	0.3	0.1
STD (C)	0.5	0.2	0.1	0.2	0.3

Average MD (%)

Treatment	T= 0 day	T= 40 days	T= 80 days	T= 120 days	T= 160 days
MA1	18.07	18.42	18.30	18.61	18.74
MA2	15.95	15.47	15.71	15.81	16.12
MA3	14.18	13.14	14.69	15.55	15.82
C1	14.06	15.34	24.15	25.94	28.36
C2	16.97	17.27	25.02	26.68	27.13
C3	13.56	16.00	23.86	25.11	25.05
Avg_all	15.47	15.94	20.29	21.28	21.87
Avg_ Maize-Amaranthr (MA)	16.07	15.68	16.23	16.66	16.90
Avg_Control (C)	14.86	16.20	24.34	25.91	26.85
STD (MA)	1.9	2.6	1.9	1.7	1.6
STD (C)	1.8	1.0	0.6	0.8	1.7

Average MC (%)

Treatment	T= 0 day	T= 40 days	T= 80 days	T= 120 days	T= 160 days
MA1	12.95	12.15	12.19	12.20	12.55
MA2	13.19	12.09	12.28	12.31	12.62
MA3	12.77	12.07	12.21	12.08	12.57
C1	13.25	12.46	12.65	12.71	12.72
C2	13.00	12.55	12.62	12.59	12.74
C3	12.99	12.49	12.56	12.70	12.76
Avg_all	13.03	12.30	12.42	12.43	12.66
Avg_ Maize-Amaranth (MA)	12.97	12.10	12.23	12.20	12.58
Avg_Control (C)	13.08	12.50	12.61	12.67	12.74
STD (MA)	0.2	0.0	0.0	0.1	0.0
STD (C)	0.1	0.0	0.0	0.1	0.0

Number of live Weevils/kg

Treatment	T= 0 day	T= 40 days	T= 80 days	T= 120 days	T= 160 days
MA1	25	36	62	64	77
MA2	25	43	82	80	84
MA3	25	38	77	60	57
C1	25	38	67	106	187
C2	25	29	62	92	151
C3	25	26	123	210	308
Avg_all	25	35	79	102	144
Avg_ Maize-Amaranthr (MA)	25	39	74	68	73
Avg_Control (C)	25	31	84	136	215
STD (MA)	0	4	11	10	14
STD (C)	0	6	34	64	82

APPENDIX C: PHYSICAL DISTURBANCE EXPERIMENT RAW DATA

Treatment	Time (days)	BCFM (%)	MC (%)	TW (Ib/bu)	MD (%)	Live / kg
Control 1	0	0.00	13.51	56.7	14.00	25
Control 2	0	0.00	13.57	56.7	14.05	25
Control 3	0	0.00	13.89	56.7	15.16	25
Disturbed 1	0	0.00	13.57	56.7	14.26	25
Disturbed 2	0	0.00	13.73	56.6	15.04	25
Disturbed 3	0	0.00	13.65	56.7	14.92	25
Control 1	40	0.48	13.25	56.6	14.24	11
Control 2	40	0.49	13.55	56.6	16.73	13
Control 3	40	0.75	13.68	56.6	17.19	9
Disturbed 1	40	0.62	13.51	56.7	14.86	10
Disturbed 2	40	0.47	13.68	56.5	15.09	6
Disturbed 3	40	0.69	13.29	56.6	16.08	3
Control 1	80	0.51	13.16	56.5	15.79	14
Control 2	80	0.81	13.38	56.5	17.06	18
Control 3	80	0.49	13.47	56.5	17.55	13
Disturbed 1	80	0.66	13.19	56.6	15.02	3
Disturbed 2	80	0.67	13.57	56.4	14.93	0
Disturbed 3	80	0.72	13.07	56.6	15.19	0
Control 1	120	0.63	13.23	56.5	17.51	31
Control 2	120	0.83	13.20	56.4	21.45	38
Control 3	120	1.01	13.64	56.1	21.21	51
Disturbed 1	120	0.87	13.21	56.6	15.09	0
Disturbed 2	120	0.40	13.31	56.4	14.83	4
Disturbed 3	120	0.69	13.10	56.4	15.28	0
Control 1	160	1.11	13.16	56.3	22.93	39
Control 2	160	0.71	13.03	56.0	22.99	41
Control 3	160	1.07	13.44	56.0	22.19	51
Disturbed 1	160	0.43	12.94	56.6	14.90	0
Disturbed 2	160	0.47	13.04	56.4	15.05	5
Disturbed 3	160	0.46	13.03	56.5	14.79	5