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Reducing losses to maize stored on farms in East Africa using hermetic storage

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by

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Above all, I am grateful to God for making this possible, and I thank my family and friends, for their patience and constant support.

DEDICATION

This dissertation is dedicated to my sister-Mrs Amina Aladi Osagie, and her family (Dr. Austin A. Osagie, Austin. S. Osagie, Rabi. F. Osagie and Amira. E. Osagie)

ABSTRACT

Significant grain and seed losses occur during maize storage in East Africa. This is due to high ambient relative humidity, and the fact that storage ecosystems and the stored maize equilibrate with ambient moisture and temperature. The result is rapid pest multiplication and mold formation in the stored maize, leading to a high spoilage rate. This is particularly important considering that a large number of farmers store their maize in open-air storage that utilizes little or no chemical preservatives. Of these pests, the most economically important are the maize weevils, and to reduce losses, a non-chemical system that naturally eliminates them was developed. Three studies aimed at “Testing time to complete adult weevil mortality in hermetic storage”, testing the “effect of hermetic storage on maize seed germination”, and “using recycled edible oil containers for hermetic maize storage” were conducted to solve these problems.

The **first study** found significant ($p<0.0001$) treatment effects. A laboratory-scale study found mean adult weevil mortality and standard error of 94.2±10.77% for hermetic treatments versus 3.1 ±4.69% for non-hermetic treatments, while a field-scale study found 96.8±3.43% mean mortality and standard error for hermetic treatments versus 3.4±3.71%, for non-hermetic treatments. In the **second study**, hermetically stored (sealed) maize seeds had 98.7 to 99.5% germination rates versus 35.0 to 72.9% for non-hermetic (open-air) storage, over the 12-month seed storage period. The conclusion is that hermetic storage preserves seed viability, even when seeds are stored under ambient (atmospheric) conditions, and with weevils. In the **third study**, market surveys found edible oil containers available for

sale and reuse as hermetic storage containers, in East African markets, and a comparison of three cleaning methods showed that oil-drain plus water at 90 to100°C plus soap is the most effective, as well as the only one that met our cleaning objectives. Leftover oils following cleaning with oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap were 0.249g, 0.142g, and 0.004g, respectively. The 0.004g from the oil-drain plus water at 90 to100°C plus soap treatment compares favorably with 0.005 to 0.006 from the control (unused experimental units (20-L HDPE containers), which had no oil contaminants. Research results, therefore, indicate that using 3g of 99.44% pure Ivory soap and hot water per gram of soybean oil contaminant is enough to clean and sanitize soybean oil contaminated 20-L HDPE containers, for safe hermetic maize storage.

CHAPTER 1: GENERAL INTRODUCTION

1.1 THESIS ORGANIZATION

The information in this dissertation is organized into five chapters. The first chapter is the general introduction, with sections on thesis organization, literature review, and general objectives. The second chapter contains a paper entitled “Testing time to complete adult weevil mortality in hermetic storage”, the third chapter contains a paper entitled “Effects of hermetic storage on maize seed germination” and the fourth chapter contains a paper entitled “Using recycled edible oil containers for hermetic maize storage”. The fifth chapter is the “General conclusions” chapter, based on the information contained in chapters two, three, and four, and answering objectives from chapter one.

Chapters two, three, and four are prepared for publication in journals and are formatted according to the guidelines for papers submitted to those journals for publication.

1.2 LITERATURE REVIEW

1.2.1 East Africa

Most maize storage, by subsistence farmers, in East Africa involves the use of open-air storage facilities (Lindblad and Druben, 1980; Wiley-Blackwell, 2004; Akaninwor and Sodje, 2005). This allows for re-wetting and related pest (weevil, mold, birds, and rodent) activities, resulting in damage to stored maize. And studies

have shown that the combined effect of maize weevils and molds alone is capable of causing up to 100% maize damage (Demissie *et al*., 2008; Weinberg, et al., 2008). This is of economic importance, considering that weevil activity introduces molds, especially since subsistence farmers lack adequate drying equipment and maize may be stored while relatively moist and warm (Mendoza et al., 1982; Bankole, et al., 2005). Also important is the fact that agriculture employs 60 to 80% of the population (Bett and Nguyo, 2007; Minot, 2008), maize accounts for 50% of caloric intake (Sinha, 2007), at least 70% of maize seeds are sourced from prior year's harvest (Gemeda, et al., 2001; Dhliwayo, *et al*., 2003), and chemical maize preservatives used in post-harvest storage are toxic, costly, and often do not work (Korunic, 1998; IRRI, 2008). For these reasons, a natural and effective method capable of reducing losses in post-harvest storage is needed. This is especially important if it allows for the reuse of containers available in the local culture, for effective storage, while preventing the economic (quantitative and qualitative) losses associated with existing pests, and if it eliminates the use of toxic chemicals, and preserves food supply.

The non-chemical, natural and effective method chosen for reducing losses to maize stored on farms in East Africa is hermetic storage. Hermetic storage is a safe, cost-effective storage method that eliminates insects and molds through the synergistic effect of O_2 depletion and CO_2 accumulation in the storage ecosystem.

1.2.1.1 Geography

Located to East of Africa (Figure 1.1), East Africa is usually divided geographically into two sub-regions: the great lakes region (Uganda, Kenya, Tanzania, Rwanda, and Burundi) and the horn of Africa (Ethiopia, Eritrea, Sudan, Djibouti, and Somalia), based on types of vegetation, availability of water, and topography (De Groote, 2002).

Figure 1.1: Africa with East Africa inset (UND, 2010).

1.2.1.2 Agroecological zones

East Africa is also, generally, divided into three major agroecological zones, based on altitude- the lowlands (from the coast up to 600 meters), the mid-altitudes (600 to 1800 meters) and the highlands (above 1600 meters) (Figure 1.2). A general

precipitation pattern of East Africa is described here, and focuses on climatic factors (temperature, precipitation) that affect maize growth. This is because maize farmers in this region also make settlement decisions based on those factors (De Groote, 2002; Worku, et al., 2002), as can be seen from the population density (Figures 1.2 and 1.3) for the region, which is driven by geography. Areas of high density include the highlands, followed by the mid-altitudes (especially around Lake Victoria). However, the lowlands are usually dry and sparsely populated, except for the coastal strip (De Groote, 2002).

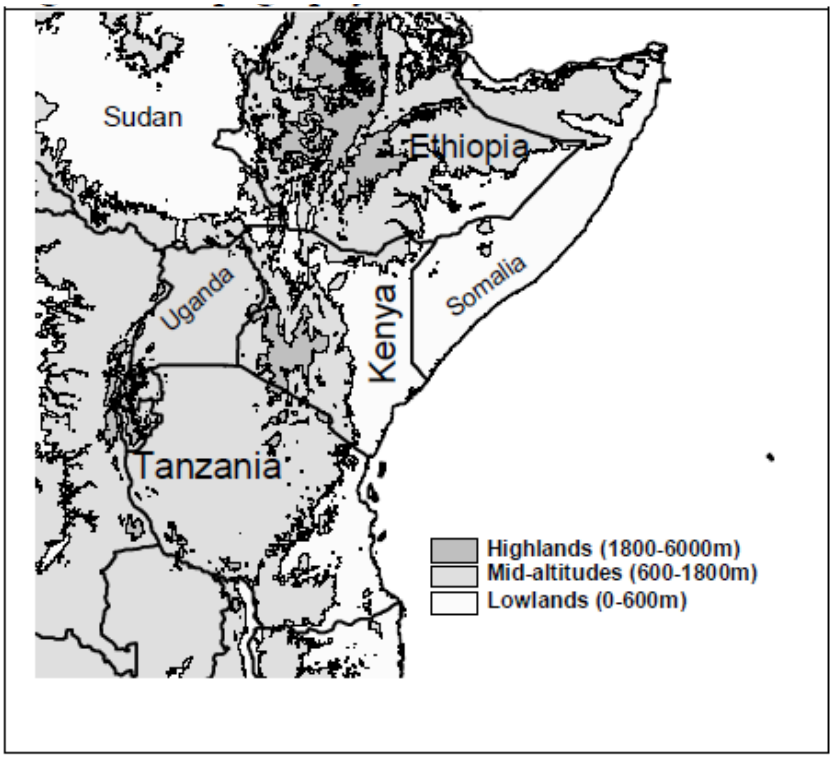

Figure 1.2: Topography of East Africa (De Groote, 2002).

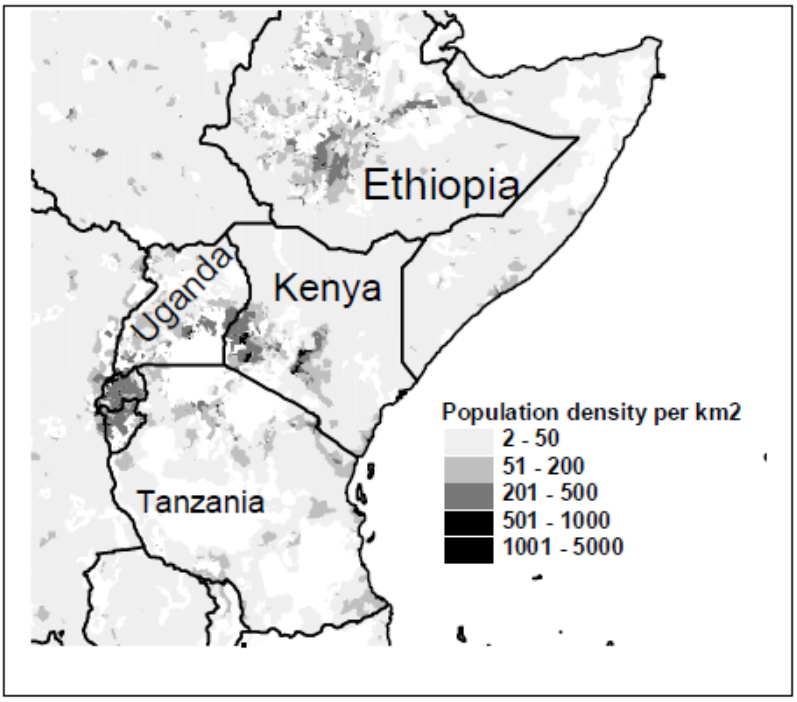

Figure 1.3: Population density of East Africa (De Groote, 2002).

1.2.1.3 Maize statistics

Maize statistics, with regards to total production, yield, area harvested, and seed quantity utilized for the year 2000 to 2008 (FAOSTAT, 2011) are presented in Table 1.1. The table shows the maize statistics for Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Sudan, and Uganda, with regards to size of area utilized for maize cultivation, yield per hectare, production volume, and amount of seed utilized in 2008-the last year for which the statistics is available.

Table 1.1: 2008 maize statistics for East Africa (FAOSTAT, 2011).

Country	Area Harvested (000 ha)	Yield (Mg/ha)	Production (000 Mg)	Seed (000 Mg)
Burundi	115.0	1.00	115.5	3.45
Djibouti	0.006	1.67	0.010	0.00
Eritrea	16.45	0.83	13.69	0.41
Ethiopia	1767	2.14	3776	44.2
Kenya	1700	1.39	2367	54.0
Rwanda	210.0	0.79	167.0	5.61
Somalia	235.0	0.42	99.00	7.05
Sudan	30.67	2.02	62.00	4.00
Uganda	862.0	1.47	1266	26.6
Tanzania	2848	1.25	3556	59.2
	778.4 (total)	**1.30 (average)**	**1142 (total)**	**20.5 (total)**

1.2.1.4 Climates of East Africa

Although there are regional differences and similarities in climatic conditions between and within East African countries (Kenya, Tanzania, Uganda and Burundi, Djibouti, Eritrea, Ethiopia, Rwanda, Sudan, and Somalia), the elements of climate (temperature, precipitation, relative humidity) are the best indicators of suitability of the different regions for maize cultivation. The primary criterion in the classification of climates is precipitation (rainfall), which also determines the seasons. These, in turn are dependent, to a large extent on temperature and relative humidity. The climate and the distribution of rainfall is described below, based on Leroux (2001). The distribution of rainfall, and the associated seasons determine the timing of rain-fed agriculture, as well as maize cultivation, harvest, and storage considerations. And the geography and rain pattern, including average quantities as a function of individual months are described below for each East African country.

Areas of little or no rainfall

These are areas with annual precipitation values between 0 and 100 mm, and include parts of deserts and some coastal strips. For instance, areas around Eastern Sahara desert, at Wadi Halfa (Sudan) experience less than 2 mm, of rainfalls, while Belet Uen and Gallacaio (Somalia) receive about 20 mm of rain annually, with fewer than 10 rain days and rear thunderstorms. And Port Sudan, northern Somalia, and the extreme eastern part of the Ogaden (Ethiopia) get about 100mm of rain.

Areas of low rainfall

These are areas with precipitation values between 100 and 500 mm, annually. They include countries in the “horn of Africa”, where 500 mm of precipitation occurs in Eastern Sudan, and northern Ethiopia (16°N). And precipitation values are below 200 mm, annually for most of Somali, the low-lying areas of Kenya (with the exception of the coastal plains), and the Galla plateau (Ethipia). Outside these large areas, exist isolated pockets where rainfall does not reach 500 mm per annum. These include Dodoma (Tanzania), parts of the dry diagonal (Kenya), and the area of Lake Magadi (at the exit from the Kenyan rift).

Areas of moderate rainfall

These areas experience annual precipitation values of between 500 and 1000 mm. The 500 mm isohyet include areas north of Raga (Sudan), Bahr el ghazal depression (Sudan), through Wau and Juba (Sudan) into lake Turkana depression (Kenya), north-eastern Uganda, the Mau plateaux (Kenya) and the Kenyan rift. The 1000 mm isohyet rounds the Ethiopian highlands, and the isolated Tanzanian

highlands, from where it passes through the plateau of Tanzania, then through lake Victoria (eastern African plateau-Tanzania, Uganda, Kenya), before forming a narrow corridor below the highlands of Rwanda and Burundi. Also included are areas along the Kipengere range, and the Mguru and Uluguru mountains (Tanzania), and the coast of Kenya, south of Lamu.

Areas of adequate rainfall

These areas experience precipitation values of between 1000 to less than 1500 mm, annually. They include southern-western Sudan, Rwanda, Burundi, two spurs passing around the Tanzanian plateau-one extending through Uganda alongside Lake Victoria into the western highlands and another, which lies across south-eastern Tanzania. This spur reaches the cost between Lamu (Kenya) and Lindi (Tanzania), and passes into the western side of Lake Nyasa (Tanzania, Malawi, Mozambique). The Ethiopian bastion is located, to the north, within the first spur and stands out from its surroundings.

Areas of high to very high rainfall

These areas experience annual precipitation exceeding 1500 mm. This threshold delimits the boundary of well-watered tropical Africa, where the essential minimum for maintaining the rainforest is 1500-2000 mm of annual rainfall, as occurs in the Congo Basin.

The Bukoba region on the western shores of Lake Victoria (Tanzania), and surrounding areas receive about 2081 mm of rainfall and experiences more than 200 storm days. The area of higher rainfall extends into the Kenyan highlands and

Tanzania. And storm activity declines eastwards from the lake, but annual rainfall amount again rises above 2000 mm within the bastion of the Ethiopian massif, at its highest, southern elevation. Annual rainfalls above 1500 mm are also associated with Rungwe massif (Tanzania), the Iringa horst (Tanzania-Mbinga district), the livingston (Tanzania) mountain and inyange range (Rwanda).

1.2.1.5 Climate in individual East African countries

Due to climate variations, between countries, in East Africa, a single city (the largest city), within each country has been chosen as a typical example (MSN weather, 2011b) of the country's climatic condition. Tables 1 to 10, below describe the temperature and precipitation of the largest city (capital) for each East African country, as a measure of how each city's climatic conditions fit the needs of maize during the growing season. The climate of each city is representative of the country in which it is located, although regional differences due to modification by relief and other climatic factors may produce slight variations, within countries, as described above. Rainfall is irregular, in lots of places, especially in countries located in the horn of Africa. Besides, reliable data is not available for some places.

Burundi-geography and climate

Burundi is an East African country located on the great lakes region, at latitude 3° 16' S and longitude 29° 18' E (MapXL, 2000). It is bordered by Rwanda, to the north and west, Congo, to the west and Tanzania to the East and south. Its capital is

Bujumbura, and Table 1.2, describes typical seasonal variations in temperature and precipitation.

Djibouti-geography and climate

Burundi is an East African country located on the horn of Africa, at latitude 11° 08' N, and longitude 42° 20'E (MapXL, 2000). Its capital is Djibouti city, and it is bordered to the north by Eritrea, to the East by the red sea, and to the west and south by Ethiopia. Table 1.3 describes typical seasonal variations in temperature and precipitation.

Table 1.2: Mean monthly precipitation and average temperatures, for Bujumbura (Burundi) (BBC, 2011a).

Climate data for Bujumbura, Burundi

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	34	32	32	31	31	31	31	33	33	33	33	34
Average high (°C)	28	28	28	28	28	29	29	30	31	30	28	28
Average low (°C)	19	19	19	19	19	18	17	18	19	20	19	19
Record low (°C)	14	15	14	15	16	13	11	13	14	14	15	16
Average precipitation (mm)	94	109	121	125	57	11	5	11	37	64	100	114

Table 1.3: Mean monthly precipitation and average temperatures, for Djibouti City (Djibouti) (BBC, 2011b).

Climate data for Djibouti city, Djibouti

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	34	34	37	38	44	47	47	47	44	39	36	34
Average high (°C)	29	29	31	32	34	37	41	39	36	33	31	29
Average low (°C)	23	24	25	26	28	30	31	29	29	27	25	23
Record low (°C)	19	18	21	21	21	23	22	22	23	21	18	17
Average precipitation (mm)	10	13	25	13	5	0	3	8	8	10	23	13

Eritrea-geography and climate

Eritrea is an East African country located in the horn of Africa, at latitudes 15° 19'N and longitudes 38° 55' E (MapXL, 2000). Its capital is Asmara, and it is bordered by Sudan and the red sea, in the north, Djibouti and red sea in the east and south, and mountains in the west. Table 1.4 describes typical seasonal variations in temperature and precipitation.

Table 1.4: Mean monthly precipitation, and average temperature for Asmara (Eritrea) (MSN weather, 2011a).

Climate data for Asmara, Eritrea

Month	J	F	M	A	M	J	J	A	S	O	N	D
Average high (°C)	31	31	33	36	38	38	38	38	38	37	34	32
Average low (°C)	22	22	24	26	28	30	30	30	28	26	24	22
Average precipitation (mm)	2.3	2.1	0.0	0.9	1.7	0.0	0.0	1.2	0.1	0.0	0.0	2.9

Ethiopia-geography and climate

Ethiopia is a country in the Horn of Africa, at latitudes 09° 02 N, and longitude 38° 42' E (MapXL, 2000). Its capital is Addis Ababa, and it is bordered by Eritrea to the north and north-east, Djibouti and Somalia to the East, Kenya to the south, and Sudan to the west and south-west. Table 1.5 describes typical seasonal variations in temperature and precipitation.

Table 1.5: Mean monthly precipitation and average temperatures, for Addis Ababa (Ethiopia) (BBC weather, 2011c).

Climate data for Addis Ababa, Ethiopia

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	28	30	29	31	33	34	31	29	27	33	27	28
Average high (°C)	24	24	25	25	25	23	21	21	22	24	23	23
Average low (°C)	6	8	9	10	10	9	10	10	9	7	6	5
Record low (°C)	2	2	3	4	4	7	7	6	3	2	1	0
Average precipitation (mm)	13	38	66	86	86	137	279	300	191	20	15	5

Uganda-geography and climate

Uganda is an East African country located at latitudes 00° 20' N, and longitude 32° 30' E (MapXL, 2000). Its capital is Kampala, and it is bordered by Kenya and Sudan to the north, Tanzania and Rwanda to the south, Congo to the west and Kenya to the East. Table 1.6 describes typical seasonal variations in temperature and precipitation.

Table 1.6: Mean monthly precipitation and average temperatures, for Kampala (Uganda) (BBC weather, 2011d).

Climate data for Kampala, Uganda

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	33	36	33	33	29	29	29	29	31	32	32	32
Average high (°C)	28	28	27	26	25	25	25	25	27	27	27	27
Average low (°C)	18	18	18	18	17	17	17	16	17	17	17	17
Record low (°C)	12	14	13	14	15	12	12	12	13	13	14	12
Average precipitation (mm)	46	61	130	175	147	74	46	86	91	97	122	99

Kenya-geography and climate

Kenya is an East African country located at latitude 01° 17’ S, and longitude 36° 48’E (MapXL, 2000). Its capital is Nairobi, and it is bordered by the rift valley and mountains to the north, Somalia and Ethiopia to the north-east, the Indian ocean to the south-east, Tanzania to the south, and lake Victoria to the south-west. Table 1.7 describes typical seasonal variations in temperature and precipitation.

Table 1.7: Mean monthly precipitation and average temperatures, for Nairobi (Kenya) (BBC weather, 2011e).

Climate data for Nairobi, Kenya

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	29	31	30	28	28	27	26	27	28	30	28	28
Average high (°C)	25	26	25	24	22	21	21	21	24	24	23	23
Average low (°C)	12	13	14	14	13	12	11	11	11	13	13	13
Record low (°C)	8	9	9	11	9	7	6	7	5	7	6	8
Average precipitation (mm)	38	64	125	211	158	46	15	23	31	53	109	86

Tanzania-geography and climate

Tanzania is an East African country located at latitudes 06 08' S, and longitude 35° 45'E (MapXL, 2000). Its capital is Dar Es Salaam, and it is bordered by Uganda to the north, Mozambique to the south, mountains to the north-east, the great lakes (Victoria, Tanganyika), and Zanzibar to the east, and an unnamed region to the west. Table 1.8 describes typical seasonal variations in temperature and precipitation.

Table 1.8: Mean monthly precipitation and average temperatures, for Dar Es Salaam. (Tanzania) (BBC weather, 2011f).

Climate data for Dar Es Salaam, Tanzania

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	35	35	36	35	33	32	32	32	33	33	34	35
Average high (°C)	31	31	31	30	29	29	28	28	28	29	30	31
Average low (°C)	25	25	24	23	22	20	19	19	19	21	22	24
Record low (°C)	21	20	21	19	18	16	16	15	16	17	19	21
Average precipitation (mm)	66	66	130	290	188	33	31	25	31	41	74	91

Rwanda-geography and climate

Rwanda is an East African country located at latitudes 01° 59'S, and longitude 30° 04'E (MapXL, 2000). Its capital is Kigali, and it is bordered by Uganda to the north, Tanzania to the east, Burundi to the south, and Congo to the west. Table 1.9 describes typical seasonal variations in temperature and precipitation.

Table 1.9: Mean monthly precipitation and average temperatures, for Kigali. (Rwanda) (BBC weather, 2011g).

Climate data for Kigali, Rwanda

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Average high (°C)	25	25	25	25	24	24	26	27	27	26	25	25
Average low (°C)	14	13	14	14	14	13	12	13	14	14	14	14
Record low (°C)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Average precipitation (mm)	111	156	140	183	164	23	7	27	63	102	110	93

Somalia-geography and climate

Somalia is an East African country located on the horn of Africa, at latitudes 02° 02'N, and longitude 45° 25'E (MapXL, 2000). Its capital is Mogadishu, and it is bordered by the gulf of Aden and Yemen to the north, Djibouti to the north-west, Kenya to the south-west, Ethiopia to the west, and the Indian ocean to the east, and south-east. Table 1.10 describes typical seasonal variations in temperature and precipitation.

Table 1.10: Mean monthly precipitation and average temperatures, for Mogadishu (Somalia) (BBC weather, 2011h).

Climate data for Mogadishu, Somalia

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	43	32	33	36	34	32	32	30	32	32	32	34
Average high (°C)	30	30	31	32	32	29	28	28	29	30	31	30
Average low (°C)	23	23	24	26	25	23	23	23	23	24	24	24
Record low (°C)	20	18	20	20	18	20	15	16	18	18	21	20
Average precipitation (mm)	0	0	0	58	58	97	64	48	25	23	41	13

Sudan-geography and climate

Sudan is an East African country located on the horn of Africa, at latitude 15° 31' N, and longitude 32° 35' E (MapXL, 2000). Its capital is Khartoum, and it is bordered by Egypt to the north, the red sea to the north-east, Eritrea and Ethiopia to the east, Kenya and Uganda, to the south-east, Congo and Central African republic to the south-west, Chad to the west, and Libya to the north-west. It is also split into east and west by the Nile river. Table 1.11 describes typical seasonal variations in temperature and precipitation.

Table 1.11: Mean monthly precipitation and average temperatures, for Khartoum (Sudan) (BBC weather, 2011i).

Climate data for Khartoum, Sudan

Month	J	F	M	A	M	J	J	A	S	O	N	D
Record high (°C)	40	44	45	47	47	48	47	43	45	45	42	40
Average high (°C)	32	34	38	41	42	41	38	37	39	40	36	33
Average low (°C)	15	16	19	22	25	26	25	24	25	24	20	17
Record low (°C)	5	7	9	12	16	19	18	18	16	17	13	7
Average precipitation (mm)	0	0	0	0	3	7	53	71	18	5	0	0

1.3 OPTIMAL CONDITIONS FOR MAIZE CULTIVATION

1.3.1 Precipitation and Maize

Desired annual precipitation for optimal maize cultivation is 500 to 800 mm (FAO, 1991a). Other authors describe marginal or reduced yield, if precipitation levels of 100 to 200 mm (per year) or drought occurs around the flowering period, and according to Heisey and Edmeades, (1998), up to 100% yield loss, can occur at 100 mm or less precipitation.

1.3.2 Temperature and maize

According to Bella, et al (2007), maize is grown most intensively in the northern hemisphere where the isotherm of July is between 21.1 and 26.7ºC, and can be grown everywhere, except for places where the growing period is too short or too cold. They described 19ºC-21ºC (June-August) as the lower threshold of the temperature optimum, as well as 24-26ºC as the optimum for the tassel phase and milk stage. And concluded that maize is not sensitive to temperature above 15ºC, during the ripening stage.

Campos, et al (2004) agrees that maize may be grown at 10 to 30ºC and at precipitation conditions as low as 200 mm, depending on variety. Since growing maize at the two ends of the temperature spectrum (10 and 30ºC) and low precipitation (200 mm), produces marginal, instead of optimal yield, it is important to recognize that growing maize under those temperature conditions require the use of abundant precipitation or the use of irrigation to prevent the exacerbation of temperature stress and stress associated with drought (Campos, et al., 2004; Lobell, et al., 2011).

1.3.3 Drought and maize

Drought refers to extended periods (months or years) when a region experiences shortage of rainfall. Regions, such as the horn of Africa, experience drought because of rainfall that is consistently below average precipitation, and also because the growing season is usually short. Drought is a normal, recurring feature of climates in parts of the world, and which often has substantial impact on the

ecosystem and agriculture of the affected region. Secondary effects include health, economic and social consequences. It usually causes reduced water quality, mass migration, internal displacement, international refugees crises, diminished crop yield and carrying capacity for livestock, desertification and erosion, famine (due to lack of water for irrigation), habitat damage (affecting both terrestrial and aquatic wildlife), malnutrition, dehydration, diseases, as well as significant damage and harm to local economies (Walker, 2004; Mengesha, 2010).

Maize is thought to be more susceptible to drought (FAO, 1991a) at flowering than other crops. According to Bella, et al (2007) maize plants are able to endure water stress associated with less than 200 mm of precipitation in places were groundwater, storage precipitation, or moisture condensation exists. But this may not be the case in drought regions, of East Africa.

1.3.4 Maize farming and agronomic conditions

The chances of complete germination and crop establishment increase under favorable soil moisture and temperature conditions. However, wherever the length of the growing season is limited by the duration of the rainy season, as occurs in the horn of Africa, early planting reduces the probability of drought during the late grain–filling stage, and delayed planting (frequently caused by labor and land preparation constraints) exacerbates agronomic problems. This often results in maize plants that are tall, prone to lodging, but having relatively fewer kernels per plant. These effects, along with the increased possibility of terminal drought stress, often result in significant yield losses (Banziger, et al., 2000, 2002). Breeding of drought-resistant

maize varieties that have only half the growing duration of the 140 days (FAO, 1991a) of regular varieties is one approach that has been utilized in the past, to overcome poor yield due to agronomic stress.

Another proven approach, aimed at improving percent seedling emergence and establishment, as well as increased yield is seed priming. Seed priming is especially beneficial for seeds that are hermetically stored, to allow for long-term, safe storage of maize seeds under harsh (hot, humid, drought) conditions, without the need for refrigeration and chemical preservatives.

1.4 POST HARVEST MAIZE LOSSES

In general, post-harvest maize losses may be classified into measurable decreases of maize grain, described as quantitative, qualitative, germinative, nutritive, and economic losses, while maize kernel damage usually describes superficial evidence of deterioration, such as insect pest holes or broken kernels. Quantitative losses refer to reduction in weight, usually resulting from pests (insects, molds, rodents), and qualitative losses include damage to or contamination of maize, usually described by comparison with quality standards. Nutritional (qualitative and quantitative) losses refer to reduction of the food value of maize, germinative losses describe a reduction in maize germination ability, while economic losses refers to a reduction in the monetary value of maize (FAO, 1991b; Bern, et al., 2008). Although Grolleaud (2002) puts post-harvest losses of food grains, due to insect infestation and mold activity, conservatively at 10–15%, combined insect and mold activity can result in up to 100% maize loss in East Africa (Demissie *et al*., 2008).

1.5 MAIZE HARVEST AND STORAGE

Maize is usually dried immediately after harvest, and is usually treated with chemical preservatives such as propionic acid, to prevent molding and rotting, before cold or warehouse storage (Villers, et al., 2008). However, several authors have proposed the use of hermetic storage, in place of chemical preservatives and cold storage, considering its many advantages (Table 1.12), which include being cost-effective, adaptable to local cultures, and environmentally friendly. It works by the synergistic effect of O_2 depletion and CO_2 accumulation, from the respiration of its contents (Yakubu, et al., 2011). This is because hermetic storage involves storage of commodities in an airtight and watertight or low permeability environment, that provides negligible or no gas exchange between the hermetic environment and external environment. And which creates a modified storage atmosphere that is lethal to storage insects and molds, maintains constant moisture and preserves stored commodity (Villers, et al., 2004; Navarro, et al., 2007; IRRI, 2004).

The increasing demand for chemical-, contaminant-, and pathogen-free, high quality maize, worldwide requires adequate maize preservation, including drying and protection from insect and microbial damage (Sinha, 1995; Weinberg, et al., 2008), in post-harvest storage. And encourages maize storage using an effective natural preservation method, such as hermetic storage.

Hermetic storage has been proven to be effective under hot and humid, tropical conditions, similar to East African storage conditions, which promote rapid grain deterioration. This is helpful to subsistence farmers, in these countries since they lack adequate equipment for drying grains (Mendoza et al., 1982), and harvested

maize is often stored while still relatively moist and warm, resulting in rapid deterioration, due to mold growth. Even where the maize is properly dried before storage, rewetting due to rain or hygroscopic maize moisture uptake from the humid environment, resulting from open-air maize storage promotes deterioration (Landers and Davis, 1986).

The use of hermetic storage, which creates a modified atmosphere, that naturally controls post-harvest stored maize pests solve these problems.

1.6 MAIZE STORAGE IN THE TROPICS

Modern and traditional approach to bulk maize storage involves storage in metal or concrete silos. Silos technology works well in temperate climates, and in developed economies, where grain aeration, cooling and related maize maintenance while in storage is possible. However, in hot, humid climates of tropical and semi-tropical regions, high humidity causes moisture condensation. This usually results in molding and spoilage of maize stored in silos, and since subsistence farmers cannot afford costs associated with silos aeration and cold storage, the use of silos for maize storage has limited application. Hermetic storage provides major advantages over conventional (metal and concrete bin silos) in addition to being as effective or more effective (Table 1.12).

Table 1.12: Comparison of hermetic to conventional (metal and concrete bin silos) storage (Villers, et al., 2008).

Item of comparison	**Hermetic ("Cocoon™") storage**	**Conventional metal or concrete bin silos**
Control measures if infestation occurs	Control by depleted O_2. Gas analyzer enables follow up on infestation level, detection of leak	Grain will have to be unloaded and treated with phosphine (PH3)
Fumigation	Not needed	Required every 6-12 weeks
Condensation at 14% MC	No, if shade is used properly	High risk storage if above 1 month and grain does not remain sufficiently dry (low moisture content (MC)
Protection from rodents	Protected	Protected
Moisture level of commodity	Remains constant	Moisture content will rise significantly
Length of storage	1 to 5 years	1-3 months depending on climate, silo material (metal or concrete), the extent of the exposure of the roof to absorb solar energy, and initial MC of the commodity
Aeration	Not needed	Required in temperate climates. Ineffective in tropics due to lack of cold nights
Life span of the structure	10-15 years	20-25 years (if metal is painted periodically against corrosion, and concrete with adequate maintenance)
Set up	Can be quickly set up at any location, indoors or outdoors	Needs concrete floor, access road, construction time

Table 1.12: Comparison of hermetic to conventional (metal and concrete bin silos) storage (Villers, et al., 2008)-continued.

Item of comparison	Hermetic ("Cocoon™") storage	Conventional metal or concrete bin silos
Mobility (ability to move/dismantle silos and move them to another area)	Excellent	Impossible once set up
Hazards	Rodents (but can easily be prevented)	Dust explosion, caking due to excess of moisture content, condensation
Safe storage duration	Proven for tropical, long term storage	Storage may not be safely extended above 1-3 months
Price per MT or Mg (investment)	US$5-US$80	US $100-250 (including infrastructure and handling equipment)
Auxiliary equipment	None	Bucket elevator, fans, "sweeper" auger
Infrastructure required	None	Road, electricity

According to Villers, et al (2008), even when commodities sufficiently dried to safe moisture contents are stored in silos, in tropical climates, they usually experience moisture condensation leading to fungal and insect growth, susceptibility to external humidity, which raises the moisture content to unsafe levels, and cross-contamination with chemical insecticides used to prevent insect infestation.

1.7 HERMETIC CONTAINER TYPES AND SIZES

Hermetic storage media utilized for storage include rigid containers (cans, 55-gallon drums, glass-canning jars), as well as flexible materials (polyethylene, polyester, laminate, cellophane, cloth, and paper) (Copeland and Mcdonald, 1995; Villers, et

al., 2008; Yakubu et al., 2011). Most rigid containers have relatively small sizes, compared to flexible (Figure 1.4 and 1.5) hermetic storage. Common, flexible storage container sizes include 60 kg to 1 tonne SuperGrainbags™, as well as large flexible storage Cocoons™ (5 to 1000 tonnes), TranSafeliners™ and Bunkers™, with sizes ranging from 5 tonnes to 30,000 tonnes (Villers, et al., 2008). The MegaCocoon™, an upgrade of the Cocoons™, has also been introduced for larger scale storage of up to 1050 tonnes. According to Villers, et al (2008), Cocoons are the most widely used form of hermeic storage, and are made from specially formulated flexible PVC, sealed with special zipper originally developed for use by astronauts. And their oxygen permeability, at 23ºC, ranges from 3 to 55 $cm^3\ m^{-2}\ day^{-1}$. SuperGrainbags have served as liners for either polypropylene or jute outer bags, and hermetic storage of wheat stored at or below its critical moisture content of 12.5 %, provides storage without significant degradation of quality, and maintained baking qualities, for up to 4 years. Also, according to the authors, the use of Bunkers in Cyprus, allowed quality preservation of barley for 3 years, with total losses of 0.66% to 0.98%, and germination rates above 88%, while storage of wheat in Cocoons and/or Bunkers reduced losses due to insects or molds to a small fraction of 1% per year. Grainpro products and other forms of hermetic storage have been utilized for successful, multiyear storage in several countries, including Australia, Bangladesh, Botswana, Brazil, Cambodia, China, Costa Rica, Cyprus, Dominican Republic, East Timor, El Salvador, Ghana, Guatemala, Honduras, Israel, India, Indonesia, Jamaica, Laos, Pakistan, Peru, Philippines, Rwanda, Sri Lanka, Sudan, Thailand, Turkey, Uganda, United States, Vietnam, and Zimbabwe.

1.8 TYPES OF HERMETIC STORAGE

Hermetic storage can be sub-classified based on how anaerobic condition in the modified hermetic storage environment is achieved. A biomodified storage environment is achieved by allowing the natural respiration of insects, molds, and store grain to use up O_2 and produce CO_2, making the hermetic environment lethal to the insects and molds (Navarro, et al., 2007; Yakubu, et al., 2011). Other implementations of hermitic storage involve rapid withdrawal of O_2 from the storage environment using a vacuum system, or rapidly flooding the environment with external CO_2 or N_2 (Villers, 2004). The ability to create a low-oxygen

Figure 1.4. Flexible hermetic storage, outdoors (Villers, et al., 2006).

modified atmosphere within a short time (few minutes to two weeks) that results in 100% insect mortality of all life stages and suppresses mold development have other advantages in addition to the ones already described. Suppressing mold development protects the stored food from contamination by cancer causing

mycotoxins (aflatoxin, ochratoxin A (OTA)) produced by molds. Hermetic storage also prevents quality losses associated with the release of free fatty acids (FFAs) in relatively high fat content commodity storage, as occurs in rice bran, brown rice, peanuts, and cocoa beans (Montemayor, 2004), due to oxidation. And the ability to store commodities in hermetic storage without the use of chemical pesticides in post-harvest storage, means reduced storage cost, as well as reduced chemical toxicity (Murdock, et. al, 2007). Yakubu, et al (2011) discovered that low-oxygen modified atmospheres can be created in biomodified atmospheres, as rapidly as necessary, through the manipulation of storage factors (number of weevils, temperature, maize moisture, container percent fill). Therefore, the choice of hermetic storage type may be dependent on type and quantity of commodity being stored, as well as cost of storage. And biogenerated, modified atmosphere is more likely to be utilized in storing non-crushable, previously dried, commodities (grains) than crushable commodities (dried fruit, tomatoes).

Figure 1.5. Grain hermetically stored in 50Kg bags lined with Super-Grainbags™ Liners (Villers, et al., 2006).

In general, hermetic storage has been used for long-term storage of **cereal grains** (rice, corn, barley, and wheat), long-term storage of a variety of **seeds** to preserve germination potential and vigor, as well as quality preservation of high-value commodities, such as cocoa and coffee.

1.9 HERMETIC STORAGE ECONOMICS

In addition to preserving seed germination at a favorable rate, hermetic storage is the cheapest form of storage compared to cold or bin silos storage, when grain or seed needs to be stored for six months or more (Villers, et al., 2008; Sabio, et al., 2009).

1.10 FACTORS INFLUENCING HERMETIC STORAGE

Factors influencing the hermetic storage environments include temperature, moisture and pests. The relative humidity, determined by the temperature and moisture is usually maintained at 60% and below (Harris and Miller, 2008), to suppress mold activity, and hermetic storage containers are usually shaded from direct sunlight, to prevent temperature buildup and the associated moisture condensation that results from increased relative humidity and water activity. This is because temperature increases cause increased relative humidity within the hermetic container, especially at higher maize moisture. Weevils are controlled by a combination of suppressing relative humidity and O_2 within the hermetic environment, while weevil mortality is aided by increased CO_2 or N_2. Mechanical fencing, which usually involves physical separation of the storage environment from the environment in which rodents live is a common approach utilized in controlling

rodent activity. Utilizing metallic containers, such as 55-gallon drums (Seck et al., 1996; Adhikarinayake, 2005), or placing double- and triple-bagged grain in such a container fences out the rodents. Double bagging involves placing grain within an airtight bag, which is tied or sealed and placed in a second bag that is also sealed, or triple bagging (Fulton, et al., 2009) involves placing the airtight grain-containing bag in two other airtight bags that sealed separately.

Most descriptions of hermetic storage are qualitative, and do not provide ways to calculate how long it takes for the O_2 within enclosed hermetic environment to be used up. The study by Yakubu, et al (2011) provided a way to quantify the remnant O_2 in a hermetic container and how long it would take for it to be used up by maize weevils, microflora, and maize respiration. The prediction takes into account, the weevil infestation level, as well as temperature and maize moisture, which are predominant factors in the rate of weevil oxygen consumption and mortality. This ability to predict time to complete mortality of adult maize weevils, which are the predominant pests (Holst, et al, 2000; Jacobs and Calvin, 2001; Demissie *et al*., 2008) means that factors involved in hermetic storage can be manipulated successfully for faster weevil mortality and reduced maize spoilage. The study looked at temperature (10 and 27°C) and maize moisture (8 and16%) extremes associated with common maize storage, and which favors optimal as well as slow weevil development. It therefore simulates hot and humid tropical conditions, as well as cold and dry temperate conditions.

1.10.1 Temperature

Temperature has a direct effect on hermetic maize storage as described above. However, the mortality effect of temperature on weevil mortality is not consistent across temperature spectrum. Nakakita and Ikenaga (1997) conducted research that involved measuring the rate of oxygen utilization of ten pre-weighed 2-week old adults of either S. zeamais or S. Oryzae released in a 15 ml respirometer flask. The flasks contained a piece of filter paper soaked in 0.1 ml of 10% KOH solution in the central cell and were covered with brass mesh. Oxygen consumption was measured at 1-hour intervals, in a Gibson respirometer placed in a temperature-controlled bath, following pre-conditioning at each temperature (30, 25, 20, 15, 10 and 5°C), for a maximum of 12 hours. The test insects were obtained from S. zeamais or S. Oryzae maintained on brown rice for more than 20 years in a culture room at 25±0.5°C and 70±5% relative humidity. They discovered that low temperatures (15°C and below) inhibited s. zeamais and s. orizae growth, while their population exploded at high temperature (25 to 30°C).

Studies conducted by Yakubu, et al (2011) discovered similar results and concluded that intermediate temperatures, which include room temperatures, have intermediate effect on s. zeamais development. Other authors also noted that the rate of insect mortality, respiration and reproduction is slower at low temperatures. And that rapid insect development occurs within a fairly narrow range of 5 to10 degrees around the optimal temperature, which, for most storage insects, is in the region of 25 to 35°C (De Lima, 1990; FAO, 1994; IRRI, 2004; Arannilewa, et al., 2006).

1.10.2 Maize moisture and hermetic storage

Storage literature (FAO, 1994; De Bruin, 2005) and hermetic storage study involving microbiological analyses (Weinberg et al., 2008) suggest rapid maize deterioration of store maize under tropical conditions. The later study, which focused on examining the effect of moisture content on the quality of maize grains in self-regulated hermetic storage, concluded that anaerobic, hermetic storage provides an excellent solution to preventing insect development. It is, therefore, capable of preventing insect damage to stored maize (Navarro, et al., 1996; Yakubu, et al., 2011) as well as mold development during storage.

1.10.2.1 Laboratory hermetic study

Overall, the hermetic study by Weinberg et al (2008) utilized maize samples at 14, 16, 18, 20 and 22% moisture contents, conditioned for 28 days in tightly wrapped plastic bags and stored in sealed containers, at 30°C, for 75 days. And was aimed at determining the effect of moisture content on the quality of maize stored under self-regulated modified atmospheres during hermetic storage. Self-regulated hermetic storage refers to storage in which combined metabolic activity of stored maize, insects, and microflora present in the hermetic atmosphere utilize O_2 and releases CO_2 that kills the insects and microflora, preserving maize quality. The research concluded that at low moistures of 14% and below, the mold count was negligible, following hermetic storage, and safe for consumption.

Experimental procedure

Maize samples preparation

Maize grain at about 14% moisture obtained from a local feed center was cleaned to remove impurities and broken kernels. It was then divided into five batches and the batches were moistened to 14, 16, 18, 20 and 22% moisture contents respectively. This was done by spraying calculated amounts of distilled water over the grains, which were spread in a thin layer on a 30-40 cm plastic tub, while thoroughly hand mixing the grain and water. 8 kg of each moistened maize samples were tightly wrapped in a plastic bag and stored for 4 weeks at 5±1°C and shaken for a few minutes everyday.

Experimental maize

Maize at the same moisture content were removed from the bags, thoroughly mixed and about 500 g was placed in each 1-L glass jars. Every jar was sealed with a screw-cap gas-tight lid and special clamps, and stored at 30°C. Each moisture content had 12 jars of maize, three of which were sampled for analysis after 15, 35, 55 and 75 days, respectively. Gas sampling was made possible by drilling a hole in the jar lids fitted with silicon-rubber septum, while the exact jar volumes were pre-determined by measuring the volume of distilled water that filled each.

Analytical procedure

Following storage, the percent germination of grains at each moisture content were determined by placing them on damp filter paper at 18°C, for 10 days. Maize samples moisture content were determined using the oven test method, by exposing

samples of maize to 105°C oven for 24 hours. Equilibrium relative humidity (e.r.h) was determined at 25°C, and pH was measured in a 10-fold aqueous extraction of 20-g samples. Ethanol and volatile fatty acid (VFA) were determined in aqueous extracts, over a temperature range of 40 to 230°C, and maize losses were evaluated according to weight loss, expressed as gas loss (g kg^{-1}), while headspace ethanol was determined with a gas chromatograph. Headspaces atmospheric gas composition was determined by withdrawing gas samples using a 3-ml gas-tight syringe, and O_2, N_2 and CO_2 concentrations.

Microbiological analysis

Microbiological evaluation involved enumeration of the total aerobic bacteria in plate count agar (Scharlau Microbiology, Barcelona, Spain), and yeasts, as well as molds on **spread-plate malt extract agar** (Difco, Detroit, MI, USA) acidified with lactic acid to pH of 4.0. The plates were incubated for 3 days at 30°C.

Statistical analysis

GLM procedure (SAS Institute Inc.,100 SAS Campus Drive, Cary, NC 27513) was utilized in the statistical analysis of the results, including analysis of variance and Duncan's multiple range test.

Results and discussion

The maize utilized in the research had initial moisture contents of 14%, 16%, 18%, 20% and 22%, corresponding to about 77.5±0.3, 85.2±0.3, 89.2±0.3, 91.5±0.4 and 92.5 ±1.2%, relative humidity respectively within hermetic storage. However,

moisture content increased, by 8-17 g kg^{-1} during hermetic storage, due to respiratory activity within the hermetic ecosystem.

The authors demonstrated the change in various atmospheric gas contents that occur within sealed maize containers at each moisture content, and suggested that the higher the moisture content, the shorter the time it took for the O_2 to be consumed and replaced with CO_2 during aerobic respiration (Yakubu, et al. 2011). This is evident from hermetic containers, where most of the O_2, in the containers with 14, 16, 18, 20 and 22% moisture content was consumed after 600, 120, 48, 24 and 12 h, respectively. They also showed that in the maize with 14 to16% moisture content CO_2 replaced only the O_2, and N_2 level initially remained constant. However, for the higher moisture maize, as more CO_2 was produced, the percentage of N_2 decreased in the sealed containers. Following the aerobic respiration phase, anaerobic respiration continued to produce CO_2. And they measured the levels of anaerobic respiration after a plateau of CO_2 level was reached and for up to 1776 h (74 days) (Figure 1.6).

Changes in hermetically store maize

The pH of the 22% moisture maize decreased from 5.8 on day 0 to 5.5 on day 75, while those of the other moisture maize remained around 6.0. Dry matter losses also increased with increasing maize moisture content. The highest concentration of major volatile products found in maize (ethanol and acetic acid) occurred in maize with higher moisture contents (20 and 22%).

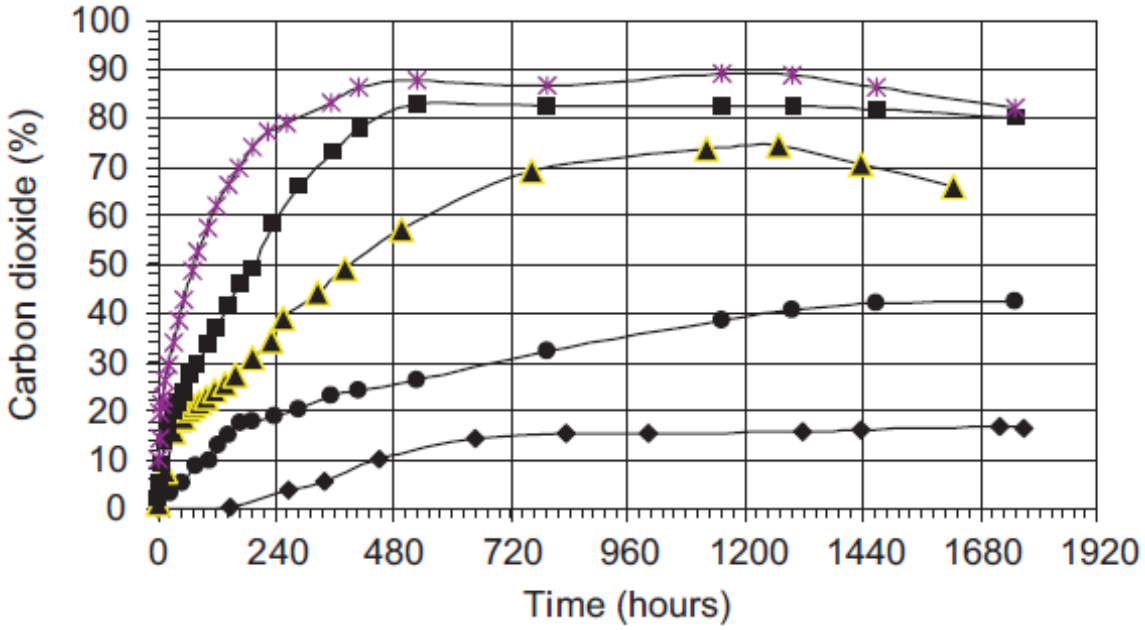

Figure 1.6. Levels of CO_2 at 14%, 16%, 18%, 20% and 22% moisture contents and 30°C during the hermetic storage period (♦, m.c. 14%; ●, m.c. 16%; ▲, m.c. 18%; ■, m.c. 20%; and, —✳— m.c. 22%) (Weinberg et al., 2008).

Ethanol was detected in the interstitial space (Table 1.13), as well as in the headspace (Table 1.14) of the hermetic container, suggesting yeast activity. Ethanol concentration is assumed to have increased during storage due to fermentation while acetic acid concentration remained constant or decreased slightly. Propionic and butyric acids were also detected, although at low concentrations (<0.3 g kg^{-1} DM).

Tables1.13 to1.17 shows summary of results of microbiological analyses. The analysis (Table 1.15) detected no visible molds in any of the treatments, indicating that hermetic storage is capable of ridding stored maize of molds. At 14 to 8% maize moisture, mold counts decreased during storage. Yeast counts also decreased, and none was found in the 14% moisture maize by the 75th day of storage, although.

However, yeast were still present on the 55th day, although bacteria in various treatments tended to decrease during storage. Overall, the population of these microorganisms were within safe limits regarding freedom from substantial spoilage ($<\log_{10}/g=4.0$). At 20 and 22% moisture content, yeasts and bacteria count were higher, tended to increase during storage, reaching population levels ($>\log_{10}/g=6.0$) usually associated with spoilage of vegetable food commodities. Interestingly, molds counts decreased rapidly at these maize moistures, and no molds were found by the 55th day of storage. This is understandable, considering that the rate of oxygen utilization and hence respiration is higher at higher maize moisture (Weinberg, et al., 2008; Yakubu, et al., 2011).

Dry matter loss

Results from the research indicate that some respiration and microbiological activity took place in intermediate moisture (15 to 18%) maize stored in hermetically sealed containers.

At 14% moisture content, almost no biological activity took place, and the stored maize retained their quality, as expected. The results indicate that the higher the moisture content, the faster the CO_2 build-up, in the hermetic containers. This CO_2 build-up is accompanied by an increase in pressure, and from the gas exchange (O_2 and CO_2) observation (Weinberg et al., 2008), it appears that at 14 and 16% moisture content, the respiration was aerobic and no excess of pressure occcured. The CO_2 concentration did not exceed 20% by volume, at those moistures. However, above 18% moisture content, a gradual increase in CO_2 concentrations

was observed that exceeded the volume of O_2 consumed, indicating that anaerobic respiration occurred (Zettler and Navarro, 2001). The jars used in these experiments were equipped with septa through which periodic gas samples were taken. During the gas sampling, pressure build-up was observed, particularly at 18-22% moisture contents. This is evident from the levels of CO_2 reaching 74%, 83% and 89% at 18, 20 and 22% moisture contents, respectively (Figure 1.6). The volume of N_2 in a completely sealed, hermetic environment was assumed by the authors to stay the same, since it is an inert gas and should not take part in the aerobic or anaerobic metabolic respiratory reactions. However, actual amount and proportion of N_2 decreased due to losses during sampling. And since the amount and proportion of CO_2 increased due to anaerobic respiration, the percentages of N_2 and CO_2 measured changed continually during the research.

The starting hypothesis of this study was that in intermediate moisture contents, maize under sealed storage conditions, had limited microbial activity resulting in the production of VFAs, which inhibits yeasts and molds that are the major spoilage microorganisms in such commodities (Moon, 1983). However, ethanol was found in higher concentrations than VFAs, which might indicate yeast activity (Table 1.16). Acetic acid was found in concentrations that were probably too low to inhibit yeasts and molds. And at higher (20 and 22%) moisture levels, large numbers of yeast and molds were found during the initial stages of storage, leading to higher dry matter losses.

Pressure buildup and relief

Gas-tight containers with high moisture maize must be equipped with pressure release valves, to reduce the pressure buildup associated with increased CO_2 volume from respiratory activities. Since small experimental jars, have rigid walls, and can withstand more pressure, the lack of a pressure release valve is not expected to have as much impact on the walls of the storage containers. However, such pressure build-up can cause the weak joints of large rigid or flexible structures to explode.

Table 1.13. Ethanol (Et) and acetic acid (HAc) contents (g kg^{-1} DM) in maize under hermetic storage (Weinberg et al., 2008).

Time (days)	**Moisture (%)**									
	14		**16**		**18**		**20**		**22**	
	Et	**HA_c**	**Et**	**HA_c**	**Et**	**HA_c**	**Et**	**HA_c**	**Et**	**HA_c**
0	0	0.5	0	0.7	0	0.7	0	0.4	0	1.0
15	0^{d}	0.2	0^{d}	0.3	0.7^{c}	0.4	1.5^{b}	0.5	2.5^{a}	0.5
35	0^{d}	0.5	$0.3^{c,d}$	0.6	1.3^{c}	0.7	2.8^{b}	0.4	3.7^{a}	0.8
55	0.1^{d}	0.5	0^{d}	0.3	2.0^{c}	0.5	2.8^{b}	0.4	4.1^{a}	0.6
75	0^{d}	0.4	$0.9^{c,d}$	0.4	$2.0^{b,c}$	0.3	$3.8^{a,b}$	0.4	5.0^{a}	0.5

For ethanol, within a row, means followed by different letters are significantly different ($p<0.05$). Propionic and butyric acids were detected at low concentrations (<0.3 g kg^{-1} DM) in some samples, with no consistent pattern.

Table 1.14. Ethanol content (mg kg^{-1} of air) in the headspace of moist maize under hermetic storage (Weinberg et al., 2008).

Time (days)	**Moisture (%)**				
	14	**16**	**18**	**20**	**22**
55	37	1124	4630	6929	7689
75	148	1279	4496	5048	6571

Table 1.15. Mold numbers (log_{10} (CFU g^{-1})) in maize under hermetic storage (Weinberg et al., 2008).

Time (days)	**Moisture (%)**				
	14	**16**	**18**	**20**	**22**
0	3.2*	4.9	4.7	4.7	6.8
15	3.3^{b}	3.1^{b}	3.7^{b}	3.7^{b}	5.2^{a}
35	2.7	1.9	2.3	0.7	2.1
55	2.5	1.0	2.4	NF	NF
75	$1.9^{a,b}$	$1.6^{a,b}$	2.2^{a}	NF^{b}	NF^{b}

Within a row, means followed by different letters are significantly different (P<0.05).

NF, not found (below the detectable level, log10 (CFU g^{-1})<2.0).

***For day 0 there was one sample only for each moisture level and they indicate mold growth during the equilibration phase at 5°C.**

Hermetic storage and silage

Silage fermentation studies suggest that at least 10 g kg^{-1} DM of VFA concentration is needed to inhibit fungi growth (Weinberg et al., 1993). However, results from this research suggest that even in the higher moisture maize not enough VFAs were produced.

Table 1.16. Yeast numbers (log_{10} ($CFUg^{-1}$)) in maize under hermetic storage (Weinberg et al., 2008).

Time (days)	**Moisture (%)**				
	14	**16**	**18**	**20**	**22**
0	2.7	NF	NF	3.5	5.4
15	2.8^{b}	2.9^{b}	2.7^{b}	3.5^{b}	5.6^{a}
35	1.5^{b}	2.4^{b}	2.4^{b}	4.7^{a}	5.3^{a}
55	1.3^{c}	1.3^{c}	$3.1^{b,c}$	$4.9^{a,b}$	6.5^{a}
75	NF^{d}	1.1^{d}	3.9^{c}	5.2^{b}	6.4^{a}

Within a row, means followed by different letters are significantly different ($P<0.05$) (for day 0 there was one sample only).

NF, not found (below the detectable level, log_{10} ($CFU\ g^{-1}$)<2.0).

This is especially because the water activity was too low to support the microbial activities of the microorganisms-***heterofermentative*** lactic acid bacteria (Troller and Stinson, 1981) or ***enterobacteria*** (Frazier and Westhoff, 1978)-that, usually produce them. This lack of sufficient VFAs enabled yeasts to develop in substantial numbers in the high moisture maize (20% and 22%) during initial stages of storage.

Table 1.17. Bacteria numbers (log_{10} ($CFUg^{-1}$)) in maize under hermetic storage (Weinberg et al., 2008).

Time (days)	Moisture (%)				
	14	**16**	**18**	**20**	**22**
0	2.9	4.4	3.8	4.5	NF
15	1.6	2.1	1.7	3.7	2.7
35	3.7^{b}	2.7^{b}	2.8^{b}	3.8^{b}	5.9^{a}
55	3.1^{c}	$3.5^{b,c}$	2.1^{c}	$5.2^{a,b}$	6.1^{a}
75	$3.0^{b,c}$	$3.2^{b,c}$	2.0^{c}	$4.8^{a,b}$	6.2^{a}

Within a row, means followed by different letters are significantly different (P<0.05) (for day 0 there was one sample only).

NF, not found (below the detectable level, $log_{10}CFUg^{-1}$<2.0).

After 35 days of storage no molds were detected, even at the highest moisture maize, probably because the O_2 was depleted very rapidly in these treatments. Molds have been found to survive in **silage**, in atmospheres with O_2 concentrations as low as 1.0% (Lisker et al., 1989). And it is possible that their utilization of O_2 is at a slower pace, in intermediate moisture maize, since molds still existed in these maize samples by the 75^{th} day of hermetic storage. For this reason, a complete analysis of mycotoxins, particularly of aflatoxin and fumonisins may be necessary, before the utilization of any maize for feed or starch extraction. Above 25% moisture content, maize may undergo lactic acid fermentation and ensiling, resulting in pH decrease (Wardinski et al., 1993; Dawson et al., 1998; Taylor and Kung, 2002). This is because ensiling is a three-step process involving the activity of aerobic microbes (resulting in their mortality), followed by anaerobic microbes, and lactic acid

fermentation. The fermentation then produces pH decreases that result in the mortality of the anaerobic microbes, and grain preservation (Bern, et al., 2008). However, maize ensiled at this moisture contents, may only be suitable for animal feeding purposes, because of the high bacterial count (Weinberg et al., 2008). According to some authors, it is possible to ensile high-moisture maize at (25 to 28%) with and without microbial and chemical additives for use as animal feed. This is because, during the ensiling fermentation of high-moisture maize, lactic acid bacteria produce organic acids (mainly lactic and acetic acids), which decreases pH to 4.0 to 4.5, to kill microbes. It is, however, necessary to protect this silage against molds, using suitable antifungal agents, since they spoil quickly upon aerobic exposure (Wardinski et al., 1993; Dawson et al., 1998; Taylor and Kung, 2002).

Conclusions

In this laboratory scale hermetic storage study, maize at intermediate and high moisture contents was stored in hermetically sealed jars without spoilage. The preservation was possible because of the synergistic effect of respired gases (O_2, CO_2), involving decreases in O_2 and accumulation of CO_2, and not due to VFAs, which were only present at very low levels. Considering that unintended fermentation and alcohol production, at intermediate and high moisture hermetic storage results in dry matter loss and increased CO_2 production, it is safer to store maize at below 14% moisture. This is also important considering that maize bacteria count increases with increasing moisture content, while negligible microbial activity occurred at 14% maize moisture.

Based on these research results, the authors proposed the avoidance of the cost associated with drying to low moistures necessary for maize storage. They further proposed the storage of maize at high moistures for ethanol production, where risk of mycotoxin presence might not be a critical issue, as against the current practice of first drying to safe storage moistures and adding water just before ethanol extraction. The authors found the results sufficiently encouraging to justify further commercial scale trials on high moisture storage of maize for ethanol production, accompanied by economical feasibility studies. They also suggested the need to undertake further studies regarding commercial scale utilization of hermetically sealed plastic containers, in order to determine the maximal safe moisture content for hermetic storage under field conditions, and to determine and justify its economical feasibility.

1.10.2.2 Field hermetic studies

Other authors have also conducted interesting and complementary studies regarding field implementations of hermetic storage. These studies considered general principles, but have also introduced other perspectives. Two of such studies were described by Aronson, et al (2005) and Harris and Miller (2008).

1.11 RODENTS AND HERMETIC STORAGE

According to Villers, et al (2008), properly designed hermetic storage is highly rodent resistant, and additional rodent resistance is provided in the case of large hermetic enclosures such as Cocoons by using tough, slippery materials such as flexible PVC (typically 0.83 mm thick), and tensioning straps, which prevent rodents from getting a tooth hold. Another, common approach, is mechanical fencing, which involves

physical separation of the maize contained in a metallic can and the exterior environment (Bern, et al., 2008).

1.12 HERMETIC STORAGE AND SEED DORMANCY

Seed dormancy is the effective delay of seed germination, under unfavorable ecological conditions. Viable seeds may therefore undergo dormancy, which allows them to preserve vigor and germinative power, while metabolically inactive-under unfavorable conditions-until favorable conditions for germination are reintroduced (Basra, 2006; Armitage and Woods, 1999; Sabio, et al., 2006). Following reversible (hermetic) seed stress (such as hypoxia), dormancy must be broken using seed conditioning techniques such as priming, to obtain seeds' natural germination rates, increase seedling stands, and crop yield (Spann, 1998; Armitage and Woods, 1999; CRC, 2001).

1.13 SEEDS

Seeds usually develop as a result of pollination of the female part (stigma) of a plant by the male part (anther) of a plant (Hill, 1995), and are an important food source (Contreras, 2011). Among some of the most economically important species are members of the *Poaceae* family (Maize, wheat, rice, etc), which contribute mostly carbohydrate, and the *Fabaceae* family (soybean, peanut, beans), which contribute mostly oil and protein to human diet.

Seeds of angiosperms (flowering plants) are generally classified into monocotyledon and dicotyledon, based on nutrient storage organs (cotyledon). In monocotyledons,

nutrients are stored in the single cotyledon and the endosperm tissue, while the radicle and hypocotyl give rise to the roots. And the epicotyl gives rise to the stem and leaves, which are usually covered by a protective sheath (the coleoptile). In dicotyledons, nutrients are stored in two enlarged cotyledons, the radicle give rise to the roots, hypocotyl to the lower stem, and the epicotyl to the leaves and upper stem (Figures 1.7).

1.13.1 Maize seed and germination

Maize is a monocotyledon of the *Poaceae* family. And its germination involves a reactivation of seed metabolic activity (Figure 1.8), which occurs in distinct stages (Figure 1.9)-activation, digestion and translocation, and seedling growth- leading to the emergence of radicle and plumule. For germination to occur, seed must be viable, quiescent (not dormant), and appropriate environmental conditions, including oxygen, temperature, moisture, and planting media must be present (Hill, 1995; Spann, 1998).

In most flowering plants having two cotyledons in the seed (eudicotyledons), a part of the developing stem, either the epicotyl (the stem above the cotyledons) or the hypocotyl (the stem below the cotyledons) elongates, forming a hook and gradually pulling the seed coat and the delicate shoot tip above the soil surface.

Germination of *dicotyledonous* seed is usually termed epigeous or hypogeous, based on the position of the cotyledons during germination. In epigeous germination, the cotyledons emerge above the soil surface, but wither and drop off after their food

stores have been used up. In hypogeous germination, the cotyledons remain below the surface and decompose after their food stores have been used up.

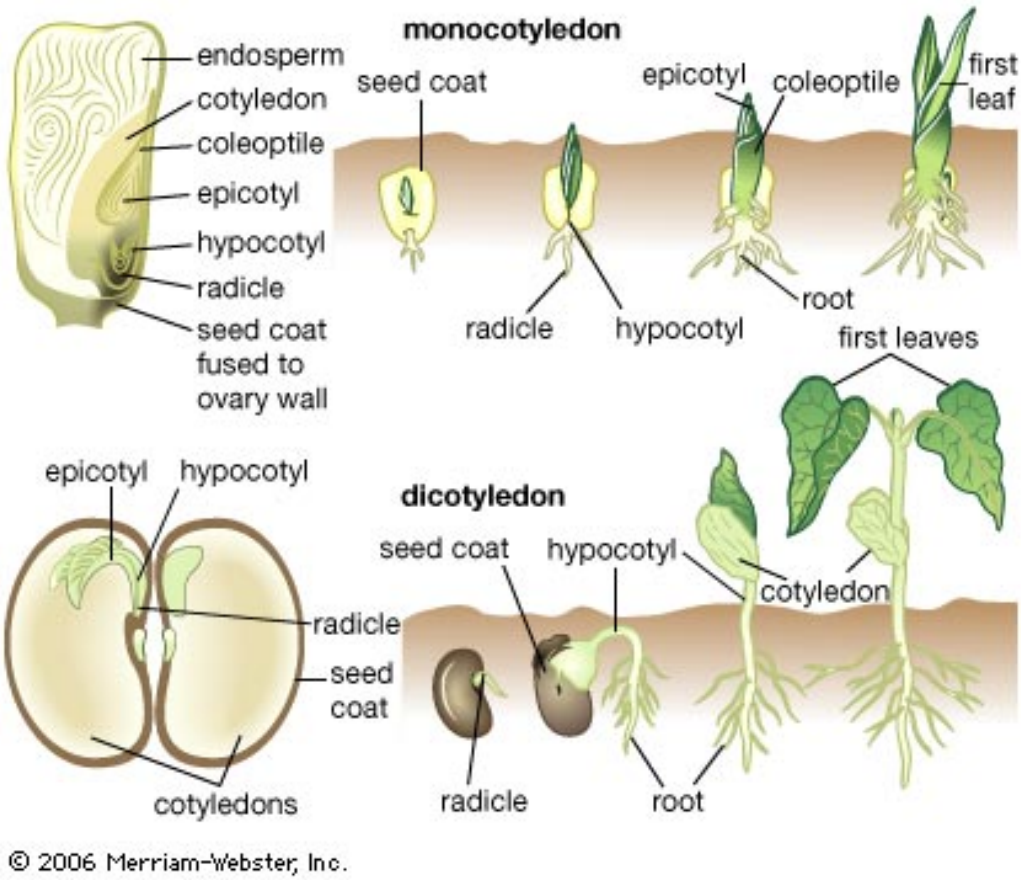

Figure 1.7. Internal structures of a monocotyledon (corn) and dicotyledon seed showing stages of germination (Merriam-Webster, 2006).

In most monocots, food is stored in the seed's endosperm (rather than the cotyledon), which is the single tubular cotyledon that elongates and draws the seed coat out of the soil. The cotyledon conducts photosynthesis, making more food, while the shoot grows up inside the tube.

When germination conditions are right, a radicle (embryonic root) emerges from the seed coat, anchoring the seed to the soil then grows and outputs lateral roots.

1.13.2 Oxygen and temperature

Seed germination requires the presence of sufficient O_2, for respiration. For this reason, soil water content must be such that it is sufficient, but does not produce waterlog, that makes oxygen inaccessible. Germination also requires that soil temperature be above freezing (0°C) but not greater than 45°C.

1.13.3 Maize moisture content and germination

A minimum state of hydration, from imbibition (seed water uptake) is necessary, within seeds for the mobilization of food, and their metabolism. This is termed colloidal swelling and is necessary for germination (Gallardo, et al., 2001; Skene, 2008). Seed is usually dried to about 5-15% moisture content, for storage. However, seeds must have 40 to 60% moisture content for germination to occur. Moisture, therefore, usually increases from 5 to15%, in dry seeds, to about 50% after the initial imbibition (Spann, 1998; Gallardo, et al., 2001). Imbibation (Figure 1.8) is a function of soil and seed osmotic potential, as well as soil potential, which depends on the presence of salts. Excess salt produces strong negative pressure, preventing water from entering the seed. This can create water stress, leading to reduced percentage germination. This situation is exacerbated when soil water supply is low, since soil osmotic potential increases greatly, and can inhibit germination, to a larger extent. And excessive water supply to the seed can result in the production of mucilage in the seed, restricting oxygen supply to the embryo (Spann, 1998).

1.13.4 Phases of germination

Phase I (Inbibition) of the germination process involves seed volume increase, due to water uptake, increased respiration, enzyme production, cell elongation and radicle emergence (Spann, 1998; Contreras, 2011). Phase II (log phase) is associated with the mobilization of materials from endosperm or coytyledons and much of the physiological activities, including protein synthesis, metabolism of storage reserves, and enzyme synthesis. Figure 1.9 describes additional detailed internal seed processes relating to germination.

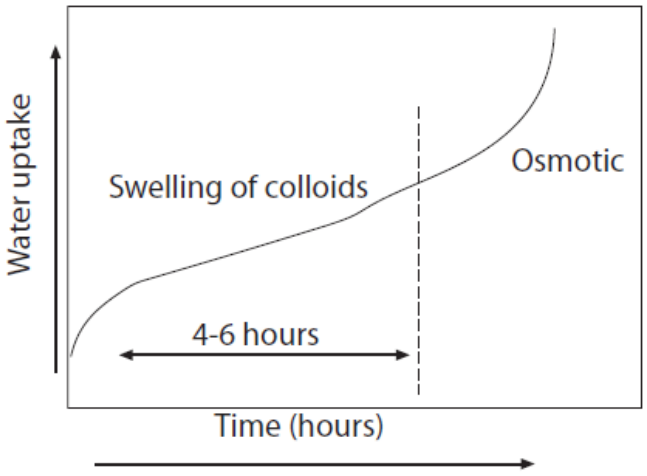

Figure 1.8. Seed water uptake (imbibation) during germination (Spann, 1998).

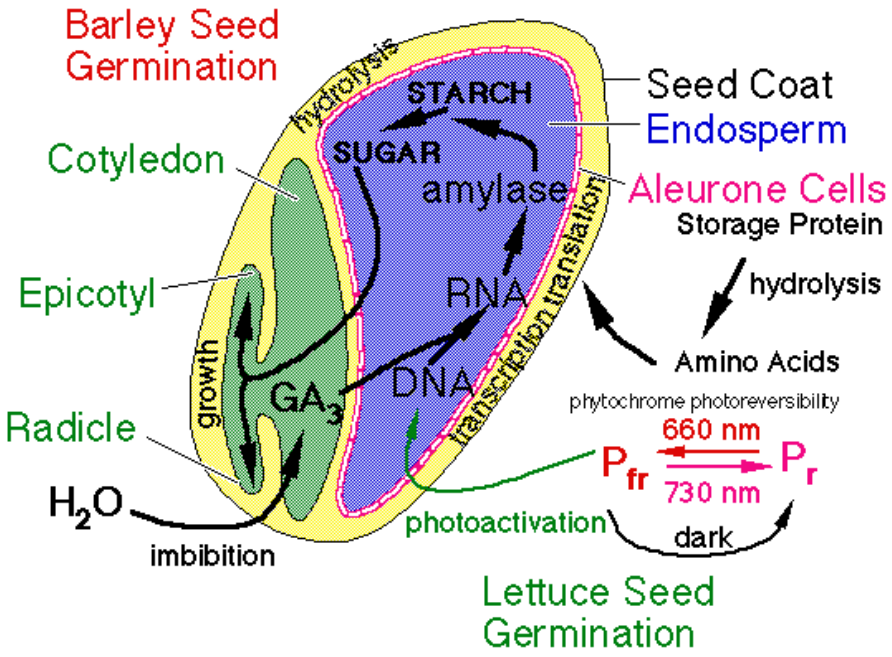

Figure 1.9. Internal seed germination processes (Koning, 1994).

for cell wall loosing occurs here. Phase III (seedling growth) is where radicle emergence and cell number increases associated with cell elongation and cell division occurs (Figure 1.10). Gallardo, et al., 2001 also described a three-phase germination process.

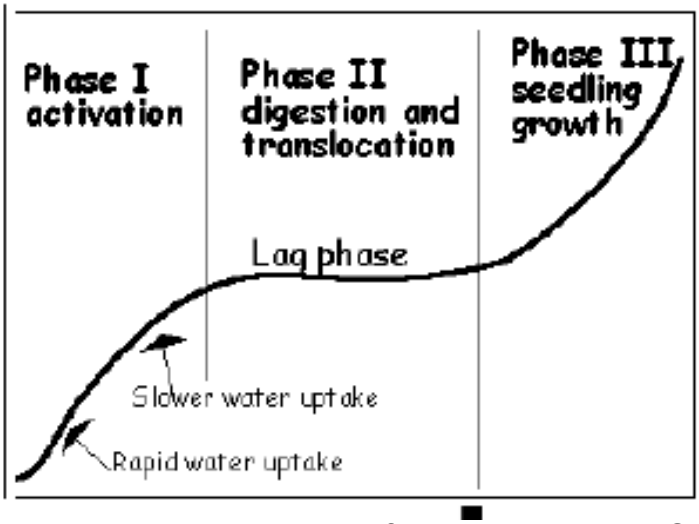

Figure 1.10. Three stages of seed germination (Spann, 1998).

1.13.5 Regulation of seed germination

The timing of seed germination is controlled by hormones, which are organic compounds that, in small concentrations, have important regulatory effects on plant and animal metabolism. Plant hormones (phytohormones) are plant growth regulators that regulate seed development, maturation, desiccation, dormancy and germination. Phytohormones reported to have regulatory effects of different physiological processes in seeds, include abscisic acid, gibberellins, ethylene, cytokinins, auxins, and brassinosteroids (Gallardo, et al., 2001; Kucera et al., 2005).

1.13.6 Hermetic storage and seed dormancy

Storage method appears to have an effect on the viability or germination rate of maize seeds, stored over time. So do temperature, maize moisture, length of storage, and genotype (texture, and pericarp characteristics).

Germination is dependent on relative humidity, which is dependent on ambient temperature and maize moisture (Copeland and Mcdonald, 1995; Basra, 2006; Desai, *et al*., 1997). And seeds often undergo dormancy, which allows them to remain viable, but metabolically inactive, under unfavorable conditions until favorable conditions for germination are reintroduced. Hence, even when seeds are sourced from harvested maize, preserved under hermetic conditions, a certain percentage of the seeds are still viable following lengthy periods of storage. This is important considering that at least seventy percent of maize seeds are sourced from prior year's maize harvest in East Africa (Gemeda, et al., 2001; Dhliwayo and Pixley, 2003). According to Hill (1995), dormant seeds are often very dry seeds that require

water absorption, to initiate the metabolic process of respiration, which causes digestion of stored food. Therefore, overcoming seed dormancy involves re-introducing the seed to favorable conditions (water, light, temperature, planting media, and oxygen).

1.13.7 Seed dormancy and abscisic acid

Most species of mother plants form seeds contain **abscisic acid,** which makes the embryo dormant until environmental conditions are favorable, for seed germination. Enzymes within the seed cause the acid to be broken down inside the embryo, usually, post-harvest. The required, inactivating, enzymes are normally present in the inactive form in the embryo until activated by seasonal or artificial low temperature. For this reason, priming using cold treatment (**stratification)** involves exposing seeds to four weeks of refrigeration or fall temperature of about 4°C, to activate the enzyme that degrades abscisic acid (ABA) in the embryo. However, because this temperature is too cold for germination to occur, the seed needs to be exposed to warm, spring-like, conditions (~25 to 27°C) even after stratification, to allow the germination process to begin. This process is termed **vernalization.** In desert plants, phenolic compounds which are inactivated by cold weather, but are also soluble in water replaces and plays the role of abscisic acid. For this reason, they are usually leached out of seeds by repeated washing and or soaking rains, to initiate germination (Spann, 1998).

1.13.8 Primary and secondary seed stress

Hermetic storage provides low-cost and viable alternative for seed storage, with favorable germination rates comparable to or better than conventional seed storage methods (De Bruin, 2005; IRRI, 2008; Sabio, et al., 2009). However, it often produces **secondary dormancy** resulting from, usually reversible, environmental stressors (oxygen and light), that can delay or prevent germination. **Primary dormancy** occurring during development and maturation results from water and salinity stress, as well as dehydration and desiccation, which can prevent germination, altogether.

1.13.9 Hypoxia

Hypoxia (low oxygen) associated with hermetic seed storage interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant grains (Benech-Arnold, 2006). However, the stress and dormancy, resulting from low oxygen levels can be removed through seed priming (Rush, 1992; Spann, 1998; Taylora, et al., 1998; Caprona, et al., 2000; CRC, 2001; Gallardo, et al., 2001; Harris, et al., 2001; Hussain, et al., 2006; Bern, et al., 2008; Contreras, 2011), following hermetic storage, before planting.

1.13.10 Seed sourcing by subsistence farmers

According to Gemeda, et al (2001), up to 97% of farmers in Ethiopia sourced their maize seed from prior year's harvest. And according to Dhliwayo and Pixley (2003) about 70% of farmers in Eastern and southern Africa obtained their seeds this way.

Quality considerations for seed selection include good grain filling, seed purity, adaptation to local conditions, being disease free and high germination rate.

1.14 RECYCLED CONTAINERS AND HERMETIC STORAGE

The use of recycled containers, which are often available for sale in the container resale market following initial use, for hermetic maize storage is promising (Boys et al., 2007; Baributsa, 2010). However, epidemiology of several food-borne illnesesses have been traced to cross-contamination by pathogens and toxins, from food contact surfaces (ASM, 2009), during storage.

The use of recycled containers for hermetic maize storage is promising (Boys et al., 2007; Baributsa, 2010). These containers are often available for sale in the container resale market, following initial use. However, epidemiology of several food-borne illnesses has been traced to cross-contamination by pathogens and toxins, from food contact surfaces (ASM, 2009), during storage. Container recycling and reuse for food storage, therefore, requires proper selection and cleaning to exclude containers with toxic contents (EPA, 2009a; 2009b).

1.14.1 Cross contamination

According to food-grade sanitary requirements, all food contact surfaces need to be smooth, impervious, free of cracks and crevices, nonporous, nonabsorbent, non-contaminating, non-reactive, corrosion resistant, durable, maintenance-free, nontoxic, and cleanable (Schmidt and Erickson, 2008). And International food laws and hazardous substance acts, forbids the use of recycled containers of hazardous substance for food packaging (Shachman, 2004) to prevent cross-contamination,

with food that may be stored within them. Based on these, the rule of thumb is to store food in materials classified as “food grade” containers (Opies, 2011). Metallic *(*stainless steel, titanium, platinum, and gold) and non- metallic *(*plastics, rubber, rubber-like materials Ceramics, and glass*)* containers that meet 3A sanitary standards (18-03 and 20-20) are classified as food grade containers (Schmidt and Erickson, 2008). Therefore, only food grade container previously used for storage of carbonated soft drinks and triglycerides, were considered for this recycling and maize storage study. But since, recycled vegetable oil containers can be cleaned using a uniform cleaning procedure (soap and saponification) worldwide, and they are more readily available than soft-drink containers, the procedure discussed here only focuses on cleaning procedures for edible oil-contaminated containers.

1.14.2 Cross-contamination and oxidation in fats and oils

The oxidation (rancidity) of fats and oils usually result from changes to their fatty acids’ chemical properties, which reduces their nutritional value. Associated with this are changes in color, taste, and smell. Factors that cause oil degradation through oxidation, that produces free radical formation, include exposure to air, light, mixing of different vegetable oil products, presence of salts, number of times oil is used, length of time oil is heated, and temperature to which it is heated (Andrikopoulos et al., 2002; Andrikopoulos, 2004; Fox and Stachowiak, 2007; Canals, et al., 2009). Oxidation occurs both during vegetable oil processing and storage (Choe and Min, 2006) and since oil has low thermo-oxidative stability, influenced by energy from factors previously described (Erhan, et al., 2006), this initiates the formation of free

radicals, which are easily formed by the removal of hydrogen atoms from monounsaturated, and polyunsaturated oils. This is because monounsaturated oils have a pair of missing hydrogen atoms and polyunsaturated oils are missing several pairs of hydrogen atoms, making them very unstable and highly reactive to oxidation. Saturated oils have a slightly higher degree of resistance to oxidation since they are chemically more stable (Asadauskas, et al., 2007; Fox and Stachowiak, 2007). Andrikopoulos et al (2002) also described the oxidative stress and deterioration that occurs in fried oil and their potential (cytotoxic, hepatotoxic, carcinogenic, mutagenic) effect(s) on health. For this reason some countries have recommendations in place for the maximum number of times vegetable oil may be used for frying in fast food and other restaurants (Andrikopoulos, 2004).

1.14.3 Free radicals and cellular damage

Free radicals are necessary for life, and play an important role in a number of biological processes, some of which are necessary for life. These include intracellular killing of bacteria by phagocytic cells, as well as cell signalling (redox) processes.

The human body employs enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase), as well as antioxidants (vitamin A, C, and E plus polyphenol) to minimize the effect of free radicals. However, excessive amounts of free radicals, such those from oxidized oil, cause cell injury, instability and death (Wang, et al., 2000) and are involved in diseases such as cancer, stroke, myocardial infarction, and diabetes. They are also implicated in other major disorders, including

arteriosclerosis, schizophrenia, alzheimer's disease, emphysema, psychosis, pigmentary melanin abnormalities, DNA mutations, deafness, arthritis and many aging and senile -related diseases (Wang, et al., 2000; Karthikeyan, et al., 2011). To preserve maize quality, while preventing cross-contamination between the stored maize and rancid, oxidized oil, a procedure was developed with the aim of cleaning edible oil containers, such that 100% of the oil is removed.

1.14.4 Edible oil container cleaning

Two related decontamination methods (saponification and soap) were considered for cleaning vegetable oil contaminated 20-L HDPE containers (“jerry cans”). In cleaning, involving saponification of fats and oil, (equation 1.1) the rate-limiting step is the alkali. Continual addition of alkali consumes all the oil, forming soap and glycerol. Considering the possible, corrosive effect on humans, and the environmental effect of alkali, as well as cost and availability, soap was employed as the primary cleaning agent to which other treatments (hot water, and oil drain from container) are compared. In addition to its already being commonly utilized for similar cleaning worldwide, soap is directly related to alkali, and molar equivalents of both can be established from saponification equations, in relation to the oil quantity to be cleaned. Hot water was used as a treatment because it is a known cleaning agent, traditionally utilized in East Africa for removing oil contaminants in containers similar to the 20-L HDPE containers utilized as experimental units, for this study. Oil drain serves as control treatment, while new 20-L HDPE container equivalents of contaminated 20-L HDPE containers serve as the control for the experimental units.

Since soap is a product of saponification (equation 1.1), general saponification principles and factors are described in detail, with a brief mention of “cleaning with soap”.

Triglycerides+3 KOH (or NaOH) +Heat –> 3 Soap+glycerol----------(1.1)

Treatments can be applied as proportions of weight, volume or as molar equivalents. Applying molar equivalents of treatments, when necessary, requires the ability to calculate molar mass and molar equivalents of treatments, applied. The procedure for achieving this is outlined below.

1.14.5 Estimating molar mass

According to Batt (2004), all the free fatty acids in oil are in the triglyceride form, the molar mass of free fatty acids in oil is about 834 g (or 278*3), 3 moles of fatty acids plus 1 mole of glycerol (molar mass=92.09) produces 1 mole of triglyceride, plus 3 moles of water. And average molar mass of soybean oil is 872.03g ((278*3)+92.09-(3*18.02)).

Based on this, estimating molar equivalents from saponification equation (Bhatt, 2004; Vaso, et al 2010), is possible, considering that there is 98% purified soap yield from soybean oil (Vaso, et al 2010), and the effective ratio of triglycerides, alkali, glycerin and soap is 1:3:1:3.

Assuming that free fatty acid (FFA) composition in soybean oil or fat is 100 kg (Bhatt, 2004), the quantity of soybean oil can be calculated as:

Quantity of soybean oil=(872.03*FFA (kg))/(278.00*3)

=(872.03*100)/(278.00*3)
=104.56 kg

1.14.6 Estimating molar equivalents

According to ACS (2011), 10 mL 6M of NaOH was required for saponification of 20 mL fat (or oil). Since the ratio of oil to alkali is 1:3, and assuming 98% soap yield, as well as 0.06 moles (6 M*0.01 liters) of NaOH, the number of moles of FFA equals 0.0588 moles ((0.06/3)*0.98 yield). This is equivalent to 49.0392g (0.0588 moles *834). Also, assuming 98% purified soap yield from soybean oil, the quantity of starting soybean can be calculated as:

=(872.03*0.049.0392)/(278.00*3)

= ~0.051 kg

And since density of distilled water equals about 1 g/cm^3, the molar equivalent of water can be calculated based on stoichiometry. However, distilled water, as treatment, can also be applied based on volume or weight equivalent of the soap treatment, since it has a 1:1 mass to volume ratio.

1.14.7 20-L HDPE containers

The 20-L HDPE containers utilized for this study have a net weight of 35 lbs (~16 kg) each, and contain 582 Fl. oz (15.88kg) 100% pure soybean oil (Columbus Foods, Chicago, Illinois). They were made out of high-density polyethylene (HDPE) plastic, with a resin classification/recycling code of 2 (ACC, 2007; Bakers & chefs, 608 S.W. 8th Street, Bentonville, AR), and which has a melting point of 130-135°C as well as a tensile strength of 4550 psi. They can withstand temperatures of 120°C

for short periods or 110°C continuously (EOS/EDS, 2000; Antec, 2001; Dow 2009; Dynalab, 2011).

1.4.8 Soybean oil

The computed average molar mass of soybean oil is 873.01 g·mol (Gonzalez, et al. 2006) or 872.03 g.mol, while the molar mass of free fatty acids in oil is about 834 g (or 278*3) mol^{-1} (Bhatt, 2004). This is because when 3 moles of fatty acids react with 1 mole of glycerol (molar mass=92.09), 1 mole of triglyceride and 3 moles of water are produced, giving an average molar mass of soybean oil equals 834 g ((278*3)+92.09-(3*18.02)) (Bhatt, 2004).

1.14.9 Soybean oil and smoke point

The temperature at which cooking oil or fat breaks down to form glycerol and free fatty acids, and produces bluish smoke is referred to as the smoke point. Refined soybean oil has a smoke point of about 257.2°C (~495°F) (Bader, 2010), while oxidized, rancid, oils have lower smoke point (Erhan, et al., 2006).

1.14.10 Saponification

Vegetable oil and animal fats (triglycerides) have a chemical propensity to undergo hydrolysis, yielding free fatty acids (FFA) and glycerols, naturally. And the addition of either of or a combination of temperature, pressure, enzymes, and strong alkali or strong acid catalysts speed up the hydrolysis (Yan, 2009). When alkalis are employed, the liberated FFA is converted into the corresponding metallic salt (soap), and the alkali catalyzed oil hydrolysis is termed saponification (soap formation). Oils

and fats also have a physical property consistent with being greasy to the touch (Schwartz and Schwartz, 1987; Lippingcot Williams & Wilkins, 2005), the presence or absence of which can be utilized in testing for complete or incomplete saponification.

In the saponification and refining of oil, it is often necessary to use excess alkali, to ensure complete saponification and coagulation of other impurities within the oil (Markley, 1951). And in the saponification of triglycerides, using an alcoholic alkali, transforms the triglyceride into the ethyl ester (alcoholysis or transesterification), which is usually the first reaction, followed by saponification. Since alcoholysis proceeds rapidly and can go to completion in minutes, at ordinary temperatures (room temperature to 60°C), it speeds up the saponification reaction, producing yields above 90% (ACS, 1920; Freedman, 1984; Vaso, et al, 2010). The soap yield from saponification is rarely 100%, due to the unsaponifiable fractions of crude oil (Yadav, 2002).

1.14.11 Saponification and temperature

Saponification can be done at both room temperature (cold saponification) and at higher temperature (warm saponification). However, saponification performed at room temperature takes relatively longer to complete. Carrying out saponification at higher temperature not only makes it proceed at a faster rate, but is likely to produce greater soap yield and cleaning effect. Researchers have demonstrated that every 10°C temperature increase increases the rate of reaction by a factor from 1.2 to 2 (Sebastião et al., 2006; ACS, 2011). And CRC (2005) successfully undertook

saponification in an enclosed container, at 80°C for 40 minutes, with occassional shaking.

1.14.12 Cleaning with soap

This study discovered that soap is capable of producing the same amount of clean as that hypothesized for alkali, when the right quantity is utilized in cleaning vegetable oil contaminated containers. Utilizing 3g of soap for each gram of soybean oil contaminant adequately decontaminated the contaminated soybean oil 20-L HDPE containers utilized in the recycling research.

1.14.13 Recycling treatment definition

Cleaning of oil-contaminated containers using water, with or without soap is common practice in East Africa (Yakubu, et al., 2011). Literature, however, suggests that cleaning, disinfecting and sanitizing containers, to prevent cross-contamination of contaminants with food stored within them is best done at high temperature, using soap and mechanical action (Knoxa and Walkera. 1947; Gangneux, et al., 2004; CRC, 2006; Helmenstine, 2011; MTL, 2011; Patwardhan and Kelkar, 2011), such as scrubbing and shaking. According to FAO (2002) heat treatment destroys oil-splitting enzymes, arrests hydrolytic rancidity and autoxidation, during vegetable oil extraction and cause oil to leach out from its container. And the use of hot water cleaning treatment is expected to produce similar advantages.

Factors considered for this research are (i) soap, at two water temperature extremes (control (45°C) and hot/boiling (90 to100°C)), (ii) water at two temperature extremes (control (45°C) and hot/boiling (90 to100°C)) and (iii) Container type (contaminated

and new). We hypothesize, therefore that cleaning with soap at high temperature (90 to100°C), with mechanical action would be enough to clean and sanitize vegetable contaminated containers enough for maize quality preservation within them. Since container surface just drained of oil gives us results to which we can compare the results of other treatments with, it would be utilized as a treatment, and because some of the oil in the 20-L HDPE containers had congealed, seemingly due to rancidity, the oil would be drained at 45°C, which is fat's melting temperature (ACS, 2011), to ensure free flow, but so as not to produce chemical changes in the oil. However, soap treatment at 45°C would not be utilized as a treatment, since it does not meet the cleaning objectives and increases cost.

1.15 RESEARCH NEED

The "*weevil mortality"* study was conducted to test earlier laboratory results, using controlled environmental tests conducted under different conditions and in different containers.

The "*seed germination*" study was undertaken because we needed to know if hermetically stored maize, containing maize weevils would provide the benefit of viable seeds, in addition to maize preservation, for food purposes. And the "*recycled container*" study was conducted since little information was found on the availability of used containers, in East Africa. A study was therefore needed on container sizes, prices, and quantity available for sale, in East Africa, as well as to establish cleaning standards, for reused edible oil containers to ensure food safety.

1.16 GENERAL OBJECTIVES

The dissertation objectives were to:

1) Test the integrity of hermetic storage and the validity of predicted time to complete mortality (PTCM) of maize weevils.
2) To determine the effects of length of hermetic storage and maize weevil infestation on maize seed germination. And to
3)
    a) Assess availability of used vegetable oil containers in East Africa suitable for hermetic storage of maize, as well as
    b) Develop procedures for cleaning the containers

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CHAPTER 2: TESTING PREDICTED TIME TO COMPLETE ADULT WEEVIL MORTALITY IN HERMETICALLY STORED MAIZE

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2.1 ABSTRACT

The ability to predict the time to 100% adult maize weevil mortality using hermetic storage is useful for design and management of hermetic storage systems. This is because the prediction, allows estimation of days until 100% adult weevil (*Sitophilus zeamais*) mortality as a function of weevil infestation level, storage temperature and maize moisture during hermetic storage. The study found significant ($p<0.0001$) temperature-moisture interaction for hermetic treatments, with 100% weevil mortality rates. The two studies presented here tests results of an earlier oxygen measurement study, using different containers and conditions not tested in the original study. The laboratory-scale study utilized maize at 12% moisture content, 216 canning jars, and 23°C chamber, and found a mean adult weevil mortality and standard error of 94.2±10.77% for hermetic treatments versus 3.1 ±4.69% for non-hermetic treatments. The field-scale study used 12 recycled 20-L HPDE containers, maize at 12.5% moisture and 23°C storage room temperature and found 96.8±3.43 mean mortality and standard error for hermetic treatments versus 3.4±3.71%, for non-hermetic. Both studies tested the results of the original study's ability to predict

time to complete adult maize weevil mortality, as well as confirmed laboratory and field efficacy of hermetic storage.

Keywords: *Maize storage, Hermetic storage, Maize weevil, 20-L HDPE containers*

2.2 INTRODUCTION

2.2.1 Staple food

Maize is a major staple food in many cultures, including East African countries, where it is a cash crop and contributes to food security and provides a source of livelihood for hundreds of thousands of farmers in the region's agriculture driven economies (Govereh, 2008; UGL, 2010). Maize accounts for at least 50% of caloric intake for people in East Africa (Sinha, 2007).

2.2.2 Post-harvest storage losses

Inadequate storage, such as open air storage in granaries leads to rapid insect growth and damage to stored maize (Lindblad and Druben, 1980; Yigezu, 2009) resulting in post-harvest storage losses (Amani, *et al*., 1992; Villers, *et al*., 2006; Darby and Caddick, 2007). According to the PHL Network (2009), about 19% of total annual maize production is lost in post-harvest storage in East Africa. And although post-harvest pests (Montemayor, 2004; Villers, 2004; SGRL, 2007; Weinberg, et al., 2008; Bern, *et al*., 2011) include insects, molds, birds and rodents, the most economically important post-harvest storage pest is the maize weevil (Jacobs, 2004; Dhliwayo and Pixley. 2003). Studies have shown that the combined effect of maize weevils and molds is capable of causing up to 100% damage to stored maize

(Demissie *et al*., 2008). According to Villers, *et al*., (2008), in tropical climates, even commodities that are initially sufficiently dried suffer from susceptibility to external humidity and moisture condensation leading to fungal and insect growth, which raises the moisture content to unsafe levels (Darby and Caddick, 2007; FAO, 2001; Weinberg, *et al*., 2008). These factors as well as the cost and toxicity of chemicals (Navarro, et al., 1994; Korunic, 1998; Villers, *et al*., 2004; 2006; Murdock, *et. al*, 2007; EPA, 2011) make natural insect control (such as hermetic storage) an attractive option (Daly, *et al*. 1998; Chapman, 1998; De Bruin, 2002; Villers, et al., 2006; Navarro, *et al*., 2007). Hermetic storage (Donahaye, *et al*., 1991; Copeland and Mcdonald, 1995; Johnson, *et al*., 2005; Fulton, *et al*., 2009) has several implementations (Donahaye, *et al*., 2007; Christopher, *et al*., 2008; IRRI, 2008), and the recycling of edible oil contaminated 55-gallon drums, for example, for long term use in hermetic storage of grains is common practice (Seck, *et al*., 1996; Lindblad and Druben, 1980; Murdock, *et al*., 2003; Adhikarinayake, 2005; Harris and Miller. 2008).

The research on which the predicted time to complete mortality (PTCM) research is based (Figure 2.1) was conducted at fixed temperatures (10°C and 27°C), maize moistures (8% and 16%), and time (4 to 28 days) (Yakubu, 2009; Yakubu et al., 2011). A field study, based on Yakubu, *et al*., (2011) stored maize *(*13.7 %), with about 90 weevils/kg, in six 10-L recycled edible oil containers at ambient temperature (~20°C), and shaded from direct sun rays. Three containers were sealed, and three had screening to allow air circulation without allowing weevils to escape. The study conducted in Uganda (East Africa) produced 100% weevil

mortality in 28 days (Figure 2.2) in the three hermetic containers (Brumm, 2011; Brumm and Bern, 2011). Although, the PTCM for that study was 14 days (Yakubu, *et al*., 2011), 28 days was utilized in the field to allow a sufficient "margin of safety" to ensure 100% weevil mortality, and reduce losses to stored maize.

2.2.3 Research need

A study is needed which will test earlier laboratory results, using controlled environmental tests conducted under different conditions and in different containers.

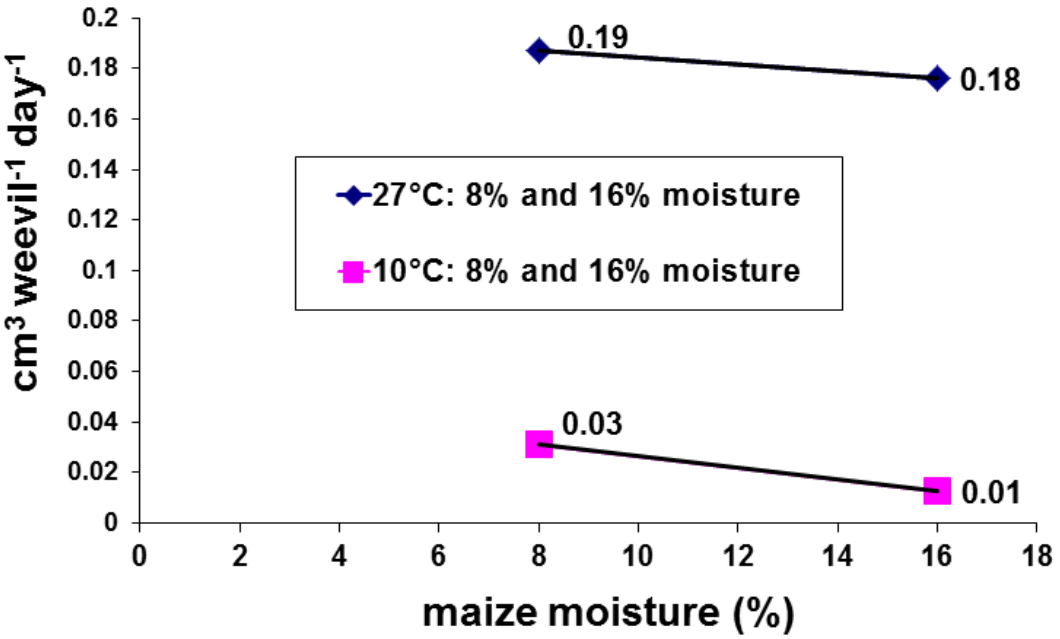

Figure 2.1. Average oxygen consumption of maize weevils in shelled maize (Yakubu, et al., 2011).

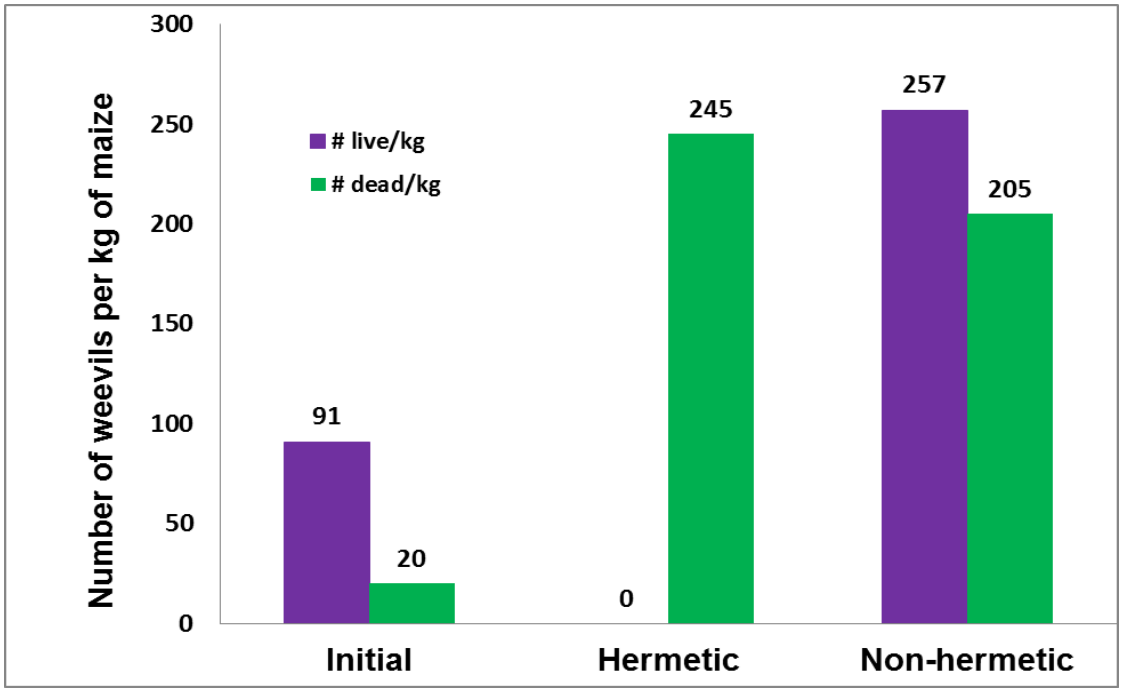

Figure 2.2: Weevil mortality in 28-days maize storage using10L recycled edible oil containers (Brumm and Bern, 2011).

2.3 OBJECTIVES

The objectives of this research were to test the predicted times to complete adult weevil mortality (1) under laboratory conditions using rigid (canning) glass jars, as well as (2) under field hermetic storage conditions using flexible (HDPE) 20-L containers.

2.4 MATERIALS AND METHODS

A laboratory-scale and a field-scale hermetic storage system were used, where the synergistic effect of O_2 depletion and CO_2 accumulation by insects, maize and microbial metabolism, is sufficient for non-chemical, post-harvest hermetic maize preservation. The study is aimed at testing the validity of the predicted days to

mortality of a previous study that defined a procedure for predicting time to complete adult weevil mortality (Yakubu, *et al*., 2011).

2.4.1 Experimental maize

Maize grain of the commercial hybrid Fontanelle *6T672* was harvested at about 14.5%[1] moisture using a 4420 Deere combine. Following harvest, maize was cleaned (Yakubu, *et al*., 2011) to remove broken maize and foreign material and stored at 4°C until use. Experimental maize moistures were measured using the 103^{o}C, 72 h oven method (ASABE, 2008). The maize which lost 2.5 moisture points during storage was used as is, for this research.

2.4.2 Experimental weevils

A stock culture of 100 adult *S*. *zeamais Motschulsky* (unsexed) obtained from the Iowa State University Entomology Departmental laboratory were placed in five unsterilized 3.74-L glass jars, with screen lids, half full of 12% moisture Fontanelle *6T672* maize. Weevils were allowed to oviposit on the maize to develop a colony. This was achieved by placing maize weevils in a growth chamber at about 27°C and at interstitial relative humidity determined by maize moisture, for two months (Arannilewa, *et al*., 2006). Weevils from this colony were used in the hermetic storage studies.

[1] All moistures are % wet basis

2.4.3 Laboratory scale testing

2.4.3.1 Objective

To test time to complete adult weevil mortality, in a laboratory scale hermetic storage study, using glass-canning jars.

2.4.3.2 Experimental containers

Each of 216, 473-mL (one-pint) Kerr canning jars (Mason Jar 61000, Jarden Home Brands, 14611 W. Commerce Road, Daleville, IN) was loaded to 90% capacity, with 0.337 kg of maize at 12% moisture and stored at 23°C along with the number of weevils (21, 17, and 14) necessary to bring about 100% weevil mortality at the desired number of days (17, 21, and 26, respectively). Hermetic tests utilized canning jars with sealed lids, while non-hermetic tests utilized jars fitted with aluminum screen lids, which allowed air passage but not weevil escape. Jars were stored in a model 13-988-126 GW, Fisher Scientific Isotemp refrigeration chamber (Thermo Fisher Scientific Inc., Waltham, MA 02454), maintained at 23^{o}C.

2.4.3.3 Treatment design

The completely randomized block treatment design had four factors (days, maize moisture, temperature, and storage type). Days (storage time) had 3 levels (17days, 21 days, and 26 days), moisture had one level (12%), temperature had one level (23°C), storage type had two levels (hermetic and non-hermetic), and there were eighteen replications (Figure 2.3).

We hypothesized that utilizing 80% and 120% of any predicted time to complete mortality (PTCM) derived from a spreadsheet developed from Yakubu et al., (2011) can predict the number of weevils needed for complete weevil mortality. And using 4 days as our PTCM, we obtained 3.2 (80% of 4 days), and 4.8 (120% of 4 days).

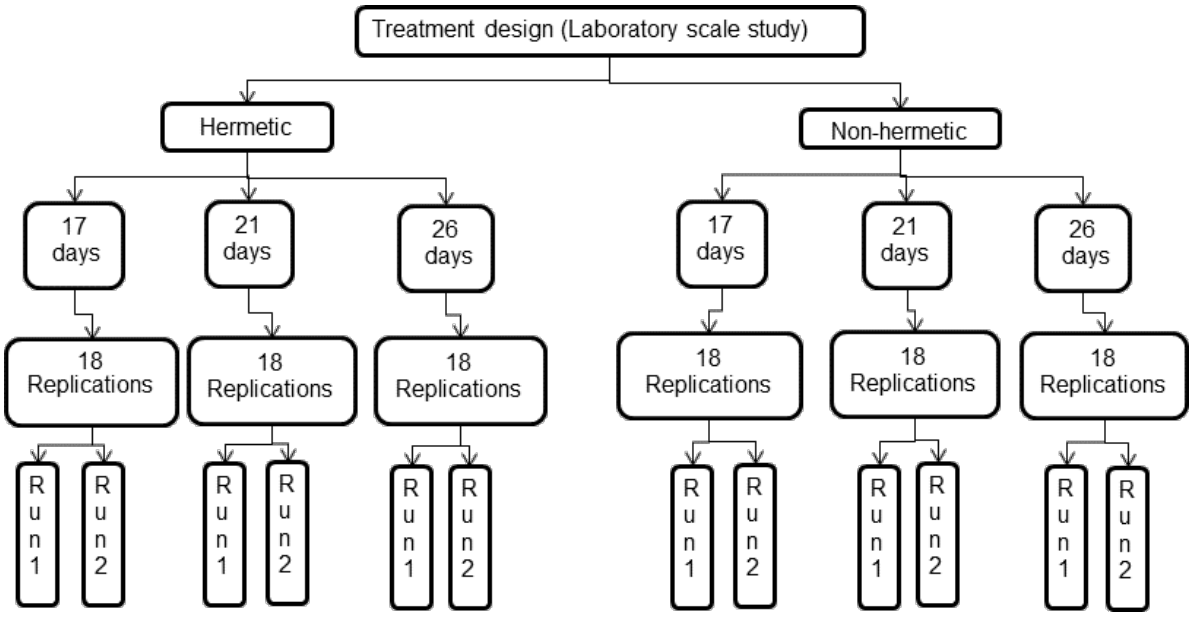

Figure 2.3. Flow chart for testing time to complete weevil mortality (laboratory study) at 12% maize moisture and 23°C.

However, we needed a PTCM that takes longer for complete mortality to occur, in order to obtain more data points, as well as improved precision.

Testing of the previous research means that we should be able to choose any three sets of values from those bounds of temperature, moisture, and time defined by Figure 2.1, such that we are able to calculate the (a) number of weevils, and (b) weevil oxygen consumption necessary for a given PTCM.

Therefore, the number of weevils necessary to bring about complete mortality were calculated for the three different PTCMs, and the sets of treatments defined

previously were replicated 18 times, in two runs and randomly assigned to positions in the 23°C temperature laboratory chambers. Runs represent the number of times the storage chamber was filled with experimental units during the conduct of this research.

2.4.4 Field scale testing

2.4.4.1 Objective

To test time to complete adult weevil mortality, in a field scale hermetic storage study.

2.4.4.2 Experimental containers

Plastic (HDPE) 20-L containers designed to hold 15.88 kg (582 fl. oz) soybean oil (Columbus Foods, Chicago, Illinois 60622) were collected from Chinese fast food restaurants in Ames and Des Moines, Iowa and cleaned using a “soak-shake-rinse” approach. This involved adding 1 L of tap water to each 20-L HDPE container, adding about 57g (2.0 oz) of ultra concentrated soap (Dawn, Procter and Gamble, Cincinnati, OH) dish soap and shaking for 5 min, until the lather filled the 20-L HDPE container interior. The containers were then covered with lids, left standing for 24 h and shaken for 5 min, at the end of the 24 h period. Their contents were emptied, thereafter, and they were each rinsed thrice. Following this, they cans were inverted and allowed to dry for 24 h before use for this research. The lids were also scrubbed, with the dish soap and sponge, upon opening before being left to dry, for the same length of time.

2.4.4.3 Treatment design

The completely randomized block treatment design consisted of three factors (day, maize moisture, and temperature). Day had one level (36 days), moisture had one level (12.5%), temperature had one level (23°C), while there were six replications (Figure 2.4).

2.4.4.4 Procedure

This (second) experiment was conducted to more closely simulate maize storage in the field, and involved storing about 15 kg maize in each of the twelve 20-L (~5-gallon) HDPE containers. Of the twelve experimental units utilized for this experiment, six were randomly assigned to non-hermetic treatments and the other six to hermetic treatments. Each one was filled with as much maize as it could contain, while resting on the floor.

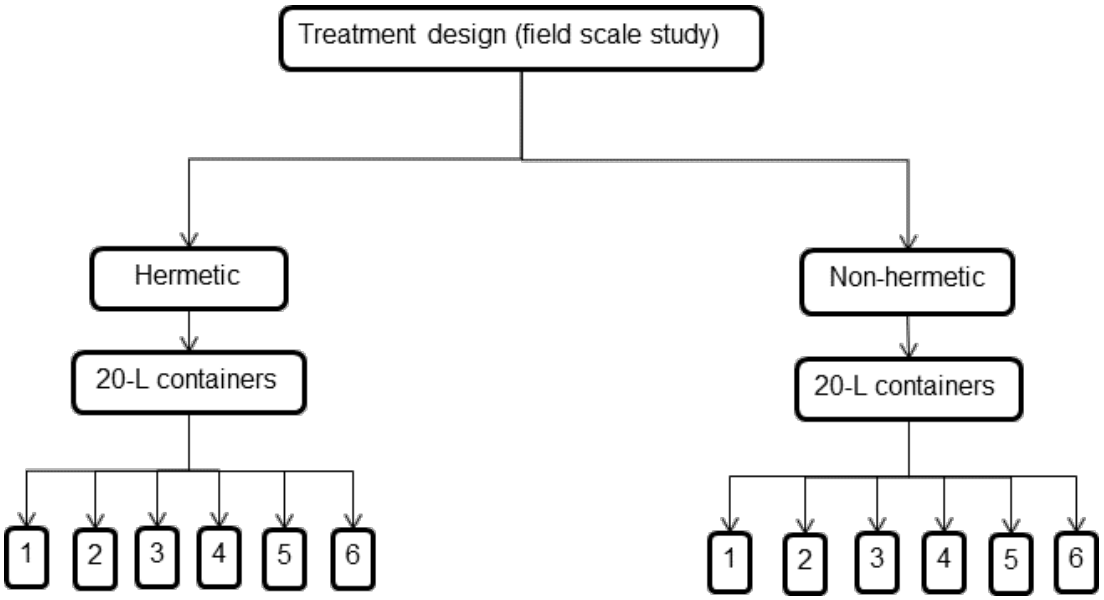

Figure 2.4. Flow chart for testing time to complete weevil mortality (Field study) at 12.5% maize moisture and 23°C.

2.4.4.5 Maize quantity and percent fill

It was noted that container volume increased when filled 20-L HDPE containers were lifted off the floor or tapped against the floor, increasing available headspace (Beals, *et al*., 2000; Roylance, 2001; Langer, 2008). Considering EU volume expansion, it was estimated that an extra 2 kg would be required to fill the EU. Based on estimated total quantity of maize (17.50 kg) required to fill this EU, a percent fill was calculated for individual EU, and the number of weevils required to use up the remainder of the oxygen was calculated, according to percent fill and maize quantity in each 20-L HDPE container. This is based on the preference for filling containers to the brim in field hermetic storage practices (Markley, 1951; Umaine, 2007; De Jaeger, *et al*., 2003; Devor, *et al*., 2007; Sohb, 2008). This provided a basis for determining the total number of weevils required to use up the O_2 and to cause complete weevil mortality. The number of weevils required to cause complete mortality was also applied to non-hermetic 20-L HDPE container, used as control treatments.

2.4.4.6 Example calculations

Step 1

Estimated additional 20L HDPE container space to be filled (kg) = A1

Estimated 100% 20L HDPE container=17.52 kg

Initial weight of maize in 20L HDPE container (kg) = A2

Estimated percent 20L HDPE container space left to fill=A3= (A1/A2)*100

Estimated percent 20L HDPE container fill=100-A3

Step 2

The "estimated percent 20L HDPE container fill" was then used to calculate number of weevils needed to use up of the100% O_2 in the 20L HDPE container.

Example

The first 20L HDPE container had 86% fill (15.40 kg). Therefore:

Number of weevils required =

$$\frac{\left(\frac{\text{atmospheric oxygen level}}{100}\right) * \left(\frac{\%void}{100}\right) * \left(\frac{\%fill}{100}\right) * (maize\ (kg) * 1387.92) + \left(1 - \frac{\%fill}{100}\right) * ((maize\ (kg) * 1387.92))}{(\#days\ to\ complete\ weevil\ mortality * weevil\ Oxygen\ utilization)} \quad \text{- - (2.1)}$$

=771 weevils=50 weevils/kg

From above, weevil oxygen utilization =0.14 cm^3 $weevil^{-1}$ day^{-1} (Yakubu, 2009), obtained from substituting known weevil numbers into equation 2.1. Number of days to complete adult weevil mortality can be calculated from the equation by similar substitution. The calculation example results presented above utilized an atmospheric O_2 level of 20.99%. Additional weevil oxygen utilization calculations that take storage maize moisture and temperature in consideration are also presented in Yakubu (2009). Using 50 weevils per kg within the storage containers, 12.5% maize moisture and 23°C temperature, it was calculated that complete weevil mortality would occur in the hermetic treatments, in about 22 days. However, an extra 14 days were added to the PTCM, in order to increase the probability of complete mortality.

Non-hermetic treatment involved filling each assigned EU with maize and weevil as above and using an aluminum screened lid. This is to simulate open-air storage, without allowing weevil escape. Therefore, using two treatments types (hermetic, non-hermetic) with six replications for each treatment type gave a total of 12 experimental units (EUs), with the total number of weevils for each 20-L HDPE container calculated according to Yakubu (2009) and counted manually.

2.4.4.7 Sealing hermetic 20-L HDPE containers

2 ply plastic bags (Iowa Prison Industries, Plastics division, Mitchellville, IA. Bag size: 33X39, MIL: 0.7; Gallon: 33; PCS: 300; LOT #: 04212009), folded 5 times were placed over the mouth of each hermetic treatment's EU, and covered with a screw-on lid, to prevent air escape, but not so tight as to damage the plastic bag. After this, one #64, crepe colored, 31/2 *1/4 in rubber band ("@ the office", 00564WM, distributed by Wal-mart stores, inc. Bentonville, AR) was wrapped around the part of the bag extending beneath the lid, four times. This was done to push the bag tightly against the neck of the EU, and prevent air exchange between the storage and external environment. A similar bag as the first one was then placed over the lid, to extend beneath it and another rubber band was used to bind the part of the bag beneath the lid tightly to the neck of the 20-L HDPE container.

2.4.4.8 Experimental chamber

The recycled 20-L HDPE containers containing maize were stored on a pallet in a room maintained at 23°C.

2.4.4.9 Post experiment weevil count

To determine number of dead weevils at the termination of the research, two empty non-hermetic jars, labeled “dead (D)” and “alive (A)”, and covered with screen and lid (with hole) where setup and assigned to each EU. Each set had the number of the 20-L HDPE container to which they were assigned written on them. And destructive sampling of EUs was used, where one EU was opened at a time and tipped over to empty its content into a 4.8-mm (12/64-in) round hole sieve, sitting over a pan, a little at a time. The sieve was then shaken to dislodge (Navarro, *et al*., 2007) the weevils into the pan, and the weevils were counted into their assigned jar, according to whether they were dead or alive (Yakubu, *et al*., 2011).

2.5 RESULTS AND DISCUSSION

2.5.1 Laboratory scale test results

This study is a follow-up laboratory-scale study of the original research (Yakubu, *et al*., 2011), which involved “predicting time to complete mortality (PTCM)”. That study found significant effects of day, storage type, temperature, moisture and oxygen on weevil mortality, for hermetically stored maize (complete data are shown in Appendix A).

2.5.2 Mortality prediction shortfalls

Weevil mortality for hermetic treatments (Table 2.2) shows a major advantage over open-air storage (non-hermetic), although weevil mortalities were not 100%. Weevil oxygen utilization rates are greater at higher temperatures (25 to 33°C) than at lower temperatures (Figures 2.1 and 2.5) and weevil mortality rate follows the same trend

(Nakakita and Ikenaga, 1997; Yakubu, *et al*., 2011). Below 25°C there is a need for time adjustment, due to the high level of variability associated with lower temperature mortality prediction (Nakakita and Ikenaga, 1997; Yakubu, 2009).

Analysis of variance results indicate that although there is significant ($p<.0001$) treatment and storage type (hermetic) effects, other main effects (day, level), as well as interaction effects (day by hermetic, block by level, and treatment by level) were not significant.

2.5.3 Adjusting predicted time to mortality

In general, the rate of oxygen depletion and weevil mortality in hermetic storage is dependent on the temperature, maize moisture content, quantity and quality of maize sample, insect population, and /or presence of molds (Krishnamurthy, *et al*., 1986). At the $p=0.05$ significance level (Dallal, 2003), the difference in mean percent mortality (Table 2.1) among days 17, 21, and 26 (hermetic) is insignificant ($p=0.5839$). The difference in mean percent mortality among days 17, 21, and 26 (non-hermetic) is also insignificant ($p=0.2005$). However, the difference in overall hermetic versus non-hermetic treatments is significant ($p<0.0001$).

Table 2.1: Days to complete weevil mortality in 12% maize stored 23°C (laboratory scale).

Storage type	Treatments (storage times)	Percent mortality
Hermetic (mean ± S.E)	17 days (80%)	94.9 ±9.10
	21 days (100%)	93.5 ±9.40
	26 days (120%)	94.1 ±13.5
Non-Hermetic (mean ± S.E)	17 days (80%)	2.25 ±3.50
	21 days (100%)	3.59 ±5.12
	26 days (120%)	3.37 ±5.26

Table 2.2 shows significant difference between hermetic and non-hermetic treatments in the laboratory-scale PTCM results, as reflected in the mean mortality for hermetic (94.20%) versus non-hermetic (3.10%) treatments.

Table 2.2: Mean adult weevil mortality for laboratory-scale PTCM study at 23°C and 12% moisture.

Experimental units: 216, 473-mL glass canning jars	
Hermetic mortality rate (%)	**Non-hermetic mortality rate (%)**
94.2±10.8	3.1 ±4.7

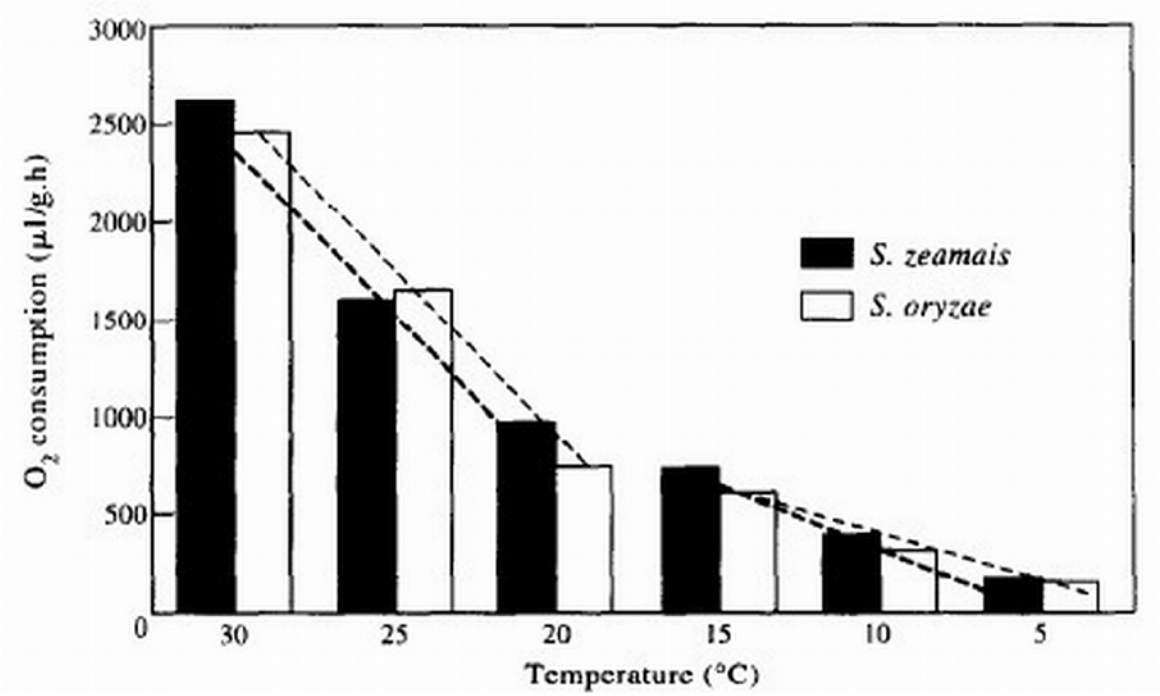

Figure 2.5. Oxygen consumption of adults of *S. zeamais* and *S. oryzae* at different temperatures (Nakakita and Ikenaga, 1997).

2.5.4 Interactions

The percentage mean adult weevil mortality differences (hermetic main effect) between hermetic and non-hermetic treatments is significant ($p<0.0001$), with an estimated mean difference of 91.1 (94.2-3.1). However, the interaction (differences of differences) result across days (Table 2.3), suggest no clear hermetic by day interaction ($p<0.5734$). This indicates that the mortality differences in hermetic treatments for all three levels of days (17, 21, and 26) are insignificant (Figure 2.6 and 2.7).

Table 2.3**:** Day by storage type (hermetic) interaction for laboratory-scale PTCM study at 23°C and 12% moisture

Storage type	**Storage time**			**Means (storage type)**
	17 days	**21 days**	**26 days**	
Hermetic	94.9	93.5	94.1	**94.2**
Non-hermetic	2.3	3.6	3.4	**3.1**
Means (storage time)	**48.6**	**48.6**	**48.8**	
Difference (storage time)	**92.6**	**89.9**	**90.7**	

2.5.5 Hermetic storage

In practice, the error margin for predicted time to complete mortality increases as the size of the storage container increases. Therefore, it is best to fill hermetic storage containers to the brim (Umaine, 2007).

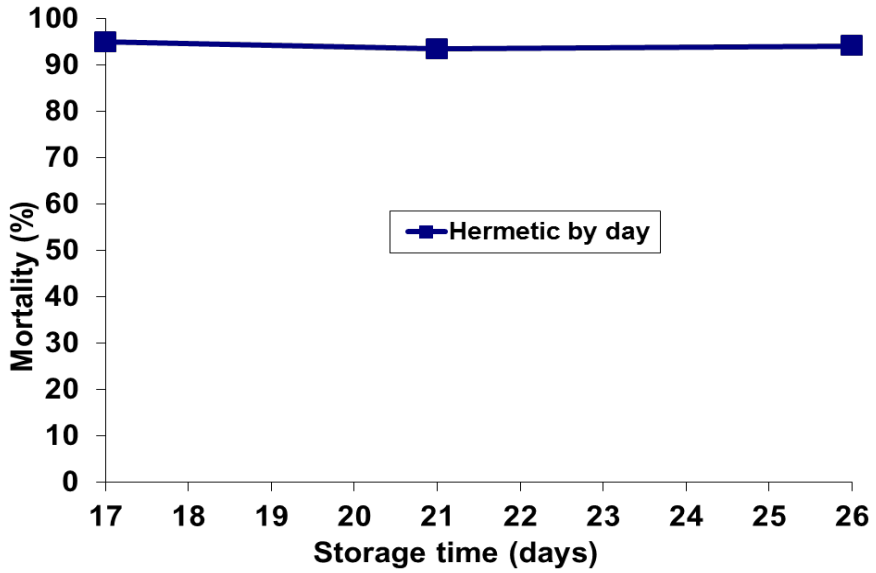

Figure 2.6. Treatment by day interactions at 23°C (***hermetic***).

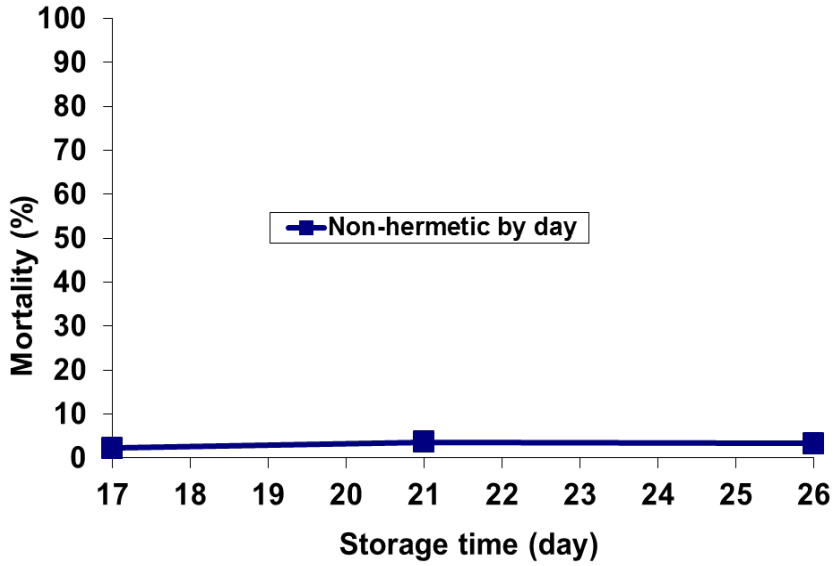

Figure 2.7. Treatment by day interactions at 23°C (***non-hermetic***).

The hermetic chamber utilized in this research had three storage levels, compared by Figure 2.8 (hermetic treatments) and Figure 2.9 (non-hermetic treatments). Both figures indicate insignificant difference between the three storage levels.

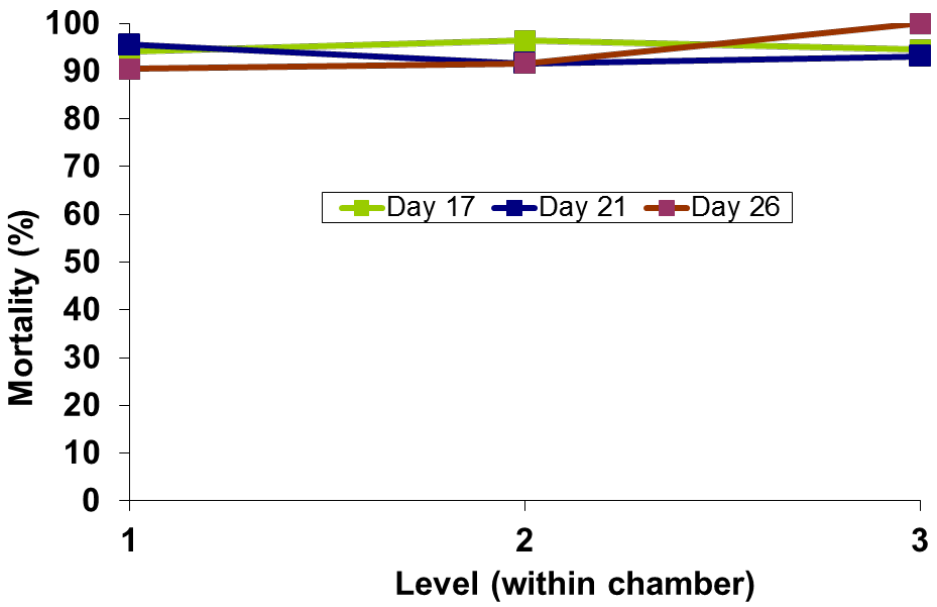

Figure 2.8. Treatment by chamber-level interactions at 23°C (for days17, 21, and 23: ***hermetic*** conditions).

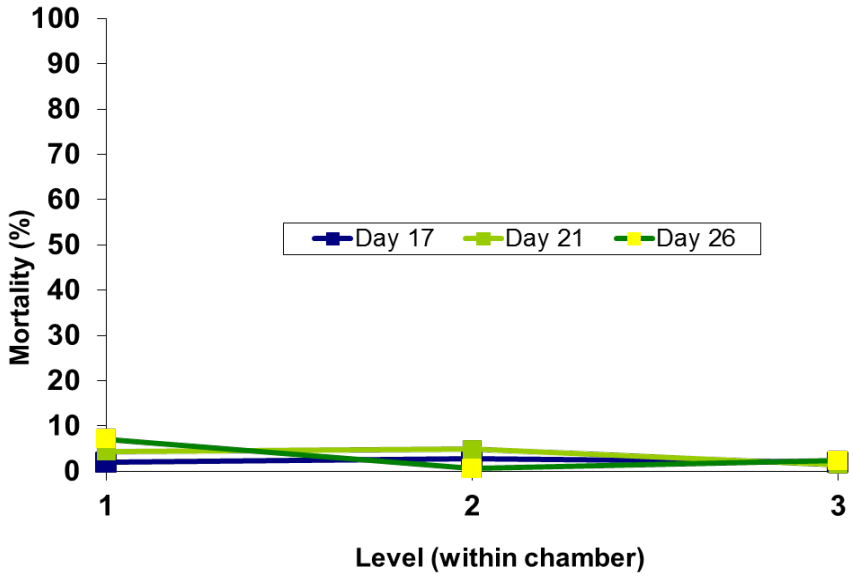

Figure 2.9. Treatment by chamber-level interactions at 23°C (for days17, 21, and 23: ***non-hermetic*** conditions).

Table 2.4 and 2.5 describe the result of research similar to those reported for laboratory scale testing (Table 2.1), but for experiments conducted under field

conditions using 12, 20-L HDPE containers, as the experimental units. This research also found significant evidence in favor of hermetic treatment over non-hermetic treatments ($p<0.0001$).

Table 2.4: Days to complete weevil mortality in HDPE 20-L containers for 12% maize stored at 23°C (field-scale).

20-L container label	Maize (kg)	Estimated % fill	Live weevils loaded	Alive	Dead	Container type	Percent Mortality
1	15.40	86	771	15	756	Hermetic	98
2	15.62	88	782	77	705	Hermetic	90
3	15.36	86	769	750	19	Non-hermetic	2
4	15.36	86	769	751	18	Non-hermetic	2
5	15.36	86	769	14	755	Hermetic	98
6	15.18	85	760	753	7	Non-hermetic	1
7	15.18	85	760	748	12	Non-hermetic	2
8	15.13	84	757	0	757	Hermetic	100
9	15.32	86	767	18	749	Hermetic	98
10	15.39	86	770	753	17	Non-hermetic	2
11	15.55	87	778	23	755	Hermetic	97
12	15.61	88	781	696	85	Non-hermetic	11

Table 2.5: Mean adult weevil mortality for Field-scale PTCM study at 23°C and 12.5% moisture.

Experimental units: 12, 20-L HDPE containers	
Mean hermetic mortality rate (%)	**Mean non-hermetic mortality rate (%)**
96.8±3.4	3.4±3.7

2.5.6 Field scale study

2.5.6.1 Weevil reproduction and temperature

Temperature may be sub-classified into optimal (25 to33^{o}C) and suboptimal (13-25^{o}C) temperatures, with regards to maize weevil activities. This is because lower temperatures reduce the rate of oxygen utilization, development, feeding, reproduction, and survival (Herrman, 1998; Nakakita and Ikenaga, 1997; PaDIL,

2009; Yakubu, *et al*., 2011). According to PaDIL (2009), complete development time for the life cycle of *S. zeamais* averaged 36 days (range 33 to 45) at 27 ± 1^{o}C, and 69 ± 3% RH.

The life cycle involves the female drilling a hole into the kernel, depositing the egg, and secreting a mucilaginous plug to enclose the egg as the ovipositor is withdrawn. However, this external evidence that the kernel is infested was negligible in both the hermetically and non-hermetically stored maize, for our research, suggesting that sub-optimal temperature may have slowed or suppressed insect reproduction. PaDIL (2009) also discovered that the maximum daily rate of fecundity, duration of development, and numbers of progeny produced were optimal at 30^{o}C and 75% RH. Since, we conducted our research at suboptimal temperature (23^{o}C) it seems like the existing eggs may require more time to hatch.

Hermetic treatments, for our research had 97 to 100% weevil mortality versus 1 to 11% mortality for non-hermetic treatments (Table 2.4). Therefore, few live weevils were remaining in the 20-L HDPE containers to which hermetic treatments were applied. Other researchers (De Bruin, 2002; Villers *et al*., 2004; Villers and Gummert, 2009) also found the presence of live insects following 12 months of hermetic storage. Villers and Gummert (2009) showed about 154 (at month zero) to 39 live insects (at month 12) or 73% decrease in live insect population in hermetic storage for 20L container (17.50 kg maize) equivalent. This may be because of late-hatching eggs, which produced weevils that were not yet dead or it may be due to residual oxygen, within hermetic containers that continued to sustain some weevil

life and prevented complete mortality (De Lima; Nakakita and Ikenaga, 1997; Fields, 2006). This seems to agree with previous authors' findings that the rate of adult insect mortality, respiration and reproduction in hermetic storage is slower at sub-optimal temperatures (De Lima; Nakakita and Ikenaga, 1997; Fields, 2006).

2.5.6.2 Packing density

Variability in 20-L HDPE container packing produced different filling rates and weights because 20-L HDPE container volume expanded with maize load and when lifted off the ground (Tsai, 1999; Roylance, 2001; Langer, 2008). Achieving packing densities close to those of farm-scale silos, to create anaerobic conditions, requires packing considerable maize kernels into a relatively small volume (Johnson, *et al*., 2005), in plastic containers.

2.6 CONCLUSIONS

Based on this research:

- Significant ($p<0.0001$) differences in mean mortality rates were recorded in hermetic (94.2%) versus non-hermetic (3.1%) laboratory scale treatments, conducted at 23°C with 12% moisture maize in 473-mL jars.
- Significant ($p<0.0001$) differences in mean mortality rates were also recorded in hermetic (96.8%)) versus non-hermetic (3.4%) field scale treatments, conducted at 23°C with 12.5% moisture maize in 20-L HDPE container.
- Insignificant interactions exist in day by hermetic ($p=0.5734$) and treatment by level ($p=0.1311$) interactions.

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CHAPTER 3: EFFECT OF HERMETIC STORAGE AND MAIZE WEEVILS ON MAIZE SEED GERMINATION

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3.1 ABSTRACT

Up to 100% seed germination loss is possible during storage by subsistence farmers. This is mostly due to high relative humidity and pest damage. Tropical relative humidity, for instance, is usually above 90% for most of the year. This is above the officially recommended seed storage relative humidity of 70% (14% moisture content) and below, and limits the ability to store seeds for extended periods in the tropics. The resulting high seed loss is due to the interaction among temperature, maize moisture content, insects and molds which accelerate seed damage. Maize weevils (*Sitophilus zeamais (Motsch.))* cause heavy damage to maize (*Zea mays L.)* during open-air storage, and although some farmers utilize insecticides for the control of the weevils, with some success, others rarely use conventional insecticides to protect their grain. Varoious authors have determined hermetic storage to be as effective as cold storage, but cheaper, in addition to being adaptable to local cultures. Therefore, this study utilized hermetic storage for the control of maize weevils, and conducted seed germination tests, following storage. The objective of the study was to determine the effectiveness of hermetic on-farm

seed storage from a seed quality preservation perspective. Laboratory studies were conducted where maize seeds in storage jars were stored in a controlled temperature (27°C) chamber for various time periods (0,4, 8, 12 months), and germination tests were conducted on the seeds under optimal growth conditions (800 mL hydration, 25°C, fluorescent lighting) in the laboratory. Hermetically stored maize seeds had 98.7 to 99.5% germination rates versus 35.0 to 72.9 for non-hermetic (open-air) storage, over the 12-month seed storage period. Treatments of particular interest are hermetic treatments with weevils, which had mean germination rates of 99.1% (at month 0), 98.7% (at month 4), 99.6% (at month 8), and 99.3% (at month 12), respectively. The conclusion is that hermetic storage preserves maize seed viability, even when seeds are stored under ambient conditions, and with weevils.

***Keyword**: Maize, weevils, hermetic seed storage, open-air, germination, seed storage.*

3.2 INTRODUCTION

Agriculture, in countries such as Ethiopia, and Somalia (East Africa) is mostly rain-fed. And post-harvest maize storage structures, such as cribs and granaries, usually made from simple materials such as wood, thatch, and mud (Lindblad and Druben, 1980; Metzeger and Muir, 1983; Vincent, et. al., 2001; Betuco. 2012) allow re-wetting of stored maize seeds. That and unreliable rainfalls (Hatibu, et al., 2003), creates a need for reliable seed storage and preservation systems to accommodate the once-a-year seed storage cycle of 8 to 9 months (De Bruin, 2005; Villers, et al.,

2008). This is important considering that at least 70 percent of maize seeds in developing countries are sourced from prior year's maize harvest (Gemeda, et al., 2001; Dhliwayo and Pixley, 2003), 50 percent of caloric intake is maize-based (Yakubu, et al., 2011), and environmental factors can easily destroy seeds (Fong and Standifer, 1969; Copeland and Mcdonald, 1995; Desai, *et al*., 1997; De Bruin, 2005; Basra, 2006).

3.2.1 Maize storage

Following maize harvest, subsistence farmers usually clean, dry and store seeds in woven jute or woven propylene bags in ordinary warehouses, since most of them cannot afford cold storage (Burden, 2003; Villers, et al., 2008). This practice usually causes rapid drop below the 85-90% "certified seed" germination rate within six months (De Bruin, 2005; Villers, et al., 2008). The use of hermetic storage has been proposed, in place of cold storage and other storage systems involving chemical preservatives, considering the many advantages of hermetic storage (Villers, et al., 2008).

3.2.2 Seeds and hermetic storage

Hermetic storage is a safe, cost-effective storage method that controls insect infestations in addition to preserving the quality of grains (Calderon and Navarro, 1980; De Bruin, 2005; Lewis, *et al*., 2005; RSAS, 2000; Villers, 2004; De Bruin, 2005; Lewis, *et al*., 2005; Sabio, et al., 2006; Villers, *et al*., 2006; Navarro, et al., 2007; Villers, et al., 2008; Weinberg et al., 2008), while allowing for pesticide-free, short-term and long-term qualitative and quantitative seed preservation, without

refrigeration, maintaining seed vigor (De Bruin, 2005; Calderon and Navarro, 1980; FAO/IPGRI, 1994; De Bruin, 2005; Sabio, *et al*., 2006; Pérez-García *et al*., 2006; Villers, *et al*., 2006; Daniel, 2007; Yakubu, et al., 2011) and pest control. Storage at low temperature (4°C (Pant and Susheela, 1977; Hernández-Muñoz, et al., 2006)) ensures greater safety margins between insect development time and break of dormancy, although hermetic storage, even at ambient temperatures, naturally eliminates insect development altogether (Yakubu, et al., 2011), and preserves germination rates (Daly, et al., 1998; Armitage and Woods, 1999; Moreno-Martinez, et al., 2000; Demissie *et al*., 2008a Poethke and Liebig, 2008;Gregg and Billups, 2009; Basra, 2006; SAFgerm, 2011). For seed germination and vigor to be maintained at close to germination rates at the unset of storage , it is important that seed moisture levels be maintained at 10-12%, regardless of storage temperature (Calderon and Navarro, 1980; Tang and Sokhansanj, 1993; FAO, 1994; Copeland and Mcdonald, 1995;;Spann, 1998; Armitage and Woods, 1999; Copeland and Mcdonald, 1995; ASAE, 2001; CRC, 2001; Basra, 2006; Armitage and Woods, 1999; Bankole, et al., 2005; De Bruin, 2005; Sabio, *et al*., 2006). Hermetic storage is capable of maintaining relative humidity that preserves seed moisture (ASTM, 1997; Lindblad and Druben, 1980; Vertucci and Roos, 1993; Walters, 1998; Smith, 1992; ASAE, 2001; Kung'u, et al., 2003; Adhikarinayake, 2005; Rickman and Aquino, 2003; De Bruin, 2005) and prevents mold growth (IBPGR, 1976; FAO, 1988; FAO/IPGRI, 1994; IPGRI, 2004; Daniel, 2007; Harris and Miller, 2008; Weinberg, et al., 2008).

3.2.3 Research need

This study was undertaken because of a need to know if hermetically stored maize containing maize weevils can preserve maize seed viability.

3.3 OBJECTIVES

The objective of this study was to determine the effects of weevils (weevils vs. no weevils), time, and storage type (hermetic vs. non-hermetic) on maize seed viability.

3.4 METHODS AND MATERIALS

To determine the effect of hermetic storage and weevils on maize seeds, the seeds were stored under hermetic and non-hermetic conditions for varying time periods, with and without weevils. Following hermetic storage, the seeds were tested for percent germination. A laboratory scale hermetic storage system employing glass-canning jars was utilized, and treatment conditions of temperature (27°C) typical of tropical maize storage temperature and safe seed storage moisture (10%), were selected. A laboratory scale seed germination system employing trays (De Geus, et al., 2008) covered with planting media (Versapak, crepe-paper), watering table, and treatment conditions of water and temperature (25°C), favorable for optimal seed germination were also selected. The randomization of treatment assignment to jars and the hermetic storage chamber, as well as the warm germination chambers was done using PROC PLAN, and PROC GLM (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC).

3.4.1 Experimental maize seed

Maize seed of the commercial hybrid 66H54 (Blue River, 27087 Timber Road, Kelley, Iowa 50134) was utilized. Maize moisture (10%) was measured using the 103^{o}C, 72-h oven method (ASABE, 2008).

3.4.2 Initial germination tests

The initial germination test for the experimental maize seed involved placing four 50 seed samples on Versapak paper laid on a tray, followed by wetting and incubation at 25°C, at the Iowa State University Seed Science Center. The 6A planting board (Hamilton, 2011), with holes matching the hybrid Blue River maize seeds, was selected and utilized in planting multiple (50) seeds at once, with equal spacing on the crepe paper. At the end of 7 days, germination rate was determined by counting the number of normal seedlings (Desai, et al., 1997; AOSA, 2010) within each 50 seed sample planted, dividing by 50, multiplying by 100, and finding the average percent germination for the four samples.

The initial germination rate for the seed (66H54, Blue river hybrids, 27087 Timber Road, Kelley, Iowa 50134) utilized in this research was 99.5%. This is because 200 (4 samples of 50) seeds were evaluated, and 199 germinated. This is impressive, considering that the seed had a grower listed germination rate of 95%, overall, and 99.5% germination rate, for pure seed (uncontaminated with other seeds).

3.4.3 Treatment design

The completely randomized block hermetic storage experimental treatment design consisted of four factors (time, maize moisture, storage temperature, and weevils). Time had four levels (0, 4, 8, and 12 months), maize moisture had one level (10%), temperature had one level (27^{o}C), and 6 replications were used. Twenty-four (4 treatments*6 replications) jars were placed in the 27^{o}C chamber during each of months 0, 4, 8, and 12 (Figure 3.1).

These conditions approximate normal seed storage moisture and East African average ambient temperature. Overall, each replication had 16 (four (hermetic, non-hermetic, weevils, no weevils) by four (0, 4, 8, 12 months)) treatment combinations, where the jars were randomly assigned to positions within a 27^{o}C chamber. It involved placing jars in the chamber at the respective months with their assigned treatments, and maintaining the chamber relative humidity at 45±2%. All jars had about 170 g of maize seeds, and jars with weevils also had 100 weevils, stored with the maize.

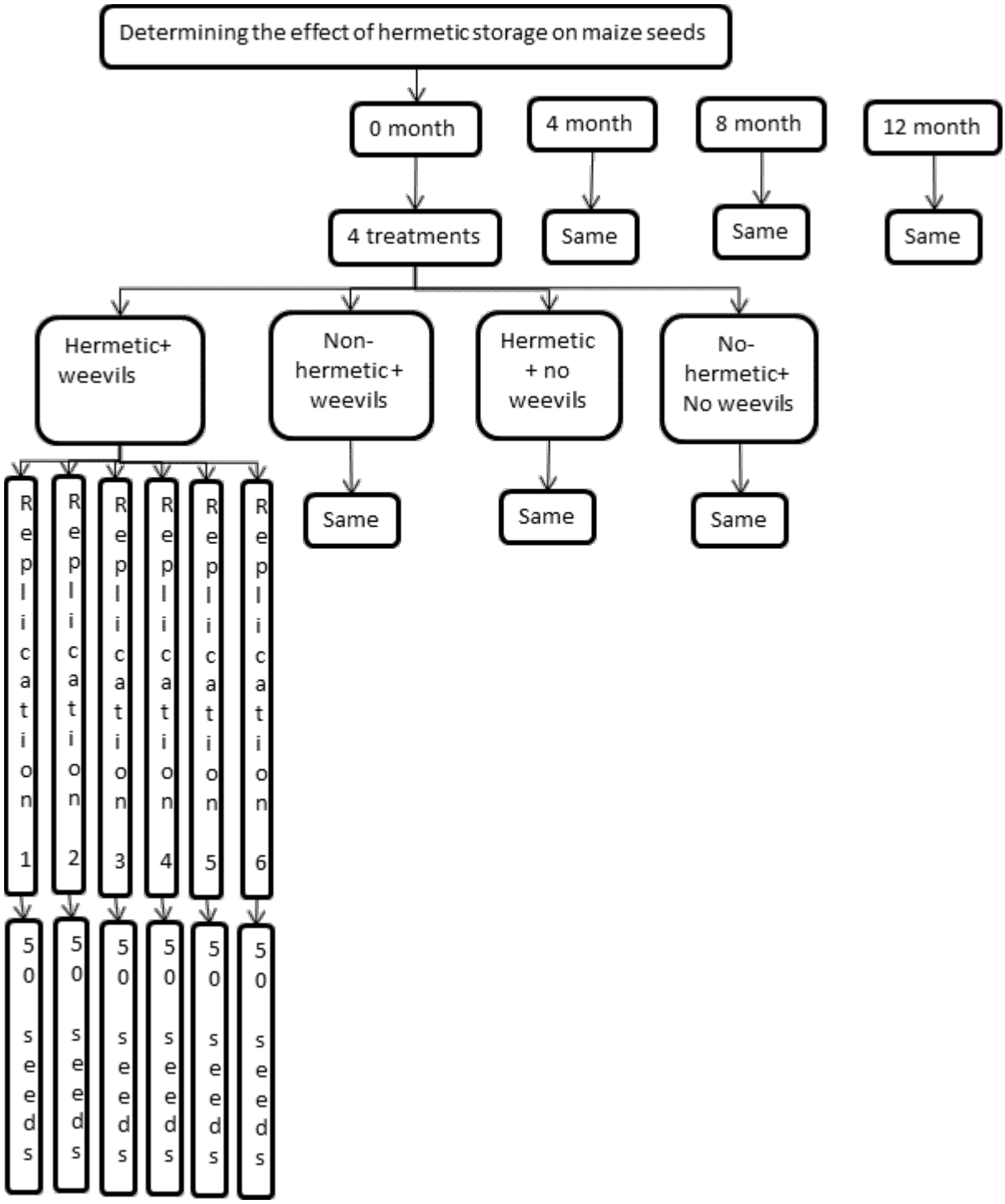

Figure 3.1: Treatment design flow chart for the hermetic seed germination study

3.4.4 Experimental weevils

A stock culture of 100 adult maize weevils (*S. zeamais Motschulsky*, unsexed), obtained from the Iowa State University Entomology Departmental laboratory were placed in five unsterilized 3.74-L glass jars, with screen lids, half full of 16.5% moisture Fontanelle 6T672 maize. Weevils were allowed to oviposit on the maize to develop a colony. This was achieved by placing jars in a rearing chamber at about 27°C and at interstitial relative humidity determined by maize moisture, for two months (Arannilewa, *et al*., 2006). Weevils from this colony were utilized in this storage study.

3.4.5 Experimental chamber

A chamber maintained at 27^{o}C was utilized in this storage experiment. A fan created air circulation within the chamber that prevents the buildup of humidity (ASAE, 1998; ASHRAE 1999; Prenger and Ling, 2010).

A humidifier controller (Dayton 1UHG3, Dayton Electric Mfg Co, 14441 W Il Route 60, Lake Forest, Ill), set at 45±2% relative humidity controlled a humidifier (RCM-832N, Kaz, Inc., 250 Turnpike Road, Southborough, MA) utilized in maintaining the seed moisture content at 10%.

3.4.6 Experimental containers

One-pint (473-mL) Kerr canning jars (Mason Jar 61000, Jarden Home Brands, 14611 W. Commerce Road, Daleville, IN) were utilized as experimental units. Jars with weevils contained 100 weevils each, so that in hermetic jars all weevils were

dead within about two days, while jars without weevils mostly retain their O_2 level. Hermetic tests utilized canning jars, as is using the hermetic canning jar lids, while non-hermetic tests utilized jars fitted with aluminum screen lids only, which allowed air passage but not weevil escape.

3.4.7 Seed priming

Following hermetic storage, seeds were preserved in cold storage (4°C), until the beginning of the germination study. This also helps to overcome dormancy, common in hermetic storage, which may prevent otherwise viable seeds from germinating. This is because pre-chilling seeds as a priming method is inferior to hydration (Cromarty, *et.al*., 1982; Hussain, et al., 2006), utilized in the germination tests.

3.4.8 Germination seed preparation

All germination tests were done at the end of the 12 months' storage period. For this reason the 24 treatment jars assigned to each of the four months (12, 8, 4, and 0) were randomly assigned to positions within the hermetic chamber at the specified months (Figure 3.1). Jars to be stored for twelve months were placed in the chamber at the onset followed by those for 8 months, four months, and 0 months, at month 12, 8, 4, and 0 respectively. At the termination of the 12-month hermetic storage period, each of the 96 storage jars was emptied onto a 4.8-mm (12/64-in) round hole sieve, one at a time. This was done to remove debris and insects before the germination tests. Following this, each jar was emptied into a pre-numbered gallon (Hefty® bag, OneZip freezer bags, 1900 West Field CT., Lake Forest, IL), and four

50-seed, samples were then counted into sterile, pre-punctured and pre-numbered bags (Whirl-Pak®, B01009WA, Nasco, 901 Janesville Avenue, Fort Atkinson, WI) sample collection bags, from each of the gallon bags. The four samples from each jar were placed in a pre-numbered Hefty bag (OneZip Freezer Bags, quart, 1900 West Field CT., Lake Forest, IL) bearing the number of the jar from which seeds were emptied. Pre-labeling 384 (96*4) sample collection bags, with their treatments and replication designations, and assigning four to each numbered quart freezer bag allowed for transfer of the hermetic storage structure to germination trays. This is because exactly 96 trays were utilized in the germination experiment, and each was labeled with the hermetic storage jar from which its samples were derived. The samples were stored in a cold-room (~4^{o}C), under airtight conditions (to prevent moisture loss), for about two weeks before planting (on crepe paper) to allow germination test logistics to be worked out.

3.4.9 Germination medium

Two, 12-ply and edge-embossed high absorbency crepe (Versa-pak, k-24, Kimberly-Clark Corporation, Neenah, WI 54957-2020; NPS Corporation Green Bay, WI 54304) germination paper was utilized as the sterile (CRC, 2007) planting media placed on the germination tray, and upon which the seeds germination tests were conducted. This is a 0.24-in-thick, crepe cellulose paper with a 5 to 7 ph range specification (ISU, 2011).

The germination study, aimed at studying seed viability, following hermetic storage involved a standard germination test.

3.4.10 Standard germination test

Germination tests were conducted at the Iowa State University Seed Science Center.The seeds were germinated on fiberglass trays (De Geus, et al., 2008), with two sheets of crepe celullose paper (k-24, Kimberly-Clark Corporation, Neenah, WI 54957-2020; NPS Corporation Green Bay, WI) moistened with about 800 mL of water (FAO, 1991; Campos, et al., 2004; Lobell, et al., 2011) on top of them. The crepe papers were moistened using a watering table (De Geus, et al., 2008) and four samples of 50 seeds, from each treatment jar (Tang and Sokhansanj, 1993; CFS, 2011), were planted on each tray. Samples were assigned to trays at random, and trays were placed inside germination carts (De Geus, et al., 2008) separated by approximately 10 cm. Carts with the trays were placed inside a growth chamber at constant 25°C, for the warm germination test (AOSA, 2010). A total of 384 (96 canning jars*4 samples of 50 seeds each) samples involving 19200 (16 treatments*4 samples*50 seeds*6 replications) seeds were evaluated using a randomized complete block design.

3.4.11 Statistics

The allocation of treatments to experimental units, in the hermetic storage setup and of sampling units to planting media in the germination test was done using a completely randomized block design. Randomization and results analysis was performed using PROC GLM (SAS version 9.0, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513).

3.5 RESULTS AND DISCUSSION

Germination studies were conducted at the termination of hermetic storage. The research tested the hypothesis that a hermetic storage system is effective for viable maize seed storage, while providing weevil control on farm, for subsistence farmers.

3.5.1 Germination study results

Hermetically stored maize seeds, with and without weevils, as well as non-hermetically stored maize without weevils have germination rates comparable to the initial maize germination rate (Table 3.1). These are also comparable to those of, or better than, maize seeds stored in cold rooms, but superior to open-air storage (De Bruin, 2005; Villers, et al., 2008; Sabio, et al., 2009).

The analysis of variance from the germination study (complete data are shown in Appendix B) shows that the six treatment replications, utilized in the research are not significantly different (Dallal, 2003) from each other ($p=0.1467$), and that the 16 treatments, within each replication, are significantly different from one another ($p<0.0001$). Significant ($p<0.0001$) main effects (hermetic, weevil, month), as well as interaction effects (hermetic by weevil, hermetic by month, weevil by month, and hermetic by weevil by month) were also recorded. Similarly, the 384 samples showed significant differences ($p<0.0001$).

This research confirmed the safety and superiority of hermetic storage (with weevils) over non-hermetic storage (with weevils) for maize seeds preservation. This is because hermetically stored maize seeds, with weevils, had 98.7 to 99.5% germination rates versus 35.0 to 72.9 for non-hermetic (open-air) storage, over the

12-months seed storage period. Treatments of particular interest are hermetic treatments with weevils (month 0), hermetic treatment with weevils (month 4), hermetic treatment with weevils (month 8), and hermetic treatment with weevils (month 12), which had mean germination rates of 99.1%, 98.7%, 99.6%, and 99.3%, respectively. These confirmed that hermetic storage preserves seed viability, even when seeds are stored under ambient (atmospheric) conditions.

Non-hermetic treatments with weevils at months 0, 4, 8, and 12 (Table 3.1) are open-air storage treatments, with conditions that are most conducive to weevil damage. Their percent germination means, along with standard errors are 99.2±0.25, 72.3±1.29, 72.9±1.28, and 35.0±1.38, respectively. At month zero (the experimental control, for “month”), no significant weevil damage occurred, due to lack of time, while weevil damage for months 4 and 8 are significant but not significantly different from each other. Non-hermetic treatment at month 12 produced the most significant weevil damage, as reflected in the low percent seed germination for that treatment. Means with the same letter (Table 3.1) are not significantly different.

Table 3.1: Treatment means and standard errors for the hermetic seed germination study

	Storage type	Time (months)			
		0	4	8	12
Weevils	Hermetic (H)	99.1^{E}±0.26	98.7^{G}±0.32	99.6^{A}±0.17	99.3^{C}±0.24
	Non hermetic (NH)	99.2^{D}±0.25	72.3^{I}±1.29	72.9^{H}±1.28	35.0^{J}±1.38
No weevils	Hermetic (H)	99.1^{E}±0.26	99.0^{F}±0.29	99.0^{F}±0.28	99.5^{B}±0.20
	Non hermetic (NH)	99.3^{C}±0.24	99.2^{D}±0.25	99.0^{F}±0.28	99.1^{E} ±0.26

In contrast, hermetic treatments with and without weevils had significantly high germination rates. This is because the weevils within hermetic treatments with weevils died within a short time due to hypoxia, and hermetic treatments without weevils had no weevils. Hence, hermetic treatments with and without weevils did not sustain high levels of seed damage, as reflected in their high mean germination rates. Hypothesis testing at 0 month, suggests no significant treatment difference ($p=0.9686$) as well, while testing at 4 months ($p=0.0055$), 8 months ($p=0.0050$), and 12 months ($p<0.0001$) suggest significance, that increases with storage time. This indicates increasing weevil damage with time, in non-hermetic storage, with weevils.

Treatment contrasts between non-hermetic treatments and hermetic treatments for 0, 4, 8, and 12 months had $p=0.6944$, $p<0.001$, $p<0.0001$, and $p<0.0001$, respectively. These, again shows that at month zero, there is no significant difference in germination between seeds stored using the two storage types

(hermetic and non-hermetic), but shows significant difference for the other three storage time periods (4, 8, 12 months).

Overall, treatment contrasts for all 16 treatments show a significant difference ($p<0.0001$). And the hypothesis of a treatment difference between all 16 also shows that there is significant difference ($p<0.0001$) between treatments.

A comparison of the four different treatment types (hermetic with weevils, non-hermetic with weevils, hermetic without weevils, and non-hermetic without weevils) shows that there is significant difference between treatments that contain weevils ($p<0.0001$), and no significant difference between treatments stored without weevils ($p=0.9089$), based on percent germination rates.

For hermetic samples (weevils vs. no weevils), the germination main effect was 0.01 percentage points (99.2 to 99.1%) for non-hermetic, samples with no weevils, and 29.4 percentage points (99.2 to 69.8%) for non-hermetic, samples with weevils.

Based on the percentage mean differences and p-values, main effects of weevils, storage type, and interaction effects were significant for non-hermetic conditions, as indicated by the low germination rates. This shows that hermetic storage does not significantly affect germination in hermetically stored maize seeds with weevils, while non-hermetic storage with weevils does.

3.5.2 Hermetic by month interactions

There was significant ($p<0.0001$) storage type (hermetic, non-hermetic) by month interaction (Figure 3.2), where the germination rate was 99.2% (hermetic) vs. 69.8 (non-hermetic). And germination rates obtained for hermetic treatments exceed the

minimum required seed “germinability” of 85-95%, for warm seed germination tests (Armitage and Woods, 1999; De Bruin, 2005).

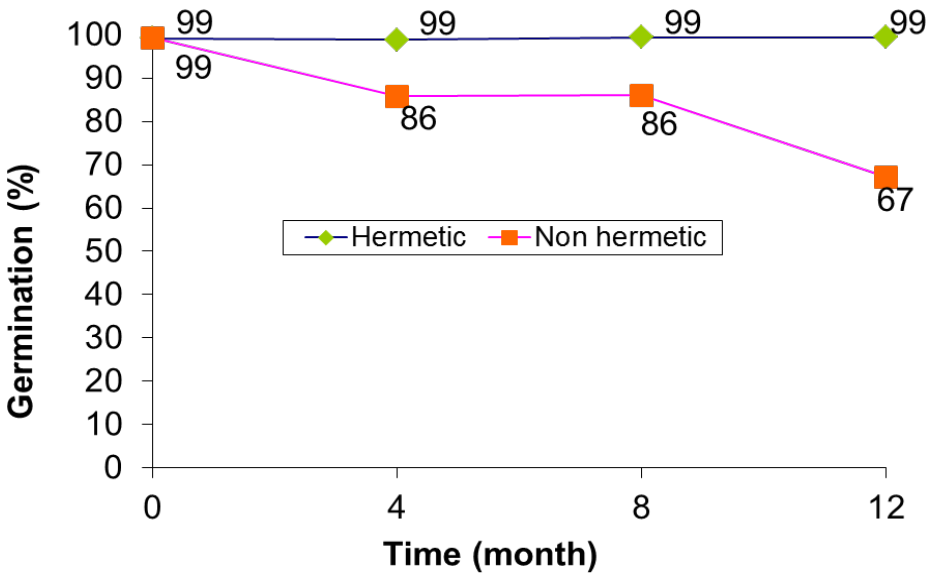

Figure 3.2: Germination plot for storage type by month interaction (averaged over 6 replications)

3.5.3 Weevil by month interaction

Figure 3.3 shows that in hermetic storage there are hermetic and weevil differences in weevil mortality due to weevil by month interactions, for storage with weevils versus storage without weevils, under hermetic and non-hermetic storage.

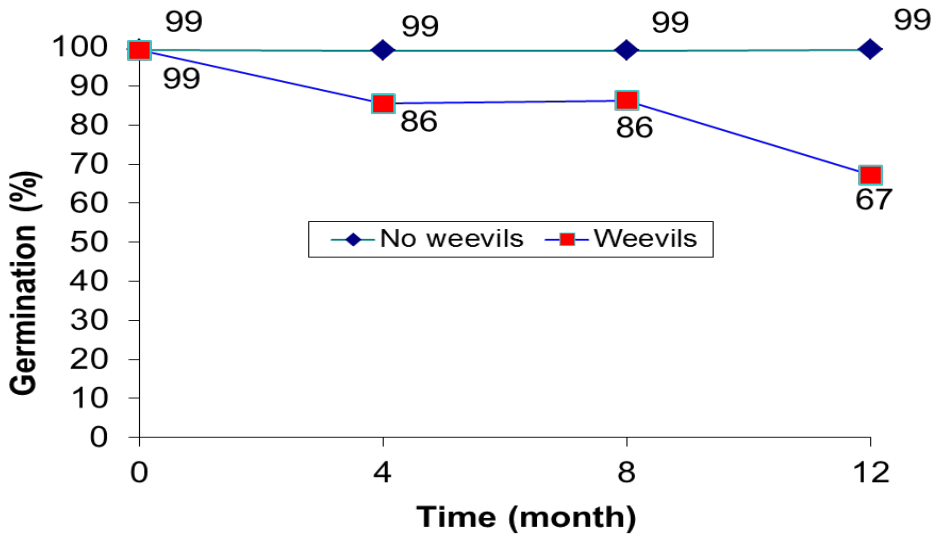

Figure 3.3: Germination plot for weevils by month interaction (averaged over 6 replications).

Figure 3.4 shows insignificant hermetic by weevil by month interaction, for hermetic treatments, while Figure 3.5 shows significant hermetic by weevil by month interaction, for non-hermetic treatments. The difference in significance, between the two, is reflected in the prediction equations (Table 3.4).

3.5.4 Hermetic by weevil by month interaction

In hermetic storage, there are hermetic, moisture and temperature differences in weevil mortality, and hence difference in maize germination rate for maize stored at 27°C, for different lengths of time (months) (Figures 3.4 and 3.5). This is due to temperature, moisture, and weevil interactions under hermetic versus non-hermetic storage. Therefore, mean percent mortality and germination rates were not the same for the different treatment combinations.

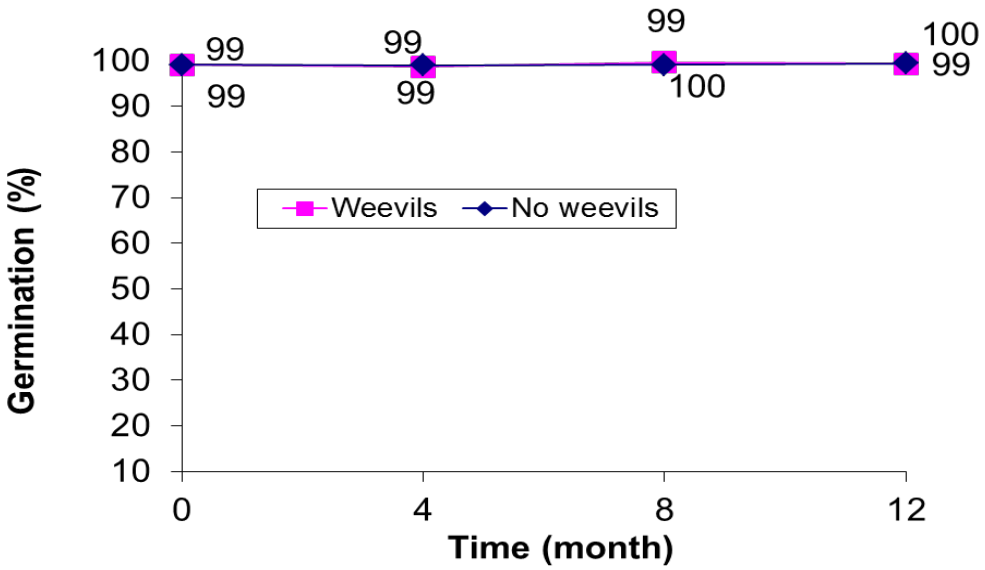

Figure 3.4: Germination plot for storage type by weevil by month interaction: ***hermetic*** (averaged over 6 replications)

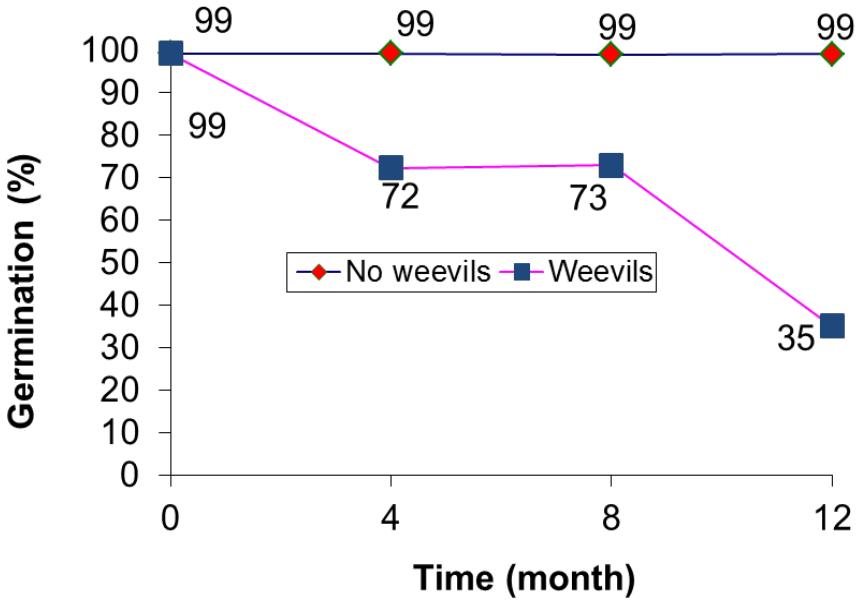

Figure 3.5: Germination plot for storage type by weevil by month interaction: ***non-hermetic*** (averaged over 6 replications).

3.5.5 Predicting percent maize germination

Equations for predicting percent maize germination are presented in Table 3.4. They are separated into “i” or prediction equations for main effects, for the four different

storage periods (0, 4, 8, and 12 months) and “ii” or prediction equations for interaction effects, for the same storage periods.

3.5.5.1 Main effects

Prediction equations for treatments “1 to 4” represent hermetic treatments, with weevils, where treatments were stored for 0, 4, 8, and 12 months, respectively. Equation “5 to 8” represents non-hermetic treatments, with weevils where treatments were stored for similar time periods. And equation “9 to12” represents hermetic treatments with no weevils, where treatments were stored for 0, 4, 8, and 12 months, respectively, while “13 to16” represents non-hermetic treatments without weevils, where treatments were also stored for similar time periods.

3.5.5.2 Interaction effects

Equations for the two curves in Figure 3.2 (H*M (hermetic) and H*M (non-hermetic)) represent hermetic by month interactions for hermetic and non-hermetic treatments. Equations for the two curves in Figure 3.3 represent weevil by month interactions (W*M (no weevils) and W*M (weevils)), for treatments without and with weevils. Figure 3.4 is represented by H*W*M (hermetic, no weevils) and H*W*M (non-hermetic, no weevils) prediction equations, which refer to (i) prediction equations for hermetic by weevil by month interactions, under hermetic conditions without weevils, as well as (ii) similar interactions under non-hermetic and without weevils conditions, respectively. The curves in Figure 3.5 represent hermetic by weevil by month interactions, under non-hermetic conditions with weevils as well as hermetic by weevil by month interactions, under non-hermetic conditions without weevils. They

are represented by prediction equations described by H*W*M (non-hermetic, no weevils) and H*W*M (non-hermetic, weevils), respectively.

For maize storage with weevils (Table 3.2), percentage mean maize germination differences between hermetic and non-hermetic treatments (hermetic main effect) is significant ($p<0.0001$), with an estimated mean difference of 29.4 (99.3-69.9).

Table 3.2: Hermetic by weevil by month interactions (weevils) for seed the germination study

Weevils	**Time (months)**				
	0	**4**	**8**	**12**	**Mean (storage type)**
Hermetic (H)	99.2	98.8	99.7	99.3	**99.3**
Non hermetic (NH)	99.2	72.3	72.9	35.1	**69.9**
Mean (time)	**99.3**	**85.6**	**86.3**	**67.2**	
Difference	**0.1**	**26.5**	**26.8**	**64.2**	

For maize storage with without weevils (Table 3.3), percentage mean maize germination differences between hermetic and non-hermetic treatments (hermetic main effect) is insignificant ($p=0.9089$), with an estimated mean difference of 0 (99.2-99.2).

Table 3.3: Hermetic by weevil by month interactions (without weevils) for seed the germination study

No weevils	Time (months)				
	0	4	8	12	Mean (storage type)
Hermetic (H)	99.2	99.0	99.1	99.5	**99.2**
Non hermetic (NH)	99.3	99.3	99.1	99.2	**99.2**
Mean (time)	**99.3**	**99.2**	**99.1**	**99.4**	
Difference	**0.1**	**0.3**	**0**	**0.3**	

3.5.6 Sample size determination

Germination tests involving four replications of 100 seeds per treatment, where treatment refers to each experimental unit or storage container, and four replications of 50 seeds per treatment is standard practice in the determination of seed viability (Tang, and Sokhansanj, 1993; CFS, 2011). However, using 4 replications of 100 seeds per container in large seed samples as is the case for this study (with 19200 seeds) produces lower experimental error, and reduces the accuracy of the test for treatment differences. It also produces huge sampling error, which does not go into testing for treatment differences. We, therefore, utilized four samples of 50 seeds for each of the 16 treatments, which produced smaller sampling error and higher experimental error, increasing testing accuracy for establishing treatment differences.

Table 3.4: Prediction equations for treatments groups and treatment factor interactions for the seed germination study

Treatments		**Prediction equation**
1 to 4	(hermetic, weevils: months 0, 4, 8, and 12)	Y=0.1417x+98.875
5 to 8	(non-hermetic, weevils: months 0, 4, 8, and 12)	Y=-19.1917x+194.642
9 to 12	(hermetic, no weevils: months 0, 4, 8, and 12)	Y=0.1083x+98.050
13 to 16	(non-hermetic, no weevil: months 0, 4, 8, and 12)	Y=-0.0667x+100.175

Interactions	**Prediction equation**
H*M (hermetic)	Y=0.0312x+99.021
H*M (non-hermetic)	Y=-2.4073x+98.996
W*M (no weevils)	Y=0.0052x+99.167
W*M (with weevils)	Y=-2.3813x+98.850
H*W*M (hermetic, no weevils)	Y=0.0271x+99.025
H*W*M (hermetic, weevils)	Y=0.035x+99.017
H*W*M (non-hermetic, no weevils)	Y=-0.0167x+99.308
H*W*M (non hermetic, weevils)	Y=-4.798x+98.683

3.5.7 Seed germination rates

Looking at Table 3.2 and Table 3.3, with respect to results of similar research conducted by other researchers (Pérez-García *et al.,* 2006; Sabio, et al., 2006), the percent germination rates obtained for some of our treatments-hermetic with and without weevils, non-hermetic with and without weevils, for months 4 to 12 are superior to results obtained by those authors. Although, there is no major difference between germination rates for the treatment groups described, germination rates for hermetic treatments with weevils are slightly higher within that group. This may be due to the precision with which time to complete weevil mortality was predicted (Yakubu, et al., 2011). It is also possible that oxygen utilization by the weevils before death, which reduced interstitial and headspace oxygen slightly may be responsible

for better seed preservation and slightly, higher germination rates in the hermetic treatment group, with weevils.

3.6 CONCLUSIONS

Based on results from this research:

- Hermetic storage can safely preserve maize seed quality
- Hermetic storage produced seed germination rates above that recommended by the “seed industry”
- Hermetically stored maize seeds, with weevils, had about 99.3% average germination rates compared to 69.9%, for open air storage. While hermetically stored maize, without weevils, had an average germination rate of about 99.2% for both hermetic and non-hermetic systems.
- Compared to non-hermetic (open air) storage, hermetic storage preserved quality for both seeds stored with and without weevils. Whereas, open-air storage only maintained seed quality for seeds stored without weevils.

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CHAPTER 4: USE OF RECYCLED CONTAINERS FOR HERMETIC MAIZE STORAGE IN EAST AFRICA

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4.1 ABSTRACT

Affordable, cost-effective, pesticide-free, and reliable maize storage containers for seed and food purposes is lacking in many subsistence farming cultures. This often results in rapid deterioration of maize, usually stored in the open-air. As a result subsistence farmers are forced to dispose of their maize for a low price right after harvest, robbing them of food for the rest of the year. The objective of the research was to develop information to enable utilization of sanitary and hermetic maize storage containers, free of edible oil and associated cancer-causing oil oxidation products, and which allows farmers to preserve their maize for as long as they wish, using locally available resources. It involved a market survey of recycled edible oil containers, conducted in East Africa (Kenya, Tanzania, and Uganda) and laboratory cleaning research, conducted at Iowa State University. The laboratory study utilized three cleaning methods (oil-drain plus water at 45˚C, oil-drain plus water at 90 to 100˚C, and oil-drain plus water at 90 to100˚C plus soap) were compared in cleaning soybean oil contaminated 20-L HDPE containers. A comparison of these methods shows that the soap and hot water treatment is the most effective, as well as the only one that meets our cleaning objectives. Leftover oils following cleaning with oil

drain, hot water, and soap plus hot water were 0.249g, 0.142g, and 0.004g, per container respectively. The 0.004g from the soap and hot water treatment compares favorably with 0.005 to 0.006g per container from the control (unused) experimental units (HDPE 20-L containers), which had no oil contaminants. Research results, therefore, indicate that using 3g of 99.44% pure Ivory soap and hot water per gram of soybean oil contaminant is enough to clean and sanitize soybean oil-contaminated 20-L HDPE containers, for safe hermetic maize storage. This is an encouraging result, since market surveys found edible oil containers available for sale and reuse in East African markets.

4.2 INTRODUCTION

Maize preservation is aimed at prolonging its storage life. However, reliable and affordable maize storage for subsistence farmers is often lacking (De Bruin, 2005; Villers, et al., 2008). A usual alternative storage method is open-air (non-hermetic) storage, which often leads to substantial loss to stored maize (O'Dowd and Dobie 1983; Holst, et al, 2000). These losses usually result from pest (insects, molds, rodents, and birds) activities (Lindblad and Druben, 1980; Demissie *et al*., 2008a; Demissie *et al*., 2008b; Gregg and Billups, 2009; PHL Network, 2009), with the most significant of them being losses due to maize weevil (*Sitophilus zeamais*) activities (Holst, et al, 2000; Demissie et al., 2008a). About 19% of total annual maize production is lost in post-harvest storage, annually in East Africa (PHL Network, 2009). And since consumers desire food that is free of chemical contaminants (Navarro, et al., 1994; CRC, 2003; Springer, 2007; Yakubu, et al., 2011), a storage

system, such as hermetic storage that provides mechanical fencing of the food storage environments from birds and rodents as well providing chemical-free hermetic insect control (Navarro, et al., 1994; CRC, 2003; Lewis, *et al*., 2005; Navarro, et al., 2007; Springer, 2007; Weinberg, et al., 2008; Glevitzky, et al., 2009; FAO, 2010; Yakubu, et al., 2011) would be beneficial to maize growers in subsistence farming cultures (Lindblad and Druben, 1980; Moreno-Martinez, et al., 2000; Gregg and Billups, 2009; Boys et al., 2007; ASM, 2009; Baributsa, 2010; Demissie et al., 2008a). This is because they would no longer be forced to sell their maize right after harvest, at low prices.

4.2.1 Epidemiology of food borne illnesses

Epidemiology of several food-borne illnesses have been traced to cross-contamination with pathogens and toxins from food contact surfaces (Schmidt and Rodrick, 2003) and toxic free radicals from oxidized edible oil (Wang, et al., 2000; Andrikopoulos et al., 2002; Andrikopoulos, 2004; Choe and Min, 2006; Asadauskas, et al., 2007; Fox and Stachowiak, 2007; Canals, et al., 2009). Container recycling and reuse for food storage, therefore, requires proper selection and cleaning to exclude containers with toxic contents (Shachman, 2004; Schmidt and Erickson, 2008; EPA, 2009a; 2009b), to protect the end-user of food products stored within them from cross-contamination and the toxic side effects of prior container contaminants (Erhan, et al., 2006; Fox and Stachowiak, 2007), and to prevent toxic loads of effluents from recycled containers from ending up in public drinking water. A

reason for which their reuse is highly regulated (EPA, 2002; Shachman, 2004; EPA, 2010a; ACRC, 2006; EPA, 2010b).

Based on the use of 55-gallon drums (Seck et al., 1996; Adhikarinayake, 2005) and other containers for long-term palm oil storage, establishing proper cleaning procedure allows the right containers to be used interchangeably for maize storage. However, due to legislation and the desire to prevent cross contamination between stored maize and chemical contaminants, only food grade containers (Shachman, 2004) previously utilized for storage of carbonated soft drinks and triglycerides (Bhatt, 2004; Ashaye and Olusoji, 2006), were considered for this recycling and maize storage research. But since soft drinks do not have uniform chemical contents (Cleveland, et al., 2001; EPA. 2006; Mercer, 2006; Malik, et al., 2006; Tsimihodimos, et al., 2009; Wiley-Blackwell, 2006; EWG, 2010CRU, 2010) only containers previously contaminated by edible oil (Lindblad and Druben,1980; Adhikarinayake, 2005; Murdock, et al., 2003; EPA, 2009c) was utilized in this research.

"Clean" refers to being free from dirt or pollution, unadulterated, sanitary or pure (Merriam-Webster, Inc. 2010). However, according to Coats (2010) and other authors (Cleveland, et al., 2001; EPA. 2006) the end-products of some chemicals are more toxic than the starting chemicals, and some chemicals cannot be cleaned, adequately. To preserve maize quality, while preventing cross-contamination between stored maize and the associated rancid oil free radicals, as well as dirt, a procedure is needed for cleaning edible oil containers such that nearly 100% of the oil is removed. This gives subsistence farmers the benefits of effective hermetic

storage, as well as improved nutrition and economy (Carroll and Fulton, 2008; Murdock, *et.al*., 2003).

4.2.2 Research need

Little information was found on the availability of used containers, in East Africa. A study was therefore needed on container sizes, prices, and quantity available for sale, in East Africa. A study was also needed to establish cleaning standards, for reused edible oil containers to ensure food safety.

4.3 OBJECTIVES

- To determine the availability of used edible oil containers in East Africa, suitable for hermetic maize storage
- To develop and test procedures for cleaning previously used edible oil containers

4.4 METHODS AND MATERIALS

This study consisted of a market survey and laboratory research.

4.4.1 Market Survey

To explore recycled edible oil container availability for hermetic storage in East Africa, a recycled container survey form (complete data are shown in Appendix E) was designed and dispatched to contacts in Uganda, Kenya, and Tanzania. The purpose of survey was for surveyors to identify containers available in selected East African markets, which can be recycled for maize storage. Containers identified were expected to be at least 5 L in capacity and airtight (no holes). Container properties to

be identified by the survey included previous or intended use (edible oil, soft drink concentrate, etc.), volume (L), material (plastic, steel, etc.), how many were in each market, as well as price. Each surveyor was expected to carry out the survey in at least three markets.

4.4.2 Laboratory research

The laboratory part of the research conducted at Iowa State University was done in two stages (Figure 4.1). The first stage involved applying three treatments (oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap) to randomly assigned experimental units (20-L HDPE containers). Experimental units utilized were previously used (contaminated) and new (uncontaminated) 20-L (~5 gallon) HDPE containers. The complementary second stage utilized the goldfisch oil extraction method to measure leftover oil quantities in the 20-L HDPE containers for use in statistical treatment analysis which allowed comparison of the three cleaning treatments.

4.4.3 Treatment definition

Cleaning of oil-contaminated containers using water, with or without soap is common practice in East Africa (Myers, 2006; Yakubu, et al., 2011). Literature, however, suggests that cleaning, disinfecting and sanitizing containers to prevent cross-contamination of food stored within them is best done at high temperature (Atlas and Snyder, 2006; Sebastião, et al., 2006), using soap and mechanical action (Knoxa and Walkera, 1947; Gangneux, et al., 2004; CRC, 2006; Helmenstine, 2011; MTL, 2011; Patwardhan and Kelkar, 2011), such as scrubbing and shaking. According to

FAO (2002) heat treatment destroys oil-splitting enzymes, arrests hydrolytic rancidity and autoxidation, during vegetable oil extraction and causes oil to leach out from its container. The use of soap plus hot water cleaning treatment is expected to produce similar advantages.

Factors considered for this research were (i) soap and water, at two water temperature extremes (control (45°C) and hot/boiling (90 to100°C)), and (ii) container history (contaminated and new (control)). We hypothesize, therefore that cleaning with soap at high temperature (90 to100°C), with mechanical action would be enough to clean and sanitize vegetable contaminated containers for maize quality preservation within them.

4.4.4 Experimental 20-L HDPE containers

The 36 experimental units (20-L HDPE containers) utilized for the research each have a net weight of about 16 kg (35 lb), and normally contain 15.88kg of oil claimed to be 100% pure soybean oil (Columbus Foods, Chicago, Illinois).

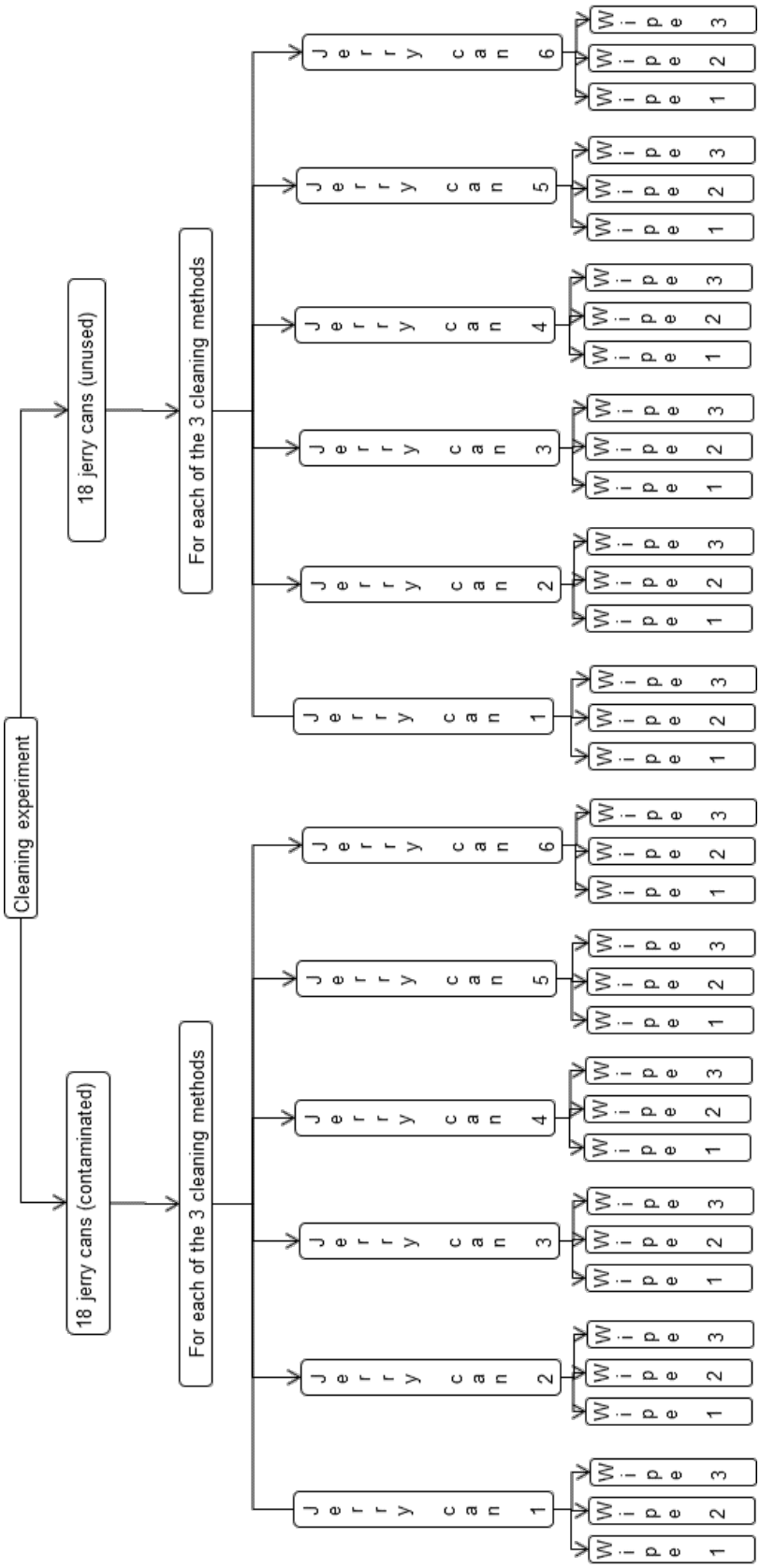

Figure 4.1: Recycling researchtreatment design flowchart

***Cleaning methods: (1) oil drain+ 45°C water, (2) oil drain+ 90-100°C water, and (3) oil drain+ 90-100°C water+soap**

They are made of high density polyethylene (HDPE) plastic, with a resin classification/recycling code of 2 (ACC, 2007; Bakers & Chefs, distributed by Sam's West, Inc. 608 S.W. 8th Street, Bentonville, AR). HDPE has a melting point of 130-135°C and tensile strength of 4550 psi, and can withstand temperatures of 120°C for short periods or 110°C continuously (EOS/EDS, 2000; Antec, 2001; Dow 2009; Dynalab, 2011).

4.4.5 Quantification of vegetable oil remnants

To obtain an estimate of the remnant quantity of oil contaminants in each of the contaminated 20-L HDPE containers, the average weight of eighteen unused (new) 20-L HDPE containers (donated by Columbus Vegetable Oils, 30 E. Oakton Street, Des Plaines, IL) was subtracted from the weight of each of the eighteen contaminated 20-L HDPE containers (donated by Ames and Des Moines area Chinese fast food restaurants).

Average weight of the new 20-L containers was 296.50 g. On average, contaminated 20-L HDPE containers assigned to the oil drain+ 45°C water, oil drain+ 90 to100°C water, and oil drain+ 90 to100°C water+soap lost weight equaling 10.45g, 14.23g, and 20.06g oil respectively before the wiping treatment application. While, on average, the contaminated 20-L HDPE containers contained 0.249g of oil (remnant oil quantity) after draining with water at 45°C, 0.142g of oil following draining with water at 90 to100°C, and 0.004g oil after draining with 90 to100°C water plus soap. In cleaning contaminated 20-L HDPE containers, to which soap plus water at 90 to100°C was employed as treatment, enough soap was added to

remove all the oil, irrespective of whether leftover oil is free flowing or bound tightly to the 20-L HDPE container internal surfaces. Therefore, the soap quantity necessary to remove nearly 100% oil contaminant was based on that. Since water at 45°C was utilized in dissolving congealed oil before draining, the fluid nature of the drained oil allowed the oil dissolved in the water to flow out, reducing the leftover oil in the drained 20-L HDPE containers.

4.4.6 Experimental soap

The preliminary soap quantification involved determining the cleansing power of Ivory soap, and quantifying the number of grams of the soap that effectively removes each gram of the vegetable oil contaminant. From the initial soap quantification experiment (complete data are shown in Appendix C), it was determined that 1g of soap removed almost 1 g of oil, from oil contaminated experimental units. However, three times that amount of soap (3g soap/gram oil) was utilized with hot water, for cleaning the 20-L HDPE containers, in order to account for variability in the history of 20-L HDPE containers collected from the field.

4.4.7 Water

The water utilized for this research was deionized (demineralized) water, which is water from which impurities, including hard water-causing minerals had been removed (Wilson, et al., 1999; Miller, et al., 2009; Lower, 2011).

4.4.8 Oil removal treatments

Stage one of the research (full run), involved adding the assigned and calculated treatment quantities, followed by the application of 3.78L of deionized water to each EU. The mixture was then shaken at the onset and at 5-minute intervals afterwards for a total of 1.5 h, and emptied. Cans were then rinsed three times and turned upside down for 48 h to allow water to flow out as well and allow drying of the 20-L HDPE container interiors to occur. The oil drain treatment involved just adding water at 45°C to assigned EUs, shaking at 5-minute intervals, and emptying at the end of the treatment application period. The hot water treatment application utilized a procedure similar to the oil drain treatment, except that only oil-drain plus water at 90-100°C was, while the soap treatment involved the addition of the calculated quantities of soap to the assigned EUs, followed by addition of 3.78L of water at 90-100°C.

4.4.9 Oil residue measurements

The second stage of the treatment involved tying about 1.7g of a disposable, absorbent piece of cheesecloth (Prym Creative, Estopilla, Prym Consumer USA, Inc., Spartanburg, SC), to the end of a wood stick and using the clothed end to wipe each of three pre-assigned 229-mm x 229-mm areas of the interior of each experimental unit (from stage one), in order to determine the level of oil still left on the can interior following each treatment application from the initial cleaning process. New cheesecloth was installed on the stick prior to each wiping. The remaining oil levels were determined by quantifying the oil content of the cheesecloths, using the

goldfisch (35001-00, LABCONCO, Kansas City, MO. 1637) hexane oil extraction method. The second stage was performed after the EUs had dried, following the initial treatment application.

4.5 RESULTS AND DISCUSSION

4.5.1 Survey results

East African Markets surveyed are Jua Kali Drum Dealers (Nairobi), Shadimum Grocers, Frere Town (Mombasa) and Musila Enterprises (Kikambala Village), in Kenya, and Mwembe (Same), Same Center (Same), Kwasakwasa (Same), Tanzania. Other markets surveyed in Tanzania, include Saidi (Nkungi), and Singida, while Markets surveyed in Uganda include Namanve Market (Mukono-Kampala), Owino Market (Kampala), and Soko Mujinga Market (Kitale). The recycled container survey identified a total of 55522 containers that met the survey criteria. However, only 42, 208 (76%) (Table 4.1) of the containers had edible oil residue or were earmarked for edible oil storage. These ranged in storage capacity from 5 to 20L, with prices ranging from $0.72 to $2.08, based on size. On average, the number of recyclable plastic containers per market were 1,142 (5-L), 967 (10-L), and 2,581 (20-L), respectively.

4.5.2 Laboratory research results

Each treatment was assigned six 20-L HDPE containers, and three wipes (interior bottom, right, and left) were taken from each of the 20-L HDPE containers.

Therefore, each of the bars in Figure 4.2 to Figure 4.8 represent the mean of the wipes from 18 (six, 20-L containers*3 wipes) 20-L HDPE container locations assigned to each of the treatments.

Figure 4.2 shows results obtained from cleaning recycled and new 20-L HPDE containers with three treatments-oil-drain plus water at 45°C (lukewarm water), oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to 100°C plus Ivory soap. It indicates a strong treatment effect, and shows that while the oil drain plus 45°C water and oil drain plus 90 to100°C water treatments left behind significant ($p<.0001$) amounts of oil, the oil-drain plus water at 90 to100°C plus soap as well as the control treatments (oil drain plus 45°C water, oil drain plus 90 to100°C, and oil drain plus 90 to100°C plus soap, applied to unused 20-L HDPE containers) had an insignificant ($p=0.8992, 0.8633, 0.8448, 0.8526$ respectively) amount of oil leftover, following cleaning treatment application.

Since analysis was done at the 0.05 significance level (Dallal, 2003), where $p \leq 0.05$ is sufficient evidence for accepting a hypothesis of treatment effect, the laboratory results conclude that there is a treatment difference between the oil-drain plus water at 45°C and oil-drain plus water at 90 to100°C (contaminated 20-L HDPE containers) treatments versus the oil-drain plus water at 90 to 100°C plus soap (contaminated 20-L HDPE containers) treatment and all the control treatments.

Any level of cross contamination between stored food and storage container surface contaminants is not acceptable (Shachman, 2004; EPA, 2009a; 2009b). The uncontaminated 20-L HDPE containers utilized in this research are reference

standards for clean 20-L containers. Based on the results, using 3g (0.031 moles) of Ivory soap (99.4% pure) to clean each gram of soybean oil contaminated 20-L HDPE containers will remove enough oil to prevent rancidity and cross-contamination of soybean oil with maize that would be store in them. This is because leftover oils from the 6 replications were not significantly different ($p=0.9218$), although there were significant treatment ($p<.0001$) and location ($p<.0001$) difference, and according to the table and Figures 4.3 to 4.6, all interactions were significant.

A total of 108 (18 wipes*6 treatments) samples were analyzed using the goldfisch oil extraction method. Therefore 54 (108/2) of the wipes were obtained from each of the contaminated and unused 20-L HDPE containers. And the remnant oil quantity (Chapter 4-Figure 2) shows the average of 18 (3 wipes/jerry*six 20-L HDPE containers) wipes obtained from six 20-L HDPE containers obtained from each set experimental units assigned to each treatment.

Table 4.1: Edible oil containers in Tanzania, Kenya and Uganda markets

	5-L plastic		10-L plastic		20-L plastic	
	Price	No. in stock	Price	No. in stock	Price	No. in stock
Kenya markets						
Market 1	-	1	-	1	-	1
Market 2	-	1	$0.60	200	$1.00	1000
Market 3	-	1	$0.80	200	-	1
Tanzania markets						
Market 1	$0.70	73	$1.80	92	$2.50	66
Market 2	$0.80	59	$1.00	64	$2.00	2004
Market 3	$0.70	48	$1.50	45	$2.00	57
Uganda markets						
Market 1	$1.00	100	$2.00	100	$3.00	100
Market 2	$0.40	10000	$1.00	8000	$2.00	20000
Market 3	-	2	-	2	-	2
Total in stock		10280		8701		23227
Average price	$0.72		$1.24		$2.08	
Average containers per market		**1142**		**967**		**2581**

1. **Not available in this market**
2. **No data**

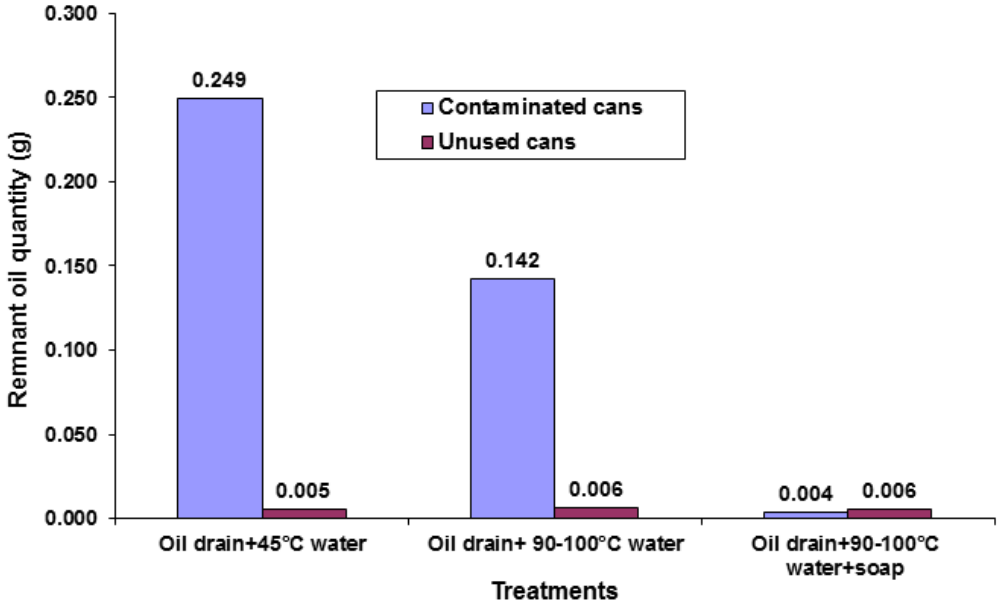

Figure 4.2: Treatment means bar chart (averaged over six replications).

Figure 4.2 indicates a strong treatment effect and shows the efficacy of Ivory soap (plus 90 to100°C water), which had the lowest oil leftover (0.004g) following cleaning of 20-L HDPE containers, in relation to oil drain (0.249g) and 90 to100°C water (0.142g) treatments.

The oil-drain plus water at 90 to100°C plus Ivory soap treatment's oil leftover value (0.004g) compares favorably with those from the control treatments, which had 0.005g, 0.006g, and 0.006g of leftover oil, for oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap, respectively.

Significant method and container effects are reflected in method (Table 4.2) and container (Table 4.3) mean differences.

Table 4.2: Method effect for the recycling research using three cleaning methods.

Method	Mean oil quantity (g)
Oil-drain+water at 45°C	0.127
Oil-drain+water at 90-100°C	0.074
Oil-drain+water at 90-100°C+soap	0.005

Table 4.3: Container effect for the recycling research using two container types.

Container	Mean oil quantity (g)
Contaminated (used)	0.132
Uncontaminated (new)	0.006

The method by container interactions (Figure 4.3), show a clear difference (p=0.0005) between the effect of cleaning methods on the two container types. For contaminated containers, cleaning with oil-drain plus water at 90 to100°C plus soap produced the cleanest containers, followed by oil-drain plus water at 90 to100°C, and oil-drain plus water at 45°C, respectively. However, there is no distinguishable difference in the oil remnant for uncontaminated containers cleaned using all three methods.

Figures 4.3 to 4.8 describe other results of this research. Bars to the left (of each pair of bars) (Figures 4.3 to Figures 4.4c) represent oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to 100°C plus soap treatments, respectively, applied to contaminated containers, while bars to the right (of each pair of bars) represent control treatments (oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap equivalents applied to unused 20-L HDPE containers.

Location by treatment interactions (Figures 4.4a, 4.4b, 4.4c) show more leftover oil for location 1 (interior, 20-L HDPE container bottom), compared to location 2 and 3 (interior, left and right 20-L HDPE container sides), which are almost alike in oil leftover levels. This is because oil usually settles to the bottom of its holding container, and the bottom is expected to hold more oil for this reason. The figures also reflect differences in treatment effects, with the oil-drain plus water at 90 to100°C plus soap treatment being the most effective. This is obvious from the leftover oil differences between contaminated 20-L HDPE containers assigned to oil drain and hot water treatments compared to those assigned to oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap control treatments, as well as oil-drain plus water at 90 to100°C plus soap treatment assigned to contaminated 20-L HDPE containers. The oil-drain plus water at 90 to 100°C plus soap treatments produced as much clean as control treatments, and since new 20-L HDPE containers and the oil-drain plus water at 90 to100°C plus soap treatments had virtually no oil, their remnant oil levels remain close to zero.

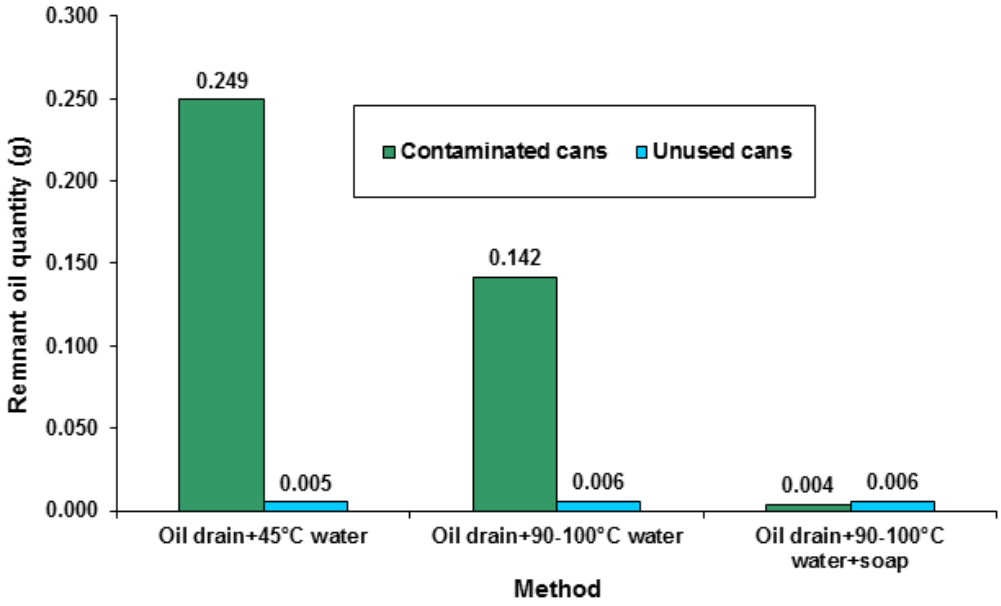

Figure 4.3: Method by container interactions (averaged over six replications).

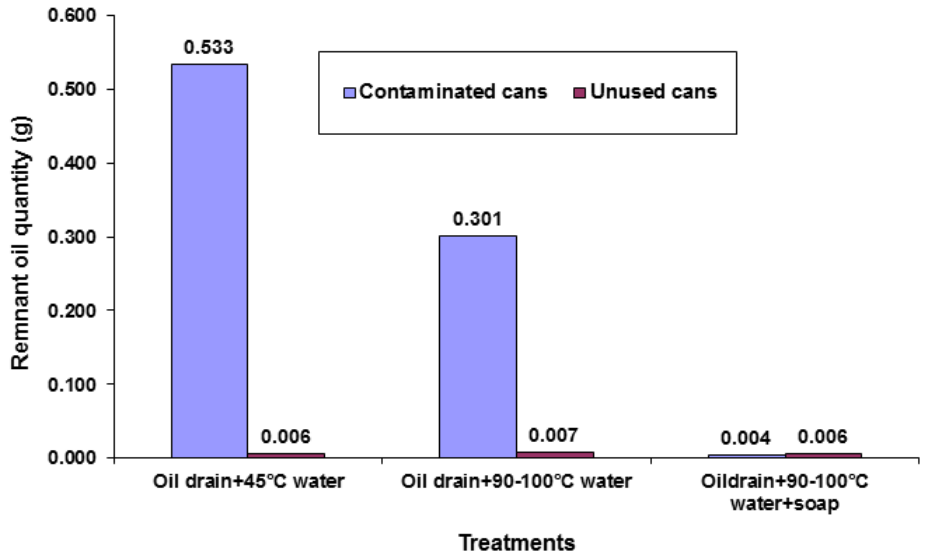

Figure 4.4a: Location by treatment interaction-location 1 (interior, can bottom, averaged over six replications).

However, the leftover oil levels for oil-drain plus water at 45˚C and oil-drain plus water at 90 to100˚C treatments are about the same, although, slightly lower for the

hot water treatments. The lack of clear trend is expected since control experimental units had no oil contaminants at the unset.

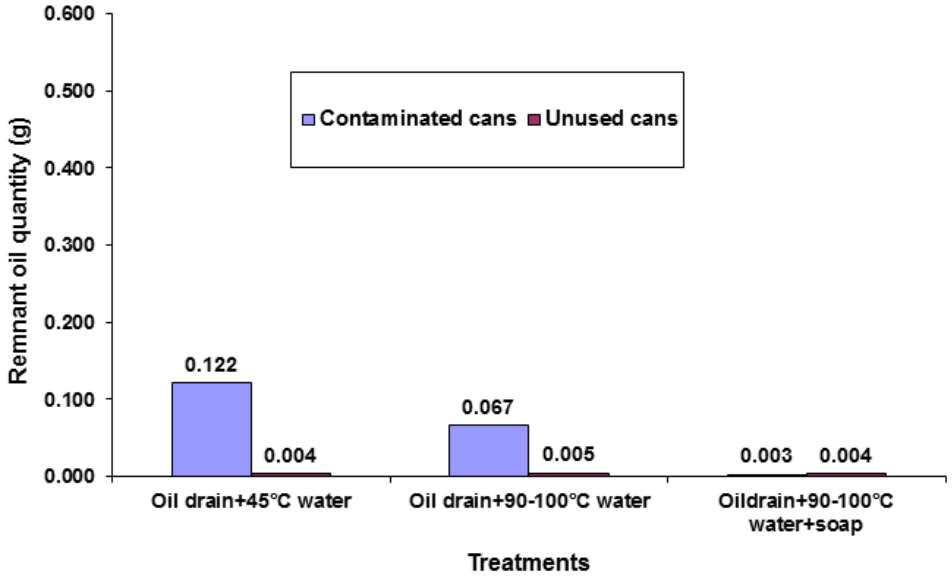

Figure 4.4b: Location by treatment interaction-location 2 (interior, left side of can, averaged over six replications).

Chapter 4-Figure 5 (location by method interaction) shows that for all methods of cleaning, location 1 (20-L HDPE container bottom, interior) had higher leftover oil than location 2 (20-L HDPE container left side, interior) and 3 (20-L container HDPE right sides, interior). And Figure 4.6, the location by method by container interaction (20-L HDPE container bottom, interior), shows a trend that indicates oil-drain plus water at 45°C, oil-drain plus water at 90 to100°C, and oil-drain plus water at 90 to100°C plus soap treatments got rid of increasing amounts of oil in that order, for contaminated 20-L HDPE containers, while the leftover oil levels for unused cans remained about the same.

Location 2 (20-L HDPE container interior, left) and 3 (20-L container interior, right) had similar remnant oil quantities for all cleaning types, for contaminated oil containers. And the leftover oils for both locations were much lower than that for location 1 (20-L HDPE container interior, bottom), when the cleaning method is oil-drain plus water at 45°C or oil-drain plus water at 90 to 100°C. Figure 4.7 and Figure 4.8 represent oil leftover quantities for 20-L container interior (left) and 20-L HDPE container interior (right), respectively for both contaminated and unused 20-L HDPE containers. For all three locations, unused cans had about the same levels of contamination.

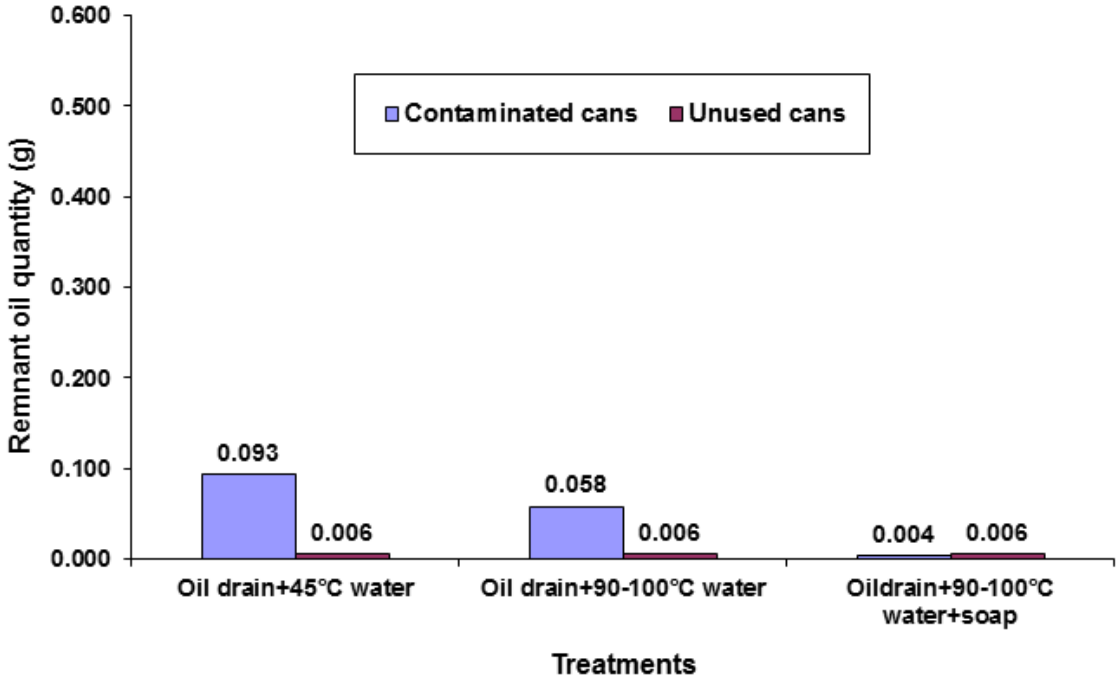

Figure 4.4c: Location by treatment interaction-location 3 (interior, right side of can, averaged over six replications).

4.5.3 Method by container interaction

To determine if method by container interactions is the same at the different levels of method, the difference of differences were calculated from Table 4.4. Oil drain plus 45°C water versus oil drain plus 90 to100°C water had 0.108 (0.244-0.136), oil drain plus 45°C water versus oil drain plus 90 to100°C water plus soap had 0.242 (0.244-0.002) and oil drain plus 90 to100°C water versus oil drain plus 90 to100°C water plus soap had 0.134 (0.136-0.002) levels of interaction, with the interaction being indicated by the different values for difference of differences. This indicates that the mean cleaning effect for the different levels of method and container were not the same and that there is interaction.

Table 4.4: Method by container interactions (with standard errors) for the recycling research.

Container	Method (remnant oil quantity (g))			
	Oil drain+45°C water	**Oil drain+ 90-100°C water**	**Oil drain+ 90-100°C water + soap**	**Container Mean**
Contaminated:	0.249+0.060	0.142+0.044	0.004+0.000	0.131
Uncontaminated:	0.005+0.001	0.006+0.001	0.006+0.001	0.006
Method mean	0.127	0.074	0.005	
Difference[a]	0.244	0.136	0.002	

[a]Difference=mean difference

A contrast of method by container interaction's average oil quantity (uncontaminated containers) (based on Table 4.4) for oil plus drain plus water at 45°C versus oil drain and water at 90 to100°C is insignificant (p=0.9452). It is also insignificant for oil drain plus water at 45°C versus oil drain plus water at 90 to100°C plus soap (p=0.6948),

but was significant for oil drain plus water at 90 to100°C versus oil drain plus water at 90-100°C plus soap (p=0.0177). All similar interactions, for uncontaminated containers were insignificant (P=1.000).

Figures 4.3, 4.4 (a, b, and c), 4.5, 4.6, 4.7, and 4.8 also show interaction effects. The interactions differ at different levels of the factors producing them, as reflected in the size of the bars representing them. For example, the combined cleaning effect of soap and hot water for instance is stronger that of either the soap or hot water alone, due to the difference of differences for the factors producing them.

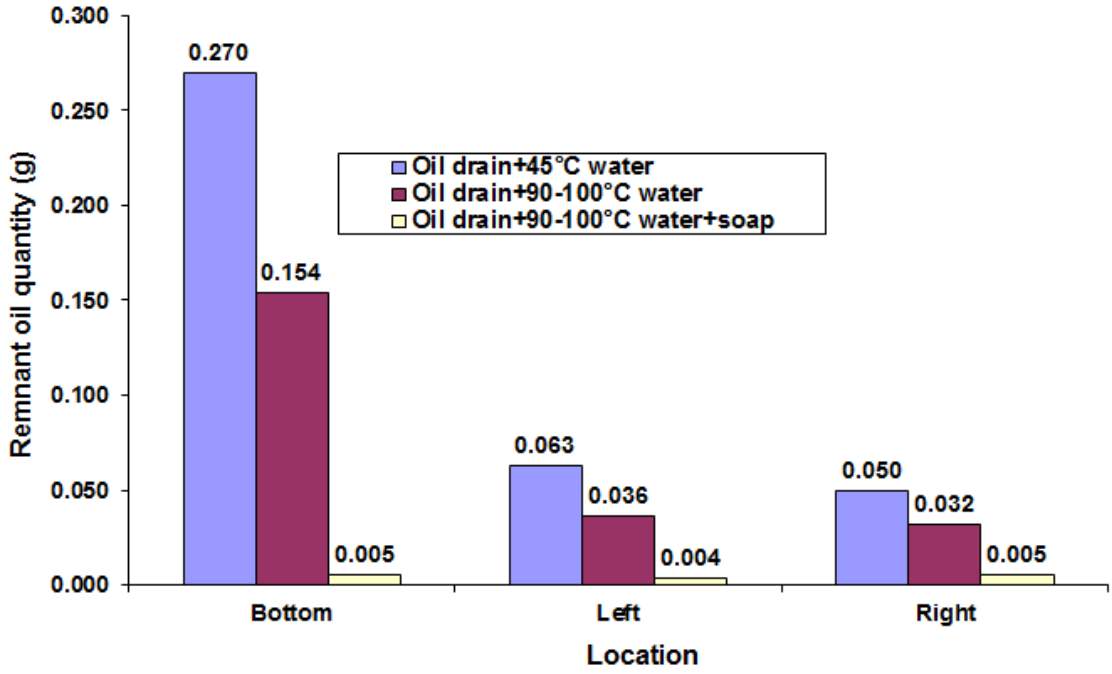

Figure 4.5: Location by method interactions (averaged over six replications).

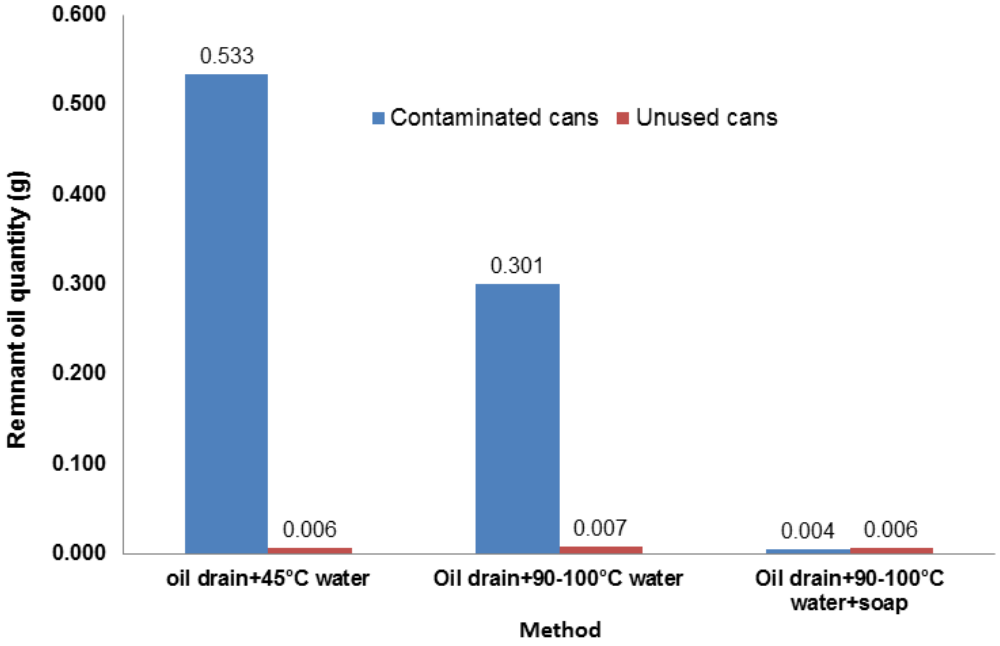

Figure 4.6: Location by method by container interactions (20-L container bottom) for contaminated EUs (averaged over six replications).

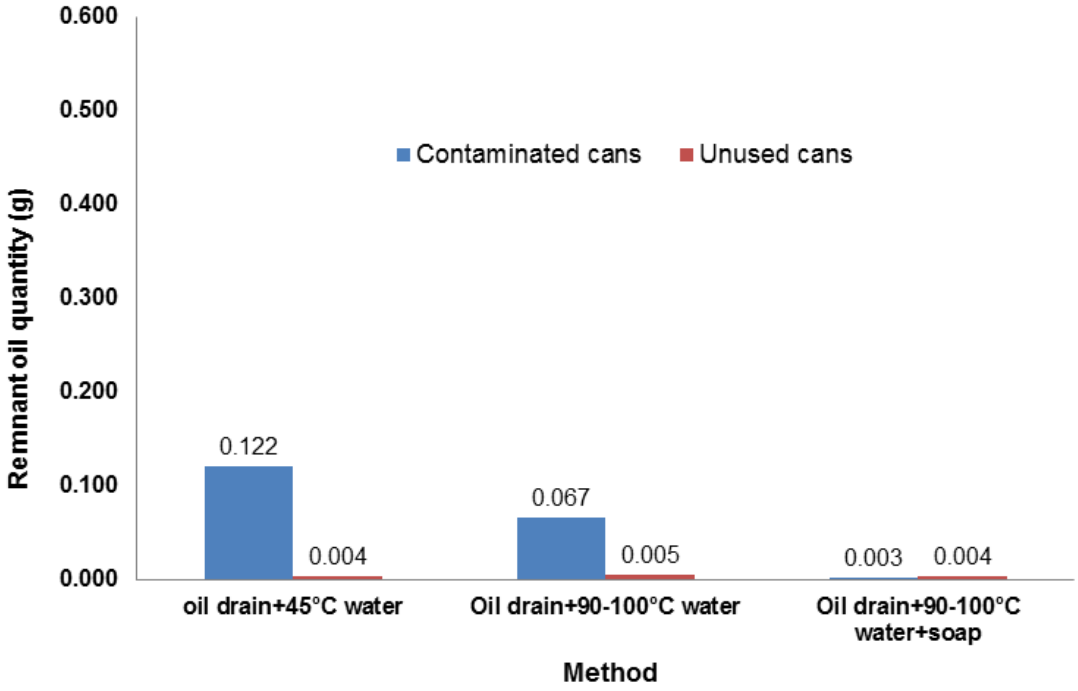

Figure 4.7: Location by method by container interactions (20-L container interior, left) for contaminated EUs (averaged over six replications).

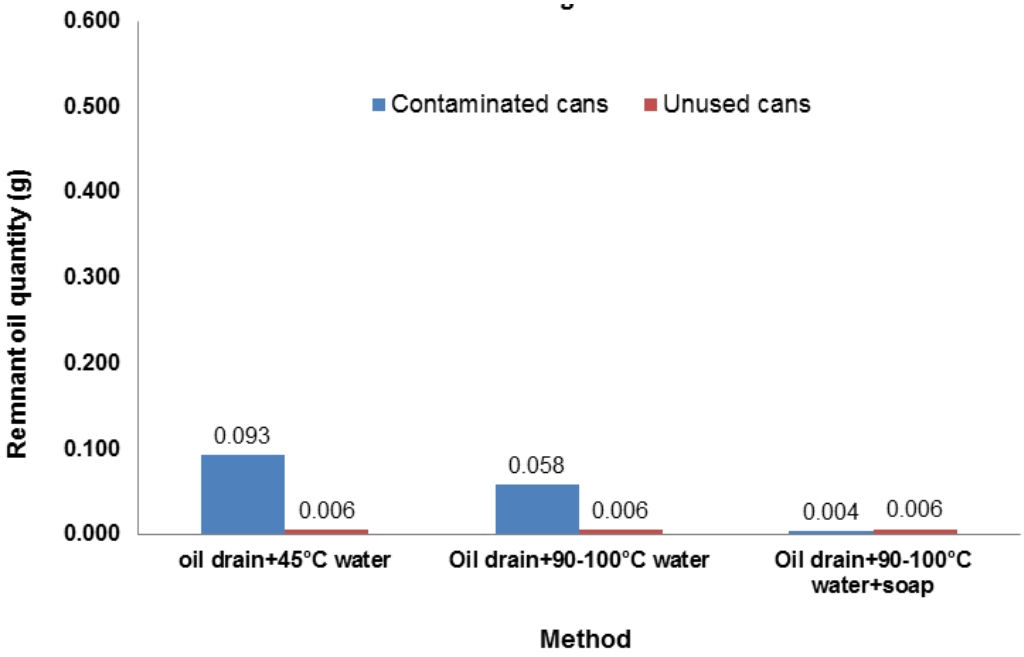

Figure 4.8: Location by method by container interactions (20-L container interior, right) for contaminated EUs (averaged over six replications).

4.6 CONCLUSIONS

Based on the results of this study, the following conclusions can be drawn:

- Affordable, edible oil containers are available in East African (Kenya, Tanzania, and Uganda) markets for use in hermetic storage. 5 to 20L, recyclable plastic containers exist with prices ranging from $0.72 to $2.08, and markets had 1,142 (5-L), 967 (10-L), and 2,581 (20-L) such containers respectively, on average per market.
- Previously used edible oil containers can be recycled following cleaning with soap plus water at 90 to100˚C, and mechanical action, for safe hermetic maize storage.

- Rancidity and associated negative health effects can be eliminated using the cleaning procedures outlined in the research, while preserving maize quality for the end-user.

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CHAPTER 5: GENERAL CONCLUSIONS

The results of the “Testing of predicted time to complete adult weevil mortality in hermetically stored maize” research confirmed that hermetic treatment is effective in field applications. This is evident in the significant ($p<0.0001$) differences between hermetic (sealed) and non-hermetic (open-air) treatments.

The “Effect of length of hermetic storage on maize seed germination”, research confirmed the safety and superiority of hermetic storage (with weevils) over non-hermetic storage (with weevils) for maize seeds preservation. This is because hermetically stored maize seeds had 98.7-99.5% germination rates versus 35.0-72.9 for non-hermetic (open-air) storage, over the 12-months seed storage period. Treatments of particular interest are hermetic treatments with weevils (month zero), hermetic treatment with weevils (month 4), hermetic treatment with weevils (month 8), and hermetic treatment with weevils (month 12), which had mean germination rates of 99.1%, 98.7%, 99.6%, and 99.3%, respectively. This confirmed that hermetic storage preserves seed viability, even when seeds are stored under ambient conditions.

The “Recycled container research” produced positive results foe the “market survey” and “laboratory cleaning” studies

5.1 MARKET SURVEY RESULTS

East African Markets surveyed are Jua Kali Drum Dealers (Nairobi), Shadimum Grocers, Frere Town (Mombasa) and Musila Enterprises (Kikambala Village), in Kenya, and Mwembe (Same), Same Center (Same), Kwasakwasa (Same),

Tanzania. Other markets surveyed in Tanzania, include Saidi (Nkungi), and Singida, while Markets surveyed in Uganda include Namanve Market (Mukono-Kampala), Owino Market (Kampala), and Soko Mujinga Market (Kitale). The recycled container survey identified a total of 55,522 containers that met the survey criteria. However, only 42,208 (76%) (Table 5.1) of the containers had edible oil residue or were earmarked for edible oil storage. These ranged in storage capacity from 5 to 20L, with prices ranging from $0.72 to $2.08, based on size. On average, the number of recyclable plastic containers per market were 1,142 (5-L), 967 (10-L), and 2,581 (20-L), respectively.

Table 5.1: Edible oil containers in Tanzania, Kenya and Uganda markets

	5-L plastic		10-L plastic		20-L plastic	
	Price	No. in stock	Price	No. in stock	Price	No. in stock
Kenya markets						
Market 1	-	1	-	1	-	1
Market 2	-	1	$0.60	200	$1.00	1000
Market 3	-	1	$0.80	200	-	1
Tanzania markets						
Market 1	$0.70	73	$1.80	92	$2.50	66
Market 2	$0.80	59	$1.00	64	$2.00	2004
Market 3	$0.70	48	$1.50	45	$2.00	57

Table 5.1: Edible oil containers in Tanzania, Kenya and Uganda markets-continued

	5-L plastic		10-L plastic		20-L plastic	
	Price	No. in stock	Price	No. in stock	Price	No. in stock
Uganda Markets						
Market 1	$1.00	100	$2.00	100	$3.00	100
Market 2	$0.40	10000	$1.00	8000	$2.00	20000
Market 3	-	2	-	2	-	2
Total in stock		10280		8701		23227
Average price	$0.72		$1.24		$2.08	
Average containers per market		**1142**		**967**		**2581**

1. **Not available in this market**
2. **No data**

5.2 LABORATORY CONTAINER RECYCLING RESULTS

Based on the results of the "Use of recycled containers for hermetic maize storage in East Africa", using 3g (0.031 moles) of Ivory soap (99.4% pure) to clean soybean oil contaminated 20-L containers will remove oil enough to prevent rancidity and cross-contamination of soybean oil with maize that would be store in them. This is because the remnant oil levels for contaminated experimental units (0.004±0.004) following application of soap treatment is not significantly different from control experimental units' remnant oil levels for oil drain, hot water and soap (0.005±0.007, 0.006±0.009,

0.006±0.001), respectively. Besides, the remnant oil level following cleaning with soap is within the margin of error. Therefore, it seems that the soap treatment successfully removed all the oil in the contaminated containers to which they were applied. For this reason it is safe to recommend that cleaning with soap (and hot water) of about the same level of purity as the one utilized in this research anywhere would successfully remove all the oil in a soybean oil contaminated container, enough to prevent rancidity and the associated health hazards, as well as preserve maize quality in storage.

These conclusions answer the dissertation's objectives of testing the integrity of hermetic storage and the validity of predicted time to complete mortality (PTCM) of maize weevils, to determine the effects of time of hermetic storage and maize weevil infestation on maize seed germination. And to (a) assess availability of used vegetable oil containers in East Africa suitable for hermetic storage of maize, as well as (b) develop procedures for cleaning the containers.

5.3 STUDIES' IMPACT

The storage solutions identified through the studies presented in this dissertation can protect food security, jobs, and local economies, using non-toxic and cost-effective food storage technologies sustainable in the local culture. This is because these technologies prevent weevil, mold, and rodent damage to stored maize, preserving food and seed supply. The ability to prevent economic (quantitative and qualitative) losses associated with existing maize pests, as demonstrated here means that farmers no longer need to dispose of their maize right after harvest. And

considering that agriculture employs 60 to 80% of the population (Bett and Nguyo, 2007; Minot, 2008), maize accounts for 50% of caloric intake (Sinha, 2007), at least 70% of maize seeds are sourced from prior year's harvest (Gemeda, et al., 2001; Dhliwayo, *et al*., 2003), and chemical maize preservatives used in post-harvest storage are toxic, costly, and often do not work (Korunic, 1998; IRRI, 2008), the results of these studies may encourage increased farming in the region.

5.4 RECOMMENDATIONS FOR FUTURE RESEARCH

Possible future research related to those presented in this dissertation includes:

a) Testing the effect of hermetic storage on molds in food storage
b) The effect of other insects (such as larger and smaller brain borer) on food stored under hermetic conditions.
c) Applying cleaning methods presented here to other storage container types.
d) Using other types of soaps for cleaning recycled containers, under conditions similar to and different from those presented in this dissertation.
e) Testing the effect of storage of other crop types and seeds under hermetic conditions.
f) Compare germination rates for seeds primed using cold storage and those primed using hydropriming.

APPENDIX A: PREDICTED TIME TO COMPLETE WEEVIL MORTALITY

Appendix A contains information relating to the "predicted time to complete mortality (PTCM)" research. Appendix A-Table 1.1 to Appendix A-Table 1.4 describes treatment, experimental designs and analysis of variance for the PTCM research.

Appendix A-Table 1.1: Predicted time to adult weevil mortality treatment design (laboratory research)

Storage type	**Storage time**		
	17 days (80% of baseline)	**21 days (baseline or 100%)**	**26 days (120% of baseline)**
Hermetic	T1	T2	T3
Non-hermetic	T4	T5	T6

Appendix A-Table 1.2 shows arrows going from experimental errors to the factors they test.

Appendix A-Table 1.2: Anova key out for Laboratory weevil mortality study at 23˚C and 12% moisture

S.V	**DF**
Treatment	5
Day	2
Herm	1
Day*Herm	2
Block	35
Level	2
Block (level)	33
Experimental error (EE): Treatment*block	175
Treatment*level	10
Treatment* block(level)	165
Corrected total	**215**
Total	**216**

Appendix A-Table 1.3: Treatment for high-density polyethylene (HDPE) 20-L containers (field research)

Storage type	Treatment
Hermetic	T1
Non hermetic	T2

A.1 ANALYSIS OF VARIANCE

Appendix A-Table 1.4 contains the "analysis of variance results" for the laboratory "**PTCM**" research.

Appendix A-Table 1.4: Anova for Laboratory scale weevil mortality study at 23°C and 12% moisture.

S.V	DF	SS	MS	F-value	Pr>F
Treatment	5	448133	89626	1211	<.0001
Day	2	1.1820	0.5910	0.01	0.9916
Herm	1	448054	448054	6403	<.0001
Day*Herm	2	78	39	0.56	0.5734
Block	35	1745	49	0.02	1.0000
Level	2	40	20	0.39	0.6799
Block (level)	33	1705	51.6	0.72	0.8666
EE: Treatment*block	175	12947	73.9		
Treatment*level	10	1101	110	1.53	0.1311
Treatment* block(level)	165	11845	71		
Corrected total	**215**	**462827**			

Appendix A-Table 1.5 contains the mean mortality results for the laboratory "**PTCM**" research, followed by additional statistical analysis related to the table.

Appendix A-Table 1.5: Days to complete weevil mortality in 12% maize stored 23°C (laboratory scale).

Storage type	Treatments (storage times)	Percent mortality
Hermetic (mean ± S.E)	17 days (80%)	94.9 ±9.10
	21 days (100%)	93.5 ±9.40
	26 days (120%)	94.1 ±13.5
Non-Hermetic (mean ± S.E)	17 days (80%)	2.25 ±3.50
	21 days (100%)	3.59 ±5.12
	26 days (120%)	3.37 ±5.26

A.2 INTERACTION ANALYSIS

A breakdown of the interaction for hermetic treatments suggest insignificance (p=0.5839, estimated difference= 0.99) for day 17 versus day 21 and 26. The estimate was calculated as a difference of differences of day 17 versus the average of days 21 and 26 or 94.97-((93.46+94.50)/2).

Interaction was also insignificant (p=0.5566, estimated difference=1.4) for day 17 versus 21, day 17 versus 26 (p=0.7182, estimated difference=0.8), and day 21 versus 26 (p=0.5566, estimated difference=0.6).

A breakdown of interactions for non-hermetic treatments among days, also suggests insignificance (p=0. 2005, estimated difference=1.2) for day 17 versus day 21 and 26, as well as insignificant (p=0.2268, estimated difference=1.3),

day 17 versus 21, day 17 versus 26 (p=0.3122, estimated difference=1.1), and day 21 versus 26 (p=0.2268, estimated difference=0.2).

There are also no clear treatment by level interaction effects ($p<0.1311$), since mortality for hermetic and non-hermetic interactions for all levels of days (17, 21, and 26) are insignificant.

APPENDIX B: SEEDS

Appendix B-Tables 1.1 to 1.5 show the treatment designations table and analysis of variance table utilized in the seed research planning and analysis. They are followed by relevant seed literature review.

Appendix B-Table 1.1**:** Seed germination research treatment design

		Time (months)			
		0	**4**	**8**	**12**
Weevils	Hermetic (H)	T_1	T_2	T_3	T_4
	Non hermetic (NH)	T_5	T_6	T_7	T_8
No weevils	Hermetic (H)	T_9	T_{10}	T_{11}	T_{12}
	Non hermetic (NH)	T_{13}	T_{14}	T_{15}	T_{16}

B.1 GERMINATION ANALYSIS OF VARIANCE KEY OUT

Appendix B-Table 1.2 shows arrows going from experimental errors to the factors they test.

Appendix B-Table 1.2: Germination anova key out for 4 samples of 50 seeds per treatment jar or germination tray.

SV	DF	DF formula
Blocks/Replications (r)	5	r-1
Treatments (t): storage jars	15	t-1
EE: Experimental errors	75	(r*t)
Corrected total samples	288	((rt(s-1))
Sampling error (SE): Seeds/samples/reps/treatment	18816	rts(subs-1)
Corrected total	**19199**	**(rts*subs)-1**
Total	**19200**	**(rts*subs)**

S=seed samples; Sub=50 seed subsamples

B.2 ANALYSIS OF VARIANCE

The analysis of variance (Appendix B: Table 1.3) shows the final results of the recycled container research, for the factors and interactions tested.

Appendix B-Table 1.3: Analysis of variance (anova) for the hermetic seed germination study

Source	DF	SS	Mean Square	F Value	Pr > F
Replications	5	204459	40892	1.69	0.1467
Treatments	15	5600316	373354	15.46	<.0001
Hermetic	1	1031067	1031067	42.69	<.0001
Weevil	1	1028138	1028138	42.57	<.0001
Month	3	619131	206377	8.54	<.0001
Herm*weevil	1	1034001	1034001	42.81	<.0001
Herm*month	3	640331	213444	8.83	<.0001
Weevil*month	3	628043	209348	8.66	<.0001
Herm*weevil*month	3	619606	206535	8.55	<.0001
Experimental error:	**75**	**1811499**	**24153**		
Residual:	**19104**	**6707850**	**1077**		
Corrected total:	**19199**	**14324124**			

B.3 TRAYS AND GERMINATION SHELVES

96 treatments*4 samples per jar = 384 total samples

16 treatments (*24 planting positions) = 384.

Total trays=384 total samples=4 samples from each treatments (per tray)

=96 (or 384/4) trays or 8 germination cabinets (96 trays on 13 trays per cabinet).

B.4 TREATMENT SAMPLES RANDOMIZATION TO TRAYS

Appendix B-Table 1.4**:** Seed (treatment) samples randomization to trays

Tray 1					Tray 2				Tray 3				Tray 4			
6	15	3	5	8	14	9	2	12	7	1	10	13	16	4	11
4	10	11	6	14	9	13	1	3	16	8	7	2	5	12	15
13	9	7	16	12	8	4	1	14	6	2	11	15	5	10	3
10	6	3	11	2	1	8	13	7	14	9	5	15	12	4	16
14	6	5	11	1	2	16	4	15	3	9	10	7	13	12	8
8	16	13	11	5	9	1	2	6	14	4	12	7	10	3	15
12	7	9	6	11	3	1	10	5	4	16	14	15	13	2	8
6	14	4	2	16	15	1	9	11	5	10	7	13	8	12	3
2	9	10	7	15	14	13	5	11	6	1	16	8	3	12	4
5	6	1	3	13	12	15	9	11	2	8	7	14	4	16	10
4	3	15	13	5	9	16	10	11	1	8	6	2	12	14	7
11	10	14	13	16	4	1	15	7	5	9	8	6	2	12	3

Appendix B-Table 1.4**:** Seed (treatment) samples randomization to trays-continued

Tray 1				Tray 2				Tray 3				Tray 4			
7	5	4	12	2	15	16	11	8	3	14	9	1	10	6	13
10	1	13	7	3	11	12	14	6	16	9	5	4	15	8	2
9	10	15	7	5	13	1	16	14	8	2	4	11	3	12	6
6	16	4	10	5	15	7	11	13	14	2	8	9	12	3	1
1	9	5	16	10	6	2	11	15	4	14	13	3	7	12	8
7	10	5	6	9	15	13	11	8	1	4	2	3	12	16	14
7	8	6	3	14	16	4	10	2	15	1	9	5	12	13	11
12	2	15	1	7	6	10	4	9	8	1	11	16	13	5	3
12	11	5	1	14	2	16	9	13	8	6	15	4	10	7	3
6	5	10	4	2	11	13	1	7	9	14	12	15	3	16	8
5	3	7	13	10	9	4	1	14	8	16	15	2	6	11	12
12	11	8	2	5	1	4	13	3	16	9	10	14	7	15	6

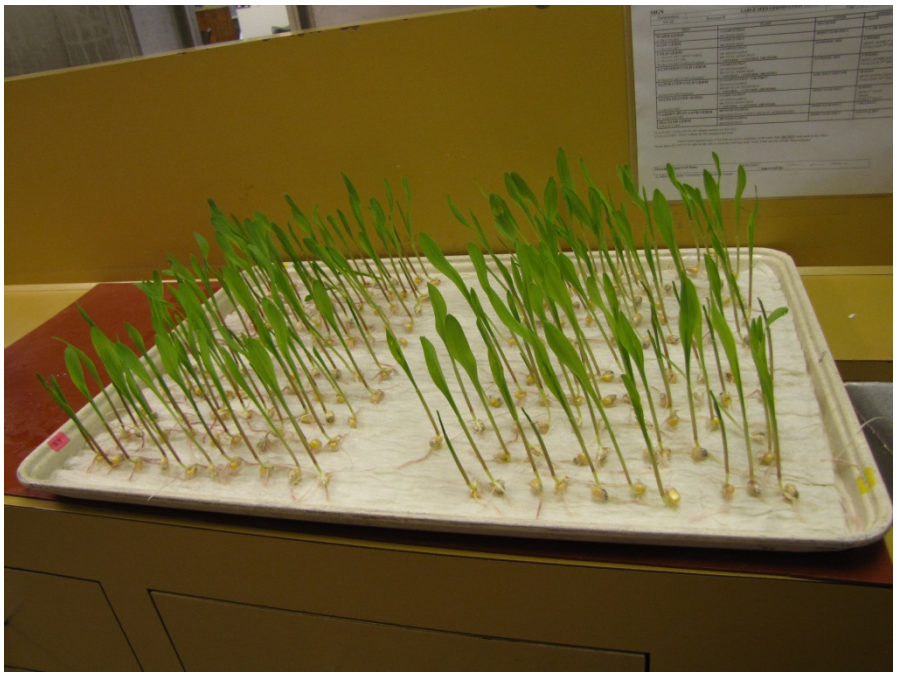
B.5 SEED PLANTING ARRANGEMENT

Appendix B-Table 1.5: Example planting arrangement (row one- Appendix B-Table 4) for the hermetic seed germination study

Tray 1		Tray 3	
Trt: 6 5o seeds	**Trt: 15** 5o seeds	**Trt: 12** 5o seeds	**Trt: 7** 5o seeds
Trt: 3 5o seeds	**Trt: 5** 5o seeds	**Trt: 1** 5o seeds	**Trt: 10** 5o seeds
Tray 2		**Tray 4**	
Trt: 8 5o seeds	**Trt: 14** 5o seeds	**Trt: 13** 5o seeds	**Trt: 16** 5o seeds
Trt: 9 5o seeds	**Trt: 2** 5o seeds	**Trt: 4** 5o seeds	**Trt: 11** 5o seeds

Appendix B-Figure 1.1A to Appendix B-Figure 1.3B show typical images of germination trays utilized in the research. Figures containing “A” represent image of seeds before germination, while Figures containing “B” represent seedlings, following germination.

Appendix B-Figure 1.1A. Typical initial germination result for treatments T1, T2, T3, T4, T5, T9, T10, T11, T12, T13, T14, T15, and T16 of the hermetic seed germination study.

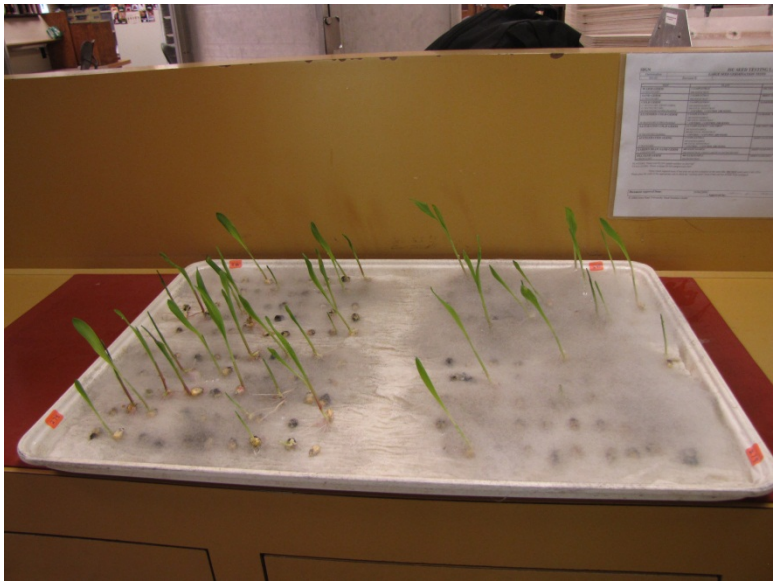


Appendix B-Figure 1.1B. Typical final germination result for treatments T1, T2, T3, T4, T5, T9, T10, T11, T12, T13, T14, T15, and T16 of the hermetic seed germination study.

Appendix B-Figure 1.2A. Typical initial germination result for treatments T6, T7, and T16 of the hermetic seed germination study.

Appendix B-Figure 1.2B. Typical final germination result for treatments T6, T7, and T16 of the hermetic seed germination study.

Appendix B-Figure 1.3A. Typical initial germination result for treatment T8, and T16 of the hermetic seed germination study.



Appendix B-Figure 1.3B. Typical final germination result for treatment T8 and T16 of the hermetic seed germination study.

B.6 FURTHER HERMETIC SEED GERMINATION ANALYSIS

For hermetic treatments, a breakdown of hermetic by weevil by month interaction effects with weevils suggests insignificance (p=0.7749, estimated difference= 0.07) for month zero versus month 4, 8, and 12. The estimate was calculated as a difference of differences of month zero versus the average of months 4, 8 and 12 or 92.2-((98.8+99.7+99.3)/3).

Interactions were also insignificant (p=0.2431, estimated difference=0.4) for month zero versus 4, month zero versus 8 (p=0.1613, estimated difference=0.5), month zero versus 12 (p=0.6406, estimated difference=0.2), month 4 versus 12 (p=0.1023, estimated difference=0.6), and month 8 versus 12 (p=0.3504, estimated difference=0.3). Interactions were, however, slightly significant for months 4 versus 8 (p=0.0103, estimated difference=0.92).

For non-hermetic treatments with weevils, a breakdown of hermetic by weevil by month interaction effects suggest significance (p<.0001, estimated difference= 39.2) for month 0 versus month 4, 8, and 12. Interaction were also significant (p<.0001, estimated difference=26.9) for month zero versus 4, month zero versus 8 (p<.0001, estimated difference=26.3), month zero versus 12 (p<.0001, estimated difference=64.2), month 4 versus 12 (p<.0001, estimated difference=37.3), and month 8 versus 12 (p<.0001, estimated difference=37.8). However interaction was insignificant for month 4 versus 8 (p=0.7195, estimated difference=0.6).

For hermetic treatments, without weevils, a breakdown of hermetic by weevil by month interaction effects suggest insignificance (p=0.9267, estimated difference=

0.03) for month zero versus month 4, 8, and 12. Interactions were also insignificant (p=0.6494, estimated difference=0.2) for month zero versus 4, month zero versus 8 (p=0.8186, estimated difference=0.1), month zero versus 12 (p=0.3633, estimated difference=0.3), month 4 versus 8 (p=0.8218, estimated difference=0.1), month 4 versus 12 (p=0.1727, estimated difference=0.5), and month 8 versus 12 (p=0.2550, estimated difference=0.4).

For non-hermetic treatments, without weevils, a breakdown of hermetic by weevil by month interaction effects, across months, suggests insignificance for month zero versus month 4, 8, and 12 (p=0.5728, estimated difference= 0.2). Interactions were also insignificant (p=0.8179, estimated difference=0.1) for month zero versus 4, month zero versus 8 (p=0.4898, estimated difference=0.3), month zero versus 12 (p=0.6452, estimated difference=0.2), months versus 8 (p=0.6452, estimated difference=0.2), month 4 versus 12 (p=0.8179, estimated difference=0.1), and month 8 versus 12 (p=0.8179, estimated difference=0.1).

APPENDIX C: RECYCLED CONTAINERS AND HERMETIC STORAGE

Appendix C contains the soap quantification, as well as trial run steps and results, for the recycled container research. This is followed by the relevant literature reviews.

C.1 TREATMENT DESIGN

Appendix C-Table 1.1: Recycled container research treatment design

	Treatments (A)		
EU (b)	**Soap**	**Hot water**	**Oil drain**
Contaminated	T1	T2	T3
New (Uncontaminated)	T4	T5	T6

C.2 EXPERIMENTAL DESIGN

C.3 ANOVA DESIGN KEY OUT

The **"**Anova design key out**"** (Appendix D-Table 1.2) shows green arrows that originate from originate experimental errors and terminate on the factors that they test.

Appendix C-Table 1.2 shows arrows going from experimental errors to the factors they test.

Appendix C-Table 1. 2: Anova design key out (Split plot) for the recycled container research

SV	DF	DF
Blocks (B)	(r-1)	**5**
Treatments (T)	(t-1)	**5**
Method (m)	(m-1)	2
Container (c)	(c-1)	1
m*c	(m-1)*(c-1)	2
Error A: Ea	**(r-1)*(t-1)**	**25**
Location (l)	(l-1)	**2**
Location*Treatment	(l-1)*(t-1)	**10**
L*m	(l-1)*(m-1)	4
w*c	(w-1)*(c-1)	2
w*m*c	(w-1)*(m-1)*(c-1)	4
Error B: Eb	**t(r-1)*(w-1)**	**60**
Corrected total (samples)	**trw(s-1)**	**107**
Total (# of samples)	**(abrw*1)-1**	**108**

C.4 METHODS AND MATERIALS

C.4.1 Soap quantification

0.1 gram Ivory soap was utilized in cleaning the oil-containated interior of three pint-sized Kerr, glass canning jars, in a soap pre-quantification exercise. However, the glass canning jars utilized in the soap prequantification excercise could only withstand the soap and water at 45°C, but shattered when the soap and hot water treatment (90-100°C) was applied. This made it impossible to obtain initial knowledge of the soap and hot water treatment effect. Therefore, soap pre-

quantification was redone using steel canisters (MS10-039-390-14, Mainstays, Bentonville, AR 72716) and both pre-quantification treatments.

The soap pre-quantification was done by weighing the canisters, pouring soybean oil into each one, allowing the oil to soil their interior, then turning them upside down to allow the excess oil to collect unto folded napkins. Once the oil had drained, additional excess oil was wiped off and canisters were weighed again. Subtracting both weights from each other gave an estimate of the weight of the oil, in the canister. A small quantity of Ivory soap (0.1 g) that was not expected to completely remove this quantity of oil was utilized in cleaning the canisters. At the end of the cleaning, the percentage of oil removed was calculated (Appendix D-Table 3 to 7). From the initial soap quantification experiment, it was determined that 1 g of soap removed most almost 1 gram of oil, from the canister. But three times that amount of soap (3 g) was utilized for cleaning the 20-L containers, to account for the variability in the history of 20-L containers collected from the field. This helped rid the EU interior completely of oil and is an acceptable practice, where a reasonable excess of material (Markley, K.S, 1951; Truman, 2009; Devor, et al., 2007; Sohb, 2008) may be utilized in cleaning oil remnants.

C.5 RESULTS AND DISCUSSION

C.5.1 Steel canister cleaning results

Using 0.1 g Ivory soap worked well in removing all the oil (~1g) from pint-sized Kerr, glass canning jars. Appendix D-Tables 3 to 7 shows that this was not the case when

0.1 gram Ivory soap and sodium palmitate were used in cleaning steel canisters, indicating that the cleaning properties of soap varies by material cleaned.
This is because the quantity of Ivory soap (99.44% pure) required to remove soybean oil contaminants from glass surfaces varied from that required to remove oil steel canisters and HDPE 20-L containers. Glass tended to require less soap than steel canisters and HDPE 20-L containers, while the steel and HDPE seemed to require about the same amount of soap for removing 1 g of oil from their surfaces.

From Appendix C-Table 1.3, the canister had about 73.57% (~1.757g) oil leftover from cleaning with 0.1 g Ivory soap. And from Appendix D-Table 4, it had about 56.00% (~1.004g) oil leftover from cleaning with 0.1 g Ivory soap.

Appendix C-Table 1.3: Steel canister cleaning with 0.1 g Ivory soap at 45°C.

Canister #	**1**	**2**	**3**	**Average**
Canister wt (g)	277.83	295.96	295.77	
Initial (Canister +oil) wt (g)	280.06	298.23	298.43	
Beginning oil wt (g)	2.23	2.27	2.66	
Final (Canister +oil) wt (g)	278.40	296.60	296.45	
Leftover oil wt (after cleaning)	1.66g =74.44%	1.63g =71.81%	1.98g =74.44%	1.757g =73.57%
Ivory soap (g)	0.1	0.1	0.1	
Temp (°C)	45	45	45	

Appendix C-Table 1.4: Steel canister cleaning with 0.1 g Ivory soap at 100°C.

Canister #	**1**	**2**	**3**	**Average**
Canister wt (g)	277.83	295.96	295.77	
Initial (Canister +oil) wt (g)	279.23	297.80	298.05	
Beginning oil wt (g)	1.40	1.84	2.28	
Final (Canister +oil) wt (g)	278.28	296.88	296.91	
Leftover oil wt (after cleaning)	0.95g =68.00%	0.92g =50.00%	1.14g =50.00%	1.004g =56.00%
Ivory soap (g)	0.1	0.1	0.1	
Temp (°C)	100	100	100	

Appendix C-Tables 1.5 to 1.6 shows steel canister cleaning results, using sodium palmitate, and indicates that the canister had 75.86% (~2.32g) oil leftover from cleaning with 0.1 g sodium palmitate.

Appendix C-Table 1.5: Steel canister cleaning with 0.1 g sodium palmitate at 45°C.

Canister #	**1**	**2**	**3**	**Average**
Canister wt (g)	277.83	295.96	295.77	
Initial (Canister +oil) wt (g)	280.45	299.01	299.43	
Beginning oil wt (g)	2.62	3.05	3.66	
Final (Canister +oil) wt (g)	278.22	296.75	296.92	
Leftover oil wt (after cleaning)	2.23g =86%	2.22g =73%	2.51g =68.58%	2.32g =75.86%
Sodium palmitate (g)	0.1	0.1	0.1	
Temp (°C)	45	45	45	

From Appendix C-Table 1.6, canister had 95.23% (~3.10g) oil leftover from cleaning with 0.1 g sodium palmitate. Since 0.1g Ivory soap removed 0.443g oil on average at 100°C (Appendix C-Table 1.7), about 0.32g of the soap was expected to remove all the oil in the canister with the least oil contamination (1.4g). Using 3 times this soap quantity (1g) removed most of the oil from the canister interior.

Appendix C-Table 1.6: Steel canister cleaning with 0.1 g sodium palmitate at 100°C.

Canister #	**1**	**2**	**3**	**Average**
Canister wt (g)	278.07	296.04	296.10	
Initial (Canister +oil) wt (g)	281.25	299.35	299.36	
Beginning oil wt (g)	3.18	3.31	3.26	
Final (Canister +oil) wt (g)	278.12	296.36	296.20	
Leftover oil wt (after cleaning)	3.13g =98.43%	2.99g =90.33%	3.16 =96.93%	~3.10g =95.23%
Sodium palmitate (g)	0.1	0.1	0.1	
Temp (°C)	100	100	100	

0.1g (of 99.44%) pure Ivory soap described (Appendix C-Table 1.7), worked well, in removing oil from oil contaminated glass canning jar interiors, during oil pre-quantification exercise, since 0.464 g of oil at 45°C, and 0.442 g of oil at 100°C, on average, were removed by 0.1g of Ivory soap from the interior of Kerr glass canning jars. However, that was not the case when this soap quantity was tested on steel canister surfaces, where it was noticed soap quantification using 0.1g Ivory soap to clean oil contaminated canister interiors removed 26.43% oil at 45°C, and 44% oil at 100°C. Palmitate on the other hand removed 24.14% oil at 45°C and 4.77% oil at 100°C (Appendix C-Table 1.3 to 1.7). This left behind about 1.76g and 1.00g of oil on average at 45°C and 100°C, respectively following cleaning with Ivory. And about 2.32g and 3.10g of oil on average at 45°C and 100°C, respectively were leftover following cleaning with palmitate (Appendix C-Table 1.7).

Appendix C-Table 1.7: cleaning results summary for Ivory soap and Sodium palmitate

Ivory soap				
Temperature (°C)	Leftover oil (%)	Leftover oil (g)	Oil removed (g)	Soap used (g)
45	73.57	1.757	((100-73.5)/100)*1.757=0.464g	0.1
100	56.00	1.004	((100-56)100)*1.004=0.443g	0.1
Sodium palmitate				
Temperature (°C)	Leftover oil (%)	Leftover oil (g)	Oil removed (g)	Soap used (g)
45	75.86	2.32	((100-75.86)/100)*2.32 =0.560	0.1
100	95.23	3.10	((100-95.23)/100)*3.10 =0.148	0.1

C.5.2 Trial run

Six quart-sized canning jars were labeled 1 to 6 and assigned to the 20-L containers that they were intended to be used with. Ivory soap was measured out based on the derived soap to oil ratio and prior knowledge of the quantity of each 20-L container's oil remnant. The measured soaps were crushed before placing them in their designated jars, following which each was filled half-way with distilled water, shaken in a gentle swirl and left standing overnight to allow for mixing of the soap and water. Three of the 20-L containers were assigned to 45°C temperature (and soap treatment), while the other three were assigned to 100°C (and soap treatment), for use in testing the cleaning effect of the derived oil to soap ratio at 45°C, as well as at 100°C (Sebastião et al., 2006, Bader, 2010, ACS, 2011).

Water was heated to 45°C and 100°C, respectively for use in the assigned 20-L containers and temperatures were confirmed using a kitchen thermometer (GT100R, TEL-TRU manufacturing company, Rochester, N.Y.). Prior to adding water to the 20-L containers, the canning jar content assigned to each 20-L container was emptied into it. 3.78L of water (at the desired temperature) was then added to each 20-L container, capped lightly (to prevent pressure buildup) and shaken to force soap lather to fill the entire 20-L container interior. Thereafter, gentle swirling motion was utilized, every 5 minutes for 1.5h, to dislodge the oil. This is because 20-L HDPE container interiors could not be scrubbed directly, due to their narrow opening. At the end of 1.5h, the 20-L containers were filled with deionized water (Wilson, et al., 1999) at the required temperature, for rinsing. Additional water was added continually to make the water overflow, and force the soap lather out of the 20-L

HDPE container. Once all the lather had left the 20-L HDPE container, the water was poured out, and 3.78L of deionized water was added to the 20-L HDPE container, shaken and emptied. Another 3.78L of deionized water was added a second time, and the 20-L container was again shaken and emptied to rinse out the soap completely. Before rinsing each 20-L HDPE container, some of its soap was used along with sponge to scrub the 20-L HDPE container's neck and cover.

The trial run confirmed that 3g (0.031 mole) of 99.44% pure Ivory soap was enough to adequately remove each gram (0.001 mole) of remnant soybean oil from their assigned 20-L HDPE containers, at 100°C.

C.5.3 Conclusion

Using about 1g of Ivory soap per gram of soybean oil contamination removed almost 1g oil from the canister interior, but to ensure complete clean, remove all the oil and avoid rancidity that may result from leftover oil, three times that amount of Ivory soap (3g) was hypothesized to completely remove each gram of oil from the steel canister interior. It was also hypothesized that the same soap to oil ratio would apply to the (HDPE) 20-L containers that needed to be cleaned in the trial and full runs of this research. This soap quantity (3g/g oil) was also expected to account for the variability associated with the history of 20-L containers collected from the field, and help rid the EU interior completely of oil.

C.5.4 Research analysis of variance

The analysis of variance (Appendix C: Table 1. 8) shows the final results of the recycled container research, for the factors and interactions tested.

Appendix C: Table1. 8: Analysis of variance table for the recycling container research.

SV	DF	SS	Mean square	F Value	Pr > F
Replications	5	0.0167	0.0030	0.28	0.9218
Treatments	5	0.9760	0.1950	16.12	<.0001
method	2	0.2740	0.1360	8.23	0.0005
container	1	0.4290	0.4290	26.00	<.0001
method*container	2	0.2740	0.1370	8.28	0.0005
Ea: replications*treatments	25	0.3030	0.0121		
Location	2	0.2990	0.1490	21.68	<.0001
location*trt	10	0.6560	0.0660	9.54	<.0001
location*method	4	0.1830	0.0457	5.61	0.0004
location*container	2	0.2910	0.1450	17.90	<.0001
location*method*container	4	0.1830	0.0456	5.61	0.0004
Eb: replications*treatments*location	60	0.4130	0.0068		
Corrected total	107	2.6620			
Total	108				

APPENDIX D: RECYCLED CONTAINER SURVEY

Presented below are survey forms and attachments (pictures), as presented by various surveyors. They are organized in alphabetical order of the countries surveyed. The contents of the survey table and comments have been left as submitted by the surveyors.

D.1 KENYA

a) Recycled container survey form

Ali Yakubu (aaa@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date: 14th October 2011

Conducted by: Anne Mukudi

Email: tinamukudi@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1) Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store: Jua Kali Drum Dealers- Factory Street-City Stadium
City: **Nairobi-Kenya.**

Appendix D: Table 1.1-Market survey form for the recycled container research

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Engine Oil	20Ltrs	Plastic	30	200=/($2)
2) Glucose	200Ltrs	Plastic	300	1,600=/($17)
3) Engine Oil	200Ltrs	Metal	2000	1,300=/($14)
4) Thinner(Solvent)	240Ltrs	Plastic	200	1,800=/($19)
5) Wine	250Ltrs	Plastic	80	2,000=/($21)
6) Paints	250Ltrs	Plastic	100	2,500=/($26)
7) Grease	200Ltrs	Metal Drums	200	1,300=/($14)

Description of the market: It is an open area, not very far from the city centre; there are other two markets near the business area, one which specializes in selling second hand/used shoes, clothes, bags, fruits and vegetables. The other Market is very large, with different trading activities. There is a mini supermarket near the trading area, and the area is a very busy area with multitude of activities going on.

Comments: The trading area I visited is a Welfare group, self help Group with 25 members all dealing in the container business. Majority of their buyer come from outside Kenya; countries like Southern Sudan, Democratic Republic of Congo, Burundi and Zaire, and the use the containers in storing petroleum products for sale, though few Kenyans also do purchase the containers but mostly for water storage, building purposes-carrying water for building and for irrigation. The welfare sometimes gets orders from the United Nation (UN) mostly requesting for the open

metal drums, which they cut it into halves, which they use it as cooking pan for the refugees in the camps, some locals also request for the open metal drums to use in food and hoof soup preparation which they sell to the people in the market. The welfare derives their source of income from container selling; they only sell containers of 20Ltrs and above. Their main source of supply for the containers comes from the Industrial area in Nairobi.

Appendix D-Figure 1.1. 208-L (55-gallon) metal drums.

Appendix D-Figure 1.2. Plastic Containers (with two emptying holes).

Appendix D-Figure 1.3. 1000L white plastic container (previously contained water purification liquid).

Appendix D-Figure1. 4. Stack of blue plastic containers (two types).

Appendix D-Figure 1.5. 20L (plastic) and 208-L (metal) containers.

b) Recycled container survey form

Ali Yakubu (aaa@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date: 18th October 2011

Conducted by: Anne Mukudi

Email: tinamukudi@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary
(3) Please attach or email pictures, where possible..

Market or Store: Shadimum Grocers, Frere Town

City: Mombasa-Kenya

Appendix D: Table 1. 2- Market survey form for the recycled container research

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Oxidizing Agent	150Ltrs	Plastic	100	500/=($5)
2) Cooking Oil	20Ltrs	Plastic	1000	120/=($1)
3) Laundry detergent	20Ltrs	Plastic	200	120/=($1)
4) Cooking oil	10Ltrs	Plastic	200	60/=($6C)
5) Fresh Juice	5Ltrs	Plastic	500	20/=($2C)
6) Drinking water	5Ltrs	Plastic	300	20/=($2C)

Description of the market: It is a shop in a densely populated area in Frere town area of Mombasa, along Mombasa/Malinda road. there are several other shops in the area dealing in a range of businesses i.e. retail shops, salons, kiosks, vegetables, meat shops, cafes, pharmacies and beauty products shops. There are also wholesale shops which deal in a variety of products.

Comments:

The shop/store I visited is a retail shop owned by a sole proprietor dealing in a variety of items including plastic containers which are mostly from products he sells. Most of the plastic container buyers are truck drivers who use them to carry diesel and end up reselling the containers in Democratic Republic of Congo and Southern Sudan. Some of the containers are used locally to store Palm wine for sale and for domestic use for water storage.

The shop owner decided to sell the containers after he realized there is a ready market for them. He sells cooking oil in small quantities and once the product is finished, he sells the containers. Quite a number of shops (there are about 10 mores shops) in the area also do sell containers. The business is profitable bearing in mind that he gets the containers for free (after the product is finished).

Appendix D-Figure 1.6. Blue plastic containers (type 2).

Appendix D-Figure 1. 7. White and yellow plastic containers.

Appendix D-Figure1. 8. Assorted (blue, black, white, yellow) plastic containers.

c) Recycled container survey form

Ali Yakubu (aaa@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date: 18th October 2011

Conducted by: Anne Mukudi

Email: tinamukudi@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store: Musila Enterprises, Kikambala Village

City: **Mombasa-Kenya**

Appendix D: Table 1.3- Market survey form for the recycled container research

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Oxidizing agent	150Ltrs	Plastic	1000	450/=($5)
2) Laundry bleach	20Ltrs	Plastic	1500	100/=($1)
3) Laundry detergent	20Ltrs	Plastic	1000	100/=($1)
4) Cooking oil	10Ltrs	Plastic	200	80/=($8C)
5) Fresh juice	5Ltrs	Plastic	500	10/=($1C)
6) Drinking water	5Ltrs	Plastic	300	10/=($1)
7) Chlorine	200Ltrs	plastic	600	450/=($5)

Description of the market: It is a shop in a village next to two beach hotels in Kikambala village, off Mombasa/Malindi road. There are other businesses mostly to do with the tourism industry around the trading area.

Comments:

The shop I visited is a retail shop owned by a couple dealing in plastic containers and curios. The couple decided to venture into the business due to the readily available plastic containers used to store detergents, bleaches, fresh juice and cooking oil at the hotels. They sell a total of approximately 3000pcs of all types of

containers in a month. Most of the plastic container buyers are wholesale customers who resell to other markets. Some of the buyers are local villagers who use it to supply palm wine.

Appendix D-Figure 1.9. Various sizes and colors of plastic containers.

Appendix D-Figure 1.10. White and yellow plastic containers.

Appendix D-Figure 1.11. 100-L and 210-L black plastic containers.

Appendix D-Figure 1.12. Stack of blue plastic containers (on raised platform).

D.2 TANZANIA

The market survey forms for Tanzania was submitted in print, and had to be scanned. The scanned forms are presented below.

RECYCLED CONTAINER SURVEY FORM

Ali Yakubu (yakubuali@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date 26.5.11 Conducted by ELIBARIKI KISIMBO Email elibariki.kisimbo@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store Mwembe **City** SAME **Country** TANZANIA

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price
1) Edible oil	10Lts	Plastic	92	2800 Tshs.
2) Edible oil	20lts	—11—	66	4000 Tshs.
3) Concentrates	20lts	—11—	12	3800 Tshs.
4) Edible oil	5Lts	—11—	73	1,100 Tshs.
5) Drinking water	20lts	—11—	88	3300 Tshs

Description of the market: This Market is Conducted only on Thursdays, it is located at Mwembe Ward- Same District.

Comments: The price of these Containers as well as other Commodities usually fluctuates.

RECYCLED CONTAINER SURVEY FORM

Ali Yakubu (yakubuali@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date 22.5.11 Conducted by ELIBARIKI KISIMBO Email elibariki.kisimbo@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store SAME CENTRE **City** SAME **Country** TANZANIA

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price
1) Soft drink	5Lts	plastic	29	2300 Tshs.
2) Edible Oil	20Lts	plastic	2004	3600 Tshs
3) Edible oil	5Lts	Plastic	59	1300 Tshs
4) Edible oil	10Lts	Plastic	64	2300 Tshs.
5)				

Description of the market: This is the Centre Market situated at Same Town and it is Conducted everyday.

Comments: The price is controlled by Demand and supply.

RECYCLED CONTAINER SURVEY FORM

Ali Yakubu (yakubuali@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date 21ST MAY 2011 Conducted by ELIBARIKI KISIMBO Email elibariki.kisimbo@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store KWASAKWASA **City** SAME **Country** TANZANIA

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price
1) Edible oil	5Lts	Plastic	48	1200 Tshs
2) Edible oil	20Lts	Plastic	57	3800 Tshs.
3) Concentrates	20 Lts	Plastic	18	4000 Tshs
4) Drinking water	20Lts	steel	25	4300 Tshs
5) Edible oil	10Lts	Plastic	45	2500 Tshs.

Description of the market: The Market is situated at Same town Kilimanjaro Region. This Market is usually Conducted on Sundays only.

Comments: The prices depends mostly on market demand. No fixed-price.

RECYCLED CONTAINER SURVEY FORM

Ali Yakubu (yakubuali@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date 3 - 2011 Conducted by Katheryn Hamilton Email K8hamilton@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary
(3)Please attach or email pictures, where possible..

Market or Store _____ **City** Singida **Country** TANZANIA

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price
1) edible oil	20 ltr	Plastic		4,500 tsh 3,000
2)				
3)				
4)				
5)				

New P 4.50
used P 3.00

Description of the market: per picture

Comments:

RECYCLED CONTAINER SURVEY FORM

Ali Yakubu (yakubuali@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date 3-2011 Conducted by Katheryn Hamilton Email K8kamilton@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary
(3)Please attach or email pictures, where possible..

Market or Store Saidi **City** Nkungi **Country** TZ

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price**
1) edible oil	20 ltr	Plastic		5000 tzs new $5.00 3500 used $3.50
2)				
3)				
4)				
5)				

Description of the market: picture

Comments:

D.3 UGANDA

a) Recycled container survey form

Ali Yakubu (aaa@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date: 20th October 2011

Conducted by: Anne Mukudi

Email: tinamukudi@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary
(3)Please attach or email pictures, where possible..

Market or Store: Namanve Market
City: **Mukono-Kampala, Uganda**

Appendix D: Table 1.4- Market survey form for the recycled container research

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Soft drinks	220	plastic	20	65,000($23)
2) Soap	100	plastic	20	20,000($7)
3) Water chemicals	1000	plastic	20	280,000($102)
4) Soap	20	plastic	20	8,000($3)
5) Soap	18	plastic	20	6000($2)
6) Paint	50	metal	20	20,000($7)
7) Soap	250	plastic	20	80,000($29)

Appendix D: Table 1.4- Market survey form for the recycled container research - continued.

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price/unit
8)Soap	200	plastic	20	60,000($21)
9) Soap	120	plastic	20	50,000($18)
10) Soft drinks	100Ltrs	Plastic	20	20,000($7)
11) Soft drinks	20Ltrs	Plastic	20	8,000($3)
12) Soft drinks	18Ltrs	Plastic	20	6,000($2)
13) Soft drinks	250Ltrs	Plastic	20	80,000($29)
14) Soft drinks	200Ltrs	Plastic	20	60,000($21)
15) Soft drinks	120Ltrs	Plastic	20	50,000($18)

Description of the market:

The market is along Jinja highway with shops surrounding them. They are sold by different people working together as friends. They mostly deal in selling the containers.

Comments:

These containers are sold by individuals who came together and begun a business to help them earn a living.

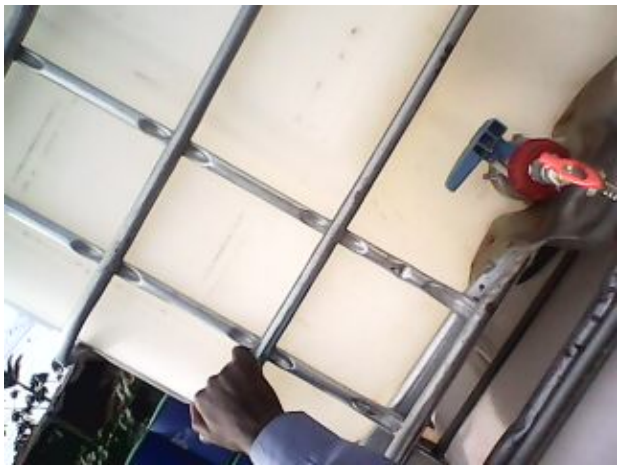
The centre does not have many dealers. They get containers from Uganda companies, import from Kenya; the containers are imported from other countries

like South Africa etc and get in to Uganda and Kenya through the Industries in the two mentioned countries.

Most of the people buy it for storing water, alcohol (Waragi) use as taps in hotels; the metals are used to make charcoal stoves and containers used as small shops. Some are taken to the northern side used by UN organizations in the camps; use some for harvesting rain water. The containers enter the market when they are completely sealed, but the traders design them according to the customers need, for example, they can put taps for the people who want to use them in hotels for washing hands.

Appendix D-Figure 1.13. Stacks of black plastic containers (on the ground).

Appendix D-Figure 1.14. Stacks of blue plastic containers (type 3).



Appendix D-Figure 1.15. Big white plastic container with fitted tap.

Appendix D-Figure 1.16. Two large blue plastic containers.

b) Recycled container survey form

Ali Yakubu (aaa@iastate.edu) Carl Bern (cjbern@iastate.edu)
Agricultural and Biosystems Engineering, Iowa State University

Date: 20th October 2011

Conducted by: Anne Mukudi

Email: tinamukudi@yahoo.com

Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store: Owino Market
City: **Kampala-Uganda**

Appendix D: Table 1.5- Market survey form for the recycled container research.

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Manufactured	200	plastic	20	60,000($21)
2) Manufactured	100	plastic	30	30,000($10)
3) Manufactured	120	plastic	20	50,000($18)
4) Cooking oil	20	plastic	100	8,000($3)
5) Cooking oil	5	plastic	100	2,000($7C)
6) Cooking oil	10	Plastic	100	4,000($2)
7) Paint	100	Metal	5	60,000($21)
8) Manufactured	65	Plastic	10	30,000($10)

Description of the market:

The market is within the city with other items being sold, like clothes both new and second hand, food stuff, new and second hand shoes.

It is the busiest market within the city with different people involved in the different business. The 5- 20Ltrs containers are sold after being cleaned and sold by the shopkeepers. The other containers are bought from the manufacturers within the country crest company while others import from Kenya and India.

Comments:

These containers are sold by shopkeepers who aim at earning a living. They get containers from Uganda companies, import from Kenya. Others are after using cooking oil manufactured in the country, the black plastic containers are manufactured in Uganda and their quality is far better than the Kenyan manufactured plastic containers. They display the containers outside the shops or put them on the shelf. Most of the people buy the containers for storing water, alcohol (Waragi), and use as taps in hotels, most of the buyers get them for home use not commercial. Some people use them for harvesting rain water.

Storage container pictures

Containers sold at “Owino Market” are similar to those sold at “Namanve Market”, therefore pictures have been omitted, here.

c) Recycled container survey form

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Date: 17th October 2011

Conducted by: Anne Mukudi

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Purpose of survey: to identify containers available in African markets that can be recycled and used for maize storage by subsistence farmers. ***Containers should be at least 5 L in capacity and airtight (no holes).***

(1)Please visit at least 3 markets (2) Please, attach extra sheets of paper as necessary (3)Please attach or email pictures, where possible..

Market or Store: Jua Kali Drum Dealers- Behind Total Petroleum Station near Soko Mujinga Market it is an open market

City: Kitale

Appendix D: Table 1.6: Market survey form for the recycled container research.

Previous/intended use (edible oil, soft drink concentrate, etc.)	**Volume (L)**	**Material (plastic, steel, etc.)**	**How many in the market**	**Price/unit**
1) Engine Oil	20Ltrs	Plastic	50	250=/($3)
2) Glucose	200Ltrs	Plastic	50	2,000=/($21)
3) Engine Oil	200Ltrs	Metal	300	1,800=/($19)
4) Thinner (Solvent)	240Ltrs	Plastic	50	2,500=/($26)
5) Wine	250Ltrs	Plastic	20	2,700=/($28))

Appendix D: Table 1.6: Market survey form for the recycled container research (continued).

Previous/intended use (edible oil, soft drink concentrate, etc.)	Volume (L)	Material (plastic, steel, etc.)	How many in the market	Price/unit
6) Paints	250Ltrs	Plastic	10	3,500=/($37)
7) Grease	200Ltrs	Metal Drums	50	2,000=/($21)
8) Cooking Oil	5Ltrs	Plastic	10,000	40=/($4C)
9) Cooking Oil	10Ltrs	Plastic	8,000	120=/($1)
10) Cooking Oil	20Ltrs	Plastic	20,000	200=/($2)

Description

Jua-kali open market is situated behind Total gas/petrol station. There are many activities going such as sell Metal boxes, jikos, karayas, metal and plastic containers opposite Jua-Kali sellers', other traders sell fruits and second hand clothes.

Comments

Kitale town is a cosmopolitan town which boarders three major countries Uganda, Sudan and Kenya. There are approximately 8 stalls which sell the containers in the market. The business is very profitable because most of the buyers are farmers and business men from the rural who own their own farms, they come to Kitale town to buy the containers and sell to the locals who buy the plastic containers and sale to small town like Kapengiria, Tongaren, Kwanza, Kiminini and Maili Nane.

The farmers buy the big plastic containers for irrigation or Zero grazing use, 20litres plastic containers are mostly used to transport milk to sell in town some people use

for water storage; a few use it to store fuel to sell in rural communities. The majority of their buyers come from Sudan and Uganda who buy big containers like 250litres and above plastic and metal which they say is very marketable. The traders get their supplies from Mombasa, Nairobi and Kisumu, Thus when selling they have to add transportation costs.

Appendix D-Figure 1.17. Plastic and metal containers (different sizes).

Appendix D-Figure 1.18. Stack of yellow, red, and white plastic containers.

Appendix D-Figure 1.19. A set of blue plastic containers .

Appendix D-Figure 1.20. Stacks of assorted metal and plastic containers.

Appendix D: Table 1.7: Market survey results analysis **f**or the recycled container research

Market	**Number of hermetic containers**
Market 1	3381
Market 2	8318
Market 3	43823
Total containers identified	**55522**

	Percent of total
Edible oil containers identified in all 3 countries	76.29
Metal drums identified in 3 countries	0.45
plastic containers identified in 3 countries	75.84

Edible oil containers identified in all 3 countries	**Number**	**Percent of total**
1) **Metal drums**	250	0.59
2) **Plastic containers**	42108	99.41
Total edible oil containers identified	**42358**	

APPENDIX E: SAS CODES

All research planning and randomization were done using “proc plan (SAS Institute Inc.,100 SAS Campus Drive, Cary, NC)”. However, only the SAS code utilized for research analysis are presented below:

E.1 SEED GERMINATION RESEARCH ANALYSIS

```
DM 'LOG;CLEAR;OUTPUT;CLEAR;';/*USE*/
OPTIONS FORMDLIM='-' NOCENTER NONUMBER NODATE; TITLE;
DATA a;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION
RESEARCH\GERMINATION-ALL.TXT';
INPUT jar level rep trt sample subsample germ herm$ weevil$ pday gshelf gcart
month tray; RUN; /*proc print data=a ;run;*/
/
*
title' FULL GERMINATION DATA';
data a;    infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION
RESEARCH\GERMINATION-ALL.TXT';
 INPUT jar    level   rep    trt      sample        subsample    germ herm$  weevil$
pday   gshelf
gcart   month tray; RUN; title 'Original values';proc sort; data=a;by  trt rep;proc print
data=a NOOBS;run; */
proc glm data=a;class jar   level   rep    trt      sample        subsample    germ
herm   weevil  pday  gshelf gcart   month tray; model germ=rep trt    rep*trt
```

```
rep*sample(trt); lsmeans trt; run;quit;
proc sort data=a; by month;  run; proc glm data=a; by month;
class jar      level  rep    trt      sample       subsample   germ herm    weevil;
model germ= rep trt  rep*trt  rep*sample(trt); lsmeans trt/stderr; MEANS trt;
run;quit; PROC SORT data=a; BY month trt; run;
PROC MEANS mean std n; BY month trt;VAR germ; by month trt;run;
proc glm data=a; class rep trt      germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month herm*weevil herm*month weevil*month
herm*weevil*month; lsmeans trt; run;quit;
title 'Percent germination values'; data percentg; set a; germ=germ*100; run;
proc sort; data=percentg; by rep trt;run;/*proc print data=percentg;run;*/
title 'General trt contrasts/GOOD'; proc glm data=percentg;
class jar      level  rep    trt      sample       subsample   germ herm    weevil;
model germ= rep trt  rep*trt  rep*sample(trt); random rep trt rep*trt
rep*sample(trt)/test; test h=trt e=rep*trt; lsmeans trt/stderr; lsmeans rep*trt;
 * trt 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16;
contrast ' trts avg: control (T5,T6, T7, T8) vs other (T1, T2,T3,T4, T9, T10, T11, T12,
T13, T14, T15, T16)/3' trt -1 -1 -1 -1 3 3 3 3 -1 -1 -1 -1 -1 -1 -1 -1; run;quit;
title 'XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX'; proc glm data=percentg;
class rep      trt      germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month herm*weevil herm*month weevil*month
herm*weevil*month; random herm*weevil herm*month weevil*month
herm*weevil*month/test; run;quit;
```

```
title 'TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT'; proc glm data=percentg;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month herm*weevil herm*month weevil*month rep*trt
herm*weevil*month;
random herm weevil month herm*weevil herm*month weevil*month rep*trt
herm*weevil*month/test; test h=herm e=rep*trt; lsmeans rep*trt; run;quit;
title 'BY MONTHS'; PROC SORT data=percentg; BY trt; run;
PROC MEANS mean std n; BY trt;VAR germ; by trt;run;
proc sort data=percentg; by month; run; proc glm data=percentg; by month;
class jar       level   rep     trt sample      subsample germ herm       weevil;
model germ= rep trt  rep*trt  rep*sample(trt); lsmeans trt/stderr; run;quit;
PROC SORT data=percentg; BY month trt; run;PROC MEANS mean std n;
BY month trt;
VAR germ; by month trt;run; title 'trt contrasts'; proc sort data=percentg;by month;
 run;  proc glm data=percentg; by month; where month=0;
class jar       level   rep     trt      sample         subsample    germ herm    weevil;
model germ= rep trt  rep*trt  rep*sample(trt);
random rep trt        rep*trt  rep*sample(trt)/test; test h=trt e=rep*trt; lsmeans
trt/stderr;
                                        * trt 1 5 9 13;
contrast 'month zero trts: control (T6) vs other (T1, T9,T13)/3:trt -1 3 -1 -1;
run;quit; proc glm data=percentg; by month; where month=4;
class jar       level   rep     trt      sample         subsample    germ herm    weevil;
```

```
model germ= rep trt rep*trt  rep*sample(trt);
random rep trt        rep*trt  rep*sample(trt)/test; test h=trt e=rep*trt; lsmeans
trt/stderr;
                                        * trt 2 6 10 14;
contrast 'month zero trts :control vs other:- p 24-6;88' trt  -1 3 -1 -1; run;quit;
proc glm data=percentg; by month; where month=8;
class jar      level  rep    trt      sample        subsample   germ herm    weevil;
model germ= rep trt rep*trt  rep*sample(trt); random rep trt    rep*trt
rep*sample(trt)/test; test h=trt e=rep*trt; lsmeans trt/stderr;
                                        * trt 3 7 11 15;
contrast 'month zero trts: control vs other:- p 24-6;88' trt -1 3 -1 -1; run;quit;
proc glm data=percentg; by month; where month=12;
class jar      level  rep    trt      sample        subsample   germ herm    weevil;
model germ= rep trt rep*trt  rep*sample(trt); random rep trt    rep*trt
rep*sample(trt)/test; test h=trt e=rep*trt;  lsmeans trt/stderr;
                                        * trt 4 8 12 16;
contrast 'month zero trts: control vs other:- p 24-6;88' trt -1 3 -1 -1; run;quit;
title 'estimating hermetic trt difference: p 113-117'; proc sort data=percentg;
by weevil; run; proc glm data=percentg;by weevil  ; class  herm;
model germ=  herm; estimate 'herm vs nh' herm 1 -1; lsmeans herm/stderr;
run;quit;
PROC SORT data=percentg; BY weevil herm; run;
PROC MEANS mean std n; BY weevil herm;VAR germ; by weevil herm;run;
title 'germination shelf and germination cart analysis'; proc sort data=percentg;
```

```
by gshelf gcart; proc glm data=percentg; class gshelf gcart;
model germ=gshelf gcart gshelf(gshelf);
contrast 'gcart1 vs gcart2' gcart 1 -1; lsmeans gshelf gcart gshelf(gshelf);run;quit;
title 'herm*month interaction:PLOTTING VALUES: '; proc sort data=percentg;
by herm  month;
proc glm data=percentg; class germ herm  month; model germ=herm  month
herm*month ; lsmeans herm herm*month; run;
title 'herm or non-hermetic:PLOTTING VALUES: '; PROC SORT data=percentg;
BY herm month; run; PROC MEANS mean std n; BY herm  month;VAR germ;
by herm  month;run;
title 'weevil or no weevil:PLOTTING VALUES: '; PROC SORT data=percentg;
BY weevil month; run;
PROC MEANS mean std n; BY weevil month;VAR germ; by weevil month;run;
title 'INTERACTIONS:PLOTTING '; proc glm data=percentg;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month herm*weevil herm*month weevil*month
herm*weevil*month;
lsmeans herm*weevil herm*month weevil*month/slice=month; lsmeans
herm*weevil*month;run;quit;
TITLE 'SLOPE ANALYSIS' title 'Regression (trts): treatments 1-4';
DATA l; input   trt   germ; datalines;
    1   99.1666667
```

```
    2   98.7500000
    3   99.6666667
    4   99.3333333
proc sort data=l; BY trt; proc reg data=l; model germ=trt /COVB; run; quit;
title 'Regression (trts): treatments 5-6'; DATA m; input   trt   germ;  datalines;
    5   99.2500000
    6   72.3333333
    7   72.9166667
    8   35.0833333

proc sort data=m; BY trt; proc reg data=m; model germ=        trt /COVB; run; quit;
title 'Regression (trts): treatments 9-12'; DATA n; input   trt   germ; datalines;
9       99.1666667
10      99.0000000
11      99.0833333
12      99.5000000
proc sort data=n; BY trt; proc reg data=n; model germ=         trt /COVB; run; quit;
 title 'Regression (trts): treatments 13-16'; DATA o; input   trt   germ; datalines;
13      99.3333333
14      99.2500000
15      99.0833333
16      99.1666667
proc sort data=o; BY trt; proc reg data=o; model germ=trt /COVB; run; quit;
title 'Regression (herm*month):  hermetic'; DATA j; input   herm    month     germ;
```

```
datalines;

H       0          99.1666667

H       4          98.8750000

H       8          99.3750000
H       12          99.4166667

proc sort data=j; BY month; proc reg data=j; model germ=      month /COVB; run;

quit;

title 'Regression (herm*month):  nonhermetic';  DATA k; input   herm    month

germ; datalines;

NH      0          99.2916667

NH      4          85.7916667

NH      8          86.0000000

NH      12          67.1250000

proc sort data=k; BY month; proc reg data=k; model germ=        month /COVB; run;

quit;

title 'Regression (weevil*month):  no weevils'; DATA h; input   weevil    month

germ; datalines;

F       0          99.2500000

F       4          99.1250000

F       8          99.0833333

F       12          99.3333333

proc sort data=h; BY month; proc reg data=h; model germ= month /COVB; run; quit;

title 'Regression (weevil*month):  with weevils'; DATA i; input   weevil    month
```

```
germ; datalines;

T        0         99.2083333

T        4         85.5416667

T        8         86.2916667
T        12         67.2083333

proc sort data=i; BY month; proc reg data=i; model germ=  month /COVB;run; quit;

title 'Regression (Herm*weevil*month): hermetic with no weevils'; DATA d; input

herm    weevil ;

month     germ; datalines;

H       F        0         99.1666667

H       F        4         99.0000000

H       F        8         99.0833333

H       F        12         99.5000000

proc sort data=d; BY month; proc reg data=d; model germ=month /COVB; run; quit;

title 'Regression(Herm*weevil*month):hermetic with weevils'; DATA e;

input herm    weevil    month     germ;  datalines;

H       T        0         99.1666667

H       T        4         98.7500000

H       T        8         99.6666667

H       T        12         99.3333333

proc sort data=e; BY month; proc reg data=e; model germ=  month /COVB; run; quit;

title 'Regression(Herm*weevil*month): nonhermetic with no weevils'; DATA f;

input herm    weevil    month     germ; datalines;
```

```
NH      F       0       99.3333333
NH      F       4       99.2500000
NH      F       8       99.0833333
NH      F       12       99.1666667
proc sort data=f; BY month; proc reg data=f; model germ= month /COVB; run; quit;
title 'Regression(Herm*weevil*month): Nonhermetic with weevils'; DATA g;
input herm    weevil    month     germ; datalines;
NH      T       0       99.2500000
NH      T       4       72.3333333
NH      T       8       72.9166667
NH      T       12       35.0833333
proc sort data=g; BY month; proc reg data=g; model germ= month /COVB; run; quit;
title 'WEEVILS/NONHERMETIC'; data a1;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\WEEVILS-
NH.TXT';
INPUT jar level rep trt sample subsample germ herm$ weevil$ pday gshelf gcart
month tray;
RUN; /*DATA aa(DROP=Obs);SET a1;RUN; */ proc sort data=a1; by trt ; proc print;
run;/* data=a1 NOOBS; */ proc glm data=a1
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month  herm*weevil*month; /*rep trt         rep*trt
rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevi*month/slice=month;
```

```
run;quit; proc glm data=a1; class rep       trt       germ herm    weevil  pday  gshelf
gcart   month;
model germ= herm*weevil*month; /*rep trt         rep*trt  rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month;
contrast 'month: 0 month vs 4, 8, 12th month' herm*weevil*month 3 -1 -1 -1;
estimate 'month: 0 month vs 4, 8, 12th month' herm*weevil*month  3 -1 -1 –
1/divisor=3 ;
contrast 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
estimate 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
contrast 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
estimate 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
contrast 'month: 0 month vs 12th month' herm*weevil*month 1 0  0 -1;
estimate 'month: 0 month vs 12th month' herm*weevil*month 1 0 0 -1 ;
contrast 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
estimate 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
contrast 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1;
estimate 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1 ;
contrast 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
estimate 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
run;quit;
title 'WEEVILS/HERMETIC'; data a2;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION
RESEARCH\WEEVILS-H.TXT';
```

```
INPUT jar level rep trt sample subsample germ herm$ weevil$ pday gshelf gcart
month tray;RUN;
/*DATA aa(DROP=Obs);SET a1;RUN; */ proc sort data=a2; by trt ;  run;/* proc print
data=a2 NOOBS;*/ proc glm data=a2;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month  herm*weevil*month; /*rep trt         rep*trt
rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month/slice=month;
run;quit; proc glm data=a2;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm*weevil*month; /*rep trt         rep*trt  rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month;
contrast 'month: 0 month vs 4, 8, 12th month' herm*weevil*month 3 -1 -1 -1;
estimate 'month: 0 month vs 4, 8, 12th month' herm*weevil*month  3 -1 -1 –
1/divisor=3 ;
contrast 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
estimate 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
contrast 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
estimate 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
contrast 'month: 0 month vs 12th month' herm*weevil*month 1 0  0 -1;
estimate 'month: 0 month vs 12th month' herm*weevil*month 1 0 0 -1 ;
contrast 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
estimate 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
```

```
contrast 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1;
estimate 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1 ;
contrast 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
estimate 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1; run;quit;
title 'NO WEEVILS/HERMETIC'; data a3;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\NOWEEVILS-
H.TXT';
INPUT  jar level rep trt sample subsample germ herm$ weevil$ pday gshelf gcart
month tray; RUN;
proc sort data=a3; by trt ;  run;/* proc print data=a3 NOOBS;*/ proc glm data=a3;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month  herm*weevil*month; /*rep trt         rep*trt
rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month/slice=month; run;quit;
proc glm data=a3; class rep         trt       germ herm    weevil  pday  gshelf gcart
month;model germ=herm*weevil*month; /*rep trt         rep*trt  rep*sample(trt);*/
random  herm*weevil*month/test;  lsmeans herm*weevil*month;
contrast 'month: 0 month vs 4, 8, 12th month' herm*weevil*month 3 -1 -1 -1;
estimate 'month: 0 month vs 4, 8, 12th month' herm*weevil*month  3 -1 -1 –
1/divisor=3 ;
contrast 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
estimate 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
```

```
contrast 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
estimate 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
contrast 'month: 0 month vs 12th month' herm*weevil*month 1 0  0 -1;
estimate 'month: 0 month vs 12th month' herm*weevil*month 1 0 0 -1 ;
contrast 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
estimate 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
contrast 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1;
estimate 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1 ;
contrast 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
estimate 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
run;quit;title 'NO WEEVILS/NON HERMETIC'; data a4;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\NOWEEVILS-
NH.TXT';
INPUT  jar level rep trt sample subsample germ herm$ weevil$ pday gshelf gcart
month tray;
RUN;/* DATA aa(DROP=Obs);SET a2;RUN;*/
proc sort data=a4; by trt ;  run;/*proc print data=a4 NOOBS; */ proc glm data=a4;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
model germ= herm weevil month  herm*weevil*month; /*rep trt         rep*trt
rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month/slice=month; run;quit;
proc glm data=a4;
class rep       trt       germ herm    weevil  pday  gshelf gcart   month;
```

```
model germ= herm*weevil*month; /*rep trt          rep*trt  rep*sample(trt);*/
random  herm*weevil*month/test; lsmeans herm*weevil*month;
contrast 'month: 0 month vs 4, 8, 12th month' herm*weevil*month 3 -1 -1 -1;
estimate 'month: 0 month vs 4, 8, 12th month' herm*weevil*month 3 -1 -1 –
1/divisor=3 ;
contrast 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
estimate 'month: 0 month vs 4th month' herm*weevil*month 1 -1 0 0;
contrast 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
estimate 'month: 0 month vs 8th month' herm*weevil*month 1 0 -1 0;
contrast 'month: 0 month vs 12th month' herm*weevil*month 1 0  0 -1;
estimate 'month: 0 month vs 12th month' herm*weevil*month 1 0 0 -1 ;
contrast 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
estimate 'month: 4 month vs 8th month' herm*weevil*month  0 1 -1 0;
contrast 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1;
estimate 'month: 4 month vs 12th month' herm*weevil*month  0 1 0 -1 ;
contrast 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1;
estimate 'month: 8 month vs 12th month' herm*weevil*month  0 0 1 -1; run;quit;
```

E.2 PREDICTED TIME TO COMPLETE ADULT WEEVIL MORTALITY RESEARCH ANALYSIS

```
DM 'LOG;CLEAR;OUTPUT;CLEAR;';/*USE*/
TITLE 'FINAL ANALYSIS'; DATA PTCM;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\PTCM.TXT';
```

```
INPUT Obs Run day trt num herm$ block level; RUN; /*proc print data=PTCM ;run;*/
PROC SORT data= PTCM;BY herm;  RUN;  /*proc print data=PTCM NOOBS; run;
QUIT;*/
PROC GLM DATA=PTCM; CLASS Run day trt num herm block level;
MODEL num=day herm day*herm;  run; quit; PROC GLM DATA=PTCM;
CLASS Run day trt num herm  block level; MODEL num=trt level  trt*level
level(block); run; quit;
PROC GLM DATA=PTCM; CLASS Run day trt num herm  block level;
MODEL num=trt level  trt*level level(block) trt*level(block);
lsmeans trt*level level(block) trt*level(block); run; quit; PROC GLM DATA=PTCM;
CLASS Run day trt num herm block level; MODEL num=day herm day*herm;
 lsmeans herm day*herm; run; quit;
PROC GLM DATA=PTCM; CLASS Run day trt num herm block level;
MODEL num=trt level trt*level level (block) trt*level(block) ;
lsmeans level level(block) trt*level;
run; quit; PROC GLM DATA=PTCM; CLASS Run day trt num herm block level;
MODEL num=trt level trt*level block(level) trt*block(level);run; quit;
PROC GLM DATA=PTCM; CLASS Run day trt herm block level;
MODEL num=trt level trt*level block(level) trt*block(level)/ss3;
RANDOM  block(level) trt*block(level); run; quit;
PROC MIXED DATA=PTCM method=type3; CLASS Run day trt herm block level;
MODEL num=trt level trt*level; RANDOM  block(level) trt*block(level); run; quit;
PROC GLM DATA=PTCM; CLASS herm day;MODEL num=day herm herm*day;
```

```
LSMEANS herm*day/stderr;  RUN; QUIT;
proc sort data=PTCM; by herm; PROC GLM DATA=PTCM;
CLASS herm; MODEL num=herm; MEANS herm/hovtest; contrast 'herm' herm 1 -1;
estimate 'Herm vs. Non-herm' herm  1 -1 ; run;quit; proc sort data=PTCM; by trt;
PROC GLM DATA=PTCM; CLASS trt; MODEL num=trt; MEANS trt/hovtest;
contrast 'treatments' trt 1 1 1 -1 -1 -1; estimate 'Herm vs. Non-herm' trt 1 1 1 -1 -1 -1
;run;quit;
DATA a;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\DATAa.TXT';
INPUT Obs Run day trt num herm$ block level; RUN; /*proc print data=a ;run;*/
PROC GLM DATA=a; CLASS Run day trt num herm block level;
MODEL num=trt; contrast 'Hermetic treatment contrasts' trt 3 -1.5 -1.5; run; quit;
proc sort; by trt; DATA b;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\DATAb.TXT';
INPUT Obs Run day trt num herm$ block level; RUN; /*proc print data=b ;run;*/
proc sort; by trt; PROC GLM DATA=b; CLASS Run day trt num herm block level;
MODEL num=trt; contrast 'Non hermetic treatment contrasts' trt 3 -1.5 -1.5;
run; quit;
Title 'LEVEL EFFECTS';
proc sort data=PTCM; by level;run; PROC GLM DATA=PTCM; CLASS Run day trt
num herm block level;
MODEL num=level; contrast 'Non hermetic treatment contrasts' level  1 0.5 0.5;run;
quit;
```

```
Title '20-L CONTAINER ANALYSIS'; DATA jcan;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION RESEARCH\jcan.TXT';
INPUT label type$ pcent; RUN; PROC GLM DATA=jcan; CLASS type pcent;
MODEL pcent=type;
lsmeans type; run; quit;
proc sort data=jcan; by type; proc print data=jcan; run; PROC GLM DATA=jcan;
CLASS type;
MODEL pcent=type; MEANS type/hovtest; contrast 'treatments' type  1 -1;
estimate 'Herm vs. Non-herm' type 1 -1 ; run;quit;
TITLE 'NON HERMETIC'; DATA NONHERMETIC;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION
RESEARCH\GERMINATION-ALL.TXT';
INPUT Obs Run day trt num herm$ block level; RUN; /*proc print data=
NONHERMETIC ;run;*/
PROC SORT DATA=NONHERMETIC; BY day; RUN;/*PROC PRINT NOOBS
DATA=NONHERMETIC; RUN;*/
PROC GLM DATA=NONHERMETIC; CLASS Run day trt num herm block level;
MODEL num= day*herm;
lsmeans day*herm;  contrast '17 days vs 21 and 26 days' day*herm 2 -1 -1;
estimate '17 days vs 21 and 26 days' day*herm 2 -1 -1/divisor=2;
contrast '17 days vs 21 days' day*herm 1 -1 0; estimate '17 days vs 21 days'
day*herm 1 -1 0;
contrast '17 days vs 26 days' day*herm 1 0 -1; estimate '17 days vs 26 days'
```

```
day*herm 1 0 -1;
contrast '21 days vs 26 days' day*herm 1 -1 0; estimate '21 days vs 26 days'
day*herm 1 -1 0;run;quit;
TITLE 'HERMETIC'; DATA HERMETIC;
infile '\\iastate.edu\cyfiles\aaa\Desktop\GERMINATION
RESEARCH\GERMINATION-ALL.TXT';
INPUT Obs Run day trt num herm$ block level; RUN; /*proc print data= HERMETIC
;run;*/
PROC SORT DATA=HERMETIC; BY day; RUN;/*PROC PRINT NOOBS
DATA=HERMETIC; RUN;*/
PROC GLM DATA=HERMETIC; CLASS Run day trt num herm block level;
MODEL num= day*herm;   lsmeans day*herm; contrast '17 days vs 21 and 26 days'
day*herm 2 -1 -1;
estimate '17 days vs 21 and 26 days' day*herm 2 -1 -1/divisor=2;
contrast '17 days vs 21 days' day*herm 1 -1 0; estimate '17 days vs 21 days'
day*herm 1 -1 0;
contrast '17 days vs 26 days' day*herm 1 0 -1; estimate '17 days vs 26 days'
day*herm 1 0 -1;
contrast '21 days vs 26 days' day*herm 1 -1 0; estimate '21 days vs 26 days'
day*herm 1 -1 0;
run;quit;
```

E.3 RECYCLED CONTAINERS RESEARCH ANALYSIS

```
DM 'LOG;CLEAR;OUTPUT;CLEAR;';
OPTIONS FORMDLIM='-' NOCENTER NONUMBER NODATE; TITLE;
DATA z; TITLE1 'RECYCLING RESEARCH-FULL RUN';
infile '\\iastate.edu\cyfiles\aaa\Desktop\z.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN;
proc print data=z;run;
PROC GLM DATA=z;CLASS EU wipe rep trt quantity method container;MODEL
quantity = trt; RUN;quit;
PROC GLM data=z; class EU wipe rep trt quantity method container;
model quantity=trt; LSMEANS trt/stderr; means trt;run;
title2 'CONTAINS ALL ERRORS AND MAIN EFFECTS';
PROC GLM data=z; class EU wipe rep trt quantity method container;
model quantity=       wipe rep trt rep*trt wipe*trt wipe*rep*trt;
lsmeans wipe rep trt rep*trt wipe*trt wipe*rep*trt; run;quit;
title2 'TEST OF HYPOTHESIS 2';
PROC GLM data=z;class EU wipe rep trt quantity method container;
model quantity=wipe rep trt rep*trt wipe*trt wipe*rep*trt;
random wipe wipe*trt wipe*rep*trt; test h=rep e=rep*trt;test h=trt e=rep*trt;
test h=wipe e=wipe*rep*trt;test h=wipe*trt e=wipe*rep*trt;run;quit;
title3 'INTERACTIONS ONLY';
PROC GLM data=z;class EU wipe rep trt quantity method container;
model quantity= method container method*container;
```

```
lsmeans method container method*container;
PROC MEANS method*container std n; VAR quantity;run; run;quit;
title4 'INTERACTIONS ONLY-#2';
PROC GLM data=z; class EU wipe rep trt quantity method container;
model quantity=wipe trt wipe*method      wipe*container wipe*method*container;
run;quit;
title5 'INTERACTIONS ONLY-#3';
PROC GLM data=z; class EU wipe rep trt quantity method container;
model quantity= wipe method container wipe*method wipe*container
method*container
wipe*method*container;
lsmeans method container method*container wipe*method wipe*method*container;
MEANS method container method*container wipe*method wipe*method*container;
run;quit;
TITLE6 'CONTRAST: NOOBS AND CONTRAST';
DATA p;   infile '\\iastate.edu\cyfiles\aaa\Desktop\p.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print data=p
;run;*/
proc print data=p;run; proc sort; by trt;
PROC GLM data=p; class EU wipe rep trt quantity method container;
model quantity= trt;lsmeans trt ;
contrast 'trt: Oil vs hot water and soap' trt 1 -0.5 -0.5;
```

```
estimate 'oil vs others' trt  1 -0.5 -0.5;run;quit;
TITLE7 'CONTRASTS NEEDED BELOW';
TITLE8 'CONTRAST: CONTaminated only';
DATA contaminated;
infile '\\iastate.edu\cyfiles\aaa\Desktop\contaminated.TXT';
INPUT EU wipe rep trt quantity method$ container$;RUN;
proc sort data=contaminated; by trt;
PROC GLM data=contaminated;class EU wipe rep trt quantity method container;
model quantity= trt ;lsmeans trt;contrast 'trt: Oil vs hot water and soap' trt 1 -0.5 -0.5;
estimate 'oil vs others' trt  1 -0.5 -0.5 ;run;quit;
TITLE 'CONTRAST: CONTAMINATED-T TEST:HOT WATER VS. SOAP';
DATA J;   infile '\\iastate.edu\cyfiles\aaa\Desktop\J.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print data=J
;run;*/
proc sort data=J; by method; proc print data=J noobs; run;/**/
proc ttest data=J; class oil-drai hot-wate ;var quantity;run;PROC GLM data=J;
class EU wipe rep trt quantity method container;model quantity= trt;lsmeans trt ;
contrast 'trt: Hot water vs soap' trt 1 -1;estimate 'hot water vs soap' trt  1 -1 ;run;quit;
TITLE9 'CONTRAST: UNCONTaminated only';DATA uncontaminated;
infile '\\iastate.edu\cyfiles\aaa\Desktop\uncontaminated.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print
data=uncontaminated ;run;*/
proc sort data=uncontaminated; by trt; PROC GLM data=uncontaminated;
```

```
class EU wipe rep trt quantity method container;
model quantity=  trt ; lsmeans trt;contrast 'trt: Oil vs hot water and soap' trt 1 -0.5 –
0.5; estimate 'oil vs others' trt  1 -0.5 -0.5 ;run;quit;
TITLE10 'CONTRAST: CONTAMINATED-T TEST:OIL VS. HOT WATER ';DATA Q;
 infile '\\iastate.edu\cyfiles\aaa\Desktop\Q.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print data=Q
;run;*/ proc sort data=Q; by trt;
proc ttest data=Q; class oil-drai hot-wate ;var quantity;run;PROC GLM data=Q;
class EU wipe rep trt quantity method container;model quantity= trt;lsmeans trt ;
contrast 'trt: Oil vs hot water' trt 1 -1;estimate 'oil vs hot water' trt  1 -1;
run;quit;
TITLE1 'CONTRAST: CONTAMINATED-T TEST:OIL VS. SOAP ';
DATA R;   infile '\\iastate.edu\cyfiles\aaa\Desktop\R.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print data=R
;run;*/
proc sort data=R; by trt; proc ttest data=R; class oil-drai soap; var quantity;run;
PROC GLM data=R;class EU wipe rep trt quantity method container;model
quantity= trt;lsmeans trt ;
contrast 'trt: Oil vs soap' trt 1 -1;estimate 'oil vs soap' trt  1 -1 ;run;quit;
TITLE2 'CONTRAST: UNCONTAMINATED-T TEST:OIL VS. HOT WATER ';
DATA S;   infile '\\iastate.edu\cyfiles\aaa\Desktop\S.TXT';
INPUT EU wipe rep trt       quantity method$ container$;RUN; /*proc print data=S
;run;*/
```

proc sort data=S; by trt; **proc ttest** data=S; class oil-drai hot-wate ;var quantity;**run**;

PROC GLM data=S;class EU wipe rep trt quantity method container;

model quantity= trt;lsmeans trt ;contrast 'trt: Oil vs hot water' trt **1 1**;

estimate 'oil vs hot water' trt **1** -**1** ;**run**;**quit**;

TITLE3 'CONTRAST: UNCONTAMINATED-T TEST:OIL VS. SOAP ';

DATA T; infile '\\iastate.edu\cyfiles\aaa\Desktop\T.TXT';

INPUT EU wipe rep trt quantity method$ container$;RUN; /*proc print data=T ;run;*/

proc sort data=T; by trt; **proc print** noobs data=T; **run**;

proc ttest data=T; class oil-drai hot-wate ;var quantity;**run**;**PROC GLM** data=T;

class EU wipe rep trt quantity method container;model quantity= trt; lsmeans trt ;

contrast 'trt: Oil vs soap' trt **1** -**1**;estimate 'oil vs soap' trt **1** -**1** ;**run**;**quit**;

TITLE 'CONTRAST: UNCONTAMINATED-T TEST:HOT WATER VS. SOAP';

DATA L; infile '\\iastate.edu\cyfiles\aaa\Desktop\L.TXT';

INPUT EU wipe rep trt quantity method$ container$;RUN; /*proc print data=L

;run;*/ **proc sort** data=L; by method; **proc print** data=L noobs; **run**;

proc ttest data=L; class oil-drai hot-wate ;

var quantity;**run**; **PROC GLM** data=L;class EU wipe rep trt quantity method

container;model quantity= trt; lsmeans trt ;contrast 'trt: Hot water vs soap' trt **1** –**1**;

estimate 'hot water vs soap' trt **1** -**1** ; **run**;**quit**;

DATA methodbycontainer; INPUT method$ quantity; DATALINES;

hot-wate 0.14202222

oil-drai 0.24949444

```
soap                    0.00385000
;RUN;
proc sort data=methodbycontainer; by method;
TITLE1 'CONTAMINATED: method by container';
PROC GLM data=methodbycontainer; class method  quantity;model quantity=
method;
contrast 'mean: oil vs Hot water' method 1 -1 0; estimate 'oil vs Hot water' method  1
-1 0;
contrast 'mean: oil vs soap' method 1 0 -1;estimate 'oil vs soap' method  1 0 -1;
contrast 'mean:  Hot water vs. soap' method 0 1 -1;estimate 'Hot water vs soap'
method  0 1 -1;run;
DATA methodbycontainer1;INPUT method$ quantity; DATALINES;
hot-wate                0.00595000
oil-drai                0.00523333
soap                    0.00564444
;RUN;
proc sort data=methodbycontainer1; by method; TITLE1 'UNCONTAMINATED:
method by container';
PROC GLM data=methodbycontainer1;class method  quantity;model quantity=
method;
contrast 'mean: oil vs Hot water' method 1 -1 0;estimate 'oil vs Hot water' method  1
 -1 0;
contrast 'mean: oil vs soap' method 1 0 -1;estimate 'oil vs soap' method  1 0 -1;
```

```
contrast 'mean:  Hot water vs. soap' method 0 1 -1;estimate 'Hot water vs soap'
method 0 1 -1;
run;
```