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Non-chemical on-farm hermetic maize storage in East Africa

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By

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ABSTRACT

A primary problem of agricultural practice, food sufficiency, and the associated economic security for several subsistence farmers and dependent population is the lack of adequate and affordable grain storage equipments. Most previous approach to maize preservation relied on the use of chemicals. However, this research attempted to define general optimum storage conditions under different temperature, moisture and time conditions, while using a non-chemical (hermetic) approach for maize preservation.

Two studies were conducted to test the efficacy of hermetic storage system in controlling oxygen supply and maize weevil population and to test the effect of maize moisture and temperature on weevil mortality. A system was designed for the first experiment to monitor the percentage weevil mortality under hermetic conditions, over time, in both low and high moistures as well as temperature combinations. The treatment jars containing maize at two moistures (6.3 and 16%) and weevils were randomly assigned to two temperature chambers (10 and 27^{O}C). The second experiment utilized oxygen sensors, a microcontroller and a computer running a Visual Basic 6.0 program to monitor the oxygen concentration within jars containing maize (at 8 and 16% moisture) and weevils, exposed to the two temperature chambers. Together, the two studies applied direct and indirect methods of weevil quantification.

Experiment one's design consisted of four factorials (time, maize moisture, temperature, and replication), with weevil mortality being the dependent variable. Days had five levels (2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 10^{th}), maize moisture had two levels (6.3% and 16%), temperature had two levels (10^{O}C and 27^{O}C), and replications had four levels.

Experiment two consisted of hermetic canning jars into which ninety weevils and about 185 g of maize, at the appropriate moisture levels were loaded. The jars were randomly assigned to the temperature chambers and connected to the data acquisition system, consisting of a computer and microcontroller used for the graphic user interface (GUI) and data acquisition.

The results indicate highly significant hermetic, temperature and moisture effects on weevil mortality, and also indicate the efficacy of hermetic storage under the conditions tested.

CHAPTER 1

GENERAL INTRODUCTION

Thesis Organization

The information presented in this thesis is organized into three chapters. The first chapter is the general introduction, with sections on the thesis organization, objectives, and literature review. The second chapter contains a paper entitled "Non chemical hermetic weevil control for on-farm maize storage in East Africa." And the third chapter is the "General conclusions" chapter, based on the information contained in chapter two, and answering objectives from chapter 1.

Chapter two was prepared for publication in the African Journal of Agricultural Research, and is formatted in accordance with the guidelines for papers submitted to that journal for publication.

Literature Review

The maize Plant

Maize (*Zea mays L. ssp. mays*) or corn (Figure 1.1a and Figure 1.1b) is a monoic annual plant belonging to the maideas tribe and the grass family of *gramineae* (*Poaceae*), with cells having 2n chromosomes (Mejía, 2008).

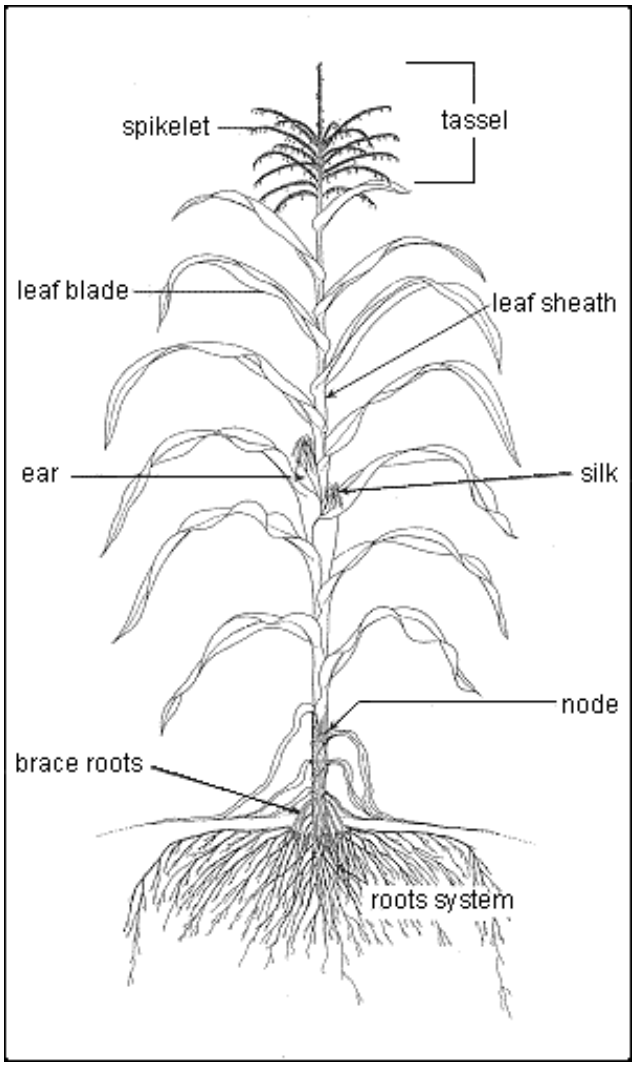

Figure 1.1a. The maize plant (IITA, 2007a)

Figure 1.1b. Maize (IITA, 2007b)

This is a tall, annual grass with overlapping sheaths and broad conspicuously distichous blades, as well as staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels). It also has pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971; CFIA, 2006).

The pollen is produced in the staminate inflorescence and the eggs are produced in the pistillate inflorescence (Figure 1.1c). Maize is normally wind pollinated, although both self and cross pollination are possible. The shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe *et al.*, 1988).

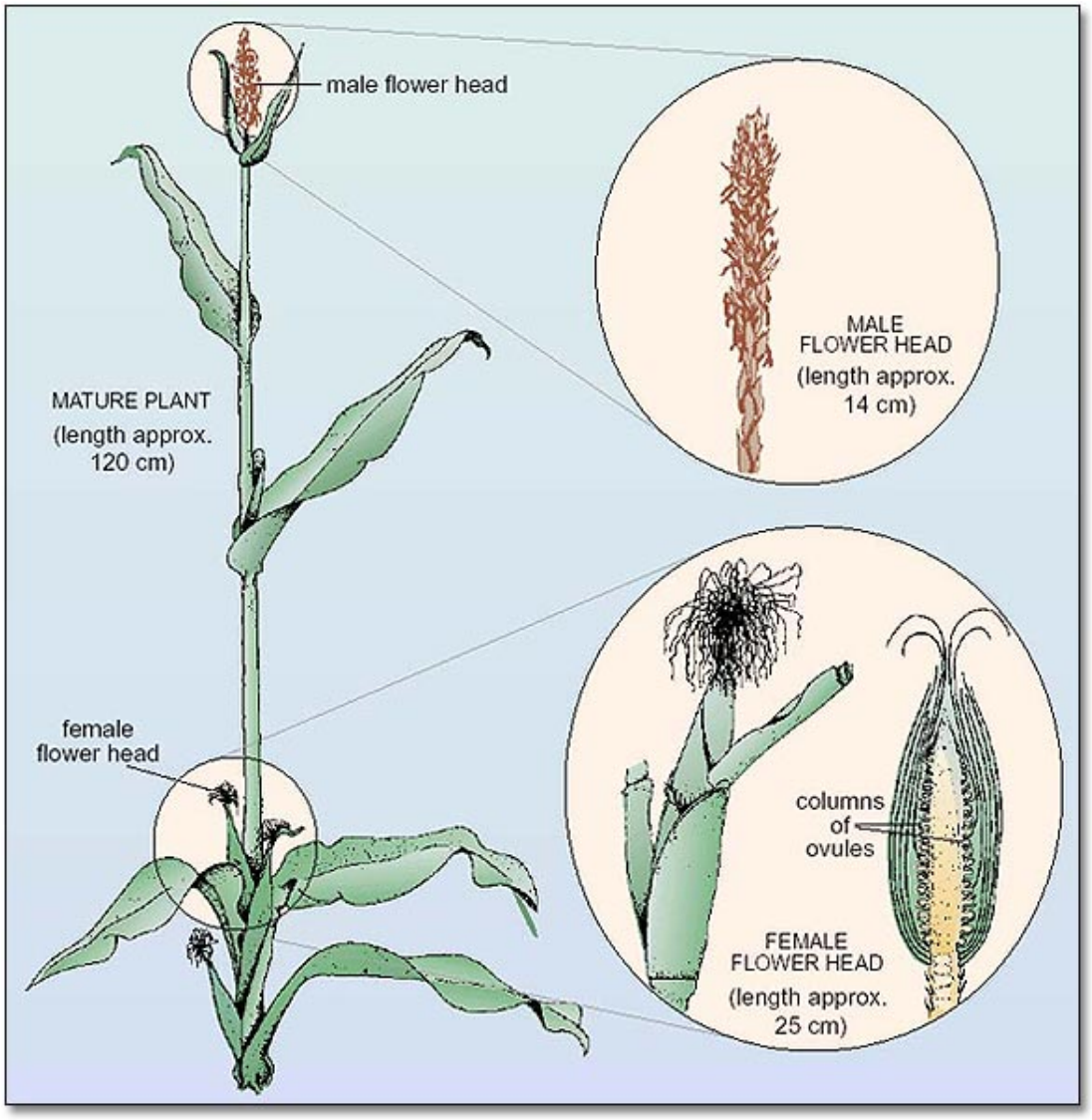

Figure 1.1c. The arrangement and structure of male and female flowers on a maize plant (Openlearn, 2008)

Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population. It has been cultivated by the indigenous peoples of North America for thousands of years, and is planted when soil temperatures are warm (greater than or equal to 10°C) (MAPAQ, 1984).

Maize Genetics

Genetic diversity exists in the domestic strains selectively bred for food. Common subspecies include Flour corn (*Zea mays var. amylacea*), Popcorn (*Zea mays var. everta),* Dent corn (*Zea mays var.; indentata)*, Flint corn (*Zea mays var. indurate)*, Sweet corn (*Zea mays var. saccharata* and *Zea mays var. rugosa),* Waxy corn (*Zea mays var. certain)*; Amylomaize (*Zea mays)*, Pod corn (*Zea mays var. tunicata Larrañaga ex A. St. Hil.)*, Striped maize (*Zea mays var. japonica*). Mejia (2008) also classified maize kernels into pop, flint, dent, floury, and sweet maize, based on endosperm characteristics and food uses.

Origin

Maize is a cereal grain derived from a direct domestication of a Mexican annual teosinte (Sanchez, et. al, 1998; Wilkes, 1967; Doebley, 1990; 2004). It spread throughout the American continents, and to the rest of the world, following European contact with the Americas, in the late 15th and early 16th century (Beadle, 1939; 1978; 1980).

Socio Economic Importance of Maize

It is widely cultivated throughout the world. Maize production (600 million Mg) exceeded rice or wheat production in 2003, and about 33 million ha of maize with a production value of more than $23 billion was planted worldwide, in 2004 (FAOSTAT, 2009).

Maize is the most important cereal crop in sub-Saharan Africa. It is high yielding, easy to process, readily digested, cheaper than other cereals and grows across a wide range of agroecological zones. Besides, every part of the maize plant has economic value-the grain, leaves, stalk, tassel, and cob can all be used to produce a large variety of food and non-food products (IITA, 2007b).

World Production

It is the most widely grown crop in the Americas, with the United States having a production of about 270 million Mg annually, and accounting for almost half of the world's harvest. Other top producers include China, Brazil, France, Indonesia, India and South Africa. It is also a major export crop (Table 1.1), and the USDA world maize production estimate for the 2007/08 harvest season is 774 million Mg (HGCA, 2008).

Maize in general is used in more ways than any other cereal. White maize in particular is preferred in developing countries as human food due to its organoleptic (Sonowola, 2001) properties. In contrast, yellow maize is used in developed countries for feeding livestock and poultry. The yellow maize is desirable, for

instance, in increasing the yellow color characteristic of the egg yolk (FAO/CIMMYT, 1997).

Table 1.1: 2004 maize export statistics by country sorted by value (FAO, 2004)

Country	Quantity (00000 Mg)	Value (00000000 US$)
United States of America	487	61.4
Argentina	107	11.9
France	61.6	14.6
Brazil	50.3	5.97
Hungary	12.4	2.75
Ukraine	12.3	1.69
India	10.7	1.56
Thailand	9.51	1.40
Germany	9.47	2.24
United Arab Emirates	4.91	0.86
South Africa	4.50	1.13
Canada	3.48	0.61
Romania	3.11	0.43
Lebanon	2.91	0.49
Austria	2.54	1.16
Belgium	2.32	0.54
Italy	1.88	0.45
Spain	1.84	0.60
Netherlands	0.82	0.57
Chile	0.63	0.71

Table 1.2 reflects volume of maize production by East African countries:

Table 1.2 : 2006-2007 maize production statistics by country sorted by value (FAOSTAT, 2009)

Country	2006	2007
	Quantity (0000 Mg)	
Ethiopia	403	400
Tanzania	337	340
Kenya	325	324
Uganda	126	126
Burundi	11.2	11.5
Somalia	9.7	9.9
Rwanda	9.2	9.0
Sudan	7.0	6.0
Eritrea	0.3	0.3
Djibouti	0.01	0.01

Maize Utilization

Maize and maize flour (cornmeal), in the form of oje, nshima, ugali, mealie pap, atole, etc are a staple food around the world. Again, popcorn is a common snack, while corn flakes, hominy, grits, and canjica are common breakfast foods. It comprises an average of 30 to 50% of the daily caloric intake of people in most southern African countries (FAO, 2001), and is a major staple food in East Africa, where per capita human consumption exceed industrial uses (Aquino *et al*., 2000; FAO/CIMMYT, 1997; IITA, 2007b).

Maize is a significant source of starch, and a feedstock for the production of corn oil, gluten, high fructose corn syrup, grain alcohol, and biofuels. It is also consumed as a vegetable, in addition to being used for livestock and dog feed, plus fish bait.

Nutritional Importance of Maize

The maize kernel (Figure 1.2a) has nutritional properties that are comparable to other cereals. Table 1.3 shows the nutritional comparison table for maize, rice and wheat (Mejía, 2008).

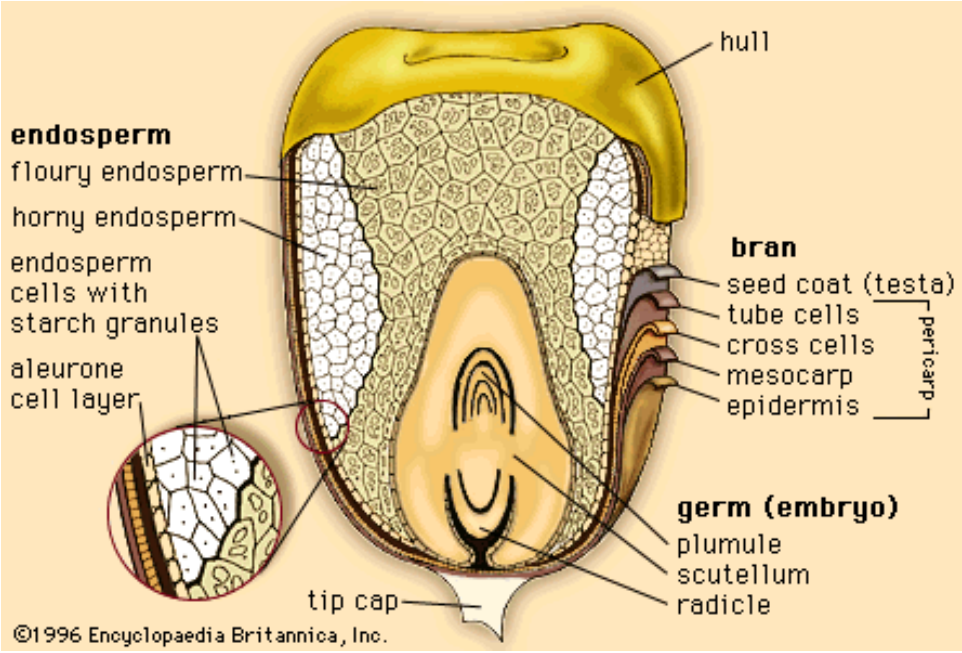

Figure 1.2a Maize kernel: outer layer and internal structure (Britannica, 1996)

The biofortification of maize through plant breeding helps prevent malnutrition (White and Broadley, 2005; WHO/FAO, 2003; Gregorio *et al*., 2000; Monasterio and Graham, 2000; Beebe *et al*., 2000), in addition to being used as a “template” for studying monocotyledonous plants (Miller, 2008).

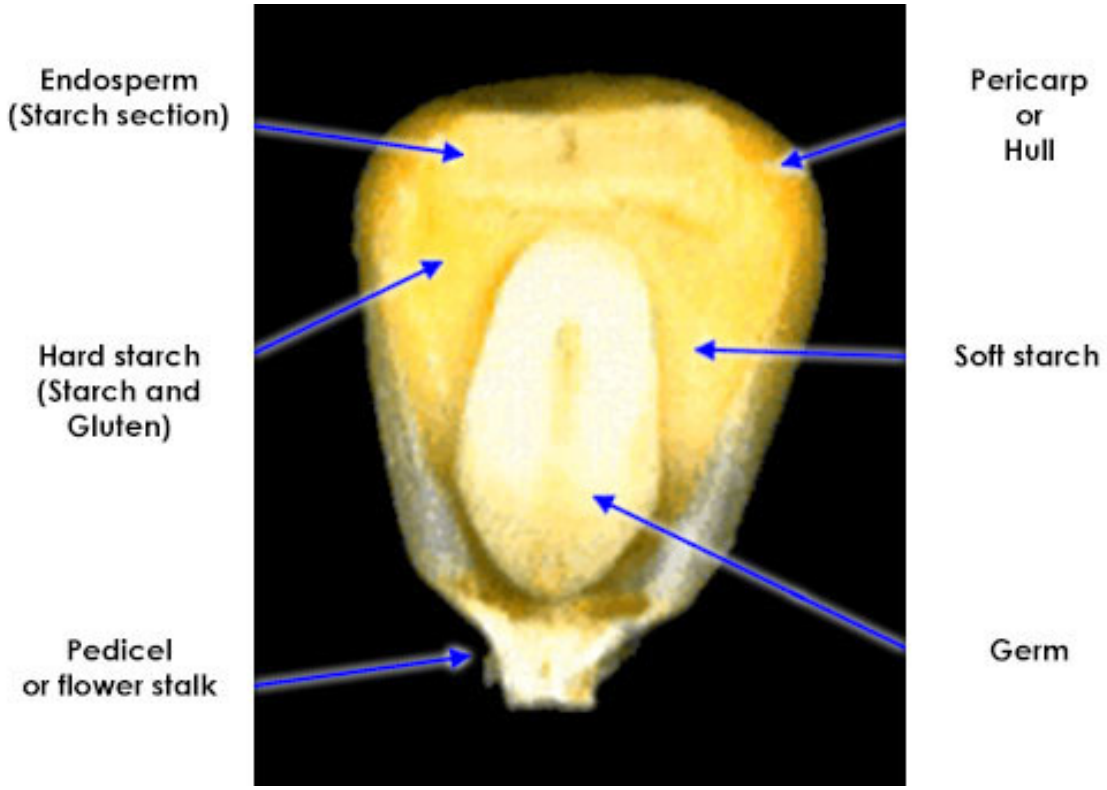

Figure 1.2 b. Parts of the maize kernel (Mejía, 2008)

Table 1.3: Nutritional composition comparison per 100 g Maize, Wheat and Rice grain sorted by value (Mejía, 2008)

Content	Maize ground meal	Wheat flour	Rice polished grain
Calories	**362**	**359**	**360**
		(g)	
Carbohydrates	74.5	74.1	78.9
Water (percent)	12	12	13
Protein	9	12	6.8
Fat	3.4	1.3	0.7
Ash	1.1	0.65	0.6
Starch fiber	1	0.5	0.2
		(mg)	
Phosphorus	178	191	140
Calcium	6	24	6
Niacin	1.9	2.0	1.5
Iron	1.8	1.3	0.8
Thiamine	0.30	0.26	0.12
Riboflavin	0.08	0.07	0.03

Maize kernel refining process

The mature maize kernel can be separated into the component parts through the milling process. Further refining extracts corn oil from the germ, leaving the remainder of the germ to combine with fiber and gluten in feed products formation. And the starch is either dried (in the wet milling process) and sold as pure starch or converted into sweeteners, alcohol, and chemicals (Mejia, 2008; CRA, 1999).

Maize storage and preservation

Grain storage and preservation takes many forms depending on the quantity of grain to be stored, the purpose of storage, and the location of the store. Maize and grain storage systems are classified as either bag or bulk storage (IRRI, 2008).

Maize grain is hygroscopic and its moisture content easily equilibrates with the surrounding air, in open-air storage (Table 1.4). This, plus the high relative humidity and temperature in the tropics, promotes the rapid infestation and multiplication of insects, molds, rodents and birds, in open-air storage (IRRI, 2006, 2008).

Table 1.4: Safe maize grain storage moisture content requirements (IRRI, 2008)

Storage period	Required moisture content for safe storage	Potential problems
2 to 3 weeks	14 – 18 %	Molds, discoloration, respiration loss
8 to 12 months	12- 13 %	Insect damage
More than 1 year	9 % or less	Loss of viability

Primary causes of stored grain spoilage include incomplete drying resulting in wet pockets, temperature variations between storage bin and the outside, and the associated moisture condensation within the bin. Other primary factors are inadequate observation and management, improper storage bin preparation, and insufficient cooling of grain after drying.

Other Storage Problems

Food crises and grain price hikes are precipitated by the lack of storage facilities, cross-country smuggling, drought, exports, rising cost of oil, biofuel subsidies in the US and Europe, the prolonged drought in Australia, as well as restrictions on the export of maize, rice and wheat by various countries. These often lead to seasonal price increases for maize, wheat, rice, and other crops (Khan, 2008; Minot, 2008).

An effective storage system suitable for use in developing nations may encourage public investment in marketing infrastructure-construction and maintenance of ports, bridges, roads, and market places; policy environments that are conducive to agricultural marketing-food assembly, transport, storage, distribution and export; policies that discourage "hoarding" or "price gouging"; reduction in internal and external barriers to trade; and the creation of certainty in the supply chain as well as improved instruments for managing risk (Minot, 2008).

Interactions of *Z. mays* with Other Life Forms

An array of diseases plague the maize crop during the growing season. These include downy mildew, rust, leaf blight, stalk and ear rots, leaf spot, and maize streak virus. Insect pests, including stem and ear borers, armyworms, cutworms, grain moths, beetles, weevils, grain borers, rootworms, and white grubs are also a great threat to the survival of maize in Africa (IITA, 2007b; CFIA, 2006**)**.

In developing countries, the interaction of *Zea mays* with the maize weevil, in post-harvest storage, is the most destructive (Lucia & Assennato, 1994 and Schneider, 1991), of all pest infestations. It results in 10-50% maize grain loss, in the tropics

and even complete destruction, in some cases (Hodges et. al, 1983; Longstaff, 1981; Keil, 1988; Henckes, 1992; Jacobs and Calvin, 2001).

Overall, 20-30% of Ethiopian stored maize is lost to *S*. zeamais infestation, while 100% maize damage has been found in maize stored for 6-8 months in the Bako region of the country (Demissie *et al*., 2008a). Mulungu *et.al*., (2007) found about 17.51% weevil damage in research involving stored maize, in Tanzania, and Demissie *et al*. (2008b) found 11-59% levels of weevil infestation in maize stored at Bako, Ethiopia.

Insect Detection and Quantification Methods

The use of carbon dioxide sensors to determine the presence and therefore the activity of insects through their respired gas, and the use of pheromones, visual lures, grain probes, insect traps, x-ray imaging, machine vision, near-infrared spectroscopy (NIR), Berlese funnel method, electrical conductance, and acoustical methods (Table 1.5) are common methods for insect detection and quantification.

Food attractants or synthetic insect pheromones (Vick *et al*., 1990) used as traps and florescent tubes, used as a lure for stored insects, are additional tools employed for luring the weevils out of the kernels for quantification, and in determining insect survival rate within storage. Since insects respond to semiochemicals (Loschiavo *et al*., 1986) at dawn, midday, dusk, at about 10–15°C, and are attracted to fluorescence, the use of the lure and trap allows for direct visual observation and counting while carbon dioxide and oxygen sensors are often used for indirect sensing.

Aggregation pheromones also exist for the lesser grain borer, *R. dominica* (Williams, *et al*., 1981), and red flour beetle *Tribolium castaneum* (Herbst) (Suzuki & Mori, 1983), and sex pheromone for the warehouse beetle, *Trogoderma variabile* (Ballion) (Cross *et al*., 1976).

Table 1.5: Detection techniques for stored grain insects (Neethirajan, *et. al*., 2007)

Insect detection methods	Pros	Cons
Grain probes and insect traps	Widely used, inexpensive, used for finding insect density	Labor intensive, limits the temporal availability of data, cannot detect internal insects, restriction in the placement of traps
Pheromones	Gives an indication of pest density	Environmental factors affects trap catches
Visual lures	Can be effective in indoor situations	Not very effective
Acoustical methods	Internal infestation can be detected	Cannot detect dead insects and infestations by early larval stages
Electrical conductance	Hidden internal infestation can be identified	Kernels with insect eggs and young larvae cannot be detected, efficiency is low compared to soft X-rays
Berlese funnel method	Cheap and commonly used method at elevators	Very slow and internal infestations cannot be identified
Near-infrared spectroscopy (NIR)	Non-destructive, rapid method, requiring no sample preparation	Cannot detect low levels of infestation, sensitive to moisture content in samples, calibration of equipment is complex and frequent
Machine vision	Effective in detecting external insects	Cannot detect internal insects

Table 1.5: Detection techniques for stored grain insects (Neethirajan, *et. al*., 2007)-continued.

Insect detection methods	Pros	Cons
X-ray imaging		Non-destructive, highly accurate, detect both internal and external insects, able to detect both live and dead insects inside grain kernels

Maize weevil

A complex of weevils including the rice (*Sitophilus oryza*), granary (*Sitophilus granarius*), and maize (*Sitophilus zeamais*) weevils are among the most destructive pests of stored grain products (grain, seeds) (Jacobs, and Calvin, 2001). These are pests of grain throughout the world, and the economic situation in a developing country like Nigeria is adversely affected by post-harvest losses resulting from weevil activities (Arannilewa *et al*., 2002). The tremendous quantitative and qualitative losses resulting from such weevil activity translates directly in into huge income losses for subsistence farmers, and has the potential to discourage farming and or raise maize and other cereal market prices.

Life Cycle

The maize weevil (Figure 1.3), *Coleoptera: Curculionidae: Sitophilus zeamais Motschulsky*, is a member of the *Sitophilus* group of weevils of the *Coleoptera* order, *Curculionidae* family and genus *Sitophilus.*

Figure 1.3. The maize weevil and weevil infested maize (Savidan, 2002)

It is a cosmopolitan insect, with yellow blotches on its forewings, and a developmental life cycle of about 28 days. The flying adults lay eggs into stored or in-field maize, and the eggs develop into larvae that feed on the grain, developing into adult weevils. According to Longstaff (1981), the adult *S. zeamais* digs a shallow pit in the maize grain coat, lays one egg, and plugs it with wax. The larva feeds on the grain, pupates within it, and emerges by tunneling through it. The emerging adult larva is capable of living for five to eight months, and each adult female lays 300-400 eggs (Ozanimals, 2009).

The combination of tropical heat, respired carbon dioxide, and the lack of oxygen in hermetic storage is lethal to all stages of insect life (eggs, pupae, larvae and adult), although the rate of insect mortality, respiration and reproduction is slower at low temperatures (IRRI, 2006; De Lima,1990). Rapid insect development occurs within a fairly narrow range of 5-10 degrees around the optimal temperature, which, for most storage insects, is in the region of 30-35°C (FAO, 1994; IRRI, 2008). But, the

optimum temperature for weevil development is about 27 °C (Arannilewa, *et al*., 2006).

Weevils in stored maize

Infested grains will usually be found heating at the surface, may be damp, and sprouting may occur (Jacobs and Calvin, 2001). These are optimum conditions for weevil growth and reproduction (IRRI, 2008), and often lead to complete destruction of grain in storage where the maize grain is undisturbed for some length of time. But, physical disturbance of the grain (Joffe, 1963), dislodges the weevil from the kernel, discourages feeding and eventually leads to weevil mortality. This is a pest control method that can potentially be used along with hermetic storage (Navarro *et al*.,1994).

Socio Economics Impact of Maize Weevils

Post-harvest damage caused by *S. zeamais* affects both quantity (nutrient loss) and quality (maize commercial grade) factors, with significant economic losses (Jordao, 1974; Boxall, 1986; Abimilho, 2002; Bern, *et. al*, 2008; Food Solutions, 2008) to the farmer or decreased benefit to the end user (reduced dry matter, mycotoxin toxicity). Table 1.6 describes allowable shelled corn storage time (SCST) in days, for different temperature and moisture combinations.

Table 1.6: Shelled corn storage time (SCST) in days (Bern, *et al.*, 2002)

Corn temp		Corn moisture % wet-basis																				
°C	°F	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
0	32	2702	1458	866	557	381	276	208	163	132	110	93	81	71	64	58	53	48	45	42	40	37
1	34	2298	1240	737	473	324	234	177	139	112	93	79	69	61	54	49	45	41	38	36	34	32
2	36	1954	1055	627	403	276	199	150	118	95	79	67	58	51	46	42	38	35	33	30	29	27
3	38	1662	897	533	342	235	169	128	100	81	67	57	50	44	39	35	32	30	28	26	24	23
4	40	1413	763	453	291	199	144	109	85	69	57	49	42	37	33	30	27	25	24	22	21	20
6	42	1202	649	385	248	170	123	93	73	59	49	41	36	32	28	26	23	22	20	19	18	17
7	44	1022	552	328	211	144	104	79	62	50	41	35	31	27	24	22	20	18	17	16	15	14
8	46	869	469	279	179	123	89	67	52	42	35	30	26	23	20	19	17	16	14	14	13	12
9	48	739	339	237	152	104	75	57	45	36	30	26	22	19	17	16	14	13	12	12	11	10
10	50	629	339	202	130	89	64	48	38	31	26	22	19	17	15	13	12	11	10	10	9	9
11	52	535	289	171	110	75	55	41	32	26	22	18	16	14	13	11	10	10	9	8	8	7
12	54	455	245	146	94	64	46	35	27	22	18	16	14	12	11	10	9	8	8	7	7	6
13	56	387	209	124	80	55	39	30	23	19	16	13	12	10	9	8	8	7	6	6	6	5
14	58	329	177	105	68	46	34	25	20	16	13	11	10	9	8	7	6	6	5	5	5	5
16	60	280	151	90	58	39	29	22	17	14	11	10	8	7	7	6	5	5	5	4	4	4
17	62	249	134	80	51	35	25	19	15	12	10	9	8	7	6	5	5	5	4	4	4	4
18	64	222	120	71	46	31	23	17	14	11	9	8	7	6	6	5	5	4	4	4	3	3
19	66	197	107	63	41	28	20	16	12	10	8	7	6	6	5	5	4	4	4	3	3	3
20	68	176	95	56	36	25	18	14	11	9	8	7	6	5	5	4	4	4	3	3	3	3
21	70	157	85	50	32	22	16	13	10	8	7	6	5	5	4	4	4	3	3	3	3	3
22	72	139	75	45	29	20	15	11	9	7	6	5	5	4	4	4	3	3	3	3	2	2
23	74	124	67	40	26	18	13	10	8	7	6	5	4	4	4	3	3	3	3	2	2	2
24	76	111	60	35	23	16	12	9	7	6	5	5	4	4	3	3	3	3	2	2	2	2
26	78	98	53	32	20	14	10	8	7	5	5	4	4	3	3	3	3	2	2	2	2	2
27	80	88	47	28	18	12	9	7	6	5	4	4	3	3	3	3	2	2	2	2	2	2
28	82	78	42	25	16	11	8	7	5	5	4	3	3	3	3	2	2	2	2	2	2	2
29	84	70	38	22	14	10	7	6	5	4	4	3	3	3	2	2	2	2	2	2	1	1
30	86	62	33	20	13	9	7	5	4	4	3	3	3	2	2	2	2	2	2	1	1	1
31	88	55	30	18	11	8	6	5	4	3	3	3	2	2	2	2	2	2	1	1	1	1
32	90	49	26	16	10	7	5	4	4	3	3	2	2	2	2	2	2	1	1	1	1	1
33	92	44	24	14	9	6	5	4	3	3	3	2	2	2	2	2	2	1	1	1	1	1
34	94	39	21	12	8	5	4	4	3	3	2	2	2	2	2	2	1	1	1	1	1	1
36	96	35	19	11	7	5	4	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1
37	98	31	17	10	6	4	4	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1
38	100	27	15	9	6	4	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1
39	102	24	13	8	5	3	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1
40	104	22	12	7	4	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
41	106	19	10	6	4	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1
42	108	17	9	6	4	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
43	110	15	8	5	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
44	112	14	7	4	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
46	114	12	7	4	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
47	116	11	6	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
48	118	10	5	3	2	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
49	120	9	5	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

The SCST assumes that allowable storage time begins at harvest, that the moistures are oven moistures (103 ^{O}C., 72 h), and that the stored maize is clean, combine run corn having 30% visible mechanical damage. It also assumes that by the end of the SCST, maximum allowable grain deterioration has occurred without a decrease in USDA grade. In addition maize variety, weather conditions, contamination with mold spores and other factors often lead to variability in the SCST prediction. The rate of maize deterioration increases with mechanical damage, and maize with fine material have shorter SCST, due to larger surface area for fungal growth (Bern, *et.al.,* 2002).

The gross biological activity in a mass of stored maize grain is usually measured by capturing and the evolved CO_2 from the grain, and assuming respiration of the mass can be modeled by the oxidation of glucose. This corresponds to an average of 7.4 g of CO_2 per kg of original dry matter, before the grade of the maize is reduced due to kernel damage. The 7.4 g/kg is equivalent to a loss of 0.5% of original maize dry matter and the deterioration of maize grain is usually expressed as percentage dry matter loss. 0.5% dry matter loss corresponds to a loss of one U.S. grade level and is accepted as the criterion for allowable storage time, defined as the Shelled Corn Storage Time (SCST) for maize.

Calculation (Example)

Situation 1: Maize is harvested at 26% and placed in a grain cart. If the average grain temperature is 70 ^{O}F, what is the SCST?

How long can the grain be stored in the holding bin?

Solution: Based on Table 1.6, SCST for 70 ^{O}F, 26% is 5 days

Situation 2: After one day, the maize above (**Situation 1**) is placed in an aerated holding bin where grain was used up at condition in the first day. How long can the grain be stored in the holding bin?

Solution: SCST at 50 ^{O}F, 26% is 19 days. But, 1day/5days (20%) of the SCST was used up at condition on the first day. Hence, only 80% of the SCST remains: (1-0.20)*19=15.2 days (Bern, et. al., 2008).

Weevil and Mold Activity

The metabolic activity of the *S. zeamais* produces additional heat and moisture within the grain storage environment that sustains the activity and proliferation of molds, such as *Aspergillus flavus.*

The mold activity in turn, increases the heat and moisture content, in addition to possibly producing deadly toxins such as aflatoxins, zearalenone, trichothecenes (DON, T-2), fumonisins, and ochratoxin. Symptoms of mycotoxin contamination in livestock include loss of appetite, poor weight gain, feed refusal, diarrhea, bleeding, unthriftiness, and death (Munkvold, *et. al*., 1997; Lewis, *et al*., 2005; Ohio State University, 2007).

Weevil Control

Post- harvest maize preservation requires a sequential and integrated weevil protection system involving drying and storage of the clean dry grain, disinfecting the storage system and controlling or preventing pest infestation during the storage period (IRRI, 2006, 2008).

Disinfesting the storage system

The control of stored grain pests traditionally relied on insecticides (malathion, fenitrothion, deltamethrin, etc) and fumigants (phosphine, carbon dioxide, etc). But consumers are increasingly demanding grain that is free from live insects and free from chemical residues (IRRI, 2008; Korunic, 1998).

Alternative, non-chemical, and low-cost storage systems designed to reduce human exposure, development of insecticide resistance and environmental and food contamination (Ebeling, 1971) include diatomaceous earth, low temperatures, modified atmospheres and mechanical impact for controlling stored grain pests (Food Solutions, 2008).

Maize Preservation

Post-harvest maize preservation aims at retaining the highest possible level of feed and food quality, until final use. And the profit associated with maize preservation is the value of the grain minus the cost of production and preservation. Therefore, if grain price goes up while preservation cost remains constant, a higher level of preservation becomes feasible and the maximum profit is derived from the preservation effort (Bern, et. al, 2008).

Respiration

All living organisms within a mass of grain carry on respiration for the biochemical oxidation of organic nutrients. This is usually modeled for the entire grain mass (grain and other living organisms) by the combustion of a carbohydrate. These

organisms include grain kernels, fungi, bacteria, insects, mites, rodents, and birds (Bern, et. al, 2008). The metabolic activity of these organisms is modeled by glucose oxidation (Bern. et. al, 2008):

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_20 + Heat\ (2834\ kJ)$ ----------(1-1)

Drying

Drying and or dehydration is the process of removing moisture from grain, to make it less hospitable to living organisms within the mass (Bern, et. al, 2008). Drying is the most widely used technology for protecting cereals from spoilage, although it can involve significant energy and equipment costs (Food Solutions, 2008). It reduces the maize moisture content, the relative humidity of the static interspace air, and either reduces or eliminates the activity and survivability of insects, and microorganisms.

Refrigeration

Forced aeration with or without applied refrigeration is one of the most used and valuable aids to grain preservation (Calderon and Barkai, 1990). This retards the respiration and metabolism of living organisms by decreasing temperature below the optimum temperature (Bern, et. al, 2008).

Ionizing Radiation

Ionizing radiation (irradiation) involves grain preservation that employs ionizing radiation to destroy bacteria, molds, and yeasts by direct hit of ionizing particles at or

near a sensitive center of the organism (Baba, et.al, 2004; Cutrubinis, et.al, 2005; Bern, et. al, 2008).

Chemical Preservatives

Chemical treatment is one of the most common means of maize preservation. Propionic and acetic acids are the most common preservative additives for maize grain, although calcium propionate, potassium sorbate, sodium propionate, sodium sorbate, sorbic acid, sodium benzoate, sulphur dioxide, citric acid, benzoic acid, salt, wood smoke, spice, sugar, condiments, and vinegar are also common preservatives (Bern, et. al, 2008).

Plant Extracts

Plant oil extracts (Table 1.7) have been used since ancient times for effective control of all stages of development of insects of stored products (Qi and Burkholder, 1981; Nezan, 1983; Adedire, 2003; Don-Pedro, 1989; 1990).

Table 1.7: Petroleum ether extract of four medicinal plants evaluated for insecticidal activities against *Sitophilus zeamais*. (Arannilewa, et. al, 2006)

Scientific name	Family	Parts used	Common name
Aristolochia	Aristolochiaceae	*rigens* Root bark	Gaping Dutchman's pipe
Allium sativum	Liliaceae	Bulbs	Garlic
Ficus exasperata	Moraceae	Leaves	Sandpaper leaf
Garcinia kola	Guttiferae	Seeds	Bitter kola

Problems Associated with Chemical Preservatives

The chemicals add to storage expenditure, and may create health and environmental hazards (Murdock, et. al, 2007; Ebeling, 1971).

Gaseous environment

Preservation is often accomplished by adjusting the gaseous environment of the grain. Flooding of the storage environment with CO_2, O_2 depletion, fermentation, the trickle ammonia process, and fumigation with methyl bromide, as well as chloropicrin are used to rid masses of grain of insects (Bern, *et. al*, 2008).

Fermentation

Chemical preservatives can be produced within the material by fermentation. Ensiling is an example of a controlled, yet encouraged, growth of microorganisms which allow the creation of unfavorable conditions for microbes (themselves inclusive) while retaining to a large extent, the nutrients being preserved (Bern, *et. al*, 2008; Alberta Agriculture and Rural Development, 2008).

High Moisture Maize Storage

The sealing of high-moisture shelled maize in airtight structures excludes external oxygen, leads to depletion of the oxygen within the storage atmosphere, anaerobic respiration, the conversion of sugar to fatty acids, and a cessation of bacterial activity in about three weeks. This leaves an oxygen-free environment conducive to the long-term preservation of maize (Bern, 1998), and forms the basis of the ensiling process.

Mechanical Isolation

Mechanical Isolation preserves grain by fencing out threatening organisms or something they need to survive (Bern, *et. al*, 2008). This principle is employed in food canning (Umaine, 2007) and hermetic storage.

Oxygen Limiting Maize Storage

Oxygen-limiting maize storage is a non-chemical maize storage system that employs oxygen impermeable packaging and prevents oxygen exchange between the stored maize and the external environment.

In general, the rate of oxygen depletion and weevil mortality is dependent on the quantity and quality of maize sample, moisture content, insect population, and or presence of molds (Krishnamurthy *et al*., 1986).

Oxygen-limiting storage (Donahaye, 1990) also prevents weevil reproduction (IRRI, 2008), and can be used with low moisture maize, since insect activities slow down at moisture contents of about 8% (FAO, 1994). It also eliminates the need for expensive and toxic chemicals, in addition to being effective for high moisture maize preservation.

This is particularly important where tropical heat and moisture promotes rapid insect multiplication and grain germination while in storage, leading to increased grain respiration and mold formation (FAO, 1994; IRRI, 2008).

Hermetic Storage and Maize Preservation.

Hermetic storage systems employs the use of airtight and watertight containers that restrict oxygen and water movement between the outside atmosphere and the stored grain in order to retain grain quality and seed viability (Murdock, et. al, 2007). The system works because insect and grain respiration reduces oxygen levels in the storage atmosphere to 5-10%, at which time insect activity ceases (IRRI, 2008). It also maintains the original storage moisture content and reduces pest damage without the need for pesticides. However, non-hermetic containers (jute or woven plastic, granaries, etc), expose the stored maize to weevil damage and moisture.

Hermetic storage practices encourage the filling of the storage container to as close to the brim (Umaine, 2007) as possible, since a large air space (headspace) to grain ratio may not allow oxygen levels to reduce to a level that will effectively control the insect population. Re-entry of oxygen through intermittent opening and closing of the storage environment is to be avoided, to discourage re-infestation by weevils (IRRI, 2008).

The simultaneous depletion of O_2 and the CO_2 accumulation resulting from weevil, grain, and fungi respiration in hermetic storage has a synergistic effect on the control of stored maize insects (Calderon and Navarro, 1979; 1980; Oxley and Wickenden, 1963; Burrell, 1968). And the lower the maize moisture content, and the associated inter-granular humidity, the higher the desiccation effect of the low O_2 (Navarro, 1978) and or elevated CO_2 concentrations (Navarro and Calderon, 1973), on the

insect population. Hence, there is high weevil mortality associated with hermetic storage under these conditions.

Sealing and Hermetic Preservation

In hermetic maize preservation, shelled and cleaned maize grain is dried to desired moisture content- 12% for seeds, 14% or less for other maize grain-then placed in an airtight and pre-cleaned container and sealed. The seal-points may be fitted closely with the use of grease, silicon, tar, or molten rubber, on the inside. The outside of the container may also be painted with paints when pervious materials (clay pots or vessels) are used (IRRI, 2008). And the longer the grain needs to be stored, the lower the required moisture content needs to be. Grain and seed stored at moisture contents above 14% may experience the growth of molds, rapid loss of viability and a reduction in eating quality.

Moisture migration within Hermetic Storage

Weevil, mold and the maize grain produce water and heat as by-products of respiration and metabolic activities. Diurnal temperature fluctuations, associated with solar radiation and rapid cooling at night, in tropical regions, causes successive moisture condensation and drying cycles at the upper grain surface. This can be remedied by placing an insulating layer of rice hulls, straw, or "felt-fiber" between the liner and the upper layer of the bagged maize or by wrapping these materials around the outside of the storage structure (IRRI, 2008).

Hermetic Storage Types

Hermitic storage is categorized as bulk or small quantity maize storage. Maize stored in small quantity usually employs the use of bags and small containers, while bulk storage employs larger storage facilities, such as silos and granaries.

Hermetic Plastic and Bag storage systems

Triple and double bagging (Figure 1.4), is a common hermetic storage method (Donahaye *et al*., 1991;IRRI, 2008) employed in the storage of maize and other grains, has proven effective in cowpeas storage in pilot tests, across West and Central Africa. It has the advantage of providing cheap storage alternatives for farmers, while increasing household income, on average by about $150 per year (Murdock, et. al, 2007).

Storage bags are usually stored in stacks under cover of a roof, in a shed, granary or under water-proof tarpaulins to improve grain preservation. However, plastic bags (PVC overliner and a polyethylene underliner) are often easily be perforated by insects and birds (SGRL, 2007), while plastic drums are easily penetrated by rodents (IRRI, 2008). IRRI demonstrates double bagging in Figure 1.4a.
Hermetic bag storage can be labor intensive, and grain spoilage resulting from the influx of oxygen and external moisture following bag perforations are common. Bartosik, *et.al* (2008) utilized a 60-m-long, 2.74-m diameter silo-bag, with 0.235-mm thick plastic cover, made of three layers-white on the outside and black on the inside, for the storage of 200 Mg of wheat. The white, hermetic, cocoon developed by MDIC (2009) is 0.83 mm thick and is resistant to degradation by UV radiation.

Grainpro SGB-HC uses woven polypropylene, reinforced with a polyethylene underliner (Figure 1.4b). The resealable and reusable 0.078 mm polyethylene bag acts as a moisture and gas barrier, as long as they are protected from puncture. And bags are custom-made to suite customer needs.

Figure 1.4a. Double-bagging (IRRI, 2008)

Figure 1.4b. Woven polypropylene SGB-HC (GrainPro, 2008)

Hermetic Bulk Storage Systems

Losses from insects, rodents, birds and moisture uptake are usually high in traditional bulk storage systems because the maize is usually stored in outdoor granaries made from woven baskets, wood, metal or concrete (Lindblad and Druben, 2008).

Large export mills (Figure 1.5) and collection houses often use metallic and concrete (or ferrocement) silos, which are better sealed and or provide hermetic storage properties (Appropedia, 2006; Smith and Boon-Long, 1970).

Related structures with hermetic properties provide protection for stored maize, plus other grains and can boost the market potentials of agriculturally based economies and export dependent nations (Mauldin, 2008), in addition to the possibility of use as a national grain reserve (PANA, 2003) or centralized warehouse storage (Donahaye *et al.,* 1991; 2001) in rural communities.

Locally available hermetic storage materials

In addition to ferrocement, 55 gallon steel barrels are excellent candidates for cheap hermetic storage in the East African sub-region. Lindblad and Druben (1980) and Adhikarinayake (2005) described the use of empty oil drums, filled to the brim, for hermetic storage. Both also provide mechanical isolation from rodents.

However, the barrels are usually contaminated by petro-chemicals, and need to be properly cleaned, to prevent cross contamination of maize stored in the barrels. Common methods for determining petro-chemicals present and measuring level of contamination involve the use of gas chromatography, followed by the use of methanol for cleaning (Turriff, et. al, 1998). The use of soaps available in the local culture, for cleaning is also common practice, although the efficacy of this method cleaning or decontamination is unknown.

Figure 1.5. Mill concrete silos (IRRI, 2008)

The development of a socio-economically acceptable storage solution that addresses the problems of local glut and price collapses (Minot, 2008) in developing nations would ensure food security for millions of people around the world. Maize accounts for 50 % of East Africa's import (Nyasa Times, 2007) and the common maize market for Eastern and Southern Africa (COMESA)'s effort to provide maize and food security for the region will receive a major boost from the use of such bulk hermetic storage facilities.

Barrels

The barrel is a unit of liquid capacity, with a value that depends on the liquid stored within it. It was traditionally made of wooden strips (staves) fitted together, in a way that eliminated gaps, when bound by metal hoops. It is originally used for bulk storage of liquids and dry goods. Wooden barrels have, however, been replaced by plastic and metal ones.

Its volume is defined according to customary law or usage. In the U.S. customary system it varies, as a liquid measure, from 31 to 42 gallons (120 to 159 L) as established by law or usage (Dictionary.com, LLC, 2009). Today, 55 gallon steel barrels are standard in the petroleum industry (The Cary Company. 2008; Farlex, 2009; General Container Corp. 2009).

Oxygen Quantification

Research involving quantification of the oxygen levels (RKI Instruments, 2007) within the hermetic storage system provides a measure of the integrity and applicability of the hermetic storage system to solving food crises (Minot, 2008). Since the respiratory and metabolic activity of insects, molds and the maize grain lower the oxygen content of the intergranular atmosphere to a level where aerobic respiration is no longer possible (Bern. et. al, 2008), the oxygen level can be quantified, using a sensor (AMIO, 2008), to determine the level of oxygen and how long it takes for the oxygen content to reach levels that support hermetic storage.

Figure 1.6 demonstrates weevil oxygen quantification principles, in hermetic storage. It is derived from research designed to study the effect of *S. zeamais* and *aspergillus chevalieri* on the oxygen level in maize stored hermetically.

The study (Moreno-Martinez et. al., 2000) utilized maize grain of hybrid AN 447, infested with 20 unsexed *S. zeamais*, stored within storage flasks (250 mL glass containing 150 g of maize) and oxygen analyzers. The jars were stored for 30 days at 26^{O}C, 16% moisture, 70% r.h., and 18±6 h L-D photoperiod, under hermetic and non-hermetic conditions to monitor the oxygen concentration, insect mortality, insect offspring, grain germination, and fungal growth.

Maize weevil mortality was recorded over 30 days, at 3 days intervals, by checking 12 jar replicates each of hermetic and non-hermetic grain. It found that oxygen was depleted to 0% in 6 days, hermetic for both treatments involving insects.

Quantification of the oxygen within the hermetic storage flasks (250-mL) was done using an electronic oxygen analyzer.

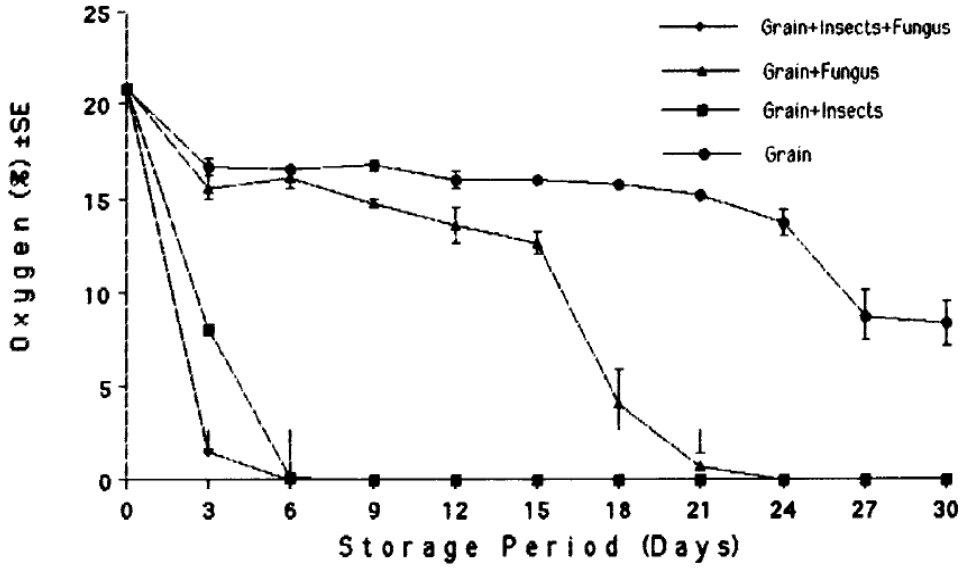

Figure 1.6: Hermetic oxygen quantification of weevil infested maize (Moreno-Martinez et. al., 2000).

The rate of oxygen utilization in treatments containing weevils was more rapid than that containing fungus and maize alone, while treatments with maize and fungus only had half the utilization rate and those with maize alone had much lower utilization rate (Figure 1.6).

It is possible to speed up the depletion of the trapped oxygen through the use of an external vacuum source, provided outside the grain storage compartment (Ross and Boykin, 1986) or to flood the storage compartment with CO_2 using an external CO_2 source. However, most hermetic studies rely on the natural respiration process of the maize grain and the weevils to create a self-sustaining hermetic storage system.

Pycnometry

Gas pycnometry (Yamagishi and Takahashi 1992; Cook *et al,* 1999) is a laboratory procedure used in measuring the volume of solids (maize) within a container (hermetic storage) through the employment of some method of gas displacement. It is based on Boyle–Mariotte's law of volume–pressure relationships, and can be done in less than 20 minutes (Kummer and Cooper 1945; McIntyre *et al* 1965; Bielders *et al.,* 1990; Marinder 1996). It can also be automated (Huang *et al.,* 1995; ISO, 1999).

Determining the maize volume allows calibration of the oxygen volume within the container. This is used to calculate the volume of oxygen consumed by a known number of weevils, within the storage ecosystem. And using the pycnometry (Micromeritics Accupyc 1330) equipment, the maize particle density within the canning jar can be determined and used to infer the air volume, for the purpose of measuring the oxygen consumption per weevil. This would help generalize the weevil study results to varying sizes of hermetic storage container.

Quantifying Weevil and Mold Damage

Quantification of the dry matter mass before and after insect activity within the maize kernels is instrumental in determining the level of nutritional loss due to the insects (Nennich and Chase, 2008).

Rodents

An effective hermetic atmosphere must also be able to withstand the gnawing activity of rodents in order to protect the stored maize from weevils. In addition to designing a rodent-proof storage system, good hygiene is important in discouraging rodent infestation. Therefore the floor and surroundings of the storage ecosystem must be kept clean at all times. The floor must be inspected regularly and crevices filled as soon as they are detected, in addition to proper record (foot prints, droppings, etc) keeping (IRRI, 2008).

GENERAL OBJECTIVES

The thesis objectives were to:

1) Describe a workable weevil protection and maize storage system for use in East Africa.
2) To determine the effect of interaction of factors such as time, oxygen level, temperature, and maize moisture content on the survivability of the maize weevil.
3) Draw a general conclusion on how the outcome of the weevil protection studies conducted meet the expected outcome of describing a non-chemical storage system that is air-tight, water-tight and rodent-proof and sustainable in the local culture.

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CHAPTER 2

NON-CHEMICAL ON-FARM HERMETIC MAIZE STORAGE IN EAST AFRICA

A paper to be submitted to the African journal of agricultural research

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ABSTRACT- *Maize (Zea mays L.) consumption makes up a significant percentage of daily calorie in-take in East Africa and adequate supply is necessary for food security for subsistence farmers, as well as domestic stability. Hermetic post-harvest storage which relies on the combination of processes that exclude oxygen, water, and pests from the storage atmosphere provides advantage over other storage systems because it effectively controls sitophilus* zeamais *(Motsch.)*, which is responsible for *damage to stored maize grain (10-50%) and eliminates the need for toxic and expensive chemicals. This paper describes the results of tests conducted on a laboratory-scale hermetic storage system. Two studies were conducted to (a) evaluate the effects of different temperatures (10 ^{o}C and 27^{o}C) and maize moistures (6.3% and 16%) on Sitophilus zeamais Motschulsky biology and mortality rate; and to (b) quantify weevil oxygen consumption. The respiration and mortality rates were affected by temperature and maize moistures, and the research found significant mean and statistical differences (at the 0.05 level) between hermetic vs. non-hermetic, 6.3% vs.16% moisture, and 10 ºC vs. 27 ºC treatment factors.* ***Study#1:***

Maize grain of the commercial hybrid Fontanelle 6T672, canning jars, and S. zeamais were utilized in the study involving hermetic and non-hermetic storage systems. Weevil mortality was affected by hermetic storage, where high mortality rates (up to 100%) were recorded compared to 2.5-7.5% for non-hermetic storage. ***Study#2:*** *Agricultural instrumentation, involving the use of oxygen sensors, a microcontroller, canning jars, 6T672 maize samples, S. zeamais, plus 10 ^{o}C and 27^{o}C temperature chambers were utilized, along with pycnometry in quantifying weevil oxygen consumption, under the different maize moistures. Weevil oxygen utilization occurred at different rates, and100% mortality was recorded in all the weevils in study #2.*

Keywords: *Maize, Hermetic, S. zeamais, Food crises, Storage, Agricultural, Instrumentation, Microcontroller*

INTRODUCTION

Maize

East Africa' s maize (*Zea mays* L. ssp. *Mays; corn*) consumption by humans (Table 2.1) far exceeds other uses (Aquino *et al*., 2000), and accounts for more than 50% of total caloric intake in local diets (Sinha, 2007). Unfortunately current, on-farm maize storage practices expose this important food source to the threat of maize weevils. East African maize production statistics, by country are shown in Table 2.2.

Table 2.1: East African average maize consumption by country (Aquino *et al*., 2000)

Country	1995-1997
	Human consumption (%)
Burundi	91
Eritrea	N/A
Ethiopia	88
Kenya	91
Rwanda	93
Somalia	89
Sudan	N/A
Tanzania	85
Uganda	64

Notes: N/A=not available

Table 2.2: East African average maize production by country (FAOSTAT, 2009)

Country	Quantity (0000 Mg)		
	2005	**2006**	**2007**
Burundi	135	112	115
Eritrea	.25	.35	.27
Ethiopia	391	403	400
Kenya	291	325	324
Rwanda	9.7	9.2	9.0
Somalia	200	9.7	9.9
Sudan	4.5	7.0	6.0
Tanzania	329	337	340
Uganda	117	126	126

Maize drying

Harvested maize in East Africa is usually sun dried or dried over wood fire. This allows drying to 12%*, or below 10% moisture, respectively (FAO/Mejia, 1991). Although sun drying potential varies depending on location, reducing moisture to as low as 6.3% is possible anywhere by use of wood heat and solar drying. Frequently, ear corn is tied by the husk and hung above a fire in a building for drying and preservation from the heat and smoke.

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*all moistures are percent wet basis

Maize deterioration

Deterioration includes the quantitative and qualitative losses associated with stored maize, due to the activities of biological, chemical and physical contaminants, leading to a decrease in value. Common indicators of deterioration are loss of dry matter, changes in color, increase in fines and broken kernels, presence of molds and mycotoxin, decrease in commercial grade, decrease in nutritive value, objectionable odor, decrease in palatability, increase in temperature, visible insects or insect-damaged grain, increase in moisture content, visible insect excreta, sprouting and decrease in viability or germination rate (Bern, *et al*., 2008)

Drying maize to below 14% moisture is recommended for preservation in East Africa (IRRI, 2006), while drying below 12% moisture inhibits development of most insects, and most do not survive at <8% moisture (FAO, 1994).

Maize grain may be held safely in cool storage (6-10°C) for up to a year, but eventual transfer to warmer conditions can create a resurgence of the temperature-suppressed infestation. However, chemical pest control, in on-farm maize storage is costly, toxic, and increases risk of weevil resistance to pesticides (Wohlgemuth, 1989; FAO, 1994).

Weevil and Mold Activity

Tropical heat, moisture and open air storage promote rapid insect multiplication, grain germination, and mold formation in stored maize (FAO,1994; Markham, 1994). And rapid insect development occurs within a fairly narrow range of 5-10 °C around

an optimal temperature, which for most storage insects, is in the region of 25-35°C (FAO, 1994; IRRI, 2006).

The extent of quantitative and qualitative losses produced by *S. zeamais* (FAO, 1985), is dependent on maize genotype, grain texture, and pericarp characteristics. Overall, 20-30% of Ethiopian stored maize kernels is lost to *S. zeamais* infestation, while 100% maize damage has been found in maize stored for 6-8 months in the Bako region of the country (Demissie *et al*., 2008a). Mulungu *et.al*., (2007) found about 17.51% weevil damage to the shelled maize kernels in research involving stored maize, in Tanzania. Demissie *et al*. (2008b) also found 11-59% levels of weevil infestation in husk covered maize stored at Bako, Ethiopia, in a separate study involving a count of the number of adult weevils per ear following one month of storage.

Control of insects in maize

The control of insects in stored maize requires an integrated approach involving an understanding of grain chemistry, and its interaction with temperature and moisture. Procedures such as drying, mechanical isolation, refrigeration, chemical treatment, and ionizing radiation are commonly employed in varying combinations for insect control.

Hermetic Storage

Hermetic storage is storage that prevents contact between stored product and external atmosphere, and where respiration within the storage ecosystem causes O_2 reduction and CO_2 accumulation. It causes suffocation and dehydration of weevils

(Navarro *et al*.,1994) and physical disturbance of maize grain (Joffe, 1963) when used along with hermitic storage can dislodge weevils from the kernel, discourage feeding, and cause weevil mortality.

A study by Moreno-Martinez et. al., 2000 utilized maize grain of hybrid AN 447, infested with 20 unsexed *S. zeamais*, stored within storage flasks (250 mL glass containing 150 g of maize) as well as oxygen analyzers, to monitor the oxygen concentration, and insect mortality.

The jars were stored for 30 days at 26^{O}C, 16% moisture, 70% r.h., and 18±6 h L-D photoperiod, and maize weevil mortality was recorded at 3-day intervals, by checking 12 jar replicates each of hermetic and non-hermetic sample. They found that oxygen was depleted to 0% in 6-9 days in the hermetic treatments, while it decreased to 8.4% after 30 days, in the non-hermetic treatment.

The rate of oxygen depletion in treatments containing weevils was more rapid than those containing fungus and maize alone, while treatments with fungus alone had half the utilization rate. Treatments with maize alone had much lower oxygen utilization rate.

Plastic bagging system

Plastic bagging employs several layers of air-tight and water-tight PVC and polyethylene bags, within which agricultural produce is stored, hermetically. Murdock currently employs heavy-duty triple-layer bags in the hermetic preservation of cow peas, in Central and Western Africa. The triple bagging procedure requires that each of the three bags be tied separately within each other, and at a one-time cost of $3

per household, this storage method has the potential to increase household income on average by about $150 per year (Carroll, and Fulton, 2008; Murdock *et.al*., 2003).

Steel and plastic containers

In addition to ferrocement, 55 gallon steel and plastic barrels are excellent candidates for cheap hermetic storage in the East African sub-region. Lindblad and Druben (1980) and Adhikarinayake (2005) described the use of empty oil drums, filled to the brim, for hermetic storage. These storage methods also provide mechanical isolation from rodents.

However, the barrels are usually contaminated by petro-chemicals, and need to be properly cleaned, to prevent cross contamination of maize stored within the barrels. Common methods for determining types of petro-chemicals present and for measuring level of contamination involve the use of gas chromatography, followed by the use of methanol for cleaning (Turriff, *et. al*, 1998). The use of soaps available in the local culture, for cleaning is common practice, although the efficacy of this method of cleaning or decontamination is unknown.

This study is aimed at providing empirical data at multiple temperatures and maize moistures that can be applied to design of effective hermetic maize storage system, under East African conditions. The goal of the system is to minimize or eliminate weevil damage, without using pesticides.

OBJECTIVE

The objectives of the research were to determine the effects and interaction of factors such as time, oxygen level, temperature, and maize moisture on the survivability of the maize weevil.

METHODS AND MATERIALS

A laboratory scale hermetic storage system was used, where weevil, mold, and maize respiration serve as an effective pest control strategy in stored maize. The research is an empirical study of weevil biology and its interaction with storage chemistry and grain properties, to establish their relationships with weevil mortality, in hermetic storage. It also employed the use of instrumentation, for the quantification of oxygen levels within the hermetic storage system, to measure the integrity and applicability of the hermetic storage system.

Treatment conditions represent different extremes of temperature (10 and 27^{O}C) and moistures (6.0-8.0%, 16%) known to impact maize weevil growth and other maize storage organisms. The treatment assignment to jars and chambers was done using PROC GLM (SAS Institute Inc.,100 SAS Campus Drive, Cary, NC 27513).

Experimental maize

Maize grain of the commercial hybrid Fontanelle *6T672* was harvested on 11/1/07 using a 4420 Deere combine. Following harvest it was cleaned to remove broken maize and foreign material and was stored at about 16.5% moisture and 4^{O}C until use.

Maize drying

Target moistures of 6.3%, 8% and 16% were chosen as moisture extremes, for this research. Moistures were confirmed using the oven test method, by exposing triplicate 30-g samples of maize to a103 ^{o}C oven for 72 hours (ASABE, 2008). Results from the oven test were 6.3% and 16%, and 8% and 16%, for the storage and oxygen quantification, respectively. A Boerner grain divider was employed in obtaining statistically representative maize quantities, for each experiment.

Stored maize was dried at the Iowa State University Department of Agricultural and Biosystems Engineering, Biomaterials Laboratory, using a small laboratory drier after harvest. Natural air was utilized for drying to 16% and 45^{O}C air for drying to 6.3%.

Experimental weevils

A stock culture of 100 adult *S. zeamais Motschulsky* (both sexes), were obtained from the Iowa State University Entomology Departmental laboratory and placed in five unsterilized 3.74-L glass jars, containing 16.5% moisture Fontanelle 6T672 maize.

The weevils were allowed to oviposit on the maize to develop a colony. This was achieved by placing them in jars covered with mesh screens and placed in a rearing chamber at about 27^{O}C at interstitial relative humidity determined by maize moisture (about 16.5%), for two months (Arannilewa,et. al., 2006). Weevils from this colony were used in the hermetic storage and oxygen quantification studies.

Experimental chambers

Two chambers maintained at 10^{o}C and 27^{o}C, respectively, were utilized in the experiments. The chambers are model 13-988-126 GW Fischer Scientific Isotemp (type R-12) refrigeration chambers (Thermo Fisher Scientific Inc., Waltham, MA 02454), with adjustable temperature controls.

Experimental containers

One-pint (473-mL) Kerr canning jars (Mason Jar 61000, Jarden Home Brands, 14611 W. Commerce Road, Daleville, IN) were utilized in both the weevil mortality and oxygen quantification experiments. In the weevil mortality experiment, each treatment jar was loaded with 350 g of maize sample and 30 weevils. In the oxygen quantification experiment, 90 weevils were loaded into each canning jar along with about 185 g of maize, at the appropriate moisture levels.

For the hermetic conditions, the Ball (Kerr) canning jars were used as is. For non-hermetic tests, solid lid inserts were replaced by aluminum screens, which allowed air passage, but not weevil escape.

Weevil mortality study

Objective

The objective of the study was to determine the effect of temperature, moisture and their interaction on weevil mortality, under hermetic and non-hermetic conditions.

Experimental design

The experimental design consisted of four factorials (time, maize moisture, temperature, and replication), with weevil mortality being the dependent variable. Days had five levels (2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 10^{th}), maize moisture had two levels (6.3% and 16%), temperature had two levels (10^{O}C and 27^{O}C), and replications had four levels. It is based partly on Moreno-Martinez *et al*., (2000), but ultimately on trial runs in which all the weevils stored in maize at 16% moisture and 27^{O}C, hermetic, died within a week.

The experimental setup employed 16 treatment jars per chamber, 2 chambers, and four replications, with each of the 128 treatment jars containing 30 weevils and 350 g of maize.

Each replication had a total of 16 treatments (10 hermetic and 6 non-hermetic) assigned to each of the 10^{o}C and 27^{o}C (Wohlgemuth, 1989; Evans, 1987) chambers. The hermetic had five levels of day and the non-hermetic had 3 levels of day, while both had two levels of maize moisture (6.3% and 16%).

Weevil mortality count was designed to be done every other day, for a period of at least one week. A redundancy was built into the design by adding an extra day of count (10^{th} day), in case the weevils at 27^{O}C did not all die by the 8^{th} day. This is because the storage experiment was designed primarily to simulate temperature in most of East African (10^{O}C and 27^{O}C), as well as the optimum temperature for weevil development.

Dead weevil features

The criteria for determining weevil mortality relied on a combination of observed common rigor mortis features. Weevils that were curled up and or had legs outstretched; lying on their side or back; immobile; unattached to maize kernels; found to flow with kernels when jar was tilted; and hard to the touch was assumed dead, especially if they retained these features when exposed to ample air supply.

Procedure

To determine number of weevil deaths, each jar from the 16 treatments (T_1-T_{16}) was examined for dead weevils on the day to which it was randomly assigned and its content was discarded. The hermetic treatment counts were, therefore, done on days 2, 4, 6, 8, and 10, while the non-hermetic treatment counts were done on days 2, 6, and 10. The number of dead weevils were recorded, from the counts and utilized in the final statistical analysis, to test for the hypothesis of a difference in weevil mortality for different temperatures and moistures, under hermetic and non-hermetic conditions.

Oxygen quantification study

Objective

The objective of this experiment was to determine oxygen depletion under different maize moisture, temperature, and hermetic storage relationships.

Experimental design

Procedure

The oxygen quantification system consisted of the environmental chambers, the Kerr storage units, and the oxygen analyzer with its data acquisition system. The data acquisition system consisted of a computer and microcontroller used for the graphic user interface (GUI) and data acquisition.

Ninety weevils were loaded into each of the Kerr hermetic canning jars along with about 185 g of maize, at the appropriate moisture levels. The jars, which were connected to a model 65 oxygen sensor (AMI, 18269 Gothard Street, Huntington Beach, CA 92648), a PMD 1408FS DAC system and a computer were randomly assigned to the environmental chambers, for oxygen quantification. A liquid-in-glass thermometer, mounted on a rubber stopper was used to monitor the temperature, and data analysis was done using PROC GLM, PROC MIXED (SAS Institute) at the $P>0.05$ significance level.

Statistical Analysis

The research found significant mean and statistical differences between hermetic vs. non-hermetic, 6.3% vs.16% moisture, and 10ºC vs. 27ºC at both moistures (Table 2.3) by comparing weevil mortality, for the 10^{th} day of study #1.

Table 2.3: Statistical factors comparison

		Storage type comparison	
		Hermetic vs. non hermetic	
Storage type			
Mean difference		55.63	
P-value[b]			<.0001
Stat. Diff.?[c]			Yes
Moisture comparison			
		6.3% vs. 16%	
Moisture			
Mean difference[a]		7.71	
P-value[b]			0.0035
Stat. Diff.?[c]			Yes
Temperature comparison			
		10°C vs. 27°C	
Mean difference[a]	**6.3% Moisture**	35.42	
	16% Moisture	48.33	
P-value[b]	**6.3% Moisture**		<.0001
	16% Moisture		<.0001
Stat. Diff.?[c]			Yes

[a] Based on percent mortality
[b] Based on two-tailed, unpaired t-tests
[c] Statistical difference at the 95% confidence interval (α=0.05)

RESULTS AND DISCUSSION

Weevil mortality results

The first study was designed to determine the effects of common tropical conditions on weevil mortality. It utilized the effect of 10 and 27°C temperature, and maize at 6.3 and 16% moisture, under hermetic and non-hermetic conditions, with

replication. The research tested the hypothesis that hermetic storage system is effective, for post-harvest weevil control, in on-farm maize preservation in East Africa. The analyses at 10^{o}C and 27^{o}C were done separately, because of the significant interaction observed between moisture and temperature test factors.

The results (Figure 2.1 and 2.2) indicate 100% weevil mortality for both moistures after six days at 27^{o}C, and 28% maximum mortality for both moistures at 10^{o}C under hermetic conditions (complete data is found in appendix). The test of significance at 27^{o}C (P=<.0001) and 10^{o}C (P=0.0004) supports the hypothesis of a slight difference in weevil mortality for the different temperature and moisture treatment combinations.

The error bars in figure 2.1 indicate the precision of the mean weevil mortality values at the various storage times, given the combined temperature, moisture and hermetic effects. Error bars are, however, not shown for figures 2.2-2.4 for brevity.

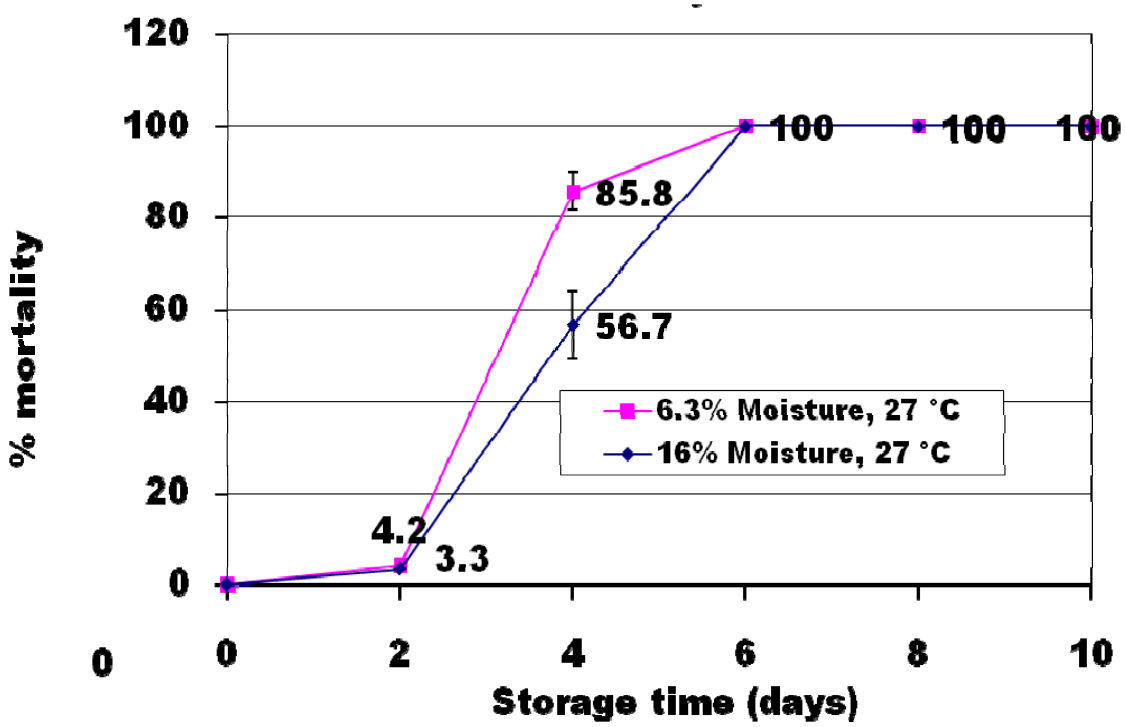

Figure 2.1: Mean mortality of *S. zeamais* hermetic storage at 27 °C

The test also provides evidence in support of the hypothesis of the efficacy of hermetic storage, especially considering that weevil mortality increases over time in the 10^{o}C treatments (Figure 2.2). The oxygen quantification research was designed to further investigate all the temperature and moisture combinations, under hermetic conditions.

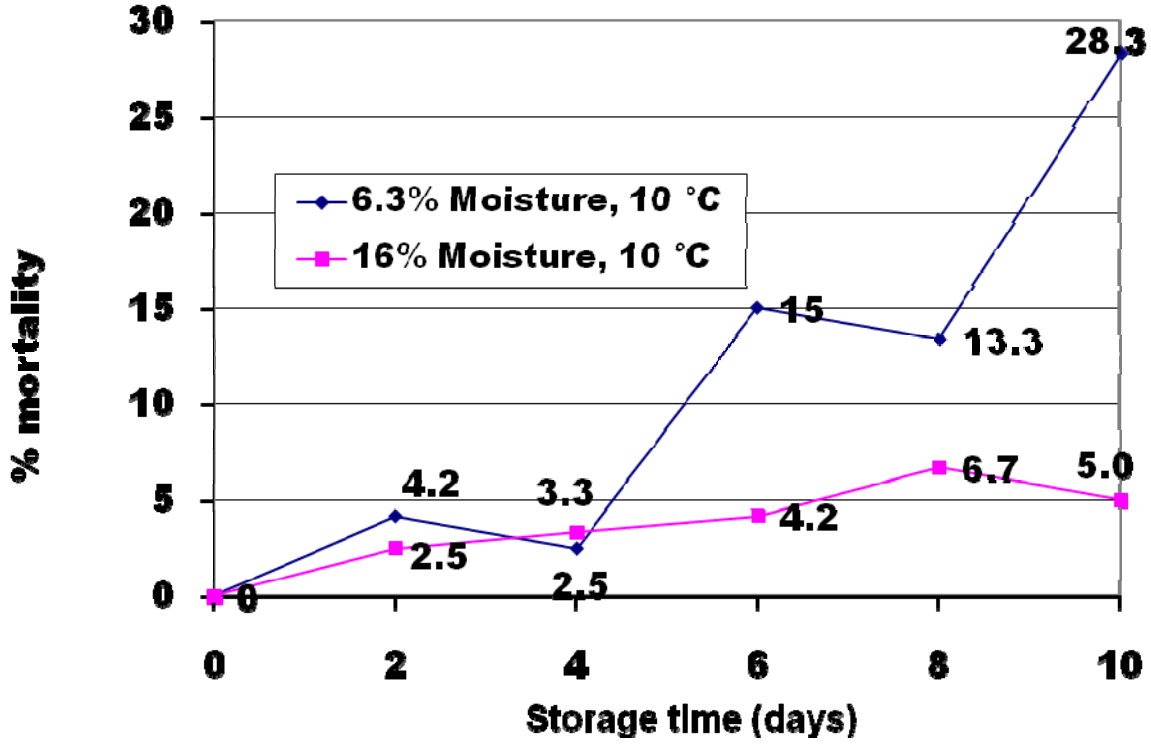

Figure 2.2: Mean mortality of *S. zeamais* hermetic storage at 10°C

Figure 2.3 and 2.4 indicate a low non-hermetic mortality rate (7.5% maximum). The slight significance in mortality rates (P=0.0345) at 27^{o}C and (P=0.0471) at 10^{o}C also accounts for a difference in treatment effect under non-hermetic conditions. And the comparison of the test of significance under hermetic (P=<.0001; 0.0004) and non-hermetic conditions (P=0.0345; 0.0471) further provides proof of the efficacy of hermetic storage when compared to non-hermetic storage. Although error bars are not displayed in figure 2.3 and 2.4, there is a higher level of variability in mortality for non-hermetic treatments.

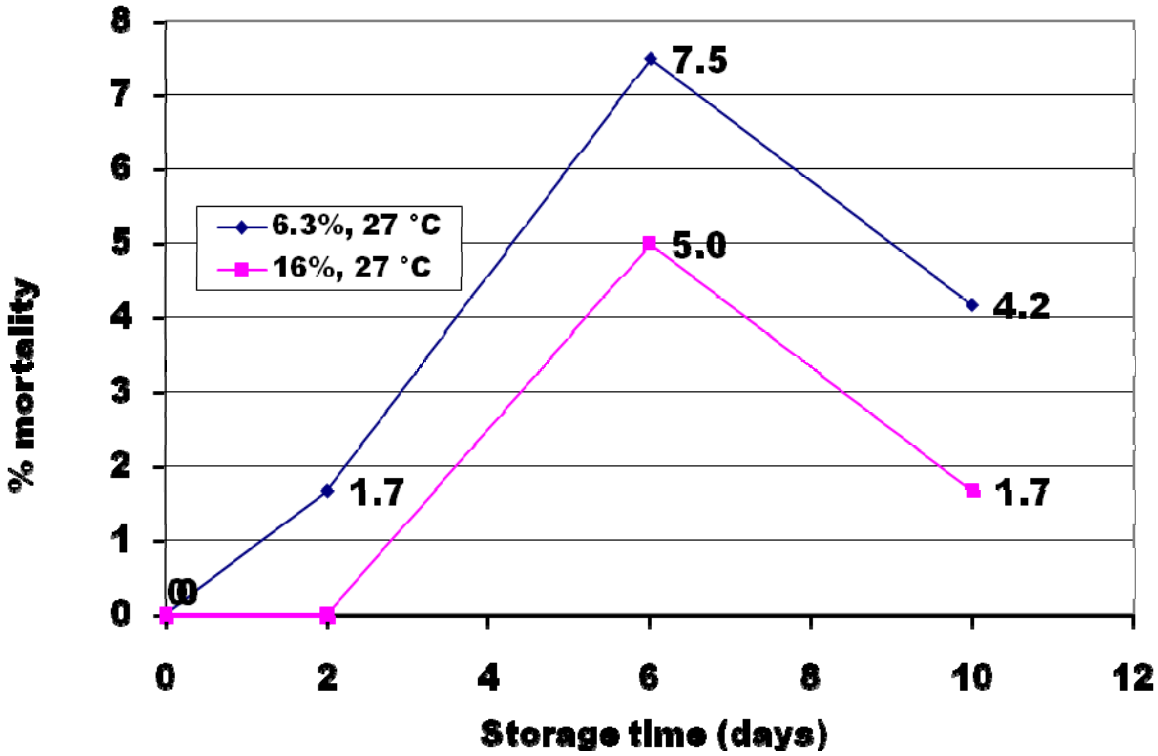

Figure 2.3: Mean mortality of *S. zeamais* non-hermetic storage at 27 °C

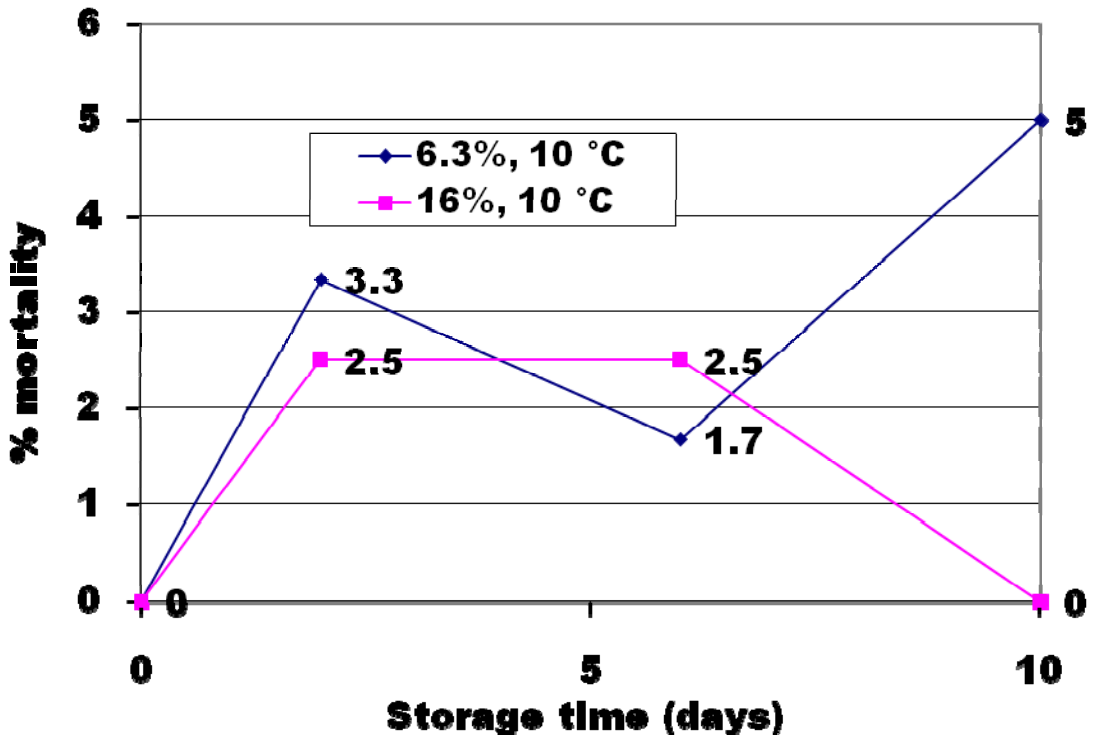

Figure 2.4: Mean mortality of *S. zeamais* non-hermetic storage at 10°C

Temperature and weevil respiration

Wohlgemuth (1989) suggested that insects and fungi of stored products are inactive at 10°C and below, but cause substantial damage at temperatures up to 35°C. Hence, losses of greater than 30% of maize stored on farm, by subsistence farmers are common (Tigar *et al*., 1994), under tropical and subtropical conditions.

Oxygen quantification results

The oxygen quantification results (Figure 2.5 and 2.6) indicate oxygen depletion, and the triplicate replication results indicate that 100% weevil mortality level is achievable at both 10 and 27^{o}C (complete data is found in appendix). However, the mortality rate is higher at 27^{o}C, since oxygen depletion is faster at that temperature

(IRRI, 2006). The weevil oxygen curves are therefore, characteristic of what is expected, for hermetic storage.

Figure 2.5 and 2.6 show the results obtained at 10°C for 8% and 16% moisture. The error bars tend to increase as percent oxygen decreases and mortality approaches 100%. The higher variability seems to be dependent on decreased sample size and oxygen levels, over time, rather than a decrease in mean precision, since standard error calculation is based on standard deviation over the square root of the sample size:

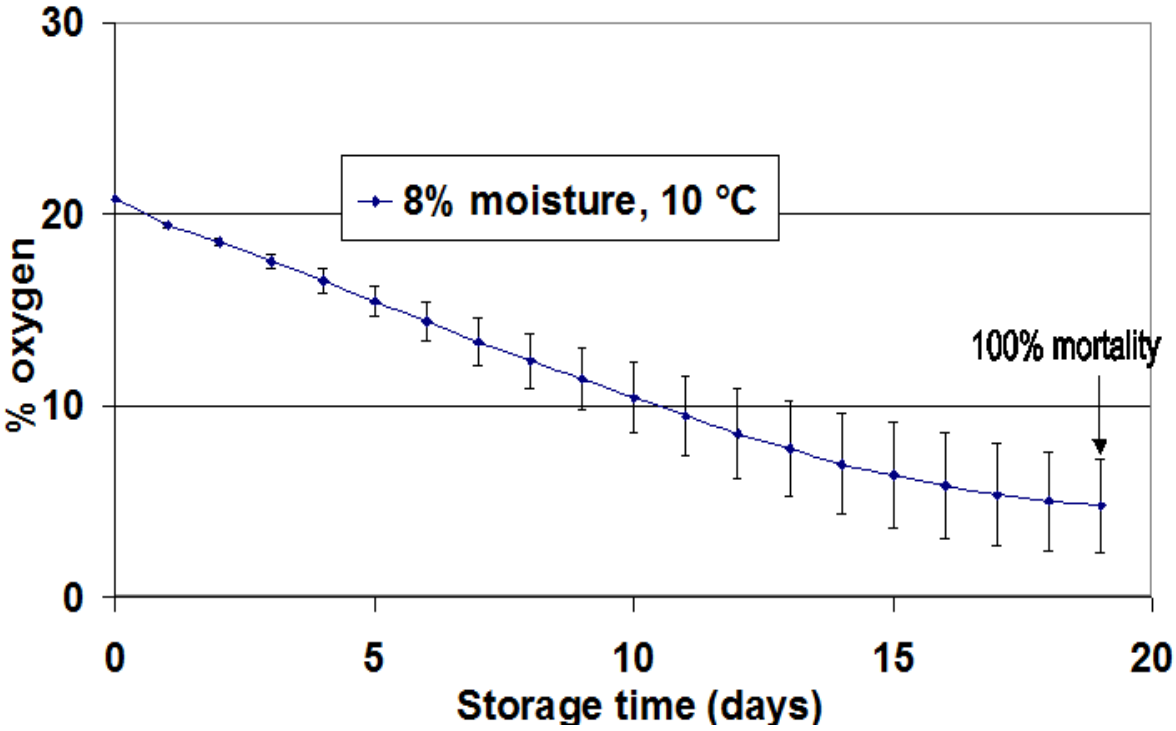

Figure 2.5: Average percentage oxygen for three replications at 8% maize moisture and 10°C

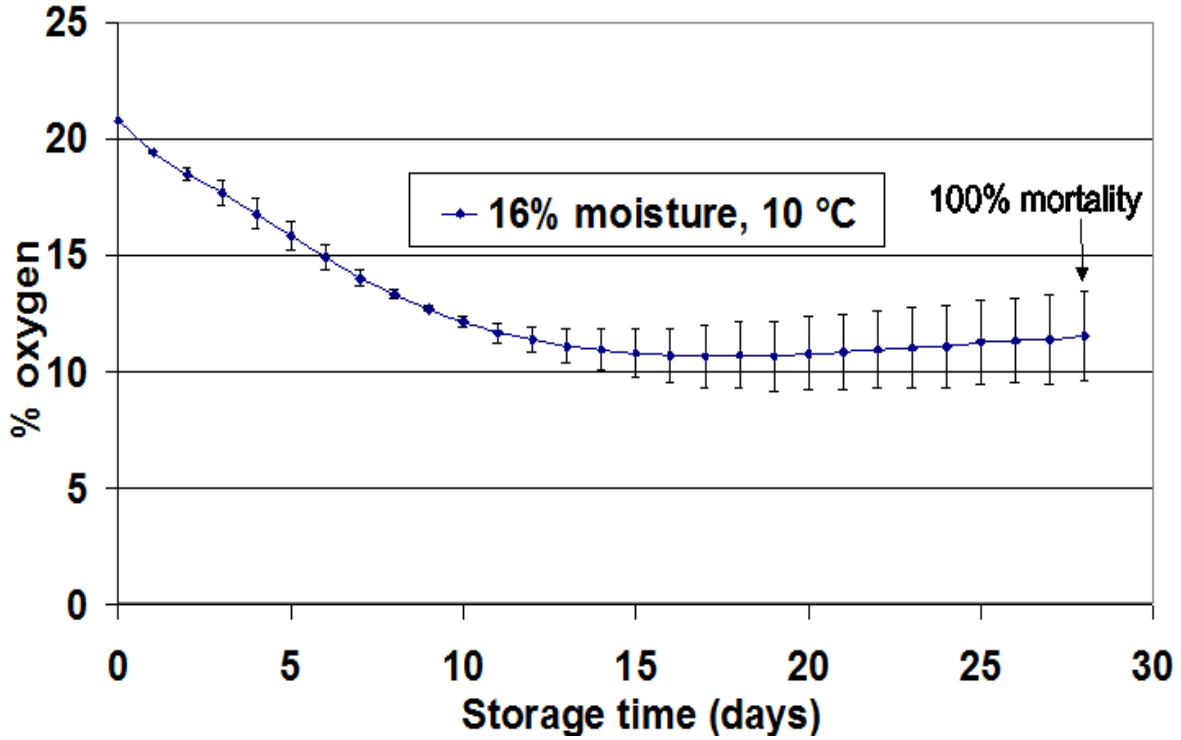

Figure 2.6: Average percentage oxygen for three replications at 16% maize moisture and 10°C

Figure 2.7 and 2.8 shows the results obtained at 27°C for 8% and 16% moisture.

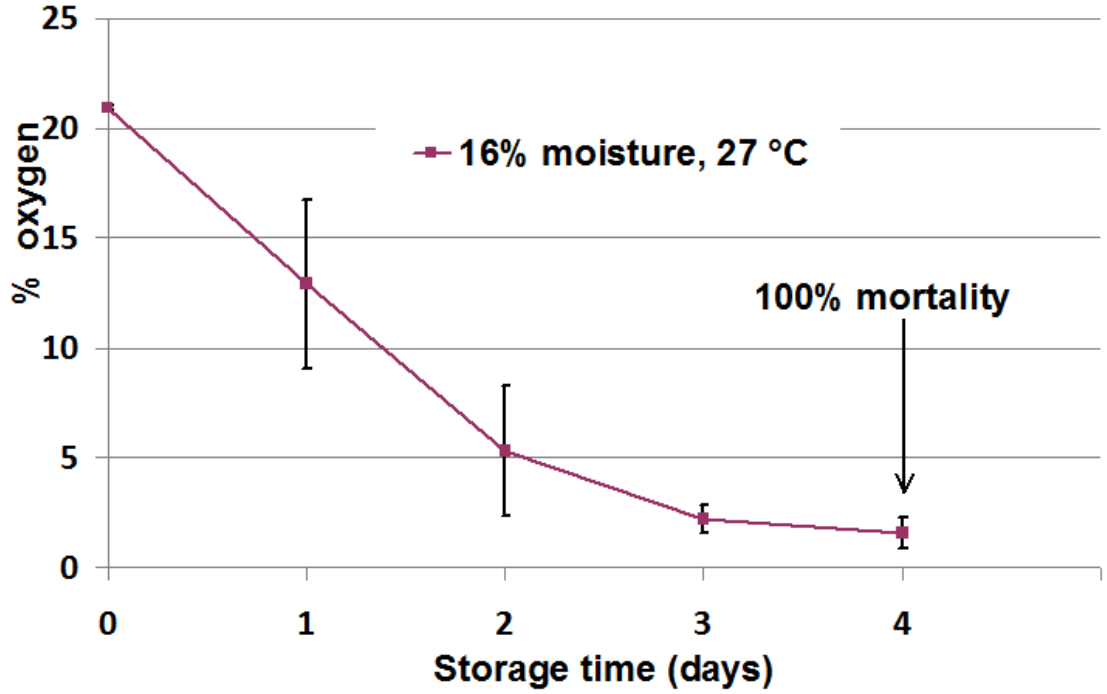

Figure 2.7: Average percentage oxygen for three replications at 16% maize moisture and 27°C

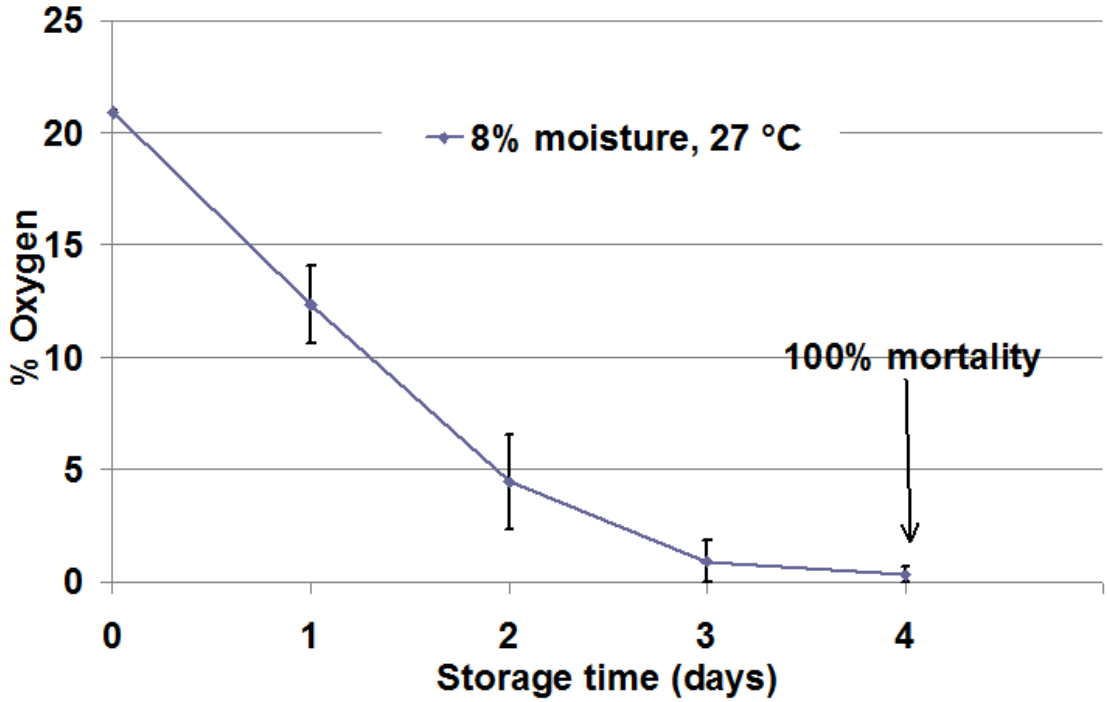

Figure 2.8: Average percentage oxygen for three replications at 8% maize moisture and 27°C

Table 2.4 also describes storage conditions and time at which 100% weevil mortality occurred.

Table 2.4: 100% weevil mortality conditions and maize densities

Storage Time (days)	Conditions	Kernel density, g/cm^3
28	16% moisture and 10 ^{O}C	1.26
19	8% moisture and 10 ^{O}C	1.24
4	16% moisture and 27 ^{O}C	1.26
4	8% moisture and 27 ^{O}C	1.24

The graphs agree with literature (Moreno-Martinez, *et al.,* 2000), since they have a downward, left to right trend and mortality occurs below 10% oxygen, except in cases of adaptation to hypoxia observed at low temperatures (10 ^{o}C). Mortality was also observed to be faster at 27 than at 10^{o}C.

Weevil adaptation to hypoxia

Hypoxia is a condition in which body tissue is starved of oxygen. Donahaye (1990) described insect adaptation to hypoxia, and the occurrence of anaerobic or partial anaerobic respiration in animals, overworked muscles, and infarcted heart muscle cells. It is a cellular last resort for energy, and animals or insect tissue cannot maintain anaerobic respiration for an extended length of time.

Based on this research results, adaptation to hypoxia is more likely to occur at low temperature (10^{O}C) and is more pronounced at higher moisture (16%), where adaptation can occur at any oxygen level (Saldıvar, et.al.,2003), indicating a

moisture effect. The fact that maize is more hygroscopic at lower moisture than at high moisture may be responsible for faster weevil mortality at the lower moisture (8%), reducing the level of hypoxic effect noticeable at low moisture compared to 16%. Weevil count following oxygen quantification, however, indicates 100% weevil mortality in all cases, although there are high variations in time to mortality at $10^{O}C$ and moisture, compared to that noticeable at $27^{O}C$. Weevils at lower moisture (8%) died sooner than the ones at high moisture (16%), in all cases.

When insects of stored products exist under refrigeration conditions, they go into hibernation and re-establish normal body functions when exposed to warmer temperatures (FAO, 1994). And under hermetic conditions, maize weevil metabolism and reproduction cease, especially at low temperatures (IRRI, 2006).

Common types of hypoxia include hypoxemic, anemic, stagnant, and histotoxic hypoxia. These involve situations of decreased oxygen, low hemoglobin count, insufficient blood flow, and tissue's inability to use O_2, respectively. Anaerobic respiration often occurs, during hypoxia, resulting in the production of hydrogen as a by-product. And oxygen analyzers, such as the one used in this research often employ zirconium, a transition element which is capable of forming oxides and hydrides.

Pycnometry results

Pycnometry was performed using Accupyc 1330 (Micromeritics, Gosford, New South Wales, Australia 2250), to obtain the kernel (particle) density, employed in obtaining the fraction of void that was utilized in calculating oxygen consumption per weevil. Kernel density was adjusted to 6.3% and 16% using the procedure described by Dorsey-Redding *et al*. (1989).

Calculation (sample):

Using triplicate samples and pycnometry, particle density for the maize was determined to be 1.26 g/cm^3. The weevil oxygen consumption per day at different moisture and temperature combinations was then calculated using the oxygen curves, pycnometry and fraction of voids (Figure 2.9; Table 2.4).

Predicting time for mortality

Figure 2.9 shows cm^3 weevil^{-1} day^{-1}, at the temperatures and moistures utilized in study #2.

1) 10% moisture at 20^{O}C =0.114 cm^3 weevil^{-1} day^{-1}, by linear interpolation (Appendix H) from Figure 2.9

Days to mortality=

$$\frac{((\text{y cm}^3(\text{O}_2)))}{\left(\#weevils * \text{x}\ \left(\text{cm}^3\ \text{weevil}^{-1}\ \text{day}^{-1}\right)\right)} \text{----------(2-1)}$$

Total air (vol) =

$$(((\frac{\%bulk}{100}) * (\frac{\%fill}{100}) * (container(vol)) + ((\frac{\%headspace}{100}) * container(vol))) \text{----------(2-2)}$$

% = O_2=20.99%

Therefore,

Total Vol (O_2) = Total air (vol)*0.2099

Weevil oxygen utilization (cm^3 $weevil^{-1}$ day^{-1}) =

$$\frac{0.2099(((\frac{\%bulk}{100})*(\frac{\%fill}{100})*(container(vol))+((\frac{\%headspace}{100})*container(vol)))}{(\#weevils*\#days))} \text{-------(2-3)}$$

Given 250 mL container with 20 weevils and 100% mortality at 6 days (Moreno-Martinez *et. al*., 2000), and assuming 90% fill, and 20.99 initial oxygen levels, weevil oxygen utilization rate is 0.20 cm^3 $weevil^{-1}$ day^{-1} (equation 2-3).

$$\%\ bulk= \frac{((\#weevil*\#days*(xcm^3\ \ weevils^{-1}\ day^{-1})-(O_{2\max}*container(vol))}{\left(O_{2\max}*\frac{\%fill}{100}*container(vol)\right)}*100 \text{ ----(2-4)}$$

Vol (air)=41.7% of maize bulk at 10% moisture, and 20 °C, using equation 2-4,

For 55 gallon barrel,

Diameter=22.5” Height=34.5”

Vol=$\pi r^2 h$=13717.47 in^3 =224789.06 cm^3

1bu. Maize=1.245 ft^3, and

13717.47 in^3 =7.93835 ft^3

One 56 lb bu.=25.40 kg maize

bu. Maize= (7.93835/1.245)

kg maize = #bu. Maize*25.40 kg

= 161.96 kg

Given: 200 weevils/kg

Total # weevils=161.96*200 = 32392 weevils

At 10% moisture and 20^{O}C,

Days = $\frac{(0.2099((0.427*224789.06)+(0.1*224789.06)))}{(32392*0.114)}$

=61 days (to 100% weevil mortality)

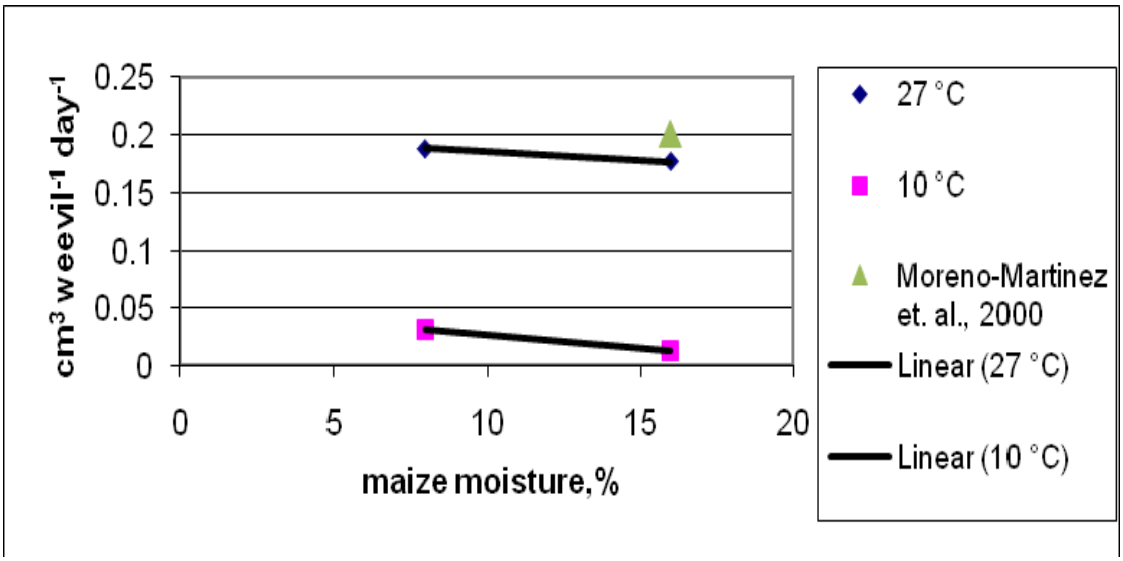

Figure 2.9: Average oxygen consumption of maize weevils in shelled maize

CONCLUSIONS

The hermetic storage, oxygen quantification and pycnometry studies show proof of concept, for the individual concepts and in support of each other that hermetic storage is effective for weevil control, in stored maize.

Significant differences were found in mean weevil mortality across days, temperatures and moistures. The research determined a high level of temperature, oxygen level, days and moisture effect on weevil mortality. It noticed a high level of interaction between moisture and temperature, and determined that hermetic storage is lethal to *S. Zeamais* survival, overall.

The study also succeeded in using the laboratory, jar-storage setup to demonstrate the efficacy of hermetic storage, especially for situations were maize storage is long-term and the maize container or store is filled to the brim.

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CHAPTER 3

GENERAL CONCLUSIONS

Storage Study

The difference in weevil mortality, between hermetic and non-hermetic maize storage is significant.

Real life hermetic storage employs processes that protect the storage container from direct sunlight, to reduce moisture condensation within the system. In practice, this is done by painting the storage exterior with a reflective paint and or placing the container under shade, especially one covered by earth mound, to provide cooling.

The focus of this research was to describe hermetic storage of maize for food purposes, especially because in my experience maize seeds are usually stored differently from maize utilized for food purposes. Besides, the bulk of stored maize in Africa and East Africa is for food purposes and the seeds are often bought from seed companies during planting seasons.

Seeds undergo dormancy, which allows them to remain viable, but metabolically inactive, under unfavorable conditions until favorable conditions for germination are reintroduced. This means that even when seeds are sourced from harvested maize preserved under hermetic conditions, a significant percentage will still be viable following several years of hermetic storage.

In general, drying to <8% moisture reduces maize respiration, and speeds up weevil mortality. When hermetic containers are filled to the brim under these

conditions, little or no moisture is given off through respiration, making moisture condensation and the resulting mold formation insignificant.

Oxygen Quantification/Weevil curves

Oxygen quantification provides an effective, indirect, and non-destructive means of monitoring weevil activity. The curves also provide proof of the efficacy of hermetic storage as an effective non-chemical weevil control system.

Interestingly, there is a high level of interaction between day, temperature and moisture, especially at 10 ^{O}C. The interaction between temperature and moisture can lead to possible insect adaptation to hypoxia, as seen in the result obtained for oxygen quantification at high moisture (16%) and 10 ^{O}C.

Since, not all such temperature and moisture combinations produce adaptation this readings sometimes are outlier, especially when weevils show adaptation for storage times that are much longer than most other readings under the similar conditions.

Seasonal temperature fluctuations can occur in some refrigerated chambers. It might therefore be necessary to construct a refrigerated chamber, with better thermostat control of the temperature or adjust original refrigeration thermostat, to accomodate this variation.

Future research

To demonstrate, the concept of hermetic storage and oxygen quantification, the jars were only filled to about 90% and 50% respectively. However, in practice, the

storage containers are usually filled to the brim, to speed up weevil mortality, and maize dried to below 12% (especially 6-8%) moisture are for storage.

Future research would dry maize to not less than 6%, to preserve viability for seed purposes. And it would conduct a germination test, following the storage period, to determine seed viability.

To obtain a common value or graph statistically representative of the weevil population, the oxygen quantification may need to be done 30 times or more times, in order to reduce the variance between samples, and hence the standard error of the sample measurements.

This is because, individual jars, had different mix of weevils of different sizes, sexes, body weight, and other possible attenuating factors. Outlier values (adaptation to hypoxia) may need to be removed from the average, to reduce error, through increased sample size. The corrected research data can then be utilized for pycnometry calculations that can be applied to any hermetic storage container.

Other future laboratory research will involve testing the hermetic properties of 55 gallon barrels (epoxy lined, and unlined), through pressure tests and oxygen depletion tests. Hermetic properties of Grain Pro's 60 kg "super grain bag" polybags will also be investigated, and the combined results of these laboratory tests will ultimately be applied to field tests, in East Africa, ands the findings will be disseminated to farmers in an agricultural extension setting.

APPENDIX A: VB SOFTWARE AND GRAPHIC USER INTERFACE (GUI)

Visual basic code

```
Public PastHour
Dim DataValue(10), vdc(10)
Public x, x1 As Integer
Dim ULStat As Integer
'Dim DataValue As Short
Private Sub Command1_Click()
'ulStart = cbDeclareRevision(CURRENTREVNUM)
'ulStart = cbErrHandling(PRINTALL, DONTSTOP)
ULStat = cbDConfigPort(1, FIRSTPORTA, DIGITALOUT)
ULStat = cbDConfigPort(1, FIRSTPORTB, DIGITALOUT)
    Timer1.Interval = 1000
    x = 0
    Timer1.Enabled = True
ULStat = cbDOut(1, FIRSTPORTA, 0)
ULStat = cbDOut(1, FIRSTPORTB, 0)
    Text12.Text = Hour(Now)
     Text3.Text = Now
     PastHour = Hour(Now)
 Open "C:\Documents and Settings\Biomaterials_LabWork\Desktop\data1.txt" For
Append As #1
```

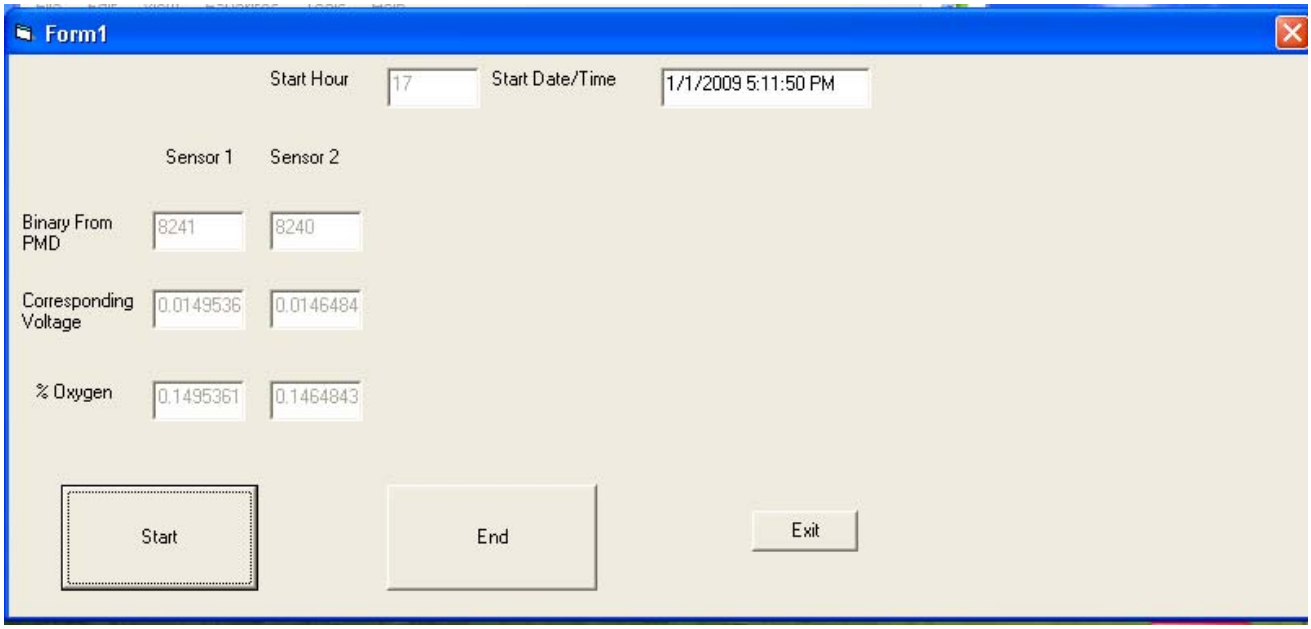
```
  Open "C:\Documents and Settings\Biomaterials_LabWork\Desktop\data2.txt" For
Append As #2
Print #1, "Voltage; ", "; PercentageOxygen; ", ";Tab(40) Date;"
Print #2, "Voltage; ", "; PercentageOxygen; ", "Tab(40); Date;"
End Sub
Private Sub Command2_Click()
    Timer1.Enabled = False
     Close #1, #2
  End Sub
Private Sub Command3_Click()
 End
End Sub
Private Sub Timer1_Timer()
      ULStat = cbAIn(1, 0, BIP2PT5VOLTS, DataValue(0))
      ULStat = cbAIn(1, 1, BIP2PT5VOLTS, DataValue(1))
      'ULStat =cbAIn(Board, Channel, Range, DataValue)
 If (Hour(Now) = PastHour + 1) Then
    Text1.Text = DataValue(0)
    Text2.Text = DataValue(1)
    vdc(0) = ((DataValue(0) / 2 ^ 14) * 5 - 2.5)
    vdc(1) = ((DataValue(1) / 2 ^ 14) * 5 - 2.5)
    Text5.Text = vdc(0)
    Text6.Text = vdc(1)
```

```
    Text10.Text = ((vdc(0) * 10))
'If Val(Text10.Text) = 0 Then Exit Sub
    Text11.Text = ((vdc(1) * 10))
'If Val(Text11.Text) = 0 Then Exit Sub
     Print #1, Text5.Text; Tab(22); Text10.Text; Tab(43); Now  'Text10.Text & " " &
     Text5.Text 'Print String (DataValue(0), "+" Now)
     Print #2, Text6.Text; Tab(22); Text11.Text; Tab(43); Now  'Text14.Text & " " &
     Text6.Text "Print String (DataValue(1), "+" Now)
      PastHour = Hour(Now)
  If PastHour <= 22 Then PastHour = PastHour + 1
Else:
 x1 = x + 1
 If Hour(Now) = 0 And x = 2 Then
    Text1.Text = DataValue(0)
    Text2.Text = DataValue(1)
    vdc(0) = ((DataValue(0) / 2 ^ 14) * 5 - 2.5)
    vdc(1) = ((DataValue(1) / 2 ^ 14) * 5 - 2.5)
    Text5.Text = vdc(0)
    Text6.Text = vdc(1)
    Text10.Text = ((vdc(0) * (10)))
    'If Val(Text10.Text) = 0 Then Exit Sub
    Text11.Text = (((vdc(1) * (10))))
    'If Val(Text11.Text) = 0 Then Exit Sub
```

```
    Print #1, Text5.Text; Tab(22); Text10.Text; Tab(43); Now
    Print #2, Text6.Text; Tab(22); Text11.Text; Tab(43); Now
  End If
      DoEvents   ' Yield to other processes.
 PastHour = Hour(Now)
    End If
End Sub
```

Graphic user interface

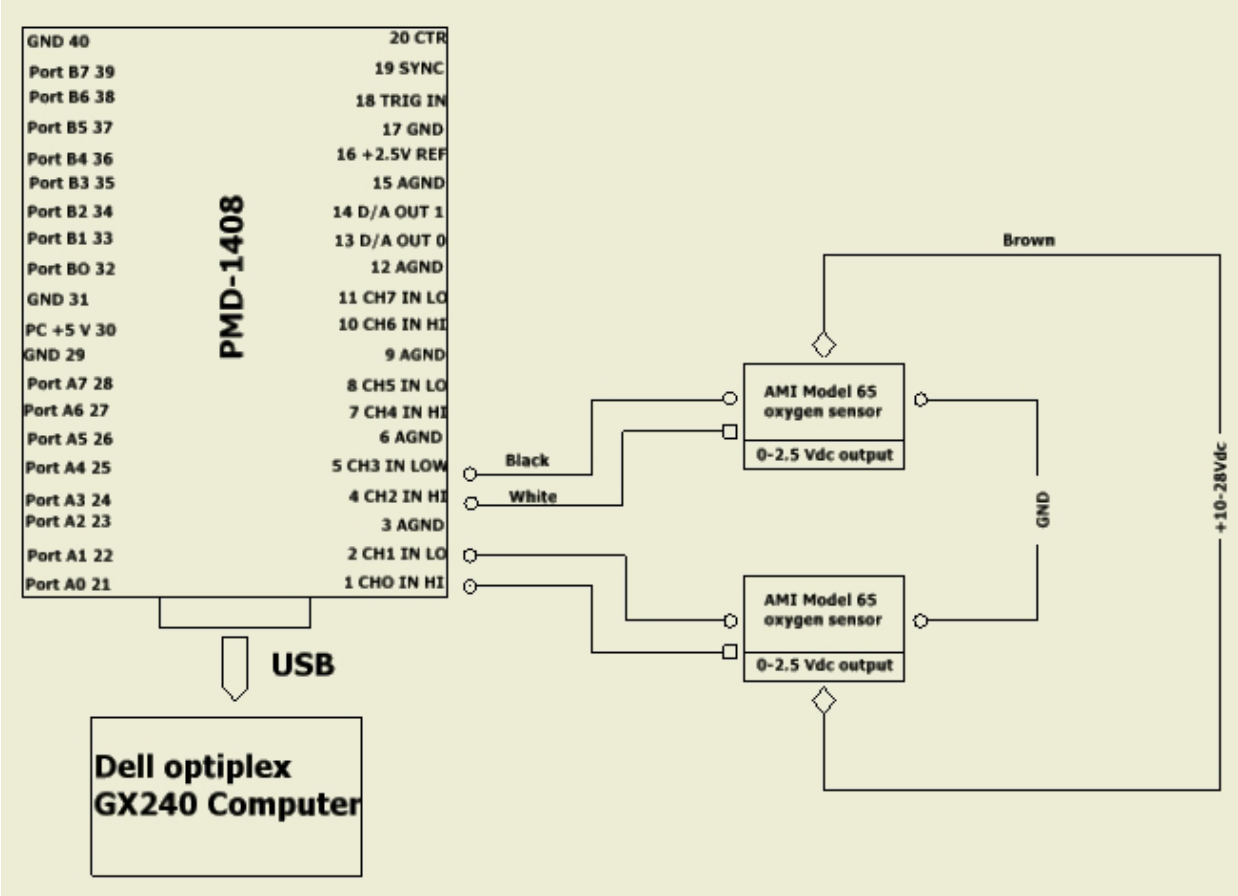
Figure 1 shows the graphic user interface utilized for data acquisition



Appendix A-Figure 1: Graphic user interface (GUI)

Oxygen quantification circuitry

Figure 2 is the circuitry utilized in the data acquisition



Appendix A-Figure 2: Oxygen quantification circuitry

APPENDIX B: STUDY 1-TREATMENT ASSIGNMENT

Treatment assignment tables are displayed below (Table 1-8)

Appendix B-Table 1: 27^{O}C Chamber 1 run 1 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
1	1	6.3%	TRT:	T 2	T 8	T 10	T 5	T6
			Jar #	1	2	3	4	5
		Hermetic						
	(27^{O}C)							
		16%		T 16	T 9	T 11	T 14	T3
				6	7	8	9	10
		6.3%	TRT:	T 4		T 15		T7
		Non-hermetic	Jar #	11		12		13
		16%	TRT	T 12		T13		T1
			Jar #	14		15		16

Appendix B-Table 2: 10^{O}C Chamber 2 run 1 treatment positions

Run	Chamber	M.C		Jar Counted Day 2	Day 4	Day 6	Day 8	Day 10
1	2 (10^{O}C)	6.3% Hermetic	TRT:	T 16	T 4	T 5	T9	T12
			Jar #	17	18	19	20	21
		16%	TRT:	T 3	T 11	T 8	T 15	T 13
			Jar #	22	23	24	25	26
		6.3% Non-hermetic	TRT:	T 2		T 7		T 6
			Jar #	27		28		29
		16%	TRT:	T 14		T 1		T 10
			Jar #	30		31		32

Appendix B-Table 3: 10^{O}C Chamber 3 run 1 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
1	3 (10^{O}C)	6.3% Hermetic	TRT:	T 12	T 1	T 10	T 11	T 14
			Jar #:	33	34	35	36	37
		16%	TRT:	T 3	T 6	T 7	T 2	T 15
			Jar#:	38	39	40	41	42
		6.3% Non-hermetic	TRT:	T 8		T 4		T 13
			Jar #:	43		44		45
		16%	TRT:	T 9		T 16		T 5
			Jar #:	46		47		48

Appendix B-Table 4: 27^{O}C Chamber 4 run 1 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
		6.3%	TRT:	T 13	T4	T11	T1	T 15
1	4		Jar #:	49	50	51	52	53
	(27^{O}C)	Hermetic						
		16%	TRT:	T2	T16	T7	T14	T9
			Jar #:	54	55	56	57	58
		6.3%	TRT:	T 6		T 3		T 8
			Jar #:	59		60		61
		Non-hermetic						
		16%	TRT:	T5		T12		T10
			Jar #:	62		63		64

Appendix B-Table 5: 27^{O}C Chamber 1 run 2 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
2	1 (27^{O}C)	6.3% Hermetic	TRT:	T 5	T 10	T12	T4	T3
			Jar #:	65	66	T67	68	69
		16%	TRT:	T 6	T13	T2	T9	T11
			Jar#:	70	71	72	73	74
		6.3% Non-hermetic	TRT:	T7		T8		T16
			Jar #:	75		76		77
		16%	TRT:	T1		T14		T15
			Jar#:	78		79		80

Appendix B-Table 6: 27^{O}C Chamber 2 run 2 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
2	2	6.3%	TRT:	T10	T13	T5	T4	T1
			Jar #:	81	82	83	84	85
	(27^{O}C)	Hermetic						
		16%	TRT	T16	T11	T9	T7	T15
			Jar #:	86	87	88	89	90
		6.3%	TRT:	T3		T8		T12
			Jar #:	91		92		93
		Non-hermetic						
		16%	TRT:	T14		T 6		T2
			Jar #:	94		95		96

Appendix B-Table 7: 10^{O}C Chamber 3 run 2 treatment positions

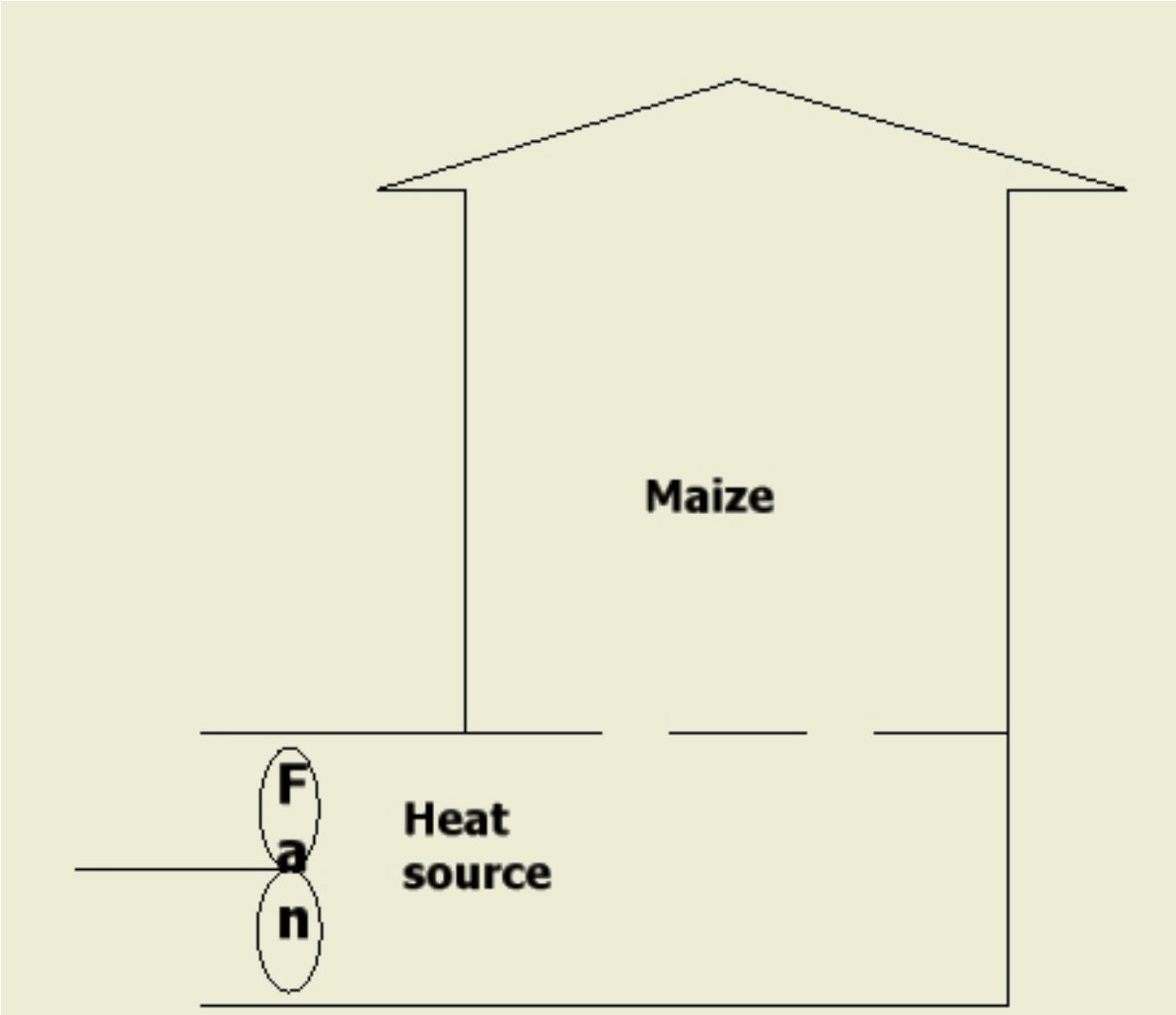
Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
2	3 (10^{O}C)	6.3% Hermetic	TRT:	T14	T6	T12	T15	T16
			Jar #:	97	98	99	100	101
		16%	TRT:	T8	T9	T11	T7	T3
			Jar#:	102	103	104	105	106
		6.3% Non-hermetic	TRT:	T1		T13		T10
			Jar #:	107		108		109
		16%	TRT:	T4		T5		T2
			Jar#:	110		111		112

Appendix B-Table 8: 10^{O}C Chamber 4 run 2 treatment positions

Run	Chamber	M.C		Jar Counted				
				Day 2	Day 4	Day 6	Day 8	Day 10
2	4 (10^{O}C)	6.3% Hermetic	TRT:	T8	T12	T3	T11	T5
			Jar #:	113	114	115	116	117
		16%	TRT:	T14	T7	T2	T9	T15
			Jar #:	118	119	120	121	122
		6.3% Non-hermetic	TRT:	T16		T10		T4
			Jar #:	123		124		125
		16%	TRT:	T13		T6		T1
			Jar #:	126		127		128

APPENDIX C: DRYING PRINCIPLE AND CALCULATIONS

Drying principles



Appendix C-Figure 1: Drying principles

Appendix C-Figure 2: Bucket grain dryer

Appendix C-Figure 3: Hair dryer (heat source)

Appendix C-Figure 4: Hair dryer support

Appendix C-Figure 5: Grain dryer and heat source

Maize moisture adjustment calculations (sample)

Maize sample size selected for drying to the required moisture was determined using equation 1-1. And the dried samples were weighed, again, following drying to ensure that the sample agreed with the predetermined weight at that moisture:

$$W_i(1-\frac{M_i}{100}) = W_f(1-\frac{M_f}{100}) \quad \text{.....(1-1)}$$

a. Drying from 16.5% to 16%:

   (1.00-0.165)*20089.14=(1-0.16) W_f

   W_f= 19969.56

b. Drying from 16.5% to 8%:

   (1.00-0.165)*20089.14=(1-0.08) W_f

   W_f= 18233

Where,

D=dry matter

W=maize weight

M=% moisture

i=initial, and

f=final

Table 1 displays the oven test results for study 1:

Appendix C-Table 1: Oven test results for study 1

Sample	6.3% Moisture Content Mass (g)	16% Moisture Content Mass (g)
1	Wet grain=30.10	Wet grain=30.10
	Dry Grain=28.17	Dry Grain=25.39
2	Wet grain=30.08	Wet grain=30.01
	Dry Grain=28.10	Dry Grain=25.24
3	Wet grain=30.05	Wet grain=30.05
	Dry Grain=28.30	Dry Grain=25.32
Average	Wet Maize=30.08	Wet Maize=30.05
	Dry Maize=28.19	Dry Maize=25.32

Oven drying calculation

Percentage moisture content wet basis values are utilized in commercial transactions involving maize. But the percentage moisture content dry basis calculation is also displayed below, for brevity.

Moisture Content (M_w) Percentage Wet Basis (% M_w (M_cW_b))

$$\% M_w = \frac{\text{wet grain mass - dry grain mass}}{\text{wet grain mass}} * 100$$

$$= \frac{(30.08-28.19)}{30.08} * 100$$

$$= \left(\frac{1.89}{30.08}\right) * 100$$

$= 6.28\%$ (for Dickey John's 8%)

Moisture Content (M_w) Percentage Dry Basis (% M_d (M_cd_b))

% M_d = (wet grain mass - dry grain mass) *100
dry grain mass

= (30.08-28.19) *100
28.19

$$= \left(\frac{1.89}{28.19}\right) * 100$$

$$= 6.70\%$$

Moisture Content (M_w) Percentage Wet Basis (% M_w (M_cW_b))

=(30.05-25.32) *100
30.05

= $$= \left(\frac{4.73}{30.05}\right) * 100$$

$= 15.74\%$ **(for Dickey John's 16%)**

Moisture Content (M_w) Percentage Dry Basis (% M_d (M_cd_b))

= (30.05-25.32) *100
25.32

$$= \left(\frac{4.73}{25.32}\right) * 100$$

$$= 18.68\%$$

Oven test results for beginning maize samples are displayed in Table 2:

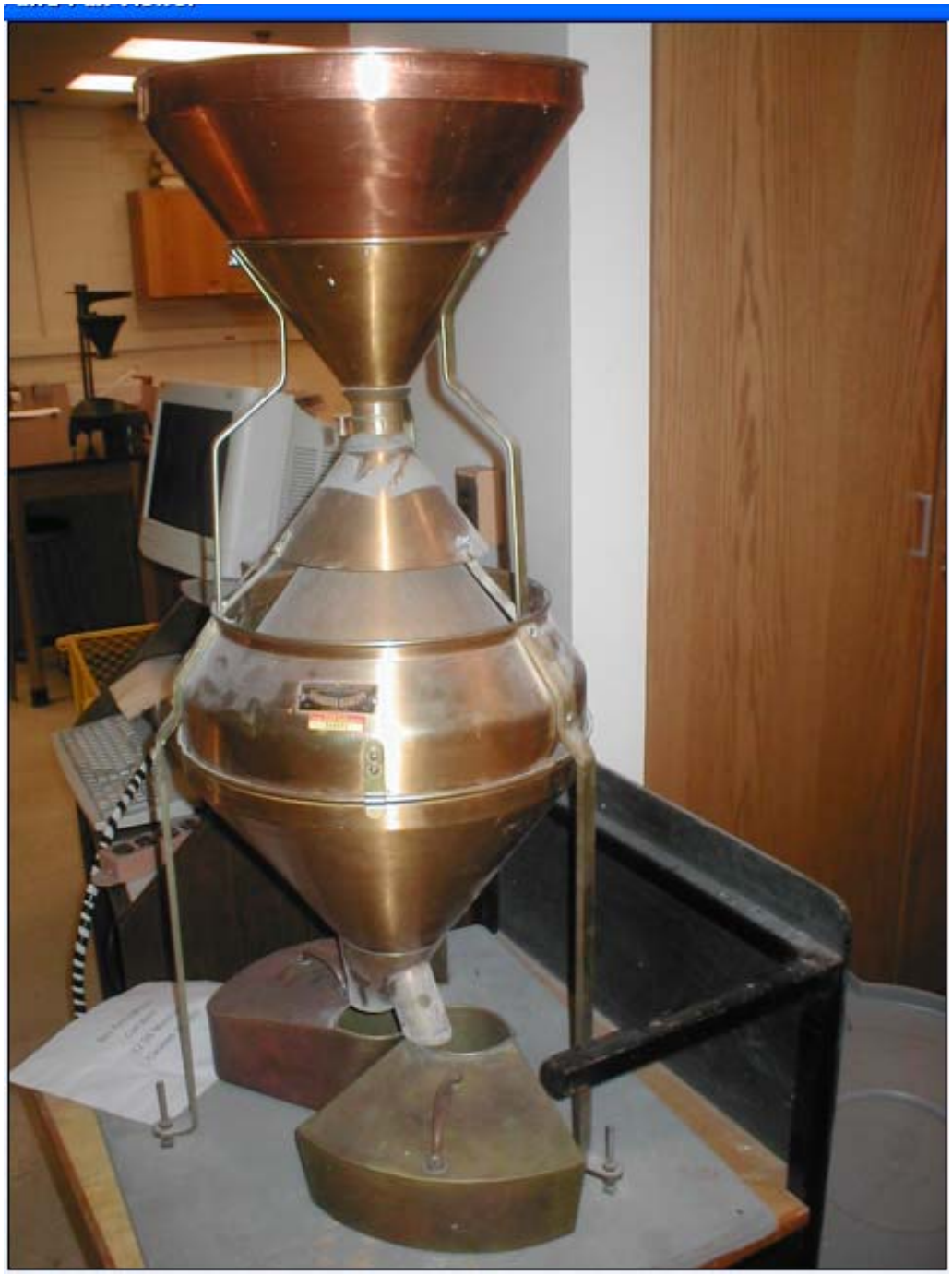
Appendix C-Table 2: Initial maize moisture determination

Sample	Start weight	End weight
1	30.19	25.21
2	30.28	25.38
3	30.06	25.01
Average	30.18	25.2
Percent moisture	16.5	

APPENDIX D: EXPERIMENTAL SETUP

Appendix D-Figure 1: Mounting thermometer on stopper

Appendix D-Figure 2: weevil and maize sieve



Appendix D-Figure 3: Boerner grain divider

Appendix D-Figure 4: Mobile workstation

Appendix D-Figure 5: Hermetic storage jars

Appendix D-Figure 6: Non-hermetic storage jars

Appendix D-Figure 7: Storage chamber at $10^{O}C$

Appendix D-Figure 8: Storage chamber at 27^{O}C

Appendix D-Figure 9: Emptying storage jar

Appendix D-Figure 10: Weevil count

APPENDIX E: STUDY 1 DATA SHEETS

Table 1 shows the records of the final maize storage weevil mortality results:

Appendix E-Table 1: weevil mortality data recording Dates: 07/09/08 to 07/17/08

Expt Name	Run	trt	Pos	Jar	Num	herm	temp	Day	mc	rep
Hermetic	1	2	1	1	2	Y	27^{O}C	2	6.3	1
Hermetic	1	8	2	2	30	Y	27^{O}C	4	6.3	1
Hermetic	1	10	3	3	30	Y	27^{O}C	6	6.3	1
Hermetic	1	5	4	4	30	Y	27^{O}C	8	6.3	1
Hermetic	1	6	5	5	30	Y	27^{O}C	10	6.3	1
Hermetic	1	16	6	6	1	Y	27^{O}C	2	16	1
Hermetic	1	9	7	7	9	Y	27^{O}C	4	16	1
Hermetic	1	11	8	8	30	Y	27^{O}C	6	16	1
Hermetic	1	14	9	9	30	Y	27^{O}C	8	16	1
Hermetic	1	3	10	10	30	Y	27^{O}C	10	16	1
Hermetic	1	4	11	11	0	N	27^{O}C	2	6.3	1
Hermetic	1	15	12	12	1	N	27^{O}C	6	6.3	1
Hermetic	1	7	13	13	0	N	27^{O}C	10	6.3	1
Hermetic	1	12	14	14	0	N	27^{O}C	2	16	1
Hermetic	1	13	15	15	0	N	27^{O}C	6	16	1
Hermetic	1	1	16	16	0	N	27^{O}C	10	16	1

Appendix E-Table 1: weevil mortality data recording-continued.

Expt Name	Run	Trt	Pos	Jar	num	herm	temp	Day	mc	Rep
Hermetic	1	16	1	17	0	Y	10^{O}C	2	6.3	1
Hermetic	1	4	2	18	2	Y	10^{O}C	4	6.3	1
Hermetic	1	5	3	19	5	Y	10^{O}C	6	6.3	1
Hermetic	1	9	4	20	1	Y	10^{O}C	8	6.3	1
Hermetic	1	12	5	21	10	Y	10^{O}C	10	6.3	1
Hermetic	1	3	6	22	0	Y	10^{O}C	2	16	1
Hermetic	1	11	7	23	1	Y	10^{O}C	4	16	1
Hermetic	1	8	8	24	1	Y	10^{O}C	6	16	1
Hermetic	1	15	9	25	4	Y	10^{O}C	8	16	1
Hermetic	1	13	10	26	0	Y	10^{O}C	10	16	1
Hermetic	1	2	11	27	0	N	10^{O}C	2	6.3	1
Hermetic	1	7	12	28	0	N	10^{O}C	6	6.3	1
Hermetic	1	6	13	29	0	N	10^{O}C	10	6.3	1
Hermetic	1	14	14	30	0	N	10^{O}C	2	16	1
Hermetic	1	1	15	31	0	N	10^{O}C	6	16	1
Hermetic	1	10	16	32	0	N	10^{O}C	10	16	1

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	trt	Pos	Jar	num	herm	temp	Day	Mc	rep
Hermetic	1	12	1	33	2	Y	10^{O}C	2	6.3	2
Hermetic	1	1	2	34	0	Y	10^{O}C	4	6.3	2
Hermetic	1	10	3	35	3	Y	10^{O}C	6	6.3	2
Hermetic	1	11	4	36	3	Y	10^{O}C	8	6.3	2
Hermetic	1	14	5	37	5	Y	10^{O}C	10	6.3	2
Hermetic	1	3	6	38	1	Y	10^{O}C	2	16	2
Hermetic	1	6	7	39	3	Y	10^{O}C	4	16	2
Hermetic	1	7	8	40	1	Y	10^{O}C	6	16	2
Hermetic	1	2	9	41	2	Y	10^{O}C	8	16	2
Hermetic	1	15	10	42	6	Y	10^{O}C	10	16	2
Hermetic	1	8	11	43	0	N	10^{O}C	2	6.3	2
Hermetic	1	4	12	44	0	N	10^{O}C	6	6.3	2
Hermetic	1	13	13	45	0	N	10^{O}C	10	6.3	2
Hermetic	1	9	14	46	0	N	10^{O}C	2	16	2
Hermetic	1	16	15	47	0	N	10^{O}C	6	16	2
Hermetic	1	5	16	48	0	N	10^{O}C	10	16	2

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	Trt	Pos	Jar	num	herm	temp	Day	mc	Rep
Hermetic	1	13	1	49	2	Y	27^{O}C	2	6.3	2
Hermetic	1	4	2	50	13	Y	27^{O}C	4	6.3	2
Hermetic	1	11	3	51	30	Y	27^{O}C	6	6.3	2
Hermetic	1	1	4	52	30	Y	27^{O}C	8	6.3	2
Hermetic	1	15	5	53	30	Y	27^{O}C	10	6.3	2
Hermetic	1	2	6	54	0	Y	27^{O}C	2	16	2
Hermetic	1	16	7	55	0	Y	27^{O}C	4	16	2
Hermetic	1	7	8	56	30	Y	27^{O}C	6	16	2
Hermetic	1	14	9	57	30	Y	27^{O}C	8	16	2
Hermetic	1	9	10	58	30	Y	27^{O}C	10	16	2
Hermetic	1	6	11	59	0	N	27^{O}C	2	6.3	2
Hermetic	1	3	12	60	0	N	27^{O}C	6	6.3	2
Hermetic	1	8	13	61	0	N	27^{O}C	10	6.3	2
Hermetic	1	5	14	62	0	N	27^{O}C	2	16	2
Hermetic	1	12	15	63	0	N	27^{O}C	6	16	2
Hermetic	1	10	16	64	0	N	27^{O}C	10	16	2

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	Trt	Pos	Jar	num	herm	temp	Day	Mc	Rep
Hermetic	2	5	1	65	0	Y	27^{O}C	2	6.3	3
Hermetic	2	10	2	66	30	Y	27^{O}C	4	6.3	3
Hermetic	2	12	3	67	30	Y	27^{O}C	6	6.3	3
Hermetic	2	4	4	68	30	Y	27^{O}C	8	6.3	3
Hermetic	2	3	5	69	30	Y	27^{O}C	10	6.3	3
Hermetic	2	6	6	70	2	Y	27^{O}C	2	16	3
Hermetic	2	13	7	71	30	Y	27^{O}C	4	16	3
Hermetic	2	2	8	72	30	Y	27^{O}C	6	16	3
Hermetic	2	9	9	73	30	Y	27^{O}C	8	16	3
Hermetic	2	11	10	74	30	Y	27^{O}C	10	16	3
Hermetic	2	7	11	75	0	N	27^{O}C	2	6.3	3
Hermetic	2	8	12	76	3	N	27^{O}C	6	6.3	3
Hermetic	2	16	13	77	0	N	27^{O}C	10	6.3	3
Hermetic	2	1	14	78	0	N	27^{O}C	2	16	3
Hermetic	2	14	15	79	4	N	27^{O}C	6	16	3
Hermetic	2	15	16	80	1	N	27^{O}C	10	16	3

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	trt	Pos	Jar	num	herm	temp	Day	Mc	Rep
Hermetic	2	10	1	81	2	Y	10^{O}C	2	6.3	3
Hermetic	2	13	2	82	0	Y	10^{O}C	4	6.3	3
Hermetic	2	5	3	83	5	Y	10^{O}C	6	6.3	3
Hermetic	2	4	4	84	3	Y	10^{O}C	8	6.3	3
Hermetic	2	1	5	85	11	Y	10^{O}C	10	6.3	3
Hermetic	2	16	6	86	1	Y	10^{O}C	2	16	3
Hermetic	2	11	7	87	0	Y	10^{O}C	4	16	3
Hermetic	2	9	8	88	2	Y	10^{O}C	6	16	3
Hermetic	2	7	9	89	1	Y	10^{O}C	8	16	3
Hermetic	2	15	10	90	0	Y	10^{O}C	10	16	3
Hermetic	2	3	11	91	2	N	10^{O}C	2	6.3	3
Hermetic	2	8	12	92	0	N	10^{O}C	6	6.3	3
Hermetic	2	12	13	93	4	N	10^{O}C	10	6.3	3
Hermetic	2	14	14	94	0	N	10^{O}C	2	16	3
Hermetic	2	6	15	95	0	N	10^{O}C	6	16	3
Hermetic	2	2	16	96	0	N	10^{O}C	10	16	3

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	Trt	Pos	Jar	num	Herm	temp	Day	Mc	rep
Hermetic	2	14	1	97	1	Y	10^{O}C	2	6.3	4
Hermetic	2	6	2	98	1	Y	10^{O}C	4	6.3	4
Hermetic	2	12	3	99	5	Y	10^{O}C	6	6.3	4
Hermetic	2	15	4	100	9	Y	10^{O}C	8	6.3	4
Hermetic	2	16	5	101	8	Y	10^{O}C	10	6.3	4
Hermetic	2	8	6	102	1	Y	10^{O}C	2	16	4
Hermetic	2	9	7	103	0	Y	10^{O}C	4	16	4
Hermetic	2	11	8	104	1	Y	10^{O}C	6	16	4
Hermetic	2	7	9	105	1	Y	10^{O}C	8	16	4
Hermetic	2	3	10	106	0	Y	10^{O}C	10	16	4
Hermetic	2	1	11	107	2	N	10^{O}C	2	6.3	4
Hermetic	2	13	12	108	2	N	10^{O}C	6	6.3	4
Hermetic	2	10	13	109	2	N	10^{O}C	10	6.3	4
Hermetic	2	4	14	110	3	N	10^{O}C	2	16	4
Hermetic	2	5	15	111	3	N	10^{O}C	6	16	4
Hermetic	2	2	16	112	0	N	10^{O}C	10	16	4

Appendix E-Table 1: weevil mortality data recording- continued.

Name	Run	Trt	Pos	Jar	num	herm	temp	Day	mc	Rep
Hermetic	2	8	1	113	1	Y	27^{O}C	2	6.3	4
Hermetic	2	12	2	114	30	Y	27^{O}C	4	6.3	4
Hermetic	2	3	3	115	30	Y	27^{O}C	6	6.3	4
Hermetic	2	11	4	116	30	Y	27^{O}C	8	6.3	4
Hermetic	2	5	5	117	30	Y	27^{O}C	10	6.3	4
Hermetic	2	14	6	118	1	Y	27^{O}C	2	16	4
Hermetic	2	7	7	119	29	Y	27^{O}C	4	16	4
Hermetic	2	2	8	120	30	Y	27^{O}C	6	16	4
Hermetic	2	9	9	121	30	Y	27^{O}C	8	16	4
Hermetic	2	15	10	122	30	Y	27^{O}C	10	16	4
Hermetic	2	16	11	123	2	N	27^{O}C	2	6.3	4
Hermetic	2	10	12	124	5	N	27^{O}C	6	6.3	4
Hermetic	2	4	13	125	5	N	27^{O}C	10	6.3	4
Hermetic	2	13	14	126	0	N	27^{O}C	2	16	4
Hermetic	2	6	15	127	2	N	27^{O}C	6	16	4
Hermetic	2	1	16	128	1	N	27^{O}C	10	16	4

Table 2 indicates a large difference in the mean weevil mortality rate between hermetic and non-hermetic storage, conditions:

Appendix E-Table 2: Mean weevil mortality at 27 ^{o}C over 3 days for each moisture

Mean hermetic weevil mortality by day					
Moisture(%)	**Day 2**	**Day 6**	**Day 10**	**mean (95% CI)**	**SEM**
6.3	1.25	30.00	30.00	20.42 (13.56-27.73)	2.67
16	1.00	30.00	30.00	20.33 (13.36-27.31)	2.94
Mean	1.13	30.00	30.00	20.38 (13.46-27.52)	N/A
Mean difference	0.25	0.00	0.00	0.08 (0.08-0.17)	N/A

Mean non-hermetic weevil mortality by day					
Moisture (%)	**Day 2**	**Day 6**	**Day 10**	**mean (95% CI)**	**SEM**
6.3	0.50	2.25	1.25	1.33 (1.31-2.69)	0.56
16	0.00	1.50	0.50	1.67(1.61-3.30)	0.35
Mean	0.25	1.88	0.88	1.00 (0.98-2.01)	N/A
Mean difference	0.50	0.75	0.75	0.50 (0.49-1.02)	N/A

Table 3 hermetic indicates significant mortality rates in all (p=<.0001) except the 2nd day (p=0.5490), of the hermetic storage.

Appendix E-Table 3: Mean weevil mortality at 27 ^{o}C, hermetic over 5 days for all moisture

Mean hermetic weevil mortality by day across all moisture			
Day	mean (95% CI)	SEM	Pr > |t|
2	1.13 (-2.68-4.93)	0.29	0.5490
4	21.38 (17.57-25.18)	4.30	<.0001
6	30.00 (26.20-33.80)	0.00	<.0001
8	30.00 (26.20-33.80)	0.00	<.0001
10	30.00 (26.20-33.80)	0.00	<.0001

The non-hermetic mortality rates (Table 4), for weevils at 27 ^{o}C, is significant on the 6th day (p=0.0008). This may be the natural weevil mortality rate, in the wild, but further investigation is required, to confirm that:

Appendix E-Table 4: Mean weevil mortality at 27 ^{o}C over 5 (hermetic) and 3 days (non-hermetic) for all moisture

Mean non hermetic weevil mortality by day across all moisture			
Day	mean (95% CI)	SEM	Pr > |t|
2	0.25 (-0.71-1.21)	0.25	0.5857
6	1.88 (0.92-2.83)	0.69	0.0008
10	0.88 (-0.08-1.83)	0.61	0.0701

Table 5, shows evidence of interaction between day and moisture content, based on across the board mean mortality difference. Hence, the mortality level increases as the length of storage period (days) increases, but at different rates, for different moisture contents:

Appendix E-Table 5: Mean weevil mortality at 10°C over 3 days for each moisture

Mean hermetic weevil mortality by day					
Moisture(%)	**Day 2**	**Day 6**	**Day 10**	**mean (95% CI)**	**SEM**
6.3	1.25	4.50	8.50	4.75 (4.42-9.04)	0.75
16	0.75	1.25	1.50	1.17 (1.16-2.38)	0.34
Mean	1.00	2.88	5.00	2.96 (2.86-5.85)	N/A
Mean Difference	0.50	3.25	7.00	3.58 (3.32-6.79)	N/A

Mean nonhermetic weevil mortality by day					
Moisture(%)	**Day 2**	**Day 6**	**Day 10**	**mean (95% CI)**	**SEM**
6.3	1.00	0.50	1.50	1.00 (0.99-2.03)	0.38
16	0.75	0.75	0.00	0.50 (0.49-1.01)	0.33
Mean	0.88	0.63	0.75	0.75 (0.74-1.53)	N/A
Mean difference	0.25	-0.25	1.50	0.50 (0.48-0.98)	N/A

Table 6 indicates higher levels of weevil mortality on the 6th to 10th day (p=0.0001-0.0003) under hermetic conditions, and lower mortality levels for non hermetic storage at 10°C:

Appendix E-Table 6: Mean weevil mortality at 10^{o}C over 5 (hermetic) and 3 days (non-hermetic) for all moisture

Mean hermetic weevil mortality by day across all moisture			
Day	**mean (95% CI)**	**SEM**	**Pr > |t|**
2	1.00 (-0.43-2.43)	0.26	0.1639
4	0.88 (-0.56-2.31)	0.39	0.2213
6	2.88 (1.44-4.31)	0.66	0.0003
8	3.00 (1.57-4.43)	0.94	0.0002
10	5.00 (3.57-6.43)	1.61	<.0001

Mean non hermetic weevil mortality by day across all moisture			
Day	**mean (95% CI)**	**SEM**	**Pr > |t|**
2	0.88 (0.12-1.63)	0.44	0.0258
6	0.63 (-0.13-1.38)	0.41	0.0974
10	0.75 (-0.0036-1.50)	0.40	0.0510

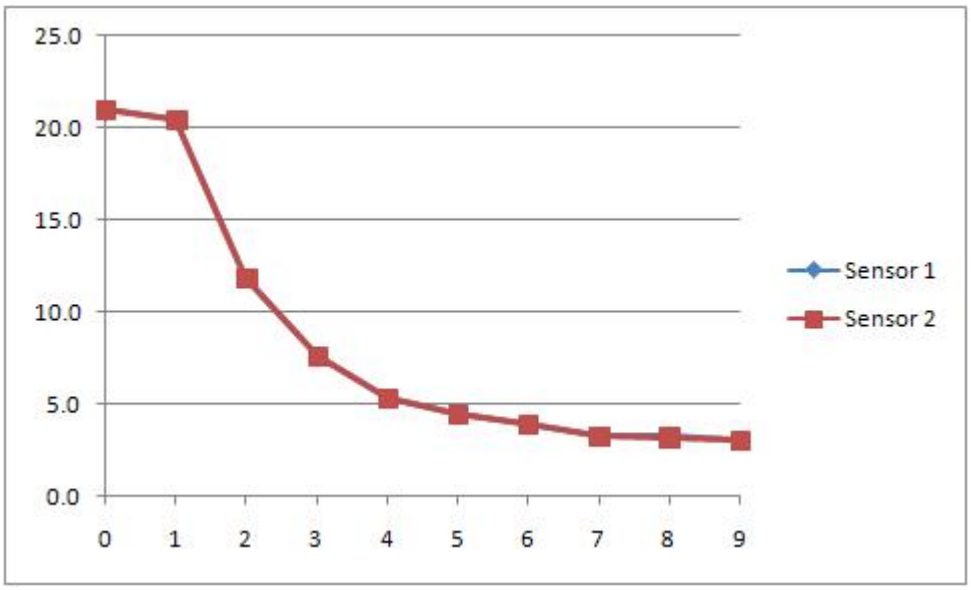
APPENDIX F: OXYGEN QUANTIFICATION

Table 1 displays the oven test results for the oxygen quantification study:

Appendix F-Table 1: Oven test results for study 2

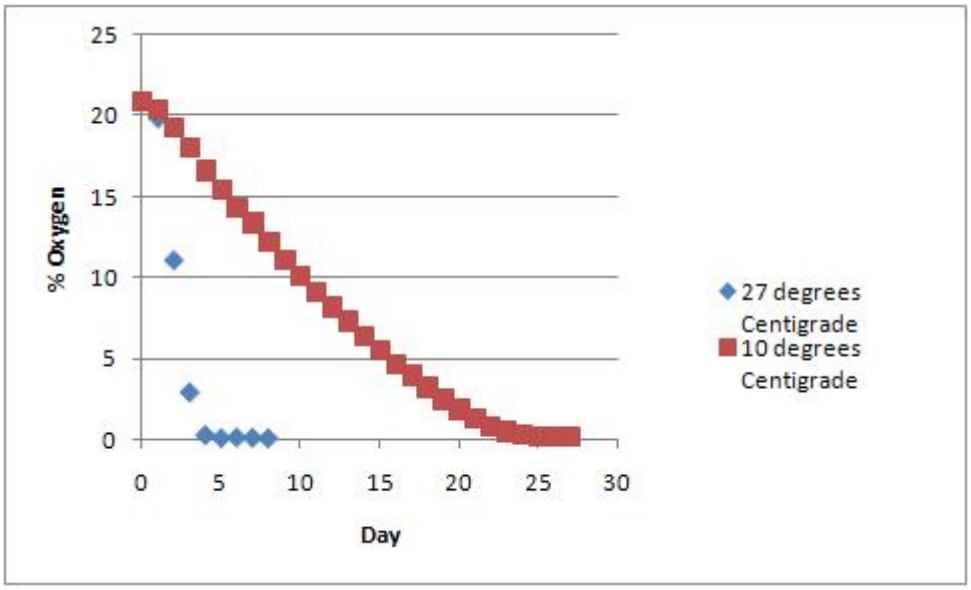
Sample	Start weight	End weight	Start weight	End weight
1	30.08	27.68	30.03	24.83
2	30.52	27.92	30.3	25.3
3	30.19	27.84	30.34	25.99
Average	30.26	27.81	30.22	25.37
Percent moisture	8%		16.04	

Sensor calibration graph (Figure 1), shows sensors calibrated to be used interchangeably:



Appendix F-Figure 1: Sensor calibration

Figure 2, shows the trial-run graph at both temperatures



Appendix F-Figure 2: Oxygen quantification trial run (16.5% moisture)

APPENDIX G: WEEVIL OXYGEN UTILIZATION

AIR PER WEEVIL (cubic centimeter of)

$$\rho_{(\text{distilled water})} = \frac{m}{V} = \frac{1g}{cm^3}$$

$$Vol_{(\text{distilled water})} = \frac{g_{(\text{distilled water})}}{1g} * cm^3 = \text{Vol (maize+interstitial air)}$$

1 Pint-Vol (pure air)= Vol (maize)

But, 1 pint = $\frac{3780}{8}$ cm^3 = 472.5 (cc)

Therefore, 472.5- Vol (air in maize+air above maize)= Vol (maize)

1 pint-Vol (water)= Vol (air above maize)

Interstitial air = Vol (water)*Fraction of Void =Vol (water)*$(1-\frac{C_b}{C_p})$

$$= 239.4* (1-\frac{0.772314 g/cm^3}{1.2601\ g/cm^3})$$

$$= 239.4*0.39=93.4$$

Total air (weevil live in)=Vol (air above maize)+Interstitial air

$$= (472-239.4)+93.4$$

$$= 32$$

Where:

C_b= bulk density (test weight) from calculation Below

C_p= particle density from ACCU 330 micrometrics

Vol (air above maize)= headspace volume

$\frac{\text{Total air (weevil live in)}}{\text{\# weevils}} * \frac{(O_{2i}-O_{2f})}{100} = cm^3$ oxygen/weevils

O_{2i}=beginning oxygen level=20.99%

O_{2f}=ending oxygen level, and

(O_{2i}- O_{2f})/100=oxygen equivalent of the proportion of air weevil utilize (since maximum oxygen in air =0.2099)

Weevil oxygen consumption/day= cc oxygen/weevils/# days (to 100% weevil mortality).

Procedure

185g maize was measured into a one pint jar. The top mark of the maize was marked on the glass jar, and the maize was emptied.

The jar was filled up to the mark, with distilled water and measured. The volume of the distilled water was then calculated using the density of distilled water.

Weight $_{(distilled\ water)}$=239.54g (equivalent of 185g maize)=239.54 cc. And

$$C_b = \frac{\text{Weight (maize)}}{\text{Vol (distilled water)}} = \frac{185g}{239.54\ cm^3}$$

$$= 0.772314 g/cm^3$$

Calculation (example)

(326.4*((20.99-0.0032)/100)/90 weevils)/4 days

=0.19 cm^3 $weevil^{-1}$ day^{-1}

Table 1 shows the result of oxygen consumption per weevil per day at each temperature and moisture:

Appendix G-Table 1: Weevil oxygen consumption at each moisture and temperature

Moisture @ 27 °C	cc/weevil/day
8	0.19
16	0.18

Moisture @ 10 °C	cc/weevil/day
8	0.03
16	0.01

Table 2 is the average values obtained from oxygen quantification at 27°C:

Appendix G-Table 2: Average values of oxygen quantification at 27°C and two moistures (8 and 16%)

Day	8% MOISTURE	16% MOISTURE
0	20.98	20.98
1	12.34	12.91
2	4.45	5.32
3	0.92	2.20
4	0.32	1.56

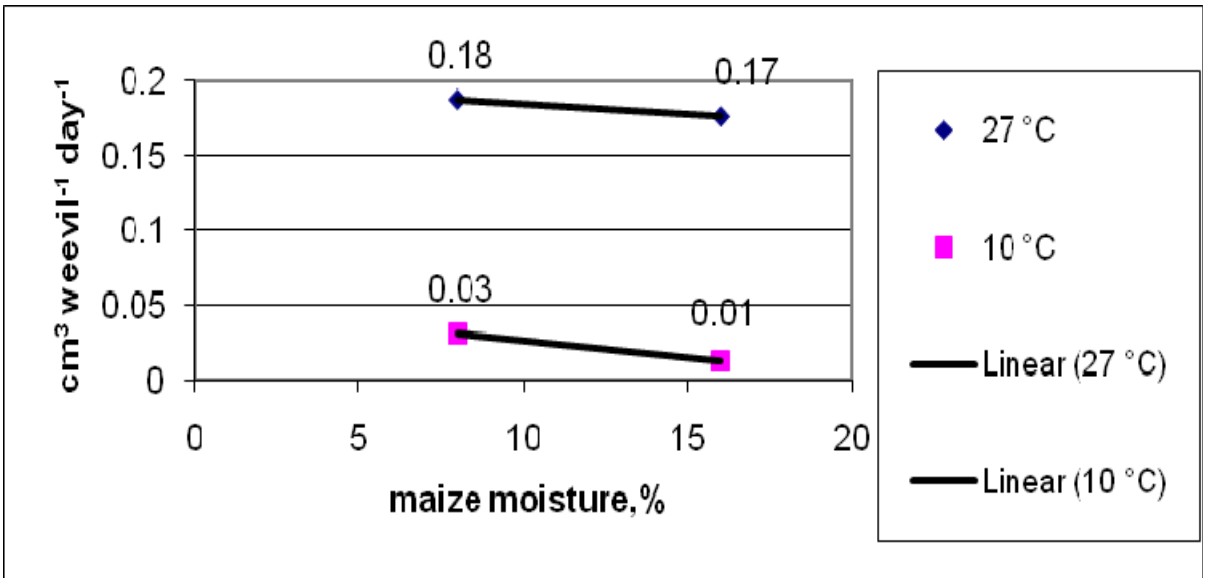
Table 3 is the average values obtained from oxygen quantification at 10°C:

Appendix G-Table 3: Average values of oxygen quantification at 10°C and two moistures (8 and 16%)

Day	8% MOISTURE	16% MOISTURE
0	20.98	20.98
1	19.43	19.42
2	18.53	18.46
3	17.54	17.68
4	16.52	16.76
5	15.44	15.84
6	14.40	14.92
7	13.34	14.00
8	12.34	13.32
9	11.38	12.69
10	10.40	12.16
11	9.469	11.66
12	8.534	11.39
13	7.764	11.10
14	6.95	10.94
15	6.37	10.79
16	5.83	10.70
17	5.34	10.67
18	5.02	10.72
19		10.67
20		10.78
21		10.85
22		10.95
23		11.03
24		11.10
25		11.27
26		11.34
27		11.38
28		11.54

APPENDIX H: WEEVIL OXYGEN GRAPH INTERPOLATION

Given figure Appendix H-Figure 1,



APPENDIX H-Figure 1: Original weevil oxygen utilization curve

To calculate weevil oxygen utilization value for 10% maize moisture, at 20^{O}C:

Step 1

Moisture (horizontal) interpolation (at 10^{O}C)

0.03 cm^3 $weevil^{-1}$ day^{-1} (8%, 10^{O}C)

-0.01 cm^3 $weevil^{-1}$ day^{-1} (16%, 10^{O}C)

0.02 cm^3 $weevil^{-1}$ day^{-1}

Going from 8% to 10%,

10%-8%=2 % (moisture difference), and linear interpolation for that moisture difference

=(2/8)*0.02 cm^3 $weevil^{-1}$ day^{-1}= 0.05 cm^3 $weevil^{-1}$ day^{-1}

Therefore, total interpolation value (8%, 10^{O}C)

=0.03-0.005= 0.025 cm^3 $weevil^{-1}$ day^{-1}

Step 2

Moisture (horizontal) interpolation (at 27^{O}C)

0.18 cm^3 $weevil^{-1}$ day^{-1} (8%, 27^{O}C)

-0.17 cm^3 $weevil^{-1}$ day^{-1} (16%, 27^{O}C)
0.01 cm^3 $weevil^{-1}$ day^{-1}

Going from 8% to 10%,

(2/8)*0.01=0.0025

Total interpolation (8%, 27^{O}C)= 0.18-0.0025=~0.177 cm^3 $weevil^{-1}$ day^{-1}

Total moisture interpolation= 0.025+0.177=0.152 cm^3 $weevil^{-1}$ day^{-1}

Step 3

Temperature (vertical) interpolation

Going from 10 to 20^{O}C=10 ^{O}C difference

10 to 27^{O}C=17 ^{O}C difference

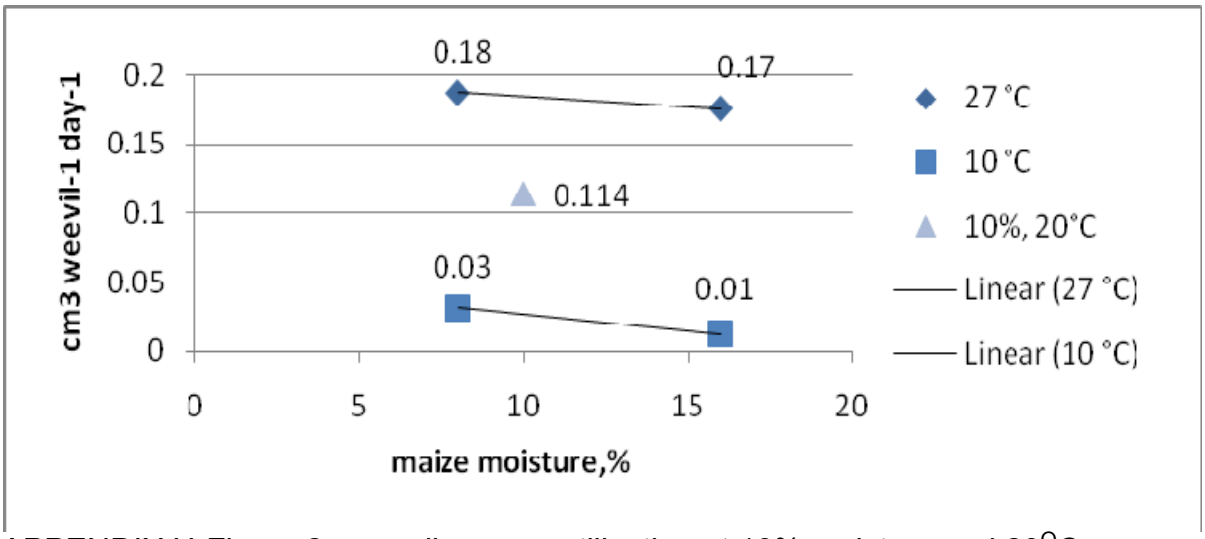
Therefore temperature interpolation=(10/17)*0.152=0.089

Step 4

Total interpolation (10%, 27^{O}C)

=Total moisture interpolation + temperature interpolation

=0.025+0.089=0.114 cm^3 $weevil^{-1}$ day^{-1}



APPENDIX H-Figure 2: weevil oxygen utilization at 10% moisture and 20^{O}C

Bulk density calculations

1 in=25.4 mm

$$\frac{25.4mm}{in}*\frac{12in}{ft}*\frac{m}{1000mm}=(\frac{0.3048in}{ft})^3=\frac{35.31ft^3}{in^3}$$

Test weight= $$\frac{56lb}{bu}*\frac{kg}{2.2lb}*\frac{bu}{1.245ft^3}*\frac{35.31ft^3}{m^3}=722\frac{kg}{m^3}=0.722\frac{g}{cm^3}$$

Bulk density= 1-fraction of voids= $1-\frac{0.722}{1.2601}$ =0.427=42.7%