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Ali Yakubu
Iowa State University, aaa@iastate.edu

Carl J. Bern
Iowa State University, cjbern@iastate.edu

Joel R. Coats
Iowa State University, jcoats@iastate.edu

Theodore B. Bailey
Iowa State University, tbbailey@iastate.edu

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Abstract

Maize (Zea mays L.) consumption makes up over half of daily caloric intake of persons in East Africa and adequate supply is necessary for food security for subsistence farmers, as well as for domestic stability. Hermetic post-harvest maize storage is an attractive non-chemical control strategy for maize weevil,Sitophilus zeamais (Motsch.), which is the principal cause of insect damage to stored maize grain. Laboratory experiments were conducted on instrumented hermetic and non-hermetic containers to measure effects of temperature (10 vs. 27°C) and maize moistures (6.3 to 16%) on maize weevil biology and mortality rate, and to quantify weevil oxygen consumption. Ten days weevil mortality was significantly higher in hermetic vs. non-hermetic storage, in 6.3% moisture maize vs. 16%, and at 27°C storage temperature vs. 10°C. Oxygen depletion results allow estimation of days to 100% adult weevil mortality as a function of weevil infestation level, storage temperature and maize moisture for East Africa conditions.

Keywords

Entomology, Statistics, Maize storage, hermetic storage, Sitofilus zeamais, maize weevil, maize deterioration

Disciplines

Agriculture | Bioresource and Agricultural Engineering | Biostatistics | Entomology

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Hermetic on-farm storage for maize weevil control in East Africa

A. Yakubu[1]*, C. J. Bern[1], J. R. Coats[2] and T. B. Bailey[3]

[1]Department of Agricultural and Biosytems Engineering, Iowa State University, Ames, Iowa 50011, USA.
[2]Department of Entomology, Iowa State University, Ames, Iowa 50011, USA.
[3]Department of Statistics, Iowa State University, Ames, Iowa 50011, USA.

Maize (*Zea mays* L.) consumption makes up over half of daily caloric intake of persons in East Africa and adequate supply is necessary for food security for subsistence farmers, as well as for domestic stability. Hermetic post-harvest maize storage is an attractive non-chemical control strategy for maize weevil, *Sitophilus zeamais* (Motsch.), which is the principal cause of insect damage to stored maize grain. Laboratory experiments were conducted on instrumented hermetic and non-hermetic containers to measure effects of temperature (10 vs. 27°C) and maize moistures (6.3 to 16%) on maize weevil biology and mortality rate, and to quantify weevil oxygen consumption. Ten days weevil mortality was significantly higher in hermetic vs. non-hermetic storage, in 6.3% moisture maize vs. 16%, and at 27°C storage temperature vs. 10°C. Oxygen depletion results allow estimation of days to 100% adult weevil mortality as a function of weevil infestation level, storage temperature and maize moisture for East Africa conditions.

Key words: Maize storage, hermetic storage, *Sitofilus zeamais,* maize weevil, maize deterioration.

INTRODUCTION

Maize

Maize (*Zea mays* L. ssp. Mays; corn) consumption by humans in East Africa far exceeds other uses and accounts for more than 50% of total caloric intake in local diets (Sinha, 2007). On small farms, hand harvesting is carried out after physiological maturity is reached, followed by drying and storage. Drying maize to below 14%[1] moisture is recommended for preservation in East Africa, while drying below 8% moisture, which kills most insects, is possible with sun drying or drying by means of wood fire or solar dryers (FAO, 1994).

Weevil and mold activity

Tropical heat, moisture and open-air storage promote rapid insect multiplication and mold formation in stored maize. Rapid insect development occurs when temperature is within 5 to 10°C of optimal temperature, which for most storage insects, is in the range of 25 to 35°C (FAO, 1994). The maize weevil (*Sitophilus zeamais*) is the principal deterioration insect of stored maize, sorghum, and other grains in the tropics (Longstaff, 1981, 1986; Jacobs and Calvin, 2001). About 96 million of the 140 million ha of maize grown annually is in the tropics, where the vast majority of the maize is stored on-farm, in storage that allows rewetting or moisture changes, and without use of chemical protectants (Lindblad and Druben, 1980; Dhliwayo and Pixley, 2003). Consequences include direct food losses and reduced future maize production for farmers, since 70% of all maize seed planted in Eastern and Southern Africa is sourced directly from previous year's harvest (Dhliwayo and Pixley, 2003).

Overall, 20 to 30% of Ethiopian stored maize is lost to *S. zeamais* infestation, while 100% damage has been found in maize stored for 6 to 8 months in the Bako region of the country (Demissie et al., 2008a). Mulungu et al. (2007) found about 18% of shelled maize with weevil damage in research involving stored maize in Tanzania,

*Corresponding author. E-mail: aaa@iastate.edu. Tel: 515 294 4894. Fax: 515-294-6633.

[1] All moistures are % wet basis.

while Demissie et al. (2008b) found levels of 11 to 59% 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. In addition, PHL Network (2009) estimated 5 year post-harvest maize losses for Tanzania at 22%, based on weight losses.

Hermetic storage

Hermetic storage isolates the storage ecosysytem from the external environment while respiration within the storage ecosystem causes O_2 reduction and CO_2 accumulation, leading to suffocation and dehydration of weevils (Navarro et al., 1994). A study by Moreno-Martinez et al. (2000) utilized 150 g samples of maize grain of hybrid AN 447 infested with 20 unsexed *S. zeamais* and stored within 250 ml glass containers, fitted with oxygen sensors. The jars were stored at 26°C, 16% moisture, 70% r.h., and 18±6 h L-D photoperiod. The research consisted of four treatments-maize grain of hybrid AN 447 (a) infested with *S. zeamais* and *Aspergillus chevalieri*, (b) infested with *S. zeamais*, (c) infected with *A. chevalieri* and (d) grain free of insects and fungus (control). Maize weevil mortality was recorded at 3 day intervals, by checking 12 jar replicates of hermetic as well as non-hermetic samples. They found that oxygen was depleted to 0% in 6 to 9 days in the hermetic treatments, while it decreased to 8.4% after 30 days in the non-hermetic treatment. Insect mortality in treatments containing only maize and insects was 100% at 9 days, and significant differences in mortality between hermetic and non-hermetic treatments were found (P≤0.01). The rate of oxygen depletion in treatments containing weevils was more rapid than those containing fungus and maize alone, while treatments with maize alone had much lower oxygen utilization rates.

Plastic bagging system

Plastic bagging employs layers of air-tight (Fulton et al., 2009) PVC and polyethylene bags within which grain is hermetically stored. Triple bagging, which involves tying three bags separately within each other, is currently employed by Purdue researchers in the hermetic preservation of cowpeas in Central and Western Africa. This storage system has the potential to increase household income of farmers on average by about $150 per year (Carroll and Fulton, 2008; Murdock et al., 2003). Studies are under way to determine plastic bag life (F. Murdock, Department of Entomology, Purdue University, West Lafayette, Indiana, personal communication).

Steel containers

Steel containers can be used for hermetic storage on farms in the East African sub-region. Lindblad and Druben (1980) and Adhikarinayake (2005) described the use of recycled steel oil drums, filled with maize, for hermetic storage and simultaneous mechanical isolation from rodents, while Murdock et al. (2003) described a cowpea bruchids-filled, metal drum fitted with a screw-type plastic sealing lid. The edges of the closure was lubricated with peanut or other cooking oil to ensure an airtight seal, and to make it easier to remove the lid following the storage period. The dry, threshed grain was stored for 6 months with minimal losses. Such containers may be contaminated by prior contents, and need to be properly cleaned to prevent cross contamination of maize stored within them. A common procedure for determining types of petro-chemicals present and for measuring the level of contamination involves methanol, hexane and other extraction methods followed by gas chromatography (Turriff et al., 1998), spectroscopy and related methods of cleanliness analysis. However, for food safety it is best to use only containers previously used to store food-grade materials, especially since international food laws and hazardous substance acts forbid use of recycled containers of hazardous substance for food packaging (Shachman, 2004). Common contaminants found in such containers include vegetable oils, which can be removed by saponification (Smith, 2009), or fruit puree and soft-drinks, cleanable by rinsing with water. The use of locally available soaps for cleaning is also common practice, although the efficacy of this method of cleaning is not well documented.

Research need

Further development of effective hermetic storage systems for maize requires more extensive quantification of the oxygen requirement of weevils within maize stored over a range of moistures and temperatures.

Objectives

The objectives of this research were to determine the effects of oxygen level, storage temperature, maize moisture, and their interaction, on the survivability of maize weevils over time in hermetic containers.

MATERIALS AND METHODS

A laboratory scale hermetic storage system employing glass jars was used, and the research employed instrumentation for quantification of oxygen levels. Treatment conditions of temperature (10°C and 27°C) and moistures (6.3, 8.0 and 16%) were selected as appropriate minimums and maximums of typical maize storage conditions in East Africa. The randomization of 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 at about 16.5% moisture using a 4420 Deere combine. Following harvest, maize was cleaned to remove broken maize and foreign material and stored at 4°C until use. Experimental maize was dried to target moistures, using a laboratory drier prior to testing. Natural air was utilized for drying to 16% and air at 45°C for drying to 8 or 6.3%. Moistures were measured using the 103°C, 72 h oven method (Asabe, 2008).

Experimental weevils

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

Experimental chambers

Two chambers maintained at 10 and 27°C, respectively, were utilized in the experiments. They are model 13-988-126 GW Fischer Scientific Isotemp refrigeration chambers (Thermo Fisher Scientific Inc., Waltham, MA 02454), with 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 canning jar was loaded with 350 g of maize and 30 adult weevils, while 90 weevils were loaded into each canning jar along with about 185 g of maize at the appropriate moisture levels in the oxygen quantification experiment. Hermetic tests utilized canning jars, as is, while non-hermetic tests utilized jars fitted with aluminum screen lids, which allowed air passage but not weevil escape.

Weevil mortality study

Objective

The objective of this study was to determine the effects of temperature and maize moisture on weevil mortality, under hermetic and non-hermetic conditions.

Experimental design

The experimental design consisted of four factorials (days, maize moisture, temperature) and replications, with weevil mortality being the dependent variable. Days had five levels (2nd, 4th, 6th, 8th, and 10th), maize moisture had two levels (6.3% and 16%), temperature had two levels (10°C and 27°C), and four replications were used. These conditions approximate those employed by Moreno-Martinez et al., (2000), although test conditions were based on results of preliminary laboratory tests.

Each replication had a total of 16 treatments (10 hermetic and 6 non-hermetic) assigned to each of the two chambers (Wohlgemuth, 1989; Evans, 1987). The hermetic jars had five levels of days and the non-hermetic had 3 levels of days, while both had two levels of maize moisture (6.3 and 16%). Each of the 128 treatment jars contained 30 weevils and 350 g of maize.

Dead weevil determination

Criteria for determining weevil mortality relied on a combination of observed rigor mortis features (Gullan and Cranston, 2000). Weevils that were curled up or had outstretched legs or found lying on their side/back or immobile or unattached to maize kernels or found to flow with kernels when jar was tilted or found to be hard to the touch or found to have any combination of these features, even when exposed to ambient air were assumed dead.

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. The hermetic treatment counts were 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 was recorded from the counts and utilized in the statistical analyses, and for testing the hypotheses of differences 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 quantify oxygen usage rates by maize weevils under different maize moisture, temperature and hermetic storage relationships.

Experimental design

The oxygen quantification (study #2) system utilized the same two environmental chambers, Kerr canning jars, and two oxygen analyzers along with their data acquisition system, which consist 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 8 or 16% moisture. The jars, which were connected to the two model 65 oxygen sensors (AMI, 18269 Gothard Street, Huntington Beach, CA 92648), a PMD 1408FS DAC system and a computer, were randomly assigned to the two environmental chambers, for oxygen quantification. Liquid-in-glass thermometers, mounted on rubber stoppers were used to monitor chamber temperatures (10 and 27°C), and recorded oxygen levels from each sensor were corrected to the average of the two sensor output values.

RESULTS AND DISCUSSION

Maize kernel density

Maize kernel density values were needed in order to calculate gas volumes within a mass of maize. Kernel densities of triplicate samples of test maize were
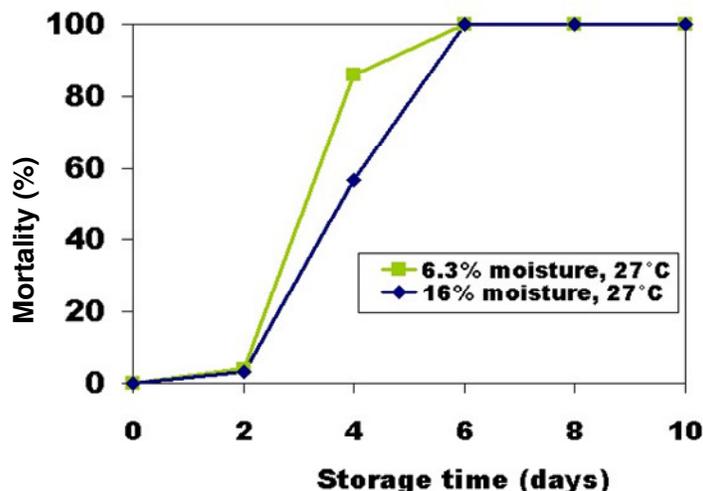
Figure 1. Mortality of *S. zeamais* hermetic storage at 27°C (averaged over four replications).
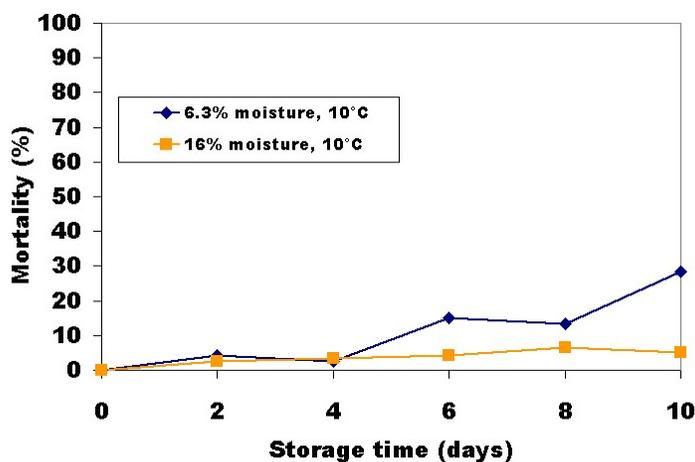

Figure 2. Mortality of *S. zeamais* at 10°C for hermetic storage (averaged over four replications).

measured using an Accupyc model 1330 pycnometer (Micromeritics, Gosford, New South Wales, Australia). Kernel densities were adjusted to 8 and 16% moisture using the procedure described by Dorsey-Redding et al. (1989). Average kernel density was 1.26 g/cm^3 at 16% moisture and 1.24 g/cm^3 at 8% moisture.

Weevil mortality results

The weevil mortality study was designed to determine effects of common limits of maize storage temperatures and maize storage moisture contents in East Africa on weevil mortality. It utilized 10 and 27°C maize storage temperatures, and maize at 6.3 and 16% moisture, under

hermetic and non-hermetic conditions, with replication. Assuming kernel density at 1.24 g/cm^3 for the 6.3% moisture maize and 1.26 g/cm^3 for the 16% moisture maize, there was about 1.27 cm^3 of oxygen for each weevil in the jar. The research tested the hypothesis that a hermetic storage system is effective for post-harvest weevil control in on-farm maize preservation in East Africa.

For hermetic storage at 27°C, weevil mortality reached 100% in six days for both the 6.3 and 16% moisture maize (Figure 1). At 10°C, weevil mortality increased over time, but reached only 28 and 5% for 6.3 and 16% moisture maize, respectively, after 10 days (Figure 2). Decreases in mortality from day 2 to 4, and from day 6 to 8 came about because four different jars were opened
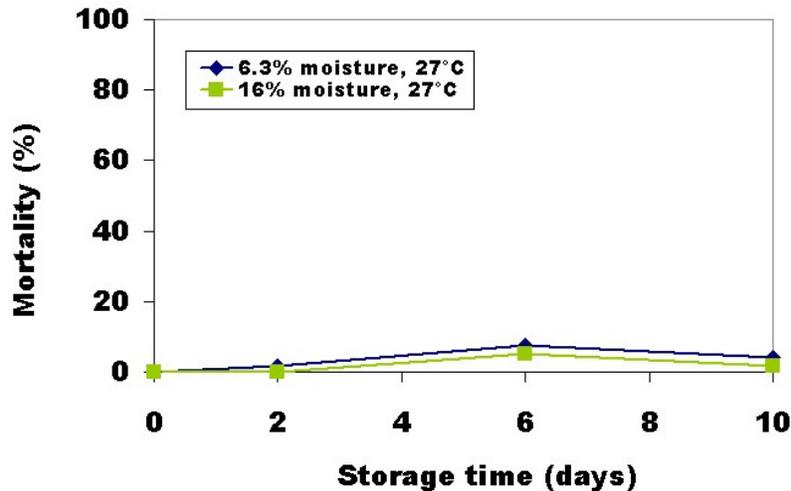
Figure 3. Mortality of *S. zeamais* at 27°C for non-hermetic storage (averaged over four replications).
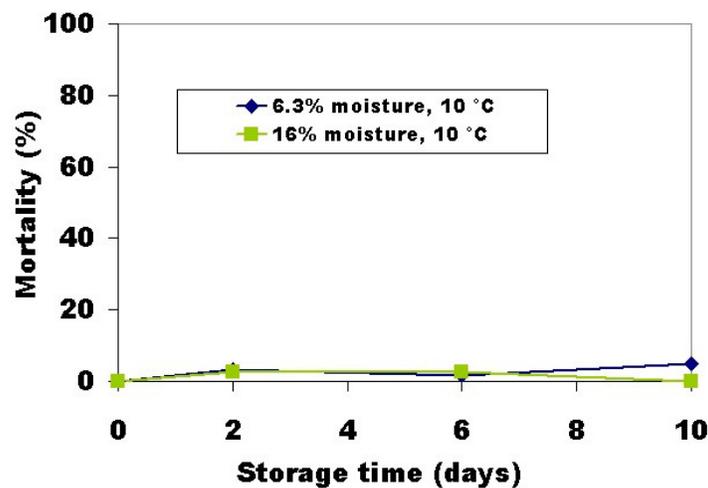
Figure 4. Mortality of *S. zeamais* at 10°C for non-hermetic storage (averaged over four replications).

and discarded after a mortality count on each sampling date.

Weevils in non-hermetic (open-air) treatments (Figures 3 and 4) have much lower mortality rates, compared to hermetic treatments. Mortality rates ranged from 0 to 5% after 10 days. As with hermetic treatments, there were instances when percent mortality decreased over time because different jars were opened, examined, and discarded on succeeding days.

Table 1 shows the ten-day mortality means and differences. For hermetic samples, the ten-day temperature main effect was 83.3% points (100 to 6.7%). Mortality at 27°C was nearly six times that at 10°C. The moisture main effect was 11.7% points (64.2 to 52.5%), with 6.3% moisture mortality being higher than 16%

moisture mortality. Based on the percentage mean differences and p-values (Table 2), main effects of temperature and moisture, and interaction effects were significant for hermetic conditions. There was a significant (p<0.0001) temperature- moisture interaction for the 27°C hermetic treatments, where the weevil mortality rate was 100%, with no associated variability (Figure 5).

For the non-hermetic samples, temperature main effect was 0.5% points (3.0 to 2.5%) and the moisture main effect was 0.9% points (1.7 to 0.8%) (Table1). Neither main effects nor interactions effects were statistically significant (Table 2) for these conditions. Weevil mortality was not affected by maize temperature or moisture content.

Table 1. Ten-day weevil mortality, percent (with standard errors).

| | | Hermetic | | | | Non-hermetic | | | |
| | | Moisture (%) | | | | Moisture (%) | | | |
		6.3	16	Mean	Diff[a]	6.3	16		Diff[a]
Temperature	10°C	28.3±4.4	5.0±5.0	16.7	23.3[b]	5.0±3.1	0.0±0.0	2.5	5.0
	27°C	100±0.0	100±0.0	100	0.0	4.2±3.1	1.7±0.9	3.0	2.5
	Mean	64.2	52.5			4.6	0.9		
	Diff[a]	71.7	95.0[b]			0.8	1.7		

[a]Diff = mean difference, [b]Significant at α = 0.05% level.

Table 2. Ten-day weevil mortality main effects and interactions.

Source		p
Hermetic	Temperature	<0.0001
	Moisture	0.0431
	Temperature*Moisture	0.0431
Non-hermetic	Temperature	0.8701
	Moisture	0.1755
	Temperature*Moisture	0.6269
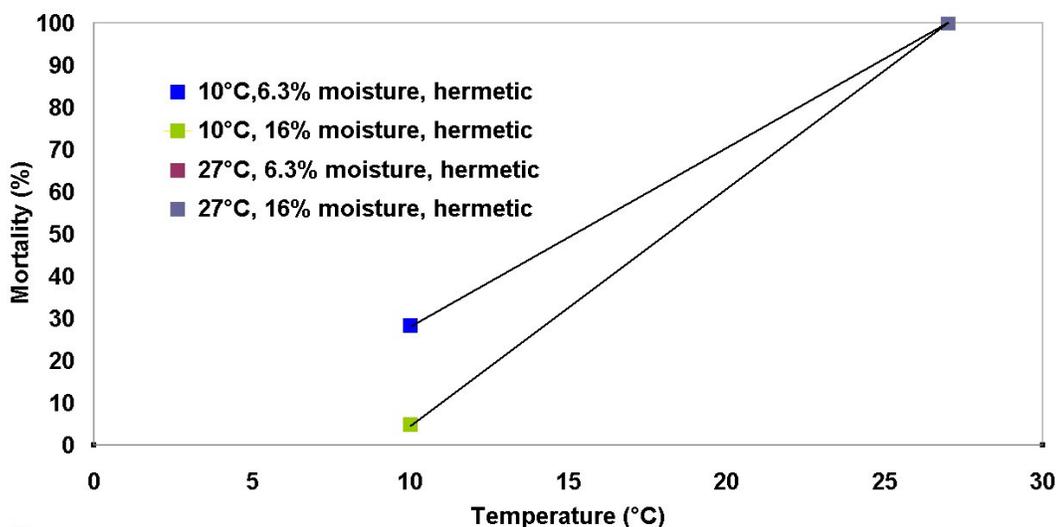
Figure 5. Temperature by moisture interaction (hermetic conditions).

Temperature and weevil respiration

Wohlgemuth (1989) suggested that insects on stored products are inactive at 10°C and below, but cause substantial damage at temperatures up to 35°C. Our results show low levels of mortality after 10 days, at 10°C, especially in 16% maize (Table 1). This suggests that there continues to be considerable activity at 10°C.

Oxygen quantification results

Oxygen quantification results (Figures 6 and 7) show that 100% weevil mortality occurred at 10 and 27°C and at both 8 and 16% moisture. Following trends observed in the mortality study, oxygen depletion was faster and 100% mortality was achieved sooner for higher maize temperatures and lower maize moistures. Assuming
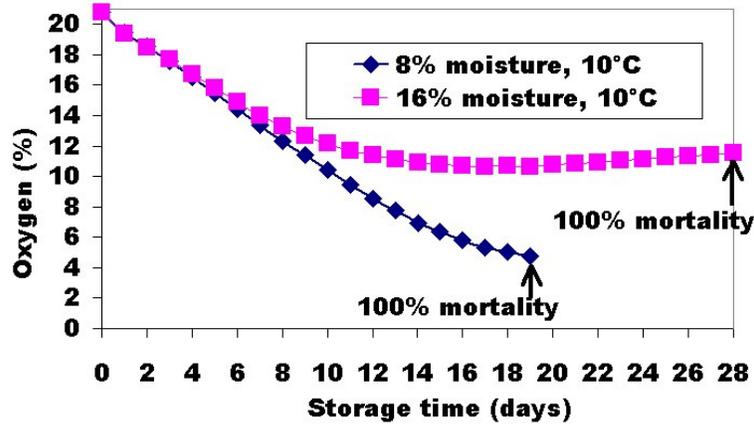
Figure 6. Percentage oxygen in hermetic storage containers at 8 and 16% maize moisture and 10°C (averaged over three replications).
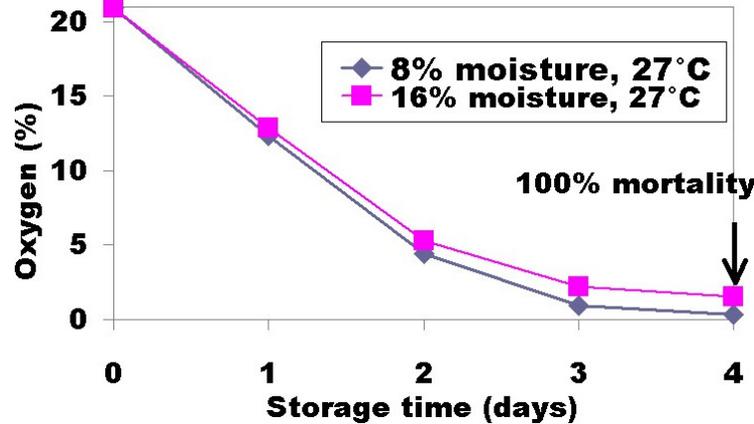

Figure 7. Percentage oxygen in hermetic storage containers at 8 and 16% maize moisture and 27°C (averaged over three replications).

kernel density at 1.24 g/cm^3 for the 8% moisture maize and 1.26 g/cm^3 for the 16% moisture maize, there was about 0.72 cm^3 of oxygen for each weevil in the jar.

Weevil adaptation to hypoxia

Hypoxia is a condition in which body tissue is starved of oxygen. Adaptation to hypoxia, and anaerobic or partial anaerobic respiration in insects, is a cellular last resort for energy, as insect tissue cannot maintain anaerobic respiration for an extended length of time (Donahaye, 1990). The flattened regions of 16% maize moisture and 10°C (Figure 6) suggest hypoxia and anaerobic respiration. Based on these research results, adaptation to hypoxia is more likely to occur at low temperature (10°C) and is more pronounced at higher moisture (16%),

where adaptation can occur at any oxygen level (Saldıvar et al., 2003).

Maize weevil oxygen consumption

Table 3 shows average time to 100% weevil mortality at each of the test conditions. Data from the oxygen quantification study were used with jar specifications and maize bulk and kernel densities, as well as weevil counts, to calculate weevil oxygen consumption for each of the four maize moisture-temperature combinations (Figure 8). Data from Moreno-Martinez et al. (2000) allowed calculation of one point (16% moisture, 26°C), and the calculated oxygen consumption value agrees well with data from experiment two. Data from experiment one allowed calculation of two points at 27°C (8 and 16%

Table 3. Average times to 100% weevil mortality.

Storage time (days)	Conditions
28	16% moisture, 10°C
19	8% moisture, 10°C
4	16% moisture, 27°C
4	8% moisture, 27°C
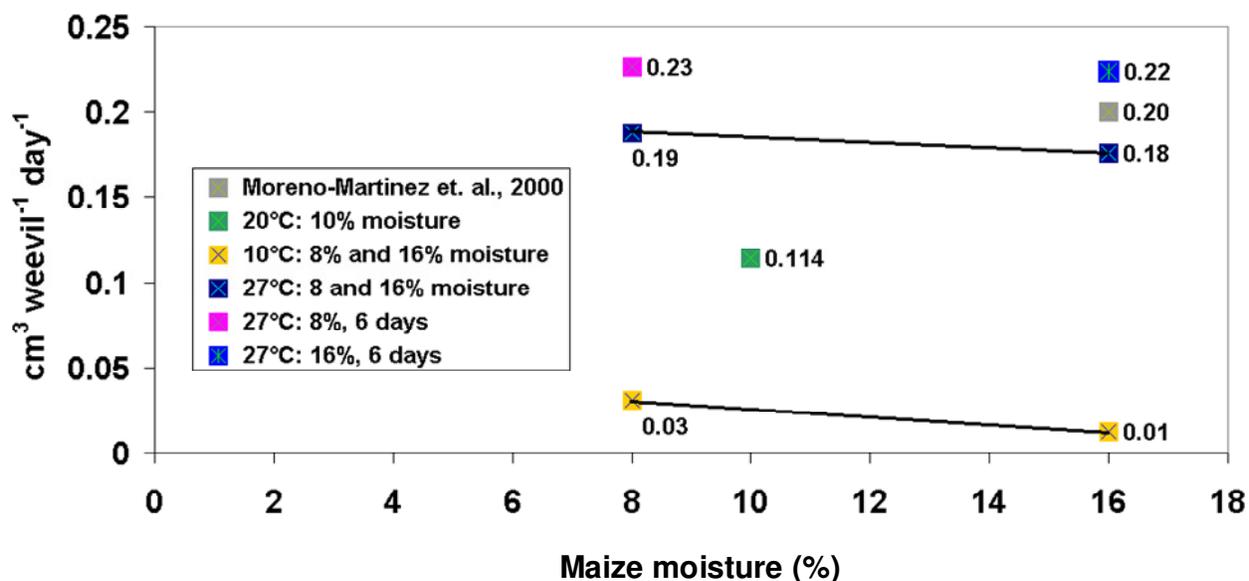
Figure 8. Average oxygen consumption of maize weevils in shelled maize.

moisture) on Figure 8. According to data trends from experiment two, oxygen consumption values should have been lower. One reason for the disagreement is that in experiment one, precise elapsed time and oxygen level at the time 100% mortality occurred were not known because of the procedures used. Maize is more hygroscopic at lower maize moisture and higher weevil stress levels occur at lower moistures than at higher moistures. Oxygen consumption was, on average, 0.015 cm^3 weevil^{-1} day^{-1} higher for weevils in 8% moisture than for weevils in 16% moisture maize. The difference, however, is not statistically significant.

Temperature by moisture interaction

Figures 5 and 8 also show that in hermetic storage, there are hermetic, moisture and temperature differences in weevil mortality at 10 vs. 27°C, as well as at 8 vs.16%, due to temperature and moisture interaction. The interactions mean that the mean percent mortality was not the same for the different levels of temperature and maize moisture.

Weevil mortality prediction

Equations for the two lines on Figure 8 are:

27°C: $Y = -0.00141x + 0.199$
10°C: $Y = -0.00234x + 0.0496$

Predicting time to 100% mortality

The area within the four points on Figure 8 (10 to 27°C, 8 to 16% moisture) includes most maize storage conditions on farms in East Africa. The graph may be used to predict time to 100% adult weevil mortality in any hermetic storage container. An example illustrates the procedure: a 225 L (55 gal) barrel contains 162 kg of maize at 10% moisture stored at 20°C, and the maize contains 100 weevils per kg. Interpolating between points (Figure 8) predicts an oxygen utilization value of 0.114 cm^3 per weevil per day. On average, weevils die when oxygen level reaches 4%. Using container and maize information, along with the calculated oxygen utilization value, the predicted time to 100% mortality is calculated

to be nine days.

Conclusions

Hermetic storage and oxygen quantification results from this research show that hermetic storage is effective for weevil control in stored maize. Weevil oxygen consumption data for temperatures and maize moisture ranges in East Africa allow prediction of the days to 100% mortality in a hermetically sealed storage container as a function of container volume, weevil concentration, maize moisture, and storage temperature.

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