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DOI: 10.5073/jka.2010.425.440

Abstract

Hermetic post-harvest maize storage can effectively control maize weevil, *Sitophilus zeamais*, which can be responsible for up to 50% damage to stored maize grain. Its use eliminates the need for toxic and expensive chemicals. Laboratory experiments were conducted on hermetic storage systems to evaluate the 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-day weevil mortality was higher in hermetic vs. non-hermetic storage, in 6.3% moisture maize vs. 16%, and at a 27°C storage temperature vs. 10°C. Oxygen depletion results allow estimation of daily weevil oxygen consumption as a function of storage temperature and maize moisture for East Africa conditions.

Keywords: Maize storage, Hermetic storage, *Sitophilus zeamais*, Maize weevil control, Maize deterioration

1. Introduction

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

Tropical heat, moisture and open-air storage promote rapid insect multiplication and mold formation in stored maize (FAO, 1994). Rapid insect development occurs when temperature is within 5 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* Motschulsky) 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 globally is in the tropics, where the vast majority of the maize is stored without chemical protectants on-farm (Lindblad and Druben, 1980). 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, 20-30% 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) also found about 18% of shelled maize with weevil damage in stored maize in Tanzania, while Demissie et al. (2008b) found levels of 11-59% weevil infestation in husk-covered maize stored at Bako, Ethiopia, after one month of storage.

Hermetic storage isolates the storage ecosystem from the external environment while respiration within the storage ecosystem causes O₂ reduction and CO₂ 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 an oxygen analyzer. The jars were stored at 26°C, 16% moisture, 70% r.h., and 18h: 6 h L:D photoperiod. 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.

The experiment also included treatments with fungus spores added and treatments without weevils. The rate of oxygen depletion in treatments containing weevils was more rapid than those containing fungi and maize alone, while treatments with maize alone had much lower oxygen utilization rates compared to fungi and maize.

Plastic bagging employs layers of air-tight and water-tight 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. With a one-time cost of \$3 per household, this storage system has the potential to increase household income on average by about \$150 per year (Murdock et al., 2003; Carroll and Fulton, 2008). Studies are under way to determine plastic bag life (Murdock, 2010).

Steel containers are excellent candidates for low-cost 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 bruchid-infested cowpea stored for 6 months in sealed drums with minimal losses. Such containers may be contaminated by petro-chemicals or something else, and need to be properly cleaned to prevent cross contamination of maize stored within them. Common methods for determining types of petro-chemicals present, and for measuring the level of contamination involve methanol extraction followed by gas chromatography (Turriff, et al., 1998). The use of locally available soaps for cleaning is also common practice, although the efficacy of this method of cleaning is not well documented.

Further development of effective hermetic storage systems for maize requires more extensive information on the oxygen requirement of weevils within maize stored over a range of moistures and temperatures. The objectives of this research were to determine the effects of oxygen level, maize temperature, maize moisture and their interaction on the survivability of maize weevils over time in hermetic containers.

2. Materials and methods

A laboratory scale hermetic storage system was used, where products of weevil, mold, and maize respiration serve as an effective pest control strategy in stored maize. The research employed instrumentation for the quantification of oxygen levels. Treatment conditions of temperature (10 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 treatment assignment to jars and chambers was done using PROC GLM (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513).

Maize grain of the commercial hybrid Fontanelle 6T672 at about 16.5% moisture was harvested using a 4420 Deere combine. Following harvest it 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 for drying to 16% and air at 45°C for drying to 8% or 6.3%. Moistures were confirmed using the oven method (103°C oven for 72 h) (ASABE, 2008).

A stock culture of 100 adult *S. zeamais* (unsexed) obtained from the Iowa State University Entomology Departmental laboratory were placed in five unsterilized 3.74-L glass jars, with screen lids, 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 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). Two chambers at 10 and 27°C were utilized in the experiments. They were model 13-988-126 GW Fischer Scientific Isotemp refrigeration chambers (Thermo Fisher Scientific Inc., Waltham, MA 02454), with temperature controls.

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 screens which allowed air passage but not weevil escape.

2.1. Weevil mortality study

The experimental design consisted of four factorials (days, maize moisture, temperature, and replication), with weevil mortality being the dependent variable. Days had five levels (2, 4, 6, 8, and 10 days), maize moisture had two levels (6.3 and 16%), temperature had two levels (10 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 (2, 6 and 10), 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.

The criteria for determining weevil mortality relied on a combination of observed rigor mortis features (Gullan and Cranston, 2000). Weevils that were curled up and/or had outstretched legs; 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 even when exposed to ambient air were assumed dead. To determine mortality, each jar from the 16 treatments (T₁-T₁₆) was examined for dead weevils on the day on 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.

2.3. Oxygen quantification study

To determine oxygen depletion under different maize moisture and temperature, 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 monitoring. Liquid-in-glass thermometers, mounted on rubber stoppers were used to monitor chamber temperatures (10°C and 27°C), and recorded oxygen levels from each sensor were corrected to the average of the two sensor output values.

3. Results and discussion

The first study consisted of testing the hermetic and non-hermetic 10 and 27°C temperatures, and maize at 6.3 and 16% moisture, under hermetic and non-hermetic conditions, with replication. At 27°C, weevil mortality reached 100% in six days for both 6.3 and 16% moisture maize (Fig. 1). At 10°C, weevil mortality increased over time, but only reached 28 and 5% for 6.3 and 16% moisture maize, respectively (Fig. 2). Decreases in mortality from day 2 to day 4, and from day 6 to day 8 came about because three different jars were opened and discarded after a mortality count on each sampling date. These results show that mortality increases more rapidly at higher maize storage temperature, and more rapidly at lower maize moisture levels.

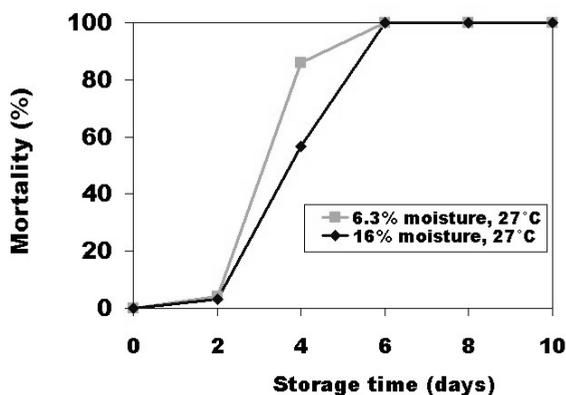


Figure 1 Mortality of *Sitophilus zeamais* hermetic storage at 27°C (averaged over three replications).

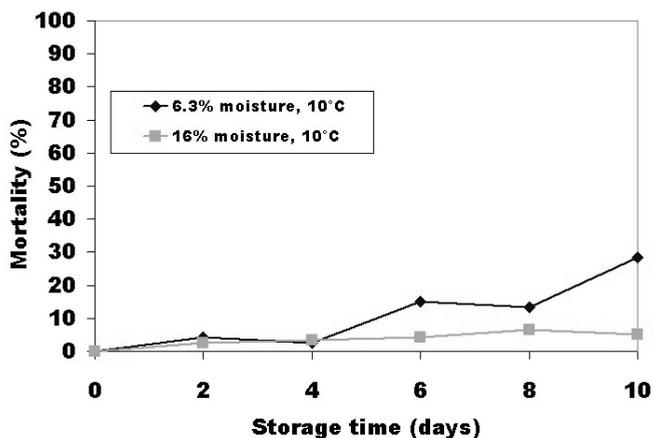


Figure 2 Mortality of *Sitophilus zeamais* hermetic storage at 10°C (averaged over three replications).

Based on Figures 3 and 4, weevils seem to have a natural mortality rate, which is dependent on natural mortality factors, irrespective of the treatment combination. The maize weevil is a vector of some predatory fungi, and has other natural enemies capable of reducing its population in open air storage (Sétamou, M. 1999; Imamura, et al., 2004; Hansen and Steenberg, 2006). Hence, a higher level of variability in mortality is associated with non-hermetic treatments.

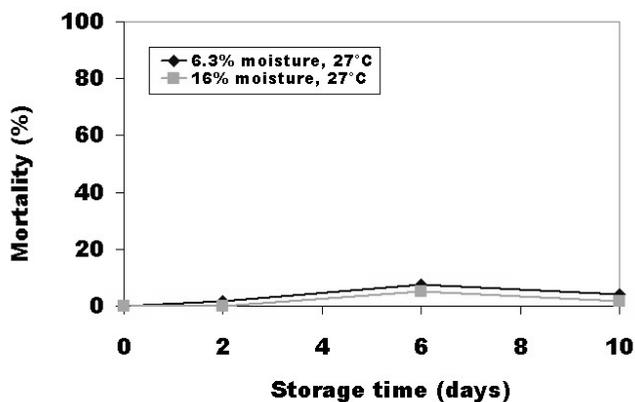


Figure 3 Mortality of *Sitophilus zeamais* non-hermetic storage at 27 °C (averaged over three replications).

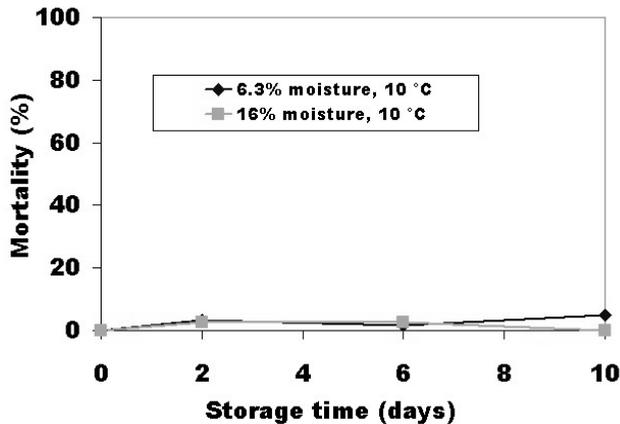


Figure 4 Mean mortality of *Sitophilus zeamais* non-hermetic storage at 10°C (averaged over three replications).

Table 1 shows mean percent mortality rates at day 10, along with standard deviations. Looking at the rows of Table 1, the three-factor (temperature by moisture by day) mean difference of differences (interaction) is more significant at 10°C than at 27°C, in both hermetic (23.3) and non-hermetic (5.0) treatments. And a comparison of the columnar differences indicates higher level of interaction at the 16% (95, 1.7) maize moisture than at 6.3% (71.7, 0.8), for both types of treatment. Interaction occurred because the mean percent mortality was not the same for the different levels of day, temperature and maize moisture.

Table 1 Mortality of *Sitophilus zeamais* after 10 d under different conditions and the differences between row and columns.

Temperature (°C)	Mortality \pm SD (%)			Non-hermetic		
	Hermetic			Grain moisture		
	6.3%	16%	Difference	6.3%	16%	Difference
10	28.3 \pm 8.8	5.0 \pm 10.0	23.3	5.0 \pm 6.4	0.0 \pm 0.0	5.0
27	100 \pm 0.0	100 \pm 0.0	0	4.2 \pm 8.3	1.7 \pm 1.9	2.5
Difference	71.7	95		0.8	1.7	

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. Our results show low levels of mortality after 10 days at 10°C, especially in 16% maize. This suggests that there continues to be considerable activity at that temperature.

Oxygen concentrations indicate that 100% weevil mortality is achievable at both 10 and 27°C and both 8 and 16% moisture content (Fig. 5, 6). Following the trends observed in the mortality study, oxygen depletion was faster and 100% mortality is achieved sooner for higher maize temperatures and lower maize moistures. Figure 6 shows results obtained at 27°C for 16% and 8% moisture.

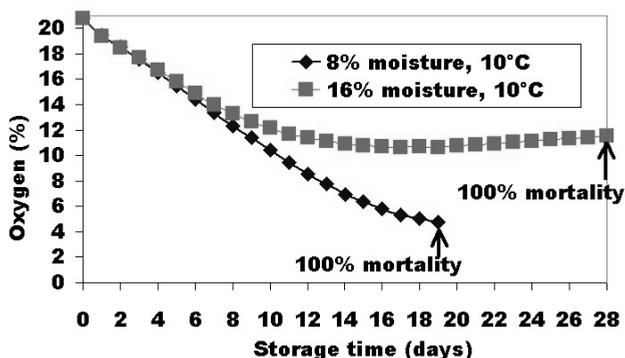


Figure 5 Average percentage oxygen for three replications at 8 and 16% maize moisture and 10°C.

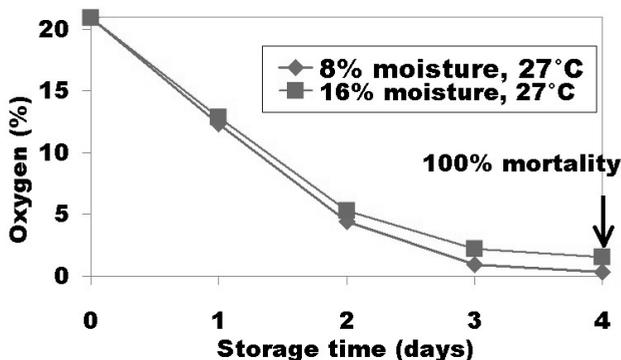


Figure 6 Average percentage oxygen for three replications at 8 and 16% maize moisture and 27°C.

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 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). Adjusted values are shown in Table 2.

Table 2 Conditions to achieve 100% mortality of *Sitophilus zeamais* and maize densities.

Storage Time (days)	Moisture (%)	Temperature (°C)	Kernel density, (g/cm ³)
28	16	10	1.26
19	8	10	1.24
4	16	27	1.26
4	8	27	1.24

Table 2 also shows average time to 100% weevil mortality at each of the test conditions. Data from studies on respiration jar volume, maize bulk and kernel densities and weevil counts were used to calculate weevil oxygen consumption for each of the four maize moisture-temperature combinations (Fig. 7). Data from the Moreno-Martinez *et al.* (2000) allows calculation of one point on Figure 7.

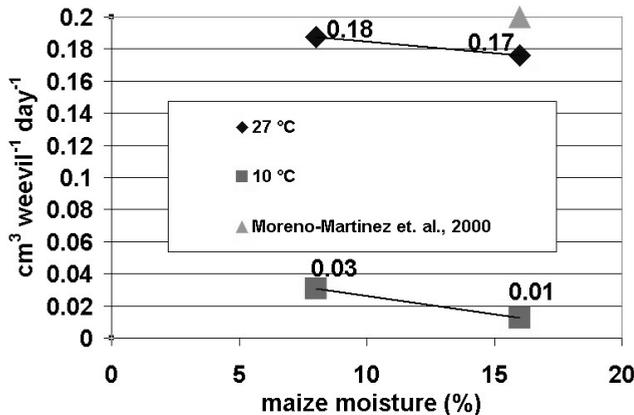


Figure 7 Average oxygen consumption of maize weevils in shelled maize.

At 16% maize moisture and 26°C, weevil oxygen consumption was 0.20 cm³ weevil⁻¹ day⁻¹. This shows good agreement with the present study. Equations for the two lines on Figure 7 are:

$$27^{\circ}\text{C}: Y = -0.00141x + 0.199; 10^{\circ}\text{C}: Y = -0.00234x + 0.0496.$$

The area within the four points on Figure 7 (10°C to 27°C, 8 to 16% moisture content) includes most maize storage conditions on farms in East Africa. The graph may be used to predict the time to 100% mortality in any hermetic storage container.

3.1 Prediction example

A 225-L (55-gal) barrel contains 162 kg of maize at 10% moisture stored at 20°C. The maize contains 100 weevils per kg. Interpolating between points on Figure 7 predicts an oxygen utilization value of 0.114 cm³ weevil⁻¹ day⁻¹. 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.

4. Conclusions

The hermetic storage and oxygen quantification studies show that hermetic storage is effective for weevil control in stored maize. Weevil oxygen consumption data allow prediction of the days to 100% mortality in a hermetically sealed storage container as a function of container volume, weevil numbers, maize moisture, and storage temperature.

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