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# GLOBAL CHALLENGES FOR THE SUCCESSFUL APPLICATION OF MA AND HERMETIC STORAGE

Shlomo Navarro*
Food Technology International Consultancy Ltd., P.O. Box 3300, Beit Yehoshua, 40591, Israel.
*Corresponding author's e-mail*: snavarro@ftic.info*

## ABSTRACT

Storage insects are aerobic organisms requiring oxygen for their survival. Therefore, they respond to altered atmospheric gas compositions containing low oxygen ($O_2$) or high carbon dioxide ($CO_2$). To have an insecticidal toxic effect, a "high-$CO_2$ atmosphere" must contain a substantial proportion of $CO_2$, often more than 60%. Insect response depends on the species, developmental stage and age, the physical conditions in the environment, exposure time, and the type of the atmospheric composition used as treatment. Lowering the r.h. increases the effectiveness of MAs. Desiccation plays a large role in the mortality of stored-product insects exposed to some MAs. To obtain good control, the temperature should be above 21°C during the application of MA. The influence of temperature over the range of 38–42°C on the effects of hypoxia and hypercarbia on insects was demonstrated. The main cause of deterioration of dry grain is insects. While the main cause of deterioration of moist grain is microflora. Therefore, hermetic storage may be addressed to dry grain or moist grain storage. Hermetic storage takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing $O_2$ and increasing $CO_2$ concentrations through respiratory metabolism. An ingress rate of 0.05%$O_2$/day is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018% over one year storage period. For dry grain storage, this level is critical since even at short storage periods of 3 to 6 months at this ingress rate, the possibility of a residual surviving insect population is eliminated at an economical threshold. This low $O_2$ ingress level, is difficult to obtain in rigid structures, but is achievable in practice using flexible liners. It could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method.

**Key words:** modified atmospheres, hermetic storage, carbon dioxide, low oxygen, stored-product insects, stored-product microflora, desiccation, respiratory metabolism

## INTRODUCTION

The beneficial effects of modified atmosphere (MA) treatment as an alternative that is safe and environmentally benign, to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored grain, oilseeds, processed commodities and packaged foods have been well documented (Navarro, 2006). Serious interest in using the technique in a practical, routine manner was not pursued until the 1970s and 80s, probably due to the success of conventional fumigants and grain protectants in controlling stored-product pests. During this period, a realization began to develop that the chemicals, if used improperly, left objectionable residues, were hazardous to apply, and that there was a potential for the development of insect resistance to them. Research was initiated during this time in Australia, in the U.S. and several other countries on the use of modified atmospheres (Ripp et al., 1984). MA and control1ed atmosphere (CA) treatments for the disinfestation of dry stored products have received increasing scientific attention during the last 32 years. Although CA has become well established for control of storage pests, its commercial use is still limited to a few countries. The widespread scientific activities on this subject resulted in several international conferences, such as the International Conferences on Control1ed Atmospheres and Fumigation in Stored Products with the report of the last meeting by Daolin et al. (2008), and the International Working Conferences on Stored-Product Protection with the report of the last meeting by Carvalho et al. (2010).

Reviewing the reports on MA and hermetic storage carried out over the last 32 years reveals that more field trials were carried out on MA, CA and fumigation than on hermetic storage using flexible containers. Only in the last several years has hermetic storage emerged as a significant alternative method of post harvest storage, particularly in tropical climate countries using several hermetic storage methods (Villers et al., 2010) in South America using the silobags (Bartosik, 2010), the use of hermetic SuperGrainbags$^{TM}$ for small farmers for rice seed since 2004 as reported by the International Rice and Research Institute (IRRI) (Rickman and Aquino, 2011) and in Africa the Purdue Improved Cowpea Storage (PICS) hermetic bags (Murdock et al., 1997; Murdock et al., 2003; Baributsa et al., 2010; Anon., 2012).

In spite of the numerous advantages of MA and hermetic storage have, these technologies still need additional field data and practical know-how. The present paper aims at describing the existing global challenges for the successful application of MA and hermetic storage.

## GASTIGHTNESS OF THE STRUCTURES

### Rigid structures

*Structural requirements:*

A fundamental requirement for the successful application of gaseous treatments to control stored-product insects is a well-sealed structure. Fumigants have been used for many years with limited requirements for structural tightness, and covering the grain bulk or the storage with plastic sheets was usually considered satisfactory. Lack of gastightness has for years been a problem for the application of fumigants in storage. The consequences of poorly sealed storages under fumigation are now more considered in view of the development of insect resistance to phosphine in poorly sealed structures (Casada and Noyes, 2001). The requirement for gastight storages for application of CAs and MAs appears to be more critical than for application of fumigants (Navarro, 1999). Therefore, before MA application, careful examination should be made of sealing requirements to obtain a standard acceptable for maintaining the gas composition over the designed exposure period.

Although practical guides for requirements for silo gastightness exist (Banks and Annis, 1977), they are very seldom implemented by the grain industry. Their specifications correspond to the pressure-decay times needed to maintain the atmospheric composition in the silos. These tests were designed to estimate the permissible limits for effectively maintaining the gas composition in the storages during the treatment (Navarro and Zettler, 2001). Comparisons of variable-pressure tests are scarce. A table was prepared to provide provisional guidelines based on the best estimates available in the literature (Navarro, 1999).

Accordingly, for example, for MA storage, with large structures of up to 500 tonnes capacity, a decay time of 5 min from 250 to 1250 Pa was regarded as satisfactory. To ensure successful application of MA in rigid structures, the grain industry should adopt the concept of sealing the structures adequately and run a suitable pressure test before MA treatment.

*Cost of sealing:*
A major challenge in the application of MA is to convert an existing structure into sufficiently gastight for the treatment (Burton, 1998). Although sufficient expertise has been gathered in countries like Australia (Newman, 2006), such expertise is lacking in many other countries that renders the initial cost sufficiently expensive to create commercial reluctance in the application of the technology. In practice, storage structures designed specifically for the application of MAs are practically nonexistent, apart from those in Australia (Ripp et al., 1984). Newman (1990) noted an increasing trend in Australia toward the use of sealed storage for dry grain, accompanied by the conversion of existing structures to sealed storage rather than construction of new installations.

In a recent study (Navarro et al., 2012a) cost of sealing of 2,400 tonnes capacity bin was 15,700 € or 6.54 €/tonne (AU$ 8.28/tonne) of grain. According to Newman (2006) "The costs of sealing a horizontal 21,800 tonne storage in 1982 was nearly AU$ 3/tonne, therefore the full cost of AU$ 64,400 amortised over 10 yr is AU$ 0.30/tonne. In 1999 the costs of sealing a storage ranged from AU$ 3.50 - 4.50 per tonne depending on the structure. Now in 2006 the costs are closer to AU$ 5 per tonne equating to AU$ 0.50/tonne over 10 yr using the previous example". This exemplifies the significant differences of sealing works carried out in a country like Australia with existing technological infrastructure and in a country that strives to initiate MA technology like Cyprus. The 2006 sealing cost in Australia was AU$ 5/tonne which may not be comparable to 2012 cost in Cyprus at AU$ 8.28/tonne. Although the costs of sealing any storage will depend entirely on the complexity of the task, the above figures may provide a perspective for the sealing challenge before MA treatment is initiated.

**Flexible structures**
Flexible structures can be used for MA/CA treatments and for the application of hermetic storage technology. However, currently, there are more flexible structures used for hermetic storage than for MA/CA storage in rigid structures (Navarro, 2006; Navarro et al., 2012b). It is assumed that flexible structures are easier to seal than rigid structures. However, gas loss through the structural membrane during gaseous treatments is an important phenomenon. Membranes of plastic permit gas permeation and gas exchange. Pressure tests, are not capable of measuring the degree of permeability losses. Since it is difficult to maintain complete gas tightness without any $O_2$ ingress into the large commercial structures, some tolerances that would permit quality preservation of the grain during hermetic storage should be established.

*Parameters for testing gas tightness for hermetic storage of grain:*
The following parameters were set for hermetic storage of cereals. Since this technology is relatively the newest and the terminology used is less elaborated, it creates much confusion of what is meant by hermetic storage of grain. This type of storage has been referred to a type of MA that can be applied for the protection of grain also termed as "sealed storage" or "air-tight storage" or "sacrificial sealed storage". This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing oxygen ($O_2$) and increasing carbon dioxide ($CO_2$) concentrations through respiratory metabolism (Navarro et al., 1994; Navarro, 2012).

Respiration of the living organisms in storage (insects, fungi, and grain) consume oxygen ($O_2$), reducing it from near 21% in air to 1 to 2% while production of carbon dioxide ($CO_2$) rises from an ambient 0.035% to near 20% or higher according to the level of moisture content. This environment kills insect and mite pests and prevents aerobic fungi from growing. Elevated $CO_2$ and depleted $O_2$ levels will generally maintain stored grain quality for long periods. Grain with excessive moisture may be invaded by lactate-forming bacteria and yeasts. The key to successful hermetic storage is air tightness and control of condensation. In modern times, storage size has increased from small family storages to large bulks representing many producers or a portion of a country's total production.

The main cause of deterioration of dry grain is insects. While the main cause of deterioration of moist grain is microflora. The grain responds differently in the ecosystem of storage when it is at intermediate moisture but close to the critical level where fungi is the dominant microflora (Navarro and Donahaye, 2005). While at higher moisture levels, the dominant microflora are; mostly yeasts and bacteria (Elepano and Navarro, 2008; Weinberg et al., 2008). Therefore, hermetic storage may be used for storing dry or moist grain (Navarro and Donahaye, 2005).

For the application of hermetic storage to dry grain an ingress rate of 0.05%$O_2$/day is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018% over one year storage period (Navarro et al., 1994). For dry grain storage, this level is critical since even at short storage periods of 3 to 6 months at this ingress rate, the possibility of a residual surviving insect population is eliminated at an economical threshold. For higher $O_2$ ingress rates, the weight loss continues to rise in proportion to the $O_2$ ingress rate and insect damage might be very significant and cannot be arrested. Ingress rates of up to 0.15%$O_2$/day can be tolerated. However, for moist grain, at higher $O_2$ ingress rates than 0.15%/day, permits grain deterioration that might lead to development of mycotoxins (Weinberg et al., 2008).

This low $O_2$ ingress level, is difficult to obtain in rigid structures, but is achievable in practice using flexible liners. It could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method. Flexible structures with higher $O_2$ ingress rates than 0.15%$O_2$/day, may be used to protect the grain from rain or increase of moisture provided the grain is dry and without any infestation. The question is whether these structures should be considered under the term of "hermetic storage" or just simply "sealed storage" without the expectations that they will develop a biogenerated atmosphere to protect the grain and use fumigation to control the insects.

**Size of the flexible structures**

Enclosures that are mostly destined for indoor hermetic storage of bagged commodities are now available in the market (PICS or Purdue Improved Cowpea Storage) (Anon., 2012; Baributsa et al., 2010; Baoua et al., 2012). The dimensions of the structure are dictated by the manageability of the stack. Unit containers in the range of 80 L to 120 L capacity named SuperGrainbags™ (SGB) exists (Villers et al., 2008; Rickman and Aquino, 2011). The SGB is a 7-layer coextruded plastic with thickness of 0.078 mm, 2.14 mL/($m^2$ 24h) permeability levels for oxygen and for water vapour of 4.28 g/($m^2$ 24h). These features of SGB maintain commodity quality, even with long transport times and in humid environments. Using the same material, the SuperGrainbag-HC™ has become available for use with mechanized loading, which handles up to a 1-tonne capacity for bags or bulk storage.

For outdoors hermetic storage of grain larger structures have been reported by Villers et al. (2008). The most widely used form of hermetic storage is the Cocoon™. It is manufactured in capacities of up to 300 tonnes. Cocoons, used for storing grain commodities, are made from specially formulated flexible 0.83 mm thick PVC with permeability to oxygen varying from 87 to 400 mL/($m^2$ 24h) and to water vapour of 8 g/($m^2$ 24h). They are sealed with an airtight zipper. A newer type of Cocoon called the MegaCocoon™ has more recently been introduced for larger scale storage of up to 1050 tonnes.

Silo Bags of 200 tonnes capacity for on-farm grain storage are used directly in the field and, with the available handling equipment, is quite simple to load and unload. This technique was originally used for silage; it involves storing dry grain in sealed plastic bags. This sealed storage method adopted in South America is used for temporary storage of dry grain and oilseeds (Bartosik 2010).

*The size factor in hermetic storages:*

Experience shows that hermetic storage works best for large structures. This is obvious from the lower surface area/volume ratio in large bulks compared with small bulks. The factor of $O_2$ ingress rate, in practice is a goal difficult to achieve. Therefore, depending on the commercially available membrane permeability, engineers should aim at designing hermetic structures of sufficiently large dimensions. To emphasize the importance of the size of the structure in hermetic storage, calculations were made assuming a permeability level of 200 $mLO_2/(m^2$ 24h) for structures of different dimensions ranging from 1 to 1,000 $m^3$ (Navarro et al., 1994). The calculations demonstrate that a tenfold increase in the volume of the bulk causes an approximate twofold decrease in the initial $O_2$ ingress rate. This indicates the importance that low-permeability liners must be preferred for hermetic storage at farm-levels in developing countries.

*Gas permeation through the membrane:*

Although insect respiration causes depletion in the $O_2$ level of the hermetic storage, to arrest insect development, a sufficiently low ingress rate $O_2$ is critical to control the insect population or to eliminate the possibility of a residual surviving insect population. Such critical residual $O_2$ level remaining in the hermetic storage structure is exemplified in Fig. 1, where insect respiration (4 insects/kg grain, each 157 μL/insect/day), the $O_2$ ingress rate, and its difference as the volume of residual $O_2$ remaining in the hermetic storage was plotted on the same graph. From Fig. 1 it is clear that the residual $O_2$ concentration would reach to about 5% in about 13.5 weeks.

This low $O_2$ ingress level is achievable in practice using flexible liners. It could serve as a guideline for $O_2$ permeability specifications of flexible liners appropriate to the hermetic storage method. For small volumes, such as bag size hermetic storage structures, a low permeability to $O_2$ is essential and for large volumes higher permeability levels can be tolerated. To exemplify such tolerances Fig. 2 was prepared that clearly shows the importance of selecting extremely low $O_2$ permeability liners when using small size (bag) hermetic storage units. According to Fig. 2, hermetic storage structures with capacities greater than 50 $m^3$ would require liners of a permeability level of 100 $mLO_2/(m^2$ day) for ingress rate of $0.05\%O_2$/day. For capacities of greater than 100 $m^3$, liners of permeability level of 400 $mLO_2/(m^2$ day) will be suitable for ingress rate of $0.15\%O_2$/day.

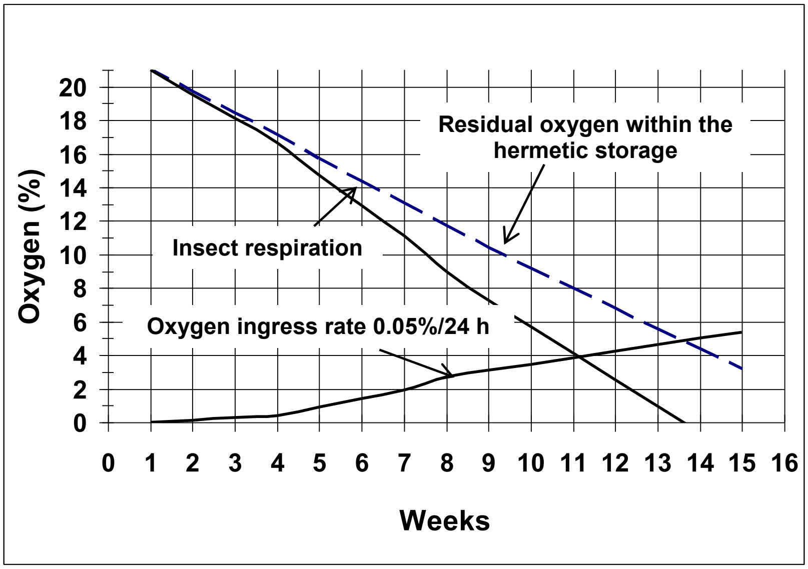


Fig. 1 - Insect respiration (4 insects/kg, each 157 μL/insect/day), $O_2$ ingress rate (0.05%/24 h), and its difference as the percentage of residual $O_2$ remaining in the hermetic storage to demonstrate the process of obtaining an $O_2$ depleted atmosphere in hermetic storage of dry grains.

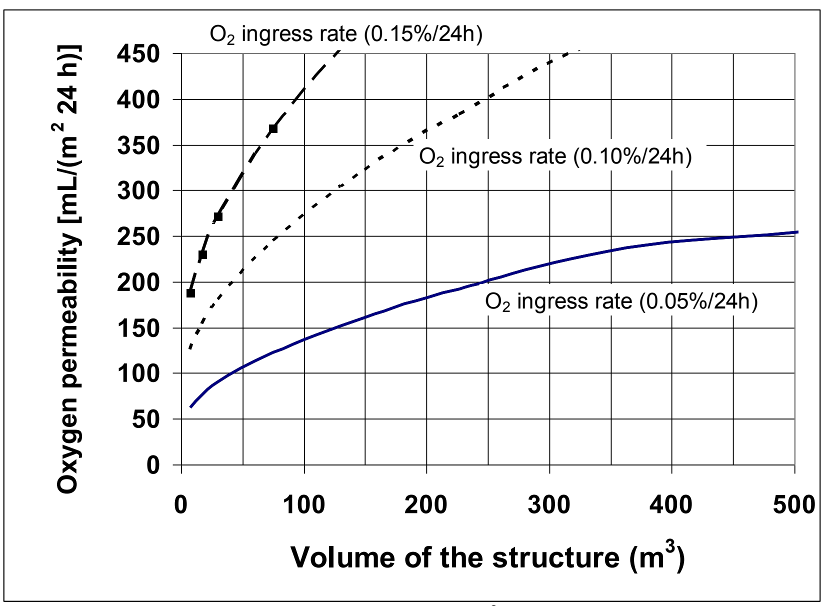


Fig. 2 – Oxygen permeability requirements [mL/(m$^2$ 24 h)] of liners in relation to various storage capacities (m$^3$) and oxygen ingress rates (%/24 h) for successful application of hermetic storage of dry grain.

*Liner durability and resistance to insects*

Flexible packaging films vary in resistance to penetration by insects. A major drawback of flexible liners is that pests leading to infestation of foods can penetrate them. The degree of pest infestation of packaged foods depends upon the pest species involved, the time of exposure to invading pests, and the prevailing environmental conditions. There are two types of insects that attack packaged products: penetrators, insects that can bore holes through packaging materials, and invaders, insects that enter packages through existing holes, such as folds and seams and air vents. *Sitophilus* spp., *Rhyzopertha dominica* (F.), *Prostephanus truncatus* (Horn), *Plodia interpunctella* (Hübner), *Lasioderma serricorne* (F.), *Callosobruscus maculatus* (F.) and *Stegobium paniceum* (L.) are some of the stored product insects that are capable of penetrating the flexible liners destined for hermetic storage of grain or pulses.

With the increase use of hermetic storage technology in bags, farmers have quickly adopted the technology. The hermetic bags provide storage opportunity to farmers and consumers interested in organic and bio products. However, liner vulnerability to insect penetration places the technology at risk. A major challenge is therefore, to explore the possibilities of preventing insect penetration through the liner to eliminate the gastightness needed for successful application of the technology.

## LETHAL ACTION OF MA ON INSECTS

### Low oxygen and anoxia

Nitrogen ($N_2$) is commonly used to produce a low-oxygen atmosphere to cause anoxia on storage insects. Generally, the lower the oxygen level, the higher the mortality. For effective control, the $O_2$ level should be <3% and preferably <1% if a rapid kill is required. Although suppression of storage-insect development was observed at about 5% $O_2$, the exposure time required to kill the insects was very long. Experiments with *Tribolium castaneum* (Herbst) in $N_2$ showed significant differences in adult mortality between 0.1 and 1.0% $O_2$. Similar experiments with *T. confusum* in $N_2$ showed a critical oxygen level at 0.9%, and >1.4% $O_2$ was found to be ineffective. The adults are generally the most susceptible to the treatment and *S. oryzae* or *R. dominica* was demonstrated to be more tolerant than *Tribolium* spp. The lowest level of tolerance to lack of $O_2$ was attained around the 1% concentration level. There are more laboratory data for *S. oryzae* than for any other stored-product pest and, except for *Trogoderma* spp.

### Effect of air relative humidity and MA

Lowering the r.h. increases the effectiveness of MAs. Results with adults of *T. confusum*, *T. castaneum*, and *Oryzaephilus surinamensis* (L.), have shown that, in atmospheres containing 99% $N_2$ (balance $O_2$), decreasing the r.h. from 68 to 9% increased the mortality from 3 to 98.5% in a 24-h exposure of the red flour beetle. These three species also exhibited a similar response to mixtures of $CO_2$ in air at lowered r.h.

Desiccation plays a large role in the mortality of stored-product insects exposed to some MAs. It was shown that when larvae, pupa, and adults of the red flour beetle were exposed to varying concentrations of $CO_2$ or $O_2$, weight loss was much higher in some of the atmospheres than in others or in air. A linear relationship of the combined effect of low $O_2$ or high $CO_2$ and r.h. in producing a lethal environment for *Ephestia cautella* pupae was shown (Navarro, 2012). In these trials the importance of the desiccation in relation to the ambient r.h.

as a result of opening the spiracles under the influence of low $O_2$ concentration was demonstrated (Navarro, 2006).

In contrast to these observations Murdock et al. (2012) attributed *C. maculatus* mortality to the dependence of the insect on carbohydrates for energy, carbohydrates must represent its main source of water. According to Murdock et al. (2012) the mode of action of hermetic storage, namely cessation of feeding, growth, development and reproduction and eventual death resulting from inadequate metabolic water due to lack of oxygen, may apply to a wide range of insect pests of stored products.

**Effect of temperature and MA**

At temperatures of 20–30°C, most species and developmental stages show >95% mortality in <10 d at both 0 and 1.0% $O_2$. *Trogoderma granarium* Everts larvae (12 d at 0% $O_2$), *S. oryzae* pupae (20 d at 0% $O_2$; >14 d at 1% $O_2$), and *Sitophilus granarius* adults (16 d at 1% $O_2$) are the only exceptions so far found. The influence of temperature on the length of time necessary to obtain good control with MAs is as important as with conventional fumigants. To obtain good control, the temperature of the grain should be above 21°C during the application of $CO_2$ (Navarro, 2006).

It was shown that, at 15.4°C, complete control of immature *R. dominica* was obtained after four weeks of exposure to 60% $CO_2$. Responses of larval, pupal, and adult stages of the nitidulid beetles *Carpophilus hemipterus* (L.) and *Urophorus humeralis* (F.) exposed to simulated burner-gas concentrations at three temperatures of 26, 30, and 35°C were reported. Comparison of exposure times showed that the effect of temperature on treatment efficacy was most pronounced at the 1% $O_2$ level, where, for the three stages of both species tested, values of $LT_{50}$ at 26°C were about half those at 35°C. However, at 3% $O_2$ and 35°C, $LT_{50}$ levels were only marginally reduced.

Eggs, larvae, pupae, and adults of *T. castaneum* to three low-oxygen concentrations at 26, 30, and 35°C were exposed. At all levels of $O_2$ (1, 2, and 3%), in typical respiration atmospheres under hermetic conditions (similar to burner-gas atmospheres), the $LT_{99}$ values at 35°C were significantly lower than those at 26°C. Work on all four development stages of *E. cautella* showed the strong influence of temperature on mortality values when the insects were exposed to $CO_2$ concentrations varying from 60 to 90% in air.

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