Chapter 11

Modified Atmospheres for the Control of Stored-Product Insects and Mites

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OVERVIEW

Background and History

Increased public concern over the adverse effects of pesticide residues in food and the environment has led to the partial replacement of fumigation by alternative control methods. Among these methods, the only one that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, is the modified atmosphere (MA) method. Modified or controlled atmospheres offer a safe and environmentally benign alternative to the use of conventional residue-producing chemical fumigants for controlling insect pests that attack stored grain, oilseeds, processed commodities, and packaged foods.

Hermetic storage of grain was practiced in ancient times in underground pits in the dry, subtropical regions of the Middle East and other dry regions of the world, such as Africa and India. Underground pits for grain storage were still used in Egypt in the 1940s, as described by Attia (1948). Very old but active hermetic storages were reported to be in operation in India (Girish, 1980) and in Yemen, Somalia, Sudan, and Egypt (Kamel, 1980). It has been suggested that, in Biblical times, Joseph employed hermetic storage for the preservation of the large grain reserves in Egypt during the seven years of plenty (Calderon, 1990).

The pioneers in the use of MAs in modern times considered the method to be an adaptation of the old principle of hermetic storage (Attia, 1948; Hyde and Daubney, 1960). One of the enthusiastic promoters of the hermetic-storage principle was the renowned French entomologist P. Vayssiere (1948). In his article in the first publication by the Food and Agriculture Organization of the United Nations on grain storage, he called hermetic storage "the process of the future for protection of foodstuffs." The scientific and practical aspects of the MA method for food preservation were reviewed intensively in the 1950s by Oxley (1948), Bailey (1955), and Hyde et al (1973). Later, Sigaut (1980) stated that, in preindustrial times, hermetic storage was probably one of the means of keeping large quantities of grain free from insect attack for significant lengths of time in areas with mild winters. He also reported that the first large-scale tests were run in underground pits in Paris from 1819 to 1830. This principle was also used on a large scale in Argentina during and after World War II, when facilities were constructed and used for the underground hermetic storage of over 2.5 million tonnes of grain (Lopez, 1946; Anonymous, 1949). More-modern concrete hermetic-storage bins built primarily for famine protection have been constructed in Cyprus and Kenya for corn storage and are continuously in operation (De Lima, 1980).

Studies in the 1860s on modifying atmospheres by adding N_2 or "burned air" to grain storages were also reported by Sigaut (1980). However, serious interest in using the technique in a practical, routine manner was not pursued until the 1950s and 1960s, probably due to the success of conventional fumigants and grain protectants in controlling stored-product pests. During this period, people began to realize that the chemicals, if used improperly, left objectionably residues, that they were hazardous to apply, and that there was a potential for the development of insect resistance to them. Research on the use of MAs was initiated during this time in Australia and in the United States and is ongoing in these and several other countries. This research has significantly restricted the use of chemicals in food. An important development stimulating further work on MA took place in the United States in 1980 and 1981. The Environmental Protection Agency approved an exemption from tolerance for CO_2, N_2, and products from an "inert" gas generator when used to control insects in raw (Federal Register, 1980) and processed (Federal Register, 1981) agricultural products.

MA and controlled atmosphere (CA) treatments for the disinfestation of dry stored products have received increasing scientific attention during the last 25 years. Although this method has become well established for control of storage pests, its commercial use is still limited to a few countries (Navarro et al, 1979; Shejbal, 1980b; Banks and Ripp, 1984; Jay and d'Orazio, 1984; Fleurat Lessard and Le Torc'h, 1987; Adler et al, 2000). Reviews on stored-product protection with MAs can be found in the publications of Bailey and Banks (1975, 1980); Jay (1980, 1984a,b), Banks (1981, 1983a,b), Annis (1987), Calderon and Barkai-Golan (1990), and Adler et al (2000).

The widespread scientific activities on this subject resulted in several international conferences, such as the International Conference on Controlled Atmospheres and Fumigation, which was held in 1980 in Rome, Italy (Shejbal, 1980a); in 1983 in Perth, Australia (Ripp et al, 1984); in 1989 in Singapore (Champ et al, 1990); in 1992 in Winnipeg, Canada (Navarro and Donahaye, 1993b); in 1996 in Nicosia, Cyprus (Donahaye et al, 1997); and in 2000 in the United States in Fresno, CA (Donahaye et al, 2001). New research findings on CAswere also reported at the International Working Conferences on Stored-Product Protection held in the United States in Savannah, GA, in 1974 (Anonymous, 1975); in Ibadan, Nigeria, in 1978 (Davis and Taylor, 1979); in Manhattan, KS, United States, in 1983 (Anonymous, 1984); in Tel Aviv, Israel, in 1986 (Donahaye and Navarro, 1987); in Bordeaux, France, in 1990 (Fleurat-Lessard and Ducom, 1991); in Canberra, Australia, in 1994 (Highley et al, 1994); in Beijing, China, in 1998 (Zuxun et al, 1999); and in York, United Kingdom, in 2002 (Credland et al, 2003). The annual research conferences on methyl bromide alternatives in the United States also provide a forum to enhance technology transfer (MBAO, 2003). These meetings served for the fruitful exchange of information among the participating scientists and the reciprocal insemination of new ideas for further research. The continuing interest in MAs led to the updating of this chapter, based on the text written by my friend, the late Ed Jay (Jay, 1984a). I wish to dedicate this chapter to his memory.

Definitions and Uses of MA

The objective of MA treatment is to attain a composition of atmospheric gases rich in CO_2 and low in O_2, or a combination of these two gases at normal or altered atmospheric pressure within the treatment enclosure, for the exposure time necessary to control the storage pests. Various terms used in reference to MA storage for the control of storage insect pests or the preservation of food have appeared in the literature to define the same method of treatment but using different means to attain the same scope of control without adversely affecting the environment. Therefore, in this section, definitions are proposed, to add clarity to the available storage-insect control methods, whether at normal atmospheric pressure or under altered atmospheric pressure.

Modified atmosphere (MA) is proposed as the general term, including all cases in which the composition of atmospheric gases or their partial pressures in the treatment enclosure have been modified to create conditions favorable for the control of storage insects. In an MA treatment, the atmospheric composition within the treated enclosure may change during the treatment period. This term comprises all the following designations.

MAs Under Normal Atmospheric Pressure

Controlled atmosphere (CA). In a CA treatment, the atmospheric composition in the treated enclosure is controlled or maintained at a level lethal to insects. The modified gas composition, usually produced artificially, is maintained unchanged by additionally generating the desired gases (CO_2 or N_2) or by further purging the storage with these gases, supplied from pressurized cylinders or otherwise (Fig. 1). These supplemental gases are introduced when their concentration in the sealed container drops to below the desired level. The CA method is intended to compensate for possible small leakages of gases, which cause the increase of the O_2 or decrease of the CO_2 content in the enclosure and which are almost impossible to avoid. Thus, the term "CA," although commonly used as the one describing the entire subject, actually has its own specific meaning.

Hermetic storage. A type of MA that can be applied for the protection of grain is "hermetic storage," termed also "sealed storage," "airtight storage," or "sacrificial sealed storage." This method takes advantage of the gases produced naturally by the respiratory metabolism of insects and commodities, using them to prevent insect development. Sufficiently sealed structures enable insects and other aerobic organisms in the commodity, or the commodity itself, to generate the MA by reducing the O_2 and increasing the CO_2 concentrations. The exposure time needed to control the insect populations and to protect the grain using hermetic storage depends on the infestation level and the activity of other aerobic organisms.

Assisted hermetic storage. Another type of hermetic storage uses exothermic gas generators, catalytic oxygen converters, or respiration gases of plant material. In this type of hermetic storage, the atmosphere is modified by the supply of gases generated outside the storage container, so that a gas composition of low oxygen (<1%) and high carbon dioxide can be achieved artificially. Exothermic gas generators burn fossil fuels to generate the low-O_2 atmosphere. Catalytic oxygen converters burn propane or butane by catalytic conversion processes without flame. Oxygen could also be removed from the air by respiration, using various plant materials or wastes placed in an external generator.

MAs Under Altered Atmospheric Pressure

Vacuum. In a low-pressure environment there is a close correlation between the partial pressure of the remaining O_2 and the rate of kill. Until recently, this treatment could be carried out only in specially constructed rigid and expensive vacuum chambers. A proposed alternative practical solution consists of the vacuum hermetic fumigation process using flexible liners. To achieve the low pressures in the flexible liners, sufficiently low pressures (25–50 mm of Hg absolute pressure) are obtained (using a commercial vacuum pump) and maintained for indefinite periods of time (Navarro et al, 2001c).

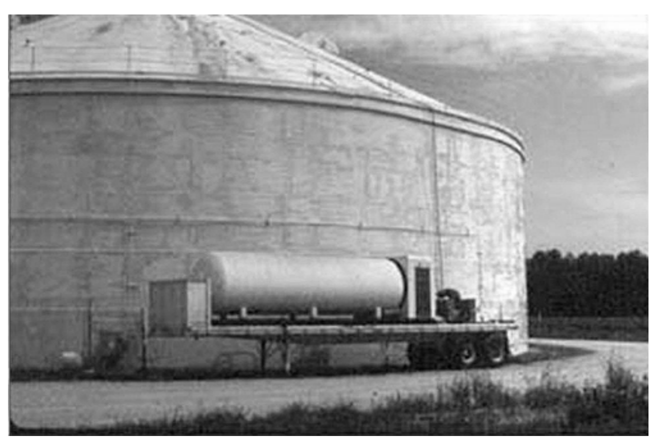

Fig. 1. Application of carbon-dioxide-based modified atmosphere in a large silo bin in Kingston, NC, U.S.A.

High-pressure carbon dioxide treatment. CO_2 treatments can be significantly shortened to exposure times measured in hours using increased pressure (10–37 bar) applied in specially designed metal chambers that can withstand the high pressures. Because of the high initial capital investment, these high-pressure-chamber treatments may be practical for high-value products such as spices, nuts, medicinal herbs, and other special commodities.

Modifying the Atmosphere of the Storage Ecosystem

Storage as an Ecosystem

Our ability to successfully apply MA in stored durable agricultural products depends on many interrelated factors (Fig. 2). To better understand their interplay, and the potential areas of intervention for improving traditional storage, the storage structure is considered an ecosystem (Sinha and Muir, 1973; Calderon, 1981). (In this section, for the sake of convenience, cereal grains and pulses serve as the example for the stored commodities.) The interrelated factors are common to all storage situations, whether they are grain in high-tech silos or grain home-stored in jute sacks. A convenient way to analyze the interactive relationships between these storage-environment factors is to consider a storage structure as the boundary that defines the environment of a community of interacting living organisms, which can be termed an "ecological system" or *ecosystem* (Sinha and Muir, 1973; Calderon, 1981).

Components of the Ecosystem

The stored grain. This is the component of principal interest to us and the one we wish to protect from insect damage. Grain in itself is a biotic factor of the system, a living organism in a state of dormancy that can remain unchanged for prolonged periods.

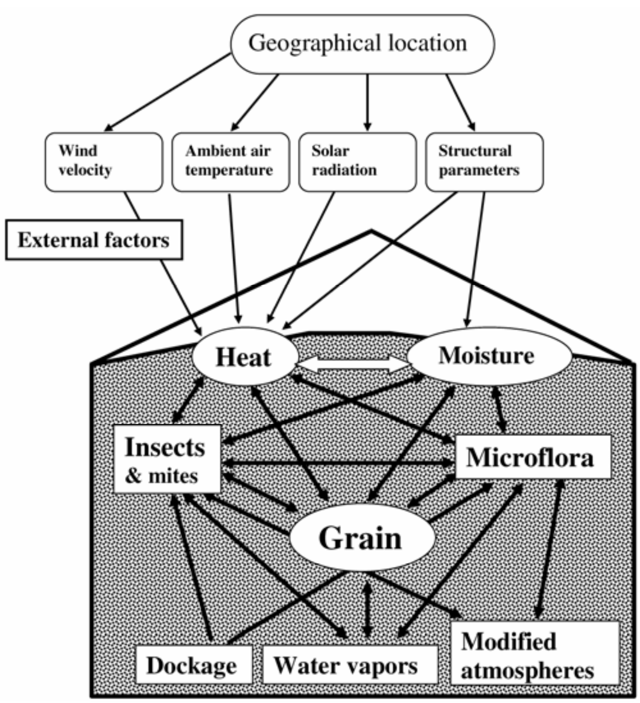

Fig. 2. External factors and the interrelated ecosystem components of grain in sealed bulks. (Compiled from Navarro and Noyes, 2002)

The storage structure. This component, which forms the boundaries of the ecosystem, is predetermined and fixed. The materials and nature of its composition and its placement are important in determining the extent to which external factors (both biotic and abiotic) affect the system. The structure must be mechanically designed to hold the grain and maintain the MA without gas loss.

Temperature. Ambient temperature is an abiotic factor that has little direct influence on grain condition but greatly influences some biotic components (insects and microflora) and therefore indirectly affects conservation of grain quality.

Humidity. Ambient humidity is an abiotic factor of the air surrounding the grains. Within the confined storage space, the moisture of the grains is important because the humidity will reach equilibrium with this moisture. The greatest influence of humidity is on molds, which begin to develop at intergranular humidities above 70%.

Atmospheric composition. Atmospheric gases that constitute air make up the third abiotic factor. The atmosphere composes about 50% of the volume of the storage structure; it fills the spaces between the grain kernels and the headspace above the grain. When there is free movement between the air inside and outside the storage structure, the composition of the atmosphere is relatively constant, consisting of about 78% nitrogen, 20.9% oxygen, 0.03% carbon dioxide, and other inert gases like argon. However, if free movement of air between the grain bulk and the outside atmosphere is restricted or completely prevented, then the biotic factors (the grain, insects, and microorganisms) may strongly alter the atmospheric composition of the ecosystem, reducing the oxygen concentration and increasing the carbon dioxide concentration.

Insects. Stored-product insects consist of a group of some 250 species (beetles and moths) characterized by their small size (which enables them to penetrate the interstices of the grain bulk), their worldwide distribution, and their feeding habits in dry environments. About six species are the major pests, and several of them attack crops in the field, thereby entering the ecosystem at the moment of loading the grain into storage. Their rates of development and population increase are strongly influenced by the temperature of the grain bulk, and their metabolic activity consumes oxygen and produces carbon dioxide, heat, and moisture, which in turn affect all biotic components of the ecosystem. Their development is suppressed and even controlled when the intergranular atmosphere is rich in carbon dioxide and deficient in oxygen.

Microorganisms. This biotic factor is composed of molds, yeasts, and bacteria. They are universally present on the grain but are inactive at humidities favorable to storage, which are those below 65% equilibrium relative humidity. When the moisture of the grain in equilibrium with 65% RH rises above a critical level, molds begin to develop.

Since both molds and insects release heat and moisture by their metabolism, they may produce temperature and moisture gradients within the stored-grain ecosystem. This, in turn, can create convection currents through the grain bulk, carrying warm, moist air from the heating region to cooler regions, where the moisture is deposited as the air cools. Such areas of condensation favor the development of microorganisms and may even cause the grain to germinate. As with insects, mold development is suppressed when the storage atmosphere is strongly modified, although this process cannot control anaerobic microorganisms.

Foreign matter. Chaff, stalks, grain dust, sand, earth, stones, dockage, etc. can either be abiotic components of the ecosystem or originate as dead parts of plants. The effects of foreign matter on the ecosystem are many. Chaff and grain dust tend to absorb moisture more rapidly than grain and present a more suitable substrate for mold development than whole grains. Many insects that are unable to penetrate sound grain are able to develop well on this material. All small particles of material tend to block the interstitial air spaces and therefore may prevent the application of control measures that rely on the penetration of MAs throughout the grain bulk to kill insect populations.

EFFECTS OF MA ON STORED-PRODUCT INSECTS AND MITES

Storage insects are aerobic organisms requiring oxygen for their survival. Therefore, they respond to altered atmospheric gas compositions containing low O_2 or high CO_2. "Low-O_2 atmospheres" typically contain less than 1% O_2, with the balance being N_2 and very low concentrations of the rest of the atmospheric gases, namely CO_2 and the inert atmospheric gases. These atmospheres function largely by their anoxic effect, and the other gases exert only a minor influence on their action. On the other hand, 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 (mainly temperature, humidity, and the partial pressure), exposure time, and the type of the atmospheric composition used as treatment.

Lethal Action of MA on Insects

Low Oxygen and Anoxia

In addition to nitrogen, which is commonly used to produce a low-oxygen atmosphere, rare gases like helium and argon have also been tested (Lindgren and Vincent, 1970; Ali Niazee, 1972) to demonstrate that they cause anoxia, with effects similar to those of nitrogen. Nitrogen is active in producing a progressive hypoxia or anoxia only when used alone at a high purity level. 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 (Navarro, 1978; Banks and Annis, 1990; Fleurat Lessard, 1990). This effect is reversed below 1% oxygen in nitrogen, where the adult rice weevils, *Sitophilus oryzae* (Navarro, 1978) showed tolerance, increasing the lethal exposure time, apparently due to closing their spiracles. In particular, *S. oryzae* adults are killed more quickly at 1.0% O_2 than at 0.1 or 2% O_2 under the same conditions (Fig. 3).

Although suppression of storage-insect development was observed at about 5% O_2 (Bailey, 1955, 1956, 1957, 1965), the exposure time required to kill the insects was very long. Experiments with *Tribolium castaneum* in nitrogen showed significant differences in adult mortality between 0.1 and 1.0% O_2 (Navarro, 1978). Similar experiments with *T. confusum* in nitrogen (Jay and Pearman, 1971; Shejbal et al, 1973; Tunc and Navarro, 1983) 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 *Rhyzopertha dominica* (the lesser grain borer) was demonstrated to be more tolerant than *Tribolium* spp. The lowest level of tolerance to lack of oxygen was attained around the 1% concentration level (Fig. 3).

Adults of the grain mite, *Acarus siro*, were exposed to various O_2 atmospheres in nitrogen. The exposure times to obtain complete mortality at 2% O_2 were 48 and 72 hr at temperatures of 26 and 15°C, respectively (Navarro et al, 1985b).

High Carbon Dioxide and Hypercarbia

Atmospheres containing about 60% carbon dioxide rapidly kill stored-product insects. At 26°C, about four days of exposure would be sufficient to kill all stages (including eggs) of most stored-product insects. When the concentration level of CO_2 was reduced to about 35% for only 10 days of exposure time, less than 1% of *T. confusum*

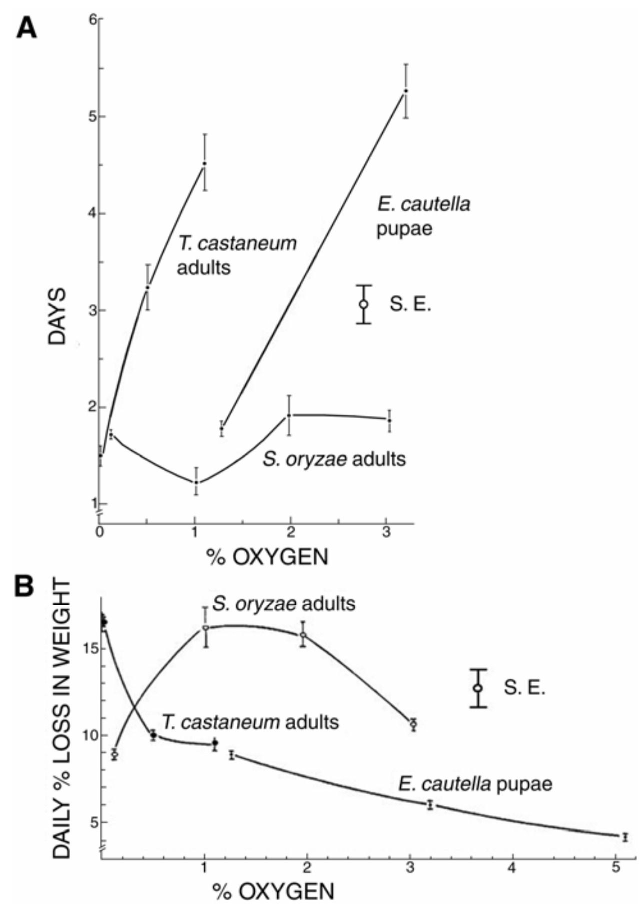

Fig. 3. Relationship between oxygen concentration and the time required for 95% mortality (A), and the effect on daily percent loss in weight (B) of three stored-product insects at 54% RH and 26°C. (Redrawn, and used with permission, from Navarro, 1978)

larvae survived the treatment (Ronai and Jay, 1982). This concentration seems to be the target level, above which toxicity of carbon dioxide occurs (Jay et al, 1970; Jay and Pearman, 1973). Laboratory tests on the major stored-product insects have shown that adults can be killed with pure CO_2 within l0–48 hr; exposure times of more than 14 days are required to kill them when the atmosphere contains less than 40% CO_2 even at temperature levels above 20°C (Kashi, 1981). The initial symptoms of carbon dioxide poisoning in insects include a narcotic effect, leading to knockdown, i.e., immobilization of the insects under carbon-dioxide-enriched atmospheres (Edwards, 1953; Ali Niazee, 1971, 1972; Edwards and Batten, 1973).

The dosage-response data for *S. oryzae*, *T. castaneum*, and *Ephestia cautella* (the almond moth) given in Table 1 illustrate the range of susceptibility within a species. There are more laboratory data for *S. oryzae* than for any other stored-product pest and, except for *Trogoderma* spp., it appears to be the most tolerant of high-CO_2 atmospheres. The minimum concentration required to give a high and rapid mortality for all developmental stages is slightly less than 40%. Eggs are significantly affected by 20% CO_2, while at >20%, adult insects are the most susceptible stage (Banks and Annis, 1990).

Diapausing *Trogoderma granarium* (Khapra beetle) larvae are the most tolerant to high-CO_2 atmospheres of any species and stage so far reported (Annis, 1987). They are tolerant of CO_2 concentrations of 60% or less in air at 25°C, and less than 95% mortality has been obtained after 25 days, the longest exposure so far tested. It appears that diapausing *Trogoderma variabile* (the warehouse beetle) larvae may have a similar response (Banks and Annis, 1990). Other *Trogoderma* spp. are also very tolerant (Jay, 1984b).

Combinations of Low Oxygen and High Carbon Dioxide

Researchers have been interested in increasing the efficacy of MA by attempting to combine very low oxygen and very high carbon dioxide concentrations. However, increasing the carbon dioxide concentration in a normal atmosphere proportionally reduces the partial pressure of the oxygen available to insects. For example, attempting to achieve a 40% CO_2 concentration at normal atmospheric pressure proportionally reduces the oxygen to about 12.6%; by the same comparison, increasing the CO_2 to 90% reduces the available oxygen to 2.1%. Gas burners or fossil burners also have the capability of generating a combination of low oxygen and high carbon dioxide. For example, a typical propane burner would produce an atmosphere of 0.5% oxygen, 13.5% CO_2, 1% nitrogen, and 1% argon. Therefore, unless a mixture of nitrogen and carbon dioxide or a gas-burner atmosphere is used, the simplest way to achieve a low-oxygen and high-CO_2 atmosphere is by using CO_2 (Storey, 1975b).

Some systematic investigations on the effect of low-O_2 atmospheres (<4% O_2 containing various proportions of CO_2, but typically with 10–20% CO_2), also known as "burner gas," were carried out. In laboratory experiments, larval, pupal, and adult stages of the nitidulid beetles *Carpophilus hemipterus* and *Urophorus humeralis* were exposed to simulated burner-gas concentrations at three temperature and gas combinations. These were 1% O_2, 85% N_2, and 14% CO_2 at 26°C; 2% O_2, 84.7% N_2, and 13.3% CO_2 at 30°C; and 3% O_2, 85% N_2, and 12% CO_2 at 35°C—all at 75% RH. For all insects submitted to the MA containing 3% O_2 at 26°C, the exposure time to produce 95% mortality was 196 hr. To obtain the same mortality level with the MA containing 1% O_2 at 35°C, 60 hr was required (Donahaye et al, 1994). Some species, e.g., *T. castaneum* as adults, were shown to be more susceptible to low-oxygen atmospheres containing CO_2 than to those containing pure N_2. The response of others, notably *Sitophilus* spp., appeared little affected by the presence of 10–20% CO_2 (Bailey and Banks, 1980). The researchers concluded that there may be some advantage in practice in using a proportion of CO_2 in low-O_2 atmospheres, although the reduction in exposure time will not be substantial if *Sitophilus* spp. are present. Calderon and Navarro (1979, 1980) and Krishnamurthy et al (1986) have claimed significant reduction in exposure times in the presence of >10% CO_2. In the case of hypoxia (2–5% O_2), when a small proportion of CO_2

TABLE 1
Approximate Time (in days) to Obtain at Least 95% Mortality Using Various Concentrations of Oxygen and Mixtures of Carbon Dioxide and Air (temperature range 20–29°C)[a]

		Oxygen (%)				Carbon Dioxide (%)			
Species	Stage[b]	0.0	1.0	2.0	3.0	20.0	40.0	60.0	80.0
Ephestia cautella	E	1.5	1.5	3.0	···	4.0	3.0	···	···
	L	1.0	0.5	···	4.0	···	···	<5.0	···
	P	2.0	1.0	<3.0	<6.0	<6.0	<3.0	<3.0	<3.0
	A	0.5	0.5	<2.0	···	···	···	<2.0	···
Sitophilus oryzae	E	9	<5.0	<7.0	14	15.5	4.5	3.5	3.5
	L	···	<6.0	···	···	>14	>7	3.0	2.0
	P	20.0	>14	>14	>14	>14	8.5	6.0	8.5
	A	4.5	5.0	>21	>21	7	3.0	1.0	1.0
Tribolium castaneum	E	2.5	1.5	3.0	4.0	>4.0	2.0	<2.0	<2.0
	L	1.5	6.5	>14	>14	>16.5	16.5	<5.0	<7.0
	P	4.0	>3.0	···	···	···	···	<5.0	<5.0
	A	1.5	6.0	>14	>14	>5.0	>14	<1.0	3.0

[a] Data from Annis (1987), Banks and Annis (1990), Navarro et al (1985c), and Tunc and Navarro (1983).
[b] E = eggs; L = larvae; P = pupae; A = adults.

(5–40%) is added to the initial mixture of N_2/O_2, the mortality rate increases considerably (Calderon and Navarro, 1979) (Fig. 4). When CO_2 is added to low-O_2 atmospheres, a synergistic effect is obvious from the significant interaction between the concentrations of these two gases (Calderon and Navarro, 1980). Even with the tolerant species, *Trogoderma granarium*, at the larval stage in 1.1–1.2% O_2, the exposure time necessary to kill 50% of the insect population (i.e., lethal time, LT_{50}) was decreased from 39 to 27 hr, when CO_2 concentrations were increased from 0 to 15% (Girish, 1978). This effect seems fairly similar to the enhancement of efficiency of fumigants by low concentrations (10–20%) of CO_2 reported by other workers (Bond and Buckland, 1978; Calderon and Leesch, 1983).

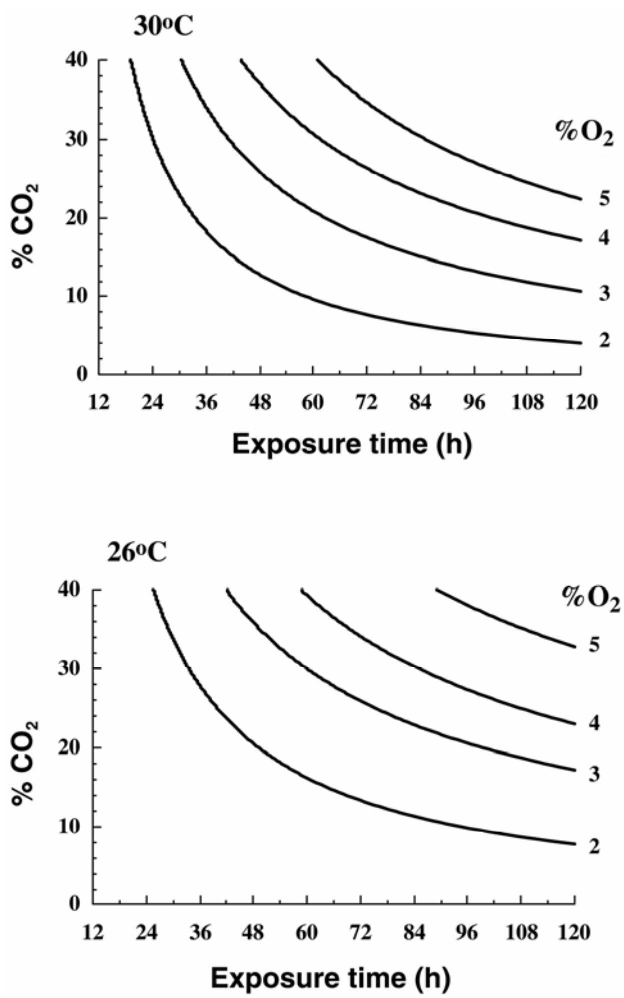

Fig. 4. Calculated exposure times and carbon dioxide levels to produce 95% mortality for *Tribolium castaneum* adults at four oxygen concentrations and two temperatures at 57% RH. (Redrawn, and used with permission, from Calderon and Navarro, 1979)

Jay (1984a) stated that, for *Sitophilus* spp., pure CO_2 is not as effective as a 60% CO_2 atmosphere (8% residual O_2). The complete lack of oxygen was found antagonistic to the toxic effect of CO_2. In a different experimental procedure, it was observed that atmospheres containing as much as 15% O_2 were lethal to *T. castaneum* and *Plodia interpunctella* larvae when about 36% CO_2 was added (Harein and Press, 1968).

Valid laboratory studies can provide guidance in selecting MA concentrations, exposure times, and optimum temperature ranges, which can be used in conducting field experiments. An example of this can be seen in Table 2, which shows the effects on mortality (based on adult emergence) of seven different MAs on one- to five-week-old immature maize weevils, *Sitophilus zeamais*, at the same temperature and relative humidity during one- to four-day exposures. Increasing the N_2 concentration from 97 to 100% greatly reduced emergence, as did increasing the CO_2 concentration from 39 to 62%. Increasing the CO_2 concentration to 99% produced less mortality than was obtained at about 60%. Such laboratory studies show that, for this species, CO_2 is more biocidal than N_2; that there is no need to increase the CO_2 concentration above 60%; and that a longer exposure is needed to obtain complete control.

TABLE 2
Mean Number of Adult Maize Weevils Emerging from Wheat Infested with One- to Five-Week-Old Immature Insects and Exposed for One to Four Days to the Indicated Atmosphere at 26.7°C and 46% RH[a]

Atmosphere[b]	Mean Emergence	Atmosphere[b]	Mean Emergence
Air (control)	26.6	39% CO_2	14.9
97% N_2	18.4	50% CO_2	9.4
99% N_2	14.5	99% CO_2	8.9
100% N_2	9.6	62% CO_2	5.8

[a] Source: Jay (1984a); used by permission.
[b] The balance of the modified atmospheres containing N_2 is O_2; the balance of the atmospheres containing CO_2 is air (N_2 plus O_2).

Interrelated Physical Environment Effects

Effect of Air Relative Humidity and MA

Laboratory studies have shown that lowering the RH increases the effectiveness of MAs. Jay et al (1971), working with adults of *T. confusum*, *T. castaneum*, and *Oryzaephilus surinamensis* (the sawtoothed grain beetle), found that, in atmospheres containing 99% N_2 (balance O_2), decreasing the RH from 68 to 9% increased the mortality from 3 to 98.5% in a 24-hr exposure of the red flour beetle. The two other insects showed a similar response to reduced relative humidity (Table 3). These three species also exhibited a similar response to mixtures of CO_2 in air at lowered relative humidities.

Desiccation plays a large role in the mortality of stored-product insects exposed to some MAs. Jay and Cuff (1981) showed that when larvae, pupa, and adults of the red flour

TABLE 3
Effect of Relative Humidity (RH) on Mortality of the Red Flour Beetle (RFB) and Confused Flour Beetle (CFB) Exposed 24 hr and Saw-toothed Grain Beetle (STGB) Exposed 6 hr to 99% N_2 and 1% O_2[a]

	% Mortality		
%RH	RFB	CFB	STGB
68	3.0	5.2	4.1
54	75.9	39.1	17.0
33	94.8	95.9	27.5
9	98.5	98.1	40.0

[a] Source: Jay (1984a); used by permission.

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. Navarro and Calderon (1974) showed a linear relationship of the combined effect of CO_2 and RH in producing a lethal environment for *E. cautella* pupae (Fig. 5). Navarro and Calderon (1980) demonstrated the strong effect of low concentrations of CO_2 and O_2 on causing mortality in *E. cautella* pupae (Fig. 6). Other laboratory studies have shown that the susceptibility of different species or strains of the same species varies considerably when insects are exposed to the same concentrations of MAs (Jay and Pearman, 1971).

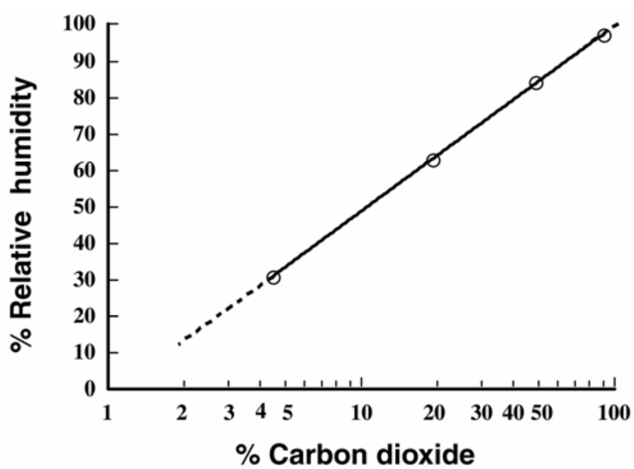

Fig. 5. Combined effect of carbon dioxide and relative humidity on the time needed to produce 95% mortality values for *Ephestia cautella* pupae after four days of exposure. (Redrawn, and used with permission, from Navarro and Calderon, 1974)

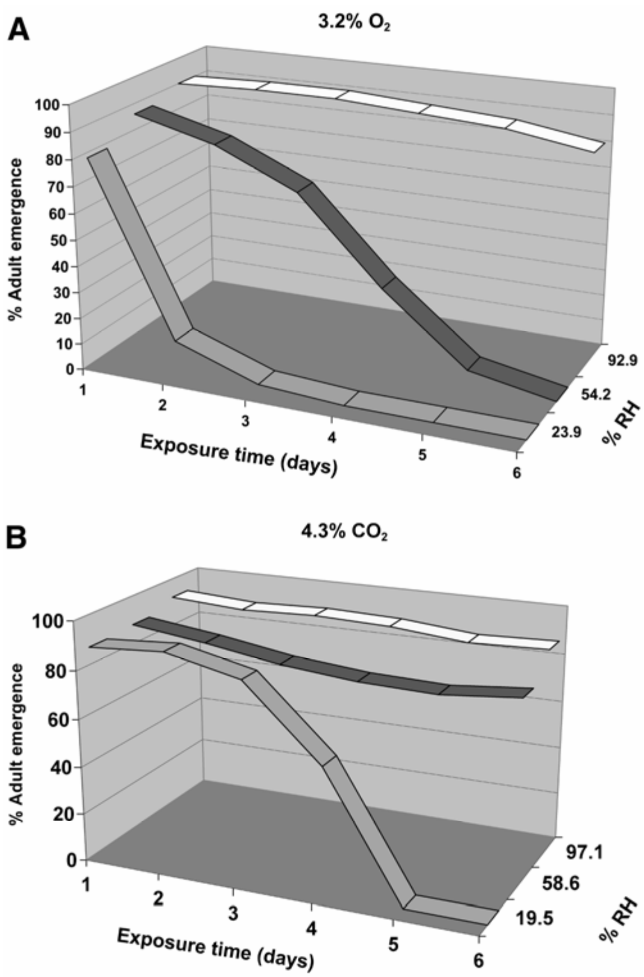

Fig. 6. Effect of oxygen (A) and carbon dioxide (B) on adult emergence from *Ephestia cautella* pupae exposed to different relative humidities at 26°C. (Adapted from Navarro and Calderon, 1980)

Effect of Temperature and MA

At temperatures of 20–30°C, most species and developmental stages show >95% mortality in <10 days at both 0 and 1.0% O_2 (Annis, 1987). *Trogoderma granarium* larvae (12 days at 0% O_2), *S. oryzae* pupae (20 days at 0% O_2; >14 days at 1% O_2), and *Sitophilus granarius* (granary weevil) adults (16 days at 1% O_2) are the only exceptions so far found (Annis, 1987).

The influence of temperature on the length of time necessary to obtain good control with MAs is as important as with conventional fumigants. Jay (1971) stated that, to obtain good control, "the temperature of the grain should be above 21°C during the application of CO_2." Navarro and Calderon (1980) compared the effect of temperature on the exposure time required to produce the mortality of adults of three storage insects in MAs (Fig. 7).

Jay (1984c) showed that, at 15.4°C, complete control of immature *R. dominica* was obtained after four weeks of exposure to 60% CO_2. Donahaye et al (1994) reported on responses of larval, pupal, and adult stages of the nitidulid beetles *C. hemipterus* and *U. humeralis* exposed to simulated burner-gas concentrations at three temperatures of 26, 30, and 35°C. 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 (Donahaye et al, 1994).

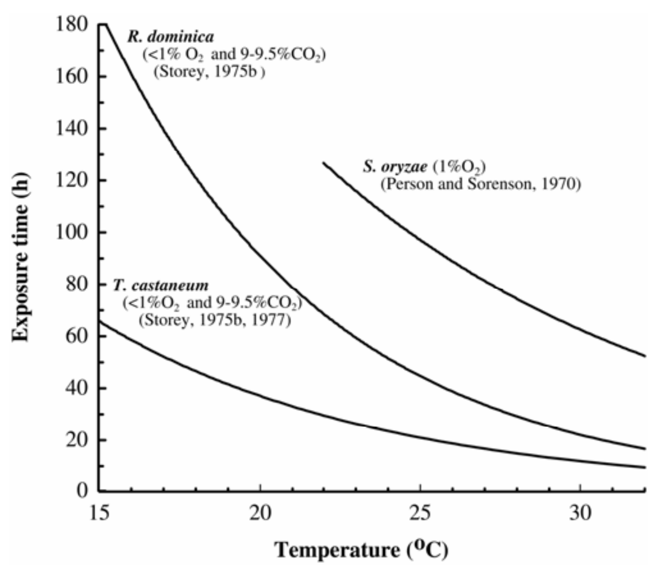

Fig. 7. Effect of temperature on exposure time required to produce 95% mortality of three species of adult stored-product insects exposed to two atmospheric gas compositions. (Adapted from Navarro and Calderon, 1980)

TABLE 4
Influence of Three Temperatures on Mortality, Expressed in LT_{99} Values (hr to obtain 99% mortality), of Four Development Stages of *Ephestia cautella* Exposed to Four CO_2 Concentrations in Air[a]

	35°C at CO_2 Concentration of				40°C at CO_2 Concentration of				45°C at CO_2 Concentration of			
Stage	**60%**	**70%**	**80%**	**90%**	**60%**	**70%**	**80%**	**90%**	**60%**	**70%**	**80%**	**90%**
Eggs	23	23	17	9	16	12	8	5	9	5	3	2
Larvae	60	27	20	12	17	9	6	6	5	4	2	2
Pupae	56	37	17	17	36	10	8	4	7	4	4	3
Adults	20	14	6	4	6	5	3	2	3	2	2	2

[a] Source: Navarro et al (2003b); used by permission.

Soderstrom et al (1992) examined the influence of temperature over the range of 38–42°C on the effects of hypoxia and hypercarbia on *T. castaneum* adults for 60-hr exposures. Although the different experimental conditions make comparison difficult, their results clearly indicate that raised temperatures could be used to reduce treatment duration. Donahaye et al (1996) exposed eggs, larvae, pupae, and adults of *T. castaneum* to three low-oxygen concentrations at 26, 30, and 35°C. 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 (Navarro et al, 2002b; 2003b) (Table 4).

Effects of Low Pressures

The mortality of insects under low pressures is caused mainly by the low partial pressure of oxygen, resulting in hypoxia (Navarro and Calderon, 1979). The partial pressure of oxygen has a decisive effect on insect mortality, while no significant function could be attributed to the low pressure itself. At 50 mm of Hg, the partial pressure of oxygen is equivalent to 1.4% O_2, this being similar to the target oxygen concentration under an MA obtained by nitrogen flushing (Fig. 8). Finkelman et al (2003b) conducted experiments in a calculated atmospheric partial pressure equivalent to an oxygen concentration of 1.3–1.8%. This oxygen level is close to the critical levels needed for insect disinfestations using the low oxygen levels achieved by displacement with nitrogen (Donahaye, 1992). The only other information available on the effects of low pressure on *E. cautella* and *T. castaneum* is that provided by Calderon et al (1966), who reported that, at 10–12 and 16–20 mm of Hg and 25°C, adults of *E. cautella* were very sensitive, and less than 1 hr of exposure was required to obtain 99% mortality, while for *T. castaneum* adults, a period of 2.7 hr was necessary.

The egg stages of *Lasioderma serricorne* (the cigarette beetle) (Bare, 1948) and *Trogoderma variabile* (Cline and Highland, 1987) were the most tolerant life stage when exposed to low pressures. In the work of Finkelman et al (2003b), the egg stage of *E. cautella* and *T. castaneum* was found to be most resistant to 55 ± 10 mm of Hg at 18°C; the times needed to obtain egg mortality of 99% were 96 and 149 hr, respectively. In contrast, the adult stage of *O. surinamensis* was the most resistant, with 164 hr being required to obtain 99% mortality. Finkelman et al (2004) showed that less than three days under 50 mm of Hg at 30°C would control all stages of *E. cautella*, *P. interpunctella*, and *T. castaneum*. The times needed to obtain 99% mortality were 45, 49, and 22 hr, respectively. The eggs of all three species were most resistant to low pressure. Mbata and Phillips (2001) exposed eggs, larvae, and pupae of *T. castaneum, P. interpunctella,* and *R. dominica* to 32.5 mm of Hg at temperatures ranging from 25 to 40°C. Eggs of *R. dominica* were most tolerant to low pressure; 134.7 and 86 hr were required for 90% mortality at 25 and 40°C, respectively.

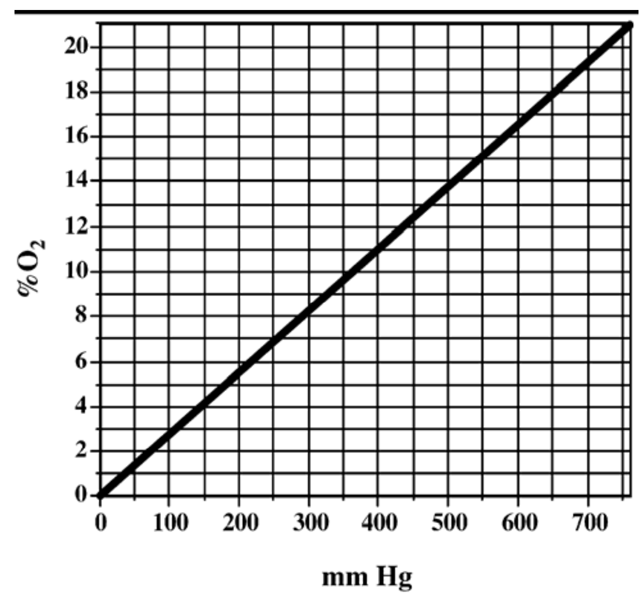

Fig. 8. Linear relationship between atmospheric pressure (mm of Hg) and oxygen concentration (%).

According to Finkelman et al (2005), for *Trogoderma granarium*, *L. serricorne*, and *O. surinamensis* at 50 mm of Hg and 30°C, the egg was the most-resistant stage, the times needed to obtain 99% mortality being 46, 91, and 32 hr, respectively. Adults of *T. granarium* and *L. serricorne* and pupae of *O. surinamensis* were the most susceptible.

Effects of High Pressure

Extremely short exposure times (a few hours) are needed to control all stages of storage insects with carbon dioxide at pressures between 10 and 37 bar. Generally, increasing the pressure reduces the lethal exposure time. Stahl et al (1985) and Stahl and Rau (1985) were the first to report that pressurized carbon dioxide is lethal to insects. Stahl and coworkers (Rau, 1985; Stahl and Rau, 1985; Stahl et al, 1985; Gerard et al, 1988a,b) tested carbon dioxide under high pressure to kill insects and microbes.

Mitsura et al (1973) were the first to show the effectiveness of compressed carbon dioxide against the cheese mite, *Tyrophagus putrescentiae*. Pohlen et al (1989), Prozell and Reichmuth (1990, 1991), Le Torc'h and Fleurat-Lessard (1991), Nakakita and Kawashima (1994), Reichmuth (1997), and Prozell et al (1997) also reported on the improved efficacy of carbon dioxide under high pressure.

Developmental stages of *L. serricorne, O. surinamensis, T. castaneum, T. confusum, Trogoderma granarium, Corcyra cephalonica* (the rice moth), *Ephestia elutella, E. cautella, P. interpunctella,* and *Sitotroga cerealella* (Angoumois grain moth) were exposed (at a temperature of 20°C) to carbon dioxide at 37 bar for 20 min, 30 bar for 1 hr, and 20 bar for 3 hr. These treatments resulted in 100% mortality of all insects. Survivors of *T. confusum* were found after treatment with 10 bar for 20 hr. Therefore, Adler et al (2000) concluded that extrapolation of laboratory results for carbon dioxide and high pressures to field situations is risky. The rate of decompression of pressurized chambers also have an adverse impact on insect mortality (Ulrichs, 1994; Ulrichs et al, 1997a,b).

Treatment with high-pressure carbon dioxide under different temperatures may result in different rates of mortality. For example, at 15°C, 95% mortality of *L. serricorne* was observed after 38.5 min of treatment, while the same level of control was achieved within 1 min at 45°C (Ulrichs, 1995).

With high pressures (20–40 bar), all types of pests and their life stages can be killed within a short time. The relatively rapid control of pests in all stages of development is based, on one hand, on the narcotic and acidifying effect induced by the high solubility of carbon dioxide in cell fluid and, on the other hand, on the destruction of the cells following the carbon dioxide pressure treatment during depressurization (Adler et al, 2000).

Biochemical Effects

The mortality of insects exposed to MAs involves a complex metabolic response. There is evidence that low-humidity environments are more effective in causing mortality (Navarro and Calderon, 1973, 1974; Navarro, 1975) and that, at low humidity, the lethal effects can be attributed to desiccation rather than to the toxic action of the MAs (Navarro, 1978). However, the inability of insects to recover after treatment was also attributed to the lack of sufficient triglycerides as substrates for energy metabolism (Navarro and Friedlander, 1974; Friedlander and Navarro, 1978, 1979a,b; Donahaye, 1991). Physiologically, organisms may react to anoxia by using alternative pathways such as the α-triglycerophosphate pathway for anaerobic energy production (Friedlander and Navarro, 1974). In this way, even at very low oxygen levels, the enormous reduction of energy consumption allows insect to survive much longer than vertebrates.

Navarro and Friedlander (1975) exposed pupae of *Cadra cautella* (the almond moth) for 24 hr to atmospheres with 10% oxygen and 20–89% carbon dioxide by volume; nitrogen made up the remaining volume. They found increasing lactate levels in the hemolymph with increasing carbon dioxide contents in the atmosphere. However, in a nitrogen atmosphere, lactate levels were found to increase sharply as soon as the oxygen level was reduced below 3% (Navarro and Friedlander, 1975). This suggested that lethal exposure times for various stored-product insects might increase sharply in nitrogen atmospheres with more than 3–4% oxygen (Lindgren and Vincent, 1970).

In another study, glucose levels in *Cadra cautella* were found to be reduced during anoxia but constant in atmospheres rich in carbon dioxide. Malate levels were increased when the insect was exposed to high concentrations of carbon dioxide (hypercarbic), but those levels decreased under low-oxygen (hypoxic) atmospheres. Citrate levels were reduced in both anoxic and hypercarbic atmospheres (Friedlander and Navarro, 1979a,b), which is probably a consequence of these atmospheres inhibiting the Kreb's cycle. Other studies by Friedlander and coworkers deal with the influence of CAs on the lipid content, water loss (Friedlander and Navarro, 1978), tissue levels of free amino acids, effects on the sorbitol pathway (Friedlander and Navarro, 1983), effects on the NADPH production (Friedlander et al, 1984), and glutathione levels (Friedlander and Navarro, 1984).

When pupae of *S. granarius* were exposed to pure nitrogen or pure carbon dioxide, lactate levels were found to increase most strongly within the first 24 hr of anoxia (Adler, 1993, 1994a,b). After 24 hr, accumulated lactate levels in CO_2-treated pupae were about one-third of those in N_2-treated pupae.

The comparatively lower lactate levels seen in *S. granarius* pupae exposed to CO_2 were attributed to acidosis caused by a combination of carbonic acid and lactic acid, which in nitrogen-treated pupae was attained by the accumulation of greater amounts of lactic acid alone (Adler, 1993). Friedlander (1984) noted that both pyruvate and lactate levels increased in pupae of *Cadra cautella* exposed to 99% nitrogen and 1% oxygen. In an atmosphere of 90% CO_2 and 10% oxygen, lactate levels in *Cadra cautella* pupae were four times higher than in pupae exposed to air, and two times higher than in pupae exposed to nitrogen. These results may indicate that the level of oxygen is important for the amounts of lactate and thus for the degree of acidosis produced by an atmosphere rich in CO_2.

Fat tissue represents a considerable proportion of the body weight of pupae and adults of the flour beetle. Villeneuve and Lemonde (1963) determined that lipids form up to 20% of the wet body weight of *T. confusum* and that they are the major source of energy during metamorphosis. Gilbert (1967) noted that triacylglycerols (TGs), which are released by hydrolysis and fatty acid production, form an important energy source for insects. These acids then release further energy by oxidation. Kennington and Cannell (1967) showed that, during exposure of *Tribolium* pupae to anoxia, lipids did not provide an energy source; however, as soon as the insects were returned to air, lipids were utilized in quantity. The accepted belief is that carbohydrate reserves in insects are utilized by the glycolytic pathway during anaerobic metabolism, but little information is available in the literature on lipid and carbohydrate metabolism under stress of hypoxia and hypercarbia.

The function of TGs in the conservation of the water balance in insects has been discussed by Edney (1967) and by Wigglesworth (1972). Friedlander and Navarro (1979a) examined the influence of CO_2 on TG metabolism in *E. cautella* pupae, to determine whether water production from TG metabolism forms a regulatory mechanism to compensate for increased water loss due to the influence of CO_2 on spiracular openings. They found that TG metabolism was not sufficient to compensate for water losses under conditions of high CO_2 concentration and low ambient humidity.

Glycogen forms one of the major carbohydrate energy reserves in insects. Friedman (1970) noted that glycogen may form from 0.01% to more than 2% of the wet weight of insects. Other important carbohydrates stored by insects are chitin and trehalose. Villeneuve and Lemonde (1965) identified carbohydrate reserves in *T. confusum* pupae. They found trehalose in considerable quantities, while the most abundant aldohexose was 6-deoxyglucose. The polysaccharides they identified by electrophoresis were similar to amylose and amylopectin and different from glycogen.

Friedlander and Navarro (1979b) measured glycogen concentrations after exposure of *E. cautella* pupae to hypoxia and hypercarbia. They found that hypercarbia increased glycogen utilization and that hypoxia made it more intensified. Glucose levels were not influenced by hypercarbia but rose significantly during exposure to hypoxia.

Physiological Effects

Resistance to MA

Resistance of stored-product insects to contact insecticides and fumigants has been well documented. Therefore, the question as to whether these insects are able to develop resistance to alternative control measures is pertinent. Bond and Buckland (1979) were the first to show that stored-product insects have the genetic potential to develop resistance to MAs. Their study obtained a threefold increase in tolerance to CO_2 by *S. granarius* after they had selected for seven generations. Similar results were obtained by Navarro et al (1985a) for resistance of *S. oryzae* to hypercarbia by selection over 10 generations.

A long-term study of the development of resistance by laboratory strains of *T. castaneum* to hypoxia and to hypercarbia was carried out by Donahaye (1990a,b) and Donahaye et al (1992a) and included investigations into the mechanisms of resistance.

Laboratory-induced resistance of a strain of *T. castaneum* to hypoxia. The laboratory-induced resistance to hypoxia by adults of *T. castaneum* was studied by Donahaye (1985, 1990a). Selection over 40 generations produced a strain resistant to an MA composed of 99.5% nitrogen and 0.5% oxygen at 95% RH, termed the "low oxygen concentration (LOC)-selected" strain. At the 40th generation, LOC-selected adults had a resistance factor (RF) of 5.2 at the LT_{50} level, in comparison with the reference laboratory strain from which the LOC strain had been selected.

It was shown that the strain that developed resistance to LOC was also more resistant to anoxia than the unselected strain. This better adaptation to anoxic conditions may have been due to more successful maintenance of energy charge or to removal of toxic end-products of glycolysis, although this was not proved experimentally. However, the physiological and biochemical adaptation of the insect to 0.5% O_2 was mainly one in which the insects were able to survive by continuing to carry out aerobic metabolism. The natural tolerance of the unselected strain to this low O_2 tension was very great, and the adaptation of the selected strain was extraordinary. At 1% O_2, the insects were active, and the LT_{50} of the unselected strain was more than 10 days. It was for this reason that, at the outset, an O_2 tension of less than 1% was chosen in spite of the difficulty in accurately maintaining such low O_2 concentrations over long periods.

Laboratory-induced resistance of a strain of *T. castaneum* to hypercarbia. The laboratory-induced resistance to hypercarbia by adults of *T. castaneum* was studied by Donahaye (1985, 1990b). Selection over 40 generations produced a strain resistant to an atmosphere containing 65% CO_2, 20% O_2, and 15% N_2 at 95% RH, termed the "high carbon dioxide concentration (HCC)-selected" strain. At the 40th generation, adults of the HCC-selected strain had an RF of 9.2 at the LT_{50} level when compared with the laboratory strain from which the HCC strain had been selected.

In his study, Donahaye (1990b) showed that weight loss occurred during exposure to HCC. Even though the exposure was carried out at 95% RH, precisely to avoid the desiccation factor, the insects were nonetheless susceptible to desiccation. The water content of the dead insect in equilibrium with 95% RH was approximately 25%, whereas this finding indicates that the live insect needs to maintain a water content of 55%.

The unselected strain was unable to control water loss. Mobilization of TG reserves was rapid but was almost exhausted within 96 hr, indicating that the attempt to mobilize water reserves was insufficient to prevent desiccation.

In sharp contrast, the HCC-selected strain was able to maintain a water balance over a much longer time period. Thus, if CO_2 acts on the nerves of the spiracular muscle (Burkett and Schneiderman, 1967), then it is possible that the resistant strain is able to overcome this and close its spiracles, while the susceptible strain cannot. Furthermore, TG reserves per insect were significantly greater in the HCC-selected strain and their mobilization was slower, indicating a better control mechanism for the maintenance of water balance.

It is suggested, therefore, that the eventual mortality of the selected strain was due to exhaustion of energy reserves and that an important aspect of the selection process is the favoring of those insects that build up greater reserves and expend them more slowly. The other resistance mechanisms, as suggested earlier, are likely to be compensatory mechanisms against the physiological and biochemical effects of hypoxia and hypercarbia, as well as spiracular control or possibly other adaptations limiting water loss.

Effect on Development

Low-oxygen atmospheres significantly retard development, this effect being particularly noticeable under slower rates of development. Nitrogen has an anesthetic effect that produces immobilization after a brief period of hyperactivity (Ali Niazee and Lindgren, 1970; Storey, 1975a). A reduction in or suspension of development occurs during the exposure period. Presumably, insects lessen their oxygen consumption to avoid the effects of anoxia. In a review article on hypoxia in marine invertebrates, Herreid (1980) developed the classic viewpoint that aerobic animals are either metabolic conformers or metabolic regulators. Physiological compensation may include a lower metabolic rate or reduction in metabolic activity during hypoxia, thus leading to conservation of energy and O_2. A positive correlation between metabolic rates and mortality due to 100% CO_2 and a negative correlation between body weights and metabolic rates were shown to exist for *S. granarius* laboratory-selected for resistance to methyl bromide (Upitis et al, 1973). These mechanisms resulted in greater body weight, extended life cycle, and lower respiratory rate in the resistant strain. Also for the LOC-selected strain, Donahaye (1990b) showed that the pupal and adult stages were significantly heavier, oviposition rate was lower, and respiration rates in air were significantly lower than those of the unselected strain. Possible physiological compensation measures during exposure that were not investigated include increased O_2 transport (including maintenance of tracheal pumping), transfer across cell membranes, or transport in the hemolymph by increased heart pumping.

During anoxia, the development of insects virtually ceases, and survival depends only on the capacity to accumulate glycolytic products and to reduce metabolism needs. However, stored-product insects placed under anoxic atmospheres suffer a loss of body water that is strongly correlated with the mortality rate (Navarro, 1978) (Fig. 3).

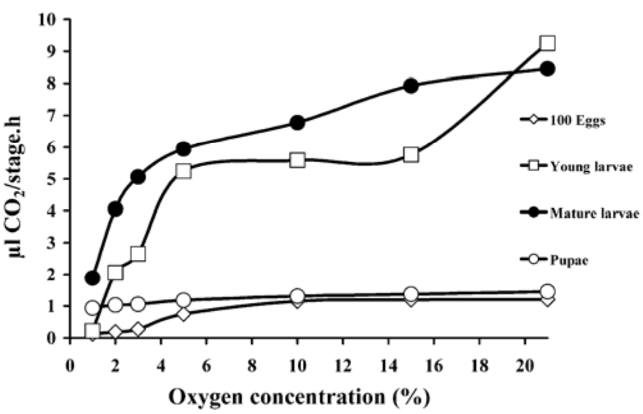

Fig. 9. Respiration rates of the development stages of *Tribolium castaneum* at different O_2 concentrations in nitrogen at 30°C and 70% RH. (Adapted from Emekci et al, 2002)

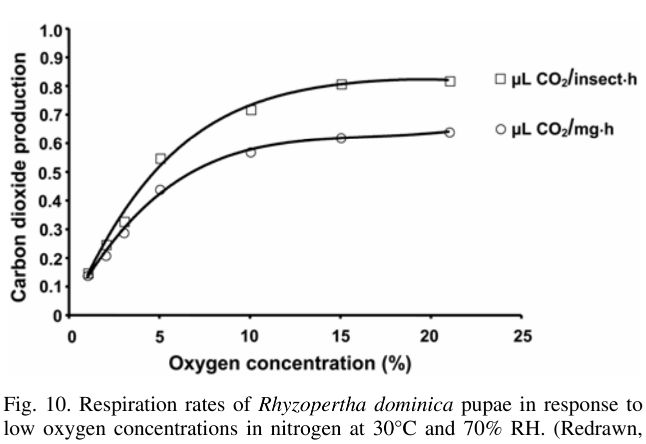

Fig. 10. Respiration rates of *Rhyzopertha dominica* pupae in response to low oxygen concentrations in nitrogen at 30°C and 70% RH. (Redrawn, and used with permission, from Emekci et al, 2004)

With sublethal periods of exposure to anoxia, a period of time is necessary, following the return to a normal atmosphere at the end of the treatment, for the recovery of vital functions. This time is needed for the recuperation of a normal aerobic metabolism (Bailey and Banks, 1980).

Respiratory Metabolism

Emekci et al (2002) showed that respiration of *T. castaneum* was suppressed at O_2 levels lower than 5% in eggs and young larvae (Fig. 9). Pupal respiration of *T. castaneum* decreased as O_2 concentrations fell. Similar results concerning respiration of *E. cautella* pupae were reported at reduced O_2 atmospheres at 26°C and 93–99% RH (Navarro and Calderon, 1979). Accordingly, CO_2 production by *E. cautella* was approximately 0.1, 0.2, and 0.35 µL/100 mg·hr^{-1} at O_2 concentrations of 1, 3, and 21%, respectively.

The increased respiration rate of *T. castaneum* adults at 3 and 5% O_2 was considered by Emekci et al (2002) as a compensatory response to the O_2 deficiency. Donahaye (1992) provided data on studies of the physiological differences between strains of adult *T. castaneum* unselected and selected for hypoxia and hypercarbia at 26°C and 95% RH. Carlson (1968), comparing different ratios of percent CO_2 and percent N_2, measured less CO_2 production (than in the control) in *T. confusum* adults at 30°C in atmospheres of 4.91, 10.15, and 14.90% O_2. His CO_2 measurements for these O_2 levels were between 1.74 and 1.88 µL/mg·hr^{-1}, similar to the results of Emekci et al (2002).

Respiration rates of immature stages of *R. dominica* increased proportionally as the O_2 concentration increased from 1 to 21% (Fig. 10). Respiration of eggs was particularly suppressed at low O_2 levels of 3% or less. CO_2 production per hour in eggs in 1% O_2 was found to be 9.7 times less than that in 21% O_2. Emekci et al (1998) obtained a similar respiratory response in *T. castaneum*, where CO_2 production of eggs in 1% O_2 was nine times less than that in 21% O_2. In young larvae, CO_2 production was adversely affected and respiration decreased by 8.2 times when the O_2 concentration decreased from 21 to 1%. Results obtained for pupae showed that *T. castaneum* respiration under the same conditions was more stable than in that of *R. dominica*, increasing by only 1.5 times, when O_2 concentrations increased from 1 to 21% (Emekci et al, 1998, 2004).

Behavioral Effects

Sublethal Effects

Many researchers have reported that high mortalities under low-O_2 conditions were obtained only after long exposures for all development stages (Calderon and Navarro, 1979; Bailey and Banks, 1980; Annis, 1987; Donahaye et al, 1996). Soderstrom and Brandl (1982) observed that 1% O_2 prevented feeding of larvae of the navel orange worm, *Amyelois transitella*. Emekci et al (2002) showed suppressed respiration at low O_2 levels, allowing *T. castaneum* to survive. In this respect, Calderon and Navarro (1980) reported that the egg stage of *T. castaneum* was more susceptible than its adult to reduced O_2

concentrations, and 100% mortality of eggs was obtained only after 96 hr at 2–4% O_2 levels. Tunc and Navarro (1983) found that exposure of newly laid up-to-one-day-old *T. castaneum* eggs to 2–4% O_2 at 26°C and 20, 50, and 95% RH caused complete mortality after 96 hr. Jay and Cuff (1981) reported that, upon exposure to ~1% O_2 at 27°C and 60% RH for 24 hr, mortalities for 16- to 20-day-old larvae, for three- to six-day-old pupae, and for two- to 14-day-old adults of *T. castaneum* were 11.2, 26, and 99.2%, respectively. Navarro (1978) obtained high mortality (95%) of *T. castaneum* adults at 1% O_2 in N_2 only after 96 hr at 26°C and 54% RH.

Calderon and Navarro (1980) reported that mortality in *R. dominica* eggs at 2, 4, and 6% O_2 in N_2 was 75, 85, and 50% after 96 hr of exposure, respectively, at 26°C and 55% RH. They also reported that adult mortality was below 10% under the same conditions. Bailey (1965) reported no mortality of *R. dominica* adults at 32°C after exposure to O_2 levels higher than 6%. As the O_2 concentration gradually decreased, mortality increased progressively, reaching 100% at 2% O_2 after 14 days of exposure. Annis and Dowsett (1993) reported that, in *R. dominica*, high mortalities for all developmental stages could be obtained only with O_2 concentrations below 1%. These results would support the view that all development stages of *R. dominica* were alive when their respiration rates were measured (Emekci et al, 2004).

Insect Response to Gas Gradients

The distribution of insects within a grain mass following a treatment of MA was studied to determine whether storage insects are able to escape the treatment or concentrate in preferred spots that permit their survival. Shejbal et al (1973) reported that adult beetles exposed to treatments with nitrogen and 0.5% oxygen tended to accumulate close to the point of gas introduction at the top of the silo. Navarro (1977) and Navarro et al (1981) studied the dispersion capacity of adults of *S. oryzae, R. dominica*, and *O. surinamensis* along a low-oxygen or a high-carbon-dioxide gradient in a wheat grain column 1 m high. *S. oryzae* and *R. dominica* did not respond to the presence of gradients and moved regardless of the presence of a low oxygen or high carbon dioxide concentration. However, the vertical distribution of adult *O. surinamensis* was inhibited by hypoxic or hypercarbic atmospheres. The authors also mentioned the accumulation of insects around leaks in the walls of a steel silo bin treated with an inert gas. In experiments with adults of *S. granarius* released into the center of a vertical grain column, Adler (1992, 1993) found a hypoxic atmosphere with 1% oxygen to be attractive to insects. This effect was markedly stronger when the gas mixture was introduced from the top rather than when it was flushed into the column from the bottom. A gas mixture with 80% nitrogen, 19% carbon dioxide, and 1% oxygen was found to have moderately repellent and arresting properties (Adler, 1992, 1993). In experiments in rectangular and circular arenas, it was found that adults of *S. granarius* preferred to stay in zones with higher oxygen levels (up to 10% oxygen in nitrogen or pressurized air) as long as the atmosphere was not made narcotic by oxygen levels below 2% or by high CO_2 levels (Adler, 1993).

Disinfestation Effect by Insect Removal from the Treated Commodity

The influence of different CAs in causing emigration of *Carpophilus* larvae from dates was compared with that of methyl bromide (MB) by Navarro et al (1989) and Donahaye et al (1991a). The treatment's effect on emigration from infested fruit is no less important than the toxic effect because established tolerances set minimum acceptance levels for the presence of both dead and live insects. To study the effectiveness in causing emigration of *Carpophilus* larvae from dates, a number of CA treatments and MB were compared by Navarro et al (1998b).

Emigration results under low atmospheric pressure and under low O_2 concentration were similar for the treatments carried out on dates and artificial feeding sites. Results of these treatments differed significantly from the control and from those of the CO_2-treated groups. *Carpophilus* larvae appear to seek out and penetrate their food substrate through cracks and crevices and then remain largely sedentary, thus creating a niche in which they feed until they begin to wander before pupation. Premature emigration from the feeding sites takes place under exposure to MB, low pressures, and low O_2 concentration. These treatments place the larva under stress, which interrupts their feeding and causes them to wander.

EFFECTS OF MA ON PRODUCT QUALITY

Germination of Seeds

Seeds stored at moisture contents in equilibrium with relative humidities below 65% are not damaged by storage fungi since these organisms require higher moisture contents (Lacey et al, 1980). Generally, the lower the moisture content and the lower the temperature, the longer the seed can be stored (Copeland and McDonald, 1985).

Seeds below their critical moisture content are not significantly affected by high-CO_2 or low-O_2 atmospheres (Banks, 1981; Fleurat-Lessard et al, 1994). However, with increasing grain-moisture contents, carbon-dioxide-rich atmospheres could reduce the physiological quality of grain by interfering with the enzymatic activity of glutamine-decarboxylase (Münzing and Bolling, 1985). Laboratory studies have demonstrated that 60% CO_2 in air at 60°C slightly reduces seed longevity compared with that in N_2-based atmospheres (Hough et al, 1971; Shejbal, 1980b). The adverse effect of CO_2 on germination of rice, maize (corn), and wheat becomes more pronounced at temperatures higher than 47°C and, from the observations carried out so far, this adverse effect may not be detectable at all below 30°C (Banks and Annis, 1990). Therefore, if preservation of germination is of primary importance, the use of CO_2-free low-O_2 atmospheres is preferable.

Hermetic storage of seeds modifies the atmospheric composition surrounding them by depleting the oxygen through the respiration of insects, molds, and seeds. The atmosphere within the storage container therefore becomes insecticidal, fungistatic, or fungicidal. Moreno et al (1988) showed that maize seeds stored at moisture contents between 15.3 and 17.7% were not invaded by fungi when stored under hermetic conditions and maintained a higher viability than seeds with similar moisture contents not stored hermetically. Corn stored for 90 days at a 15.3% moisture content maintained a viability of 95% under hermetic conditions, compared to viability that dropped to 43% in samples kept under nonhermetic conditions (Moreno et al, 1988).

Under the high humidity of the tropics, microflora commonly invade stored seeds (Mendoza et al, 1982). Therefore, the problems of maintaining seed viability in storage have always been an important concern to farmers and seed growers in the developing countries due to inadequate storage facilities. Under hermetic-storage conditions, storage insects can develop a storage atmosphere lethal to themselves before they cause damage to the germination of seeds. In Israel, plastic structures have been developed suitable for long-term storage systems, as well as for intermediate storage of grain in bags or bulk for cooperatives and subsistence farmers (Navarro et al, 1990). For small-scale applications, these plastic structures use flexible liners (Navarro et al, 2002a). Predictive models have been developed to determine the response of insects to gastightness levels (Navarro et al, 1994).

Corn Storage Trials in Thailand and in the Philippines

Viability of corn stored under hermetic (148 days of storage) and nonhermetic (120 days of storage) conditions in the Philippines did not significantly change between the initial and final samples. The temperature inside the control stack kept under a nonhermetic tarp was 28°C, and temperatures under the hermetic conditions were in the range of 29–31°C. In the hermetic storage, the CO_2 level rapidly increased to 12%, while the O_2 concentration sharply declined to around 7% over the first two weeks of storage. On the basis of viability tests and insect infestations, storage under hermetic conditions was considered successful, in comparison with the control stacks (Navarro and Caliboso, 1996; Navarro et al, 1998a) (Table 5).

Paddy Storage Trials in the Philippines and in Cambodia

To test the viability of paddy rice stored under hermetic and nonhermetic conditions, two trials were carried out in the Philippines, one lasting for 117 days and the other for 187 days (Navarro and Caliboso, 1996; Navarro et al, 1997a). Viability of paddy stored under hermetic conditions did not change significantly during the trials. Only a slight reduction was observed in the viability of paddy in the control.

The tests in Cambodia lasted for 223 days, during which a slight increase in moisture content was observed under the hermetic conditions and a significant increase of 2 percentage points of moisture content (from 13 to 15%) in the control stack. This significant increase in moisture content apparently influenced the sharp decrease in viability of the paddy in the control stack, from 95% at the start of the trial to 66% at the end of the trial. However, the hermetic seal preserved the germination capacity, and the drop was only from 97 to 91% for the same period (S. Bunna, *personal communication,* CARDI, Phnom Penh, Cambodia, 2001) (Table 5).

Wheat Storage Trials

To test the viability of wheat stored under hermetic conditions in Israel, two trials were carried out, with storage periods of 1,440 and 450 days and only under hermetic conditions. In trial I, the viability of wheat changed only slightly, from the initial 99% to 97% at the end of 1,440 days. In trial II, the wheat viability dropped slightly, from an initial level of 97% to 91% at the end of the storage

TABLE 5
Viability of Maize (Corn), Paddy, and Wheat Stored Under Hermetic and Nonhermetic Conditions[a]

Commodity Country	Storage Condition	Length of Trial (days)	Initial Germination (%)	Germination at End of Trial (%)	Moisture Content at Start of Trial (%)	Moisture Content at End of Trial (%)
Maize						
Thailand						
Trial I	Hermetic	90	97	98	12.4	12.2
	Nonhermetic	90	97	95	12.4	11.5
Trial II[b]	Hermetic	280	97	81		
	Nonhermetic	280	98	0	12.2	13.6
Philippines[c]	Hermetic	148	94	92	12.2	13.6
	Nonhermetic	120	88	87	13.2	13.6
Paddy					12.0	12.0
Philippines						
Trial I	Hermetic	117	98	99	12.1	12.3
	Nonhermetic	117	98	98	9.7	11.6
Trial II[c]	Hermetic	183	94	93	10.8	10.6
	Nonhermetic	117	95	92	12.2	12.8
Cambodia[d]	Hermetic	223	97	91	13	14
	Nonhermetic	223	95	66	13	15
Wheat						
Israel						
Trial I	Hermetic	1,440	99	97	10.6	10.7
Trial II[c]	Hermetic	450	97	91	11.4	11.4

[a] Source: Navarro et al (2002a); used by permission.
[b] Data from Sukprakarni et al (1998).
[c] Data from Navarro and Caliboso (1996).
[d] Data from S. Bunna, CARADI, Phnom Penh, Cambodia, *personal communication* (2001).

period. In both trials, insect populations were successfully controlled; the average CO_2 concentrations ranged between 10 and 15% (Navarro and Caliboso, 1996) (Table 5).

Product Quality Preservation

Several trials were performed to determine the quality changes of treated cocoa beans and hazelnuts (Ziegleder, 1991). Accelerated tests for storability under high-pressure CO_2 were simulated at 35°C for three months. The tested qualities of cocoa beans did not change significantly. Only treated hazelnuts showed a tendency to turn rancid earlier than untreated samples. All other tested criteria of these products remained unchanged. Similar findings were reported for many other food products exposed to high-pressure CO_2 (Gerard et al, 1988a,b, 1990; Pohlen et al, 1989).

Data about the comparative effects of low-O_2 and high-CO_2 atmospheres on end-use parameters are very limited. Generally, low-O_2 atmospheres preserve quality better than air (Duff et al, 1986; Richard-Molard et al, 1987), but there are some indications that the effects of CO_2-rich atmospheres should be checked before use in unfamiliar conditions. For instance, Shejbal (1980b) warns of (unspecified) detrimental effects on quality. Wheat held for long periods under unfavorable storage conditions and high-CO_2 atmospheres yielded flour with a much longer mixing time than that stored under the same O_2 concentration in N_2 only. CO_2 was at one time suspected of causing increased yellowing of rice in storage, but laboratory studies suggest that this is not so (Bason et al, 1987; Banks and Annis, 1990).

Donahaye et al (2001) reported on quality preservation of paddy rice stored in stacks of 13.4–31.9 tonnes (t) in flexible enclosures outdoors for 78–183 days. The quality of the paddy was compared with that of three control stacks (5.3–5.6 t capacity) held under tarpaulins in the open for 78–117 days. The trials were conducted at the NAPHIRE compound, Nueva Ecija, the Philippines. The enclosures consisted of heavy-duty polyvinyl chloride (PVC)-based sheeting sufficiently gastight to control insect infestations. Initial and final samples were taken to determine changes in paddy quality; insect infestation, fungal infection, milling recovery, head rice, yellow kernels, brokens, germination, and weight loss were analyzed. Percent milling recovery and levels of yellowing in the gastight stacks showed no significant change. The levels of head rice and brokens were preserved in seven out of nine stacks. A decrease in percent of head rice in two stacks was attributed to the biological aging phenomenon. The two control stacks showed a decrease in head rice and an increase in brokens. Rice yellowing was very pronounced in one of the control stacks.

Sorption of Carbon Dioxide

Jay (1980) found that sorption of CO_2 by grain makes the gas effective against insect species whose immature stages feed inside the kernel. The adsorption mechanism of CO_2 into the grain has been found to be very similar to that observed in sorption of gases by charcoal and silica gel (Mitsuda et al, 1973). The sorption phenomenon causes the removal of some molecules of gas from the free space in the treated enclosure, which causes a progressive lowering of the concentration (partial pressure) of the gas in the free space.

Effect on Changes in Pressure of the Treated Container

Banks et al (1980) observed an initial rapid decay of concentration shortly after CO_2 was purged into large bins containing wheat. This initial rapid decay seemed to be associated with sorption of the CO_2 by the grain. Characteristics of CO_2 sorption by stored wheat were studied by Cofie-Agblor et al (1993, 1995) and by Yamamoto and Mitsuda (1980). Typical reduced pressures created by sorption in the gastight containers were shown by Navarro (1997b). Since the amount of CO_2 sorbed is proportional to the amount of grain in the container at a given partial pressure of CO_2 and temperature, the resulting reduced pressure is also proportional to the void space of the system. Therefore, the drop in pressure can represent the experimental conditions only when the grain bulk volume occupies 93% of the total container capacity. Under these conditions, the lowest pressure recorded was 520 mm of Hg at 15°C (Navarro, 1997b) (Fig. 11). The sorption rate changed inversely with the temperature of the wheat, and the highest absolute pressure of 606 mm of Hg was obtained at 30°C. Mitsuda and Yamamoto (1980) reported that a 0.8-L container filled with grain (apparently rice) developed a negative pressure of 0.27 kg/cm^2 after seven days. This negative pressure in terms of absolute pressure at standard temperature and pressure conditions was calculated to be about 555 mm of Hg.

Corrosion Potential of Carbon Dioxide

Carbon dioxide reacts with moisture during bulk-grain treatment until an equilibrium is established between dissolved and atmospheric carbon dioxide. It is reported that carbon dioxide is absorbed into new concrete structures (Spratt, 1975), and, in moist concrete walls, carbonic acid formed from carbon dioxide could corrode metal structures.

CO_2 reacts with basic calcium compounds in new concrete to give calcium carbonate (Hamada, 1968). The pro-

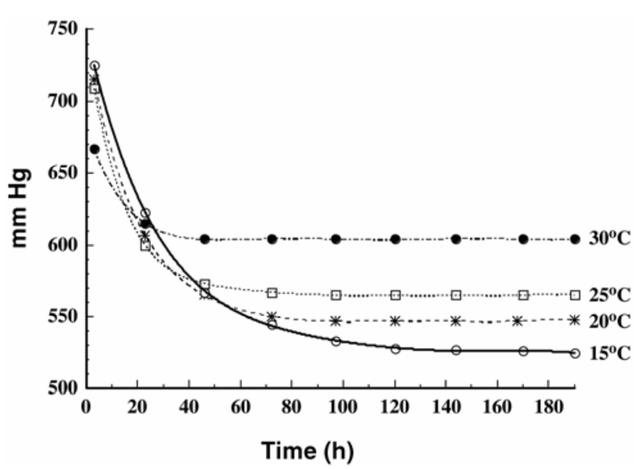

Fig. 11. Pressure decay due to sorption of CO_2 in gastight containers filled to 93% capacity with wheat at four temperatures, with an initial CO_2 concentration of 99.8% and an initial pressure of 768 mm of Hg. (Redrawn, and used with permission, from Navarro, 1997b)

cess, known as carbonation, has no parallel in N_2-based atmospheres. Carbonation can potentially lead to corrosion of reinforcing steel in concrete buildings, such as silos, with consequent weakening or failure. However, Banks and McCabe (1988) argued that, although carbonation occurs in concrete storages, it does not appear to affect them adversely. In new concrete structures, the carbonation reaction can take up substantial quantities of CO_2, leading to increased usage of CO_2 to create a given high-CO_2 atmosphere. It has been calculated (Banks and McCabe, 1988) that a new 2,800-t concrete bin can take up about 7 t of CO_2, about 2.5 times the quantity normally used to give a 70% CO_2 atmosphere when the structure contains wheat. The exact quantity taken up depends on the structural design and concrete specifications. A pressure reduction of more than 3 kPa in 0.5 hr has been observed (Banks and McCabe, 1988) in a well-sealed bin, attributable to CO_2 uptake by the concrete of the structure.

GENERATION AND APPLICATION OF MA

Generation of MA

The objective of MA treatment is to attain atmospheric gases rich in CO_2 and low in O_2, or a combination of these two gases within the storage enclosure or treatment chamber, for the time necessary to control the storage pests. At present, the most widely used source for production of such atmospheric gas compositions is tanker-delivered liquefied CO_2 or N_2. The availability and suitability of this means of gas supply must be questioned when the gases are transported over long distances from an industrial production area to the storage site. Therefore, alternative potential methods of generating MAs should also be considered.

From Tankers

When the target MA gas composition is <1% O_2 or a high CO_2 concentration, a commonly used method is to supply N_2 or CO_2 from pressurized tankers. The practical aspects of purging grain storages have been described by Guiffre and Segal (1984) for CO_2 and by Banks et al (1980) for N_2 and CO_2. A significant portion of the cost of applying MAs generated from tankers is for transportation and on-site purging. Bulk liquid gas is transported in conventionally insulated road tankers.

For large-scale application of N_2 or CO_2, vaporizers are essential. These vaporizers consist of a suitably designed receptacle with a heating medium (electricity, steam, diesel fuel, or propane), a hot-water-jacketed super-heated coil, and forced or natural draft. A forced-draft-type of vaporizer with electrical super heating was found to be convenient (Guiffre and Segal, 1984).

From Exothermic Gas Generators

For on-site generation of MAs by combustion of hydrocarbon fuel to produce a low-O_2 atmosphere containing some CO_2, commercial installations (termed "exothermic gas generators" or "gas burners") are available. Such equipment was originally designed for MA storage of fresh fruits. It allows the presence of ~2–3% O_2 and removes CO_2 through scrubbers. Therefore, its use in the grain industry requires several adaptations, such as adjusting the equipment to obtain an O_2 level of <1%, taking full advantage of the CO_2 generated, and removing excessive humidity from the generated atmosphere. Combustion of propane yields approximately 13% CO_2 and combustion of butane yields approximately 15% CO_2. The MA generated is more toxic than is an N_2 atmosphere deficient in O_2. This is because the presence of CO_2 in the MA causes hypercarbia; hypercarbia and hypoxia together are synergistic in their effect on insect mortality (Calderon and Navarro 1979, 1980; Bell, 1984; Navarro and Jay, 1987). Equipment has been designed to operate with open-flame burners, with catalytic burners, and as an internal combustion system. Full-scale field trials using open-flame burners (Storey, 1973; Fleurat-Lessard and Le Torc'h, 1987) and catalytic burners (Navarro et al, 1979) to provide a low-O_2 gas mixture have proven successful. Open-flame burners are capable of producing high gas-flow rates at a low O_2 tension. Consequently, the generated MA can be applied directly to purge the treated enclosure. On the other hand, catalytic systems reduce the O_2 concentration in the atmosphere by a fixed fraction during passage through the catalyst and therefore preferably should be used in a recirculation system. The development of a modified internal-combustion engine for MA generation has been reported (Banks, 1984a). In spite of its advantages over the open-flame and catalytic burners as an easily operated, transportable, and independent system, information on field application of such a combustion system is lacking.

From On-Site N_2 Generators

Commercial equipment, also termed "pressure-swing adsorption" systems, using the process of O_2 adsorption from compressed air passed through a molecular sieve bed, is available (Zanon, 1980). For continuous operation, a set of two adsorbers is provided; these operate sequentially for O_2 adsorption and regeneration. N_2 at a purity of 99.9% can be obtained through regulation of inlet airflow. Equipment is now manufactured that is rated to supply an outlet flow rate of 120 m^3/hr at an outlet purity of 98% N_2. However, in view of the high capital investment involved, it would seem wise to undertake a long-term cost-benefit analysis to explore the justification of using these installations.

Biogeneration of MAs

Two principal forms of biogeneration of MAs exist, namely, "hermetic storage" and "assisted hermetic storage."

Hermetic storage. A high level of gastightness is required for a structure to be suitable for hermetic storage of dry grain. The effect of restricted air supply on storage insects was studied by Oxley and Wickenden (1963), who suggested that, in leaky structures, it is necessary to increase the rate of O_2 consumption to a level at which insect infestation cannot persist. Burrell (1980) concluded that sealing infested grain using hermetic storage to kill the insects can be satisfactory for a heavy infestation in warm grain but is likely to prove uneconomical for light infestations or when the grain is cool, because of the long storage period required before complete kill is obtained. To obtain complete control of all insects in order to eliminate the danger of renewed infestation of grain removed from the hermetic container, Banks (1984a) proposed a possible solution of increasing consumption of O_2 by artificially infesting the grain with insects. Similarly, Burrell (1980) intentionally wetted a small region of the stored commodity. Because part of the commodity is sacrificed for the generation of the MA, Banks (1984a) has termed this type of storage "hermetic storage with sacrificial areas." However, experience has shown us that, for hermetic storage in flexible plastic storage systems under subtropical climatic conditions, hermetic storage of grain continues to offer an excellent solution, provided there is a certain degree of tolerance of the presence of insects at critical areas in the storage structure (e.g., at the grain surface, where moisture condensation is likely to occur). At the end of long-term hermetic storage, when the unloaded grain is destined for immediate consumption, the risk of spreading insect infestation was found to be negligible. The success of insect control by hermetic storage treatments is comparable to that of conventional fumigants (~99.9% kill), and losses due to insect activity are minimal (0.15% loss in weight for a storage period of 15 months) (Navarro et al, 1984).

Assisted hermetic storage. The term "assisted hermetic storage" was introduced by Banks (1984a) to define a process in which MA generation is assisted by a biogenerator source without sacrificing the commodity. Using a similar approach, Calderon et al (1981) examined the possibility of generating an MA by inoculating wet rice bran. The best-known working example of assisted hermetic storage is that used in China (Lu, 1984). With this method, removal of O_2 is achieved by recirculating storage gases through a closed system containing racks of moist grain and bran infected with a particular mold culture. This MA generation system merits further attention to explore potential applications at locations where a regular supply of industrial gases is nonexistent or economically unfeasible.

Potential Systems for the Generation of MA

Less commercialized, or potential, methods for the generation of MAs have been discussed by Banks (1984a). They include extraction of N_2 from the low-O_2 exhaust stream produced by the combustion of hydrocarbons in air (Zanon, 1980), catalytic oxidation of ammonia, hydrogen combustion, direct electrolytic or catalytic removal of O_2, removal of O_2 by chemical reaction, producer gas combustion systems to generate CO_2, combustion of methane derived from fermentation, burning carbon-containing materials in air, burning coal or charcoal in O_2, and production of CO_2 from fermentation.

Methods for Applying Modified Atmospheres

Prerequisites for Application to Grain Stored in Bulk

Choice of atmospheric gas composition. A simple and descriptive graphical presentation to illustrate the relationship between exposure period, O_2 and CO_2 concentrations (at normal atmospheric pressure), and the mortality of different insect life stages was compiled from the literature by Annis (1987). In his review, he proposed provisional dosage regimes at grain temperatures of 20–29°C.

A summary of these dosage regimes is given in Table 6, which shows that the use of an atmosphere with less than 1% O_2 requires considerably longer exposure times than 80% CO_2 atmospheres to kill insect populations other than *Trogoderma granarium*. The basis for preparing these regimes was the time response of the most tolerant developmental stage of the most tolerant insect species. In the absence of *Trogoderma granarium*, a low-O_2 regime should be based on the response of *S. oryzae* pupae, while the CO_2 regimes should be based on *T. castaneum* adults and larvae (Annis, 1987; Navarro and Jay, 1987).

The dosage regimes presented in Table 6 should be viewed as very generalized recommendations. More recently published information (Navarro and Jay, 1987; Reichmuth, 1997; Bell et al, 2003) indicates that further work is needed to enable precise dosage recommendations to be established for the application of MAs for the major stored-product insects under the wide range of intrinsic and extrinsic factors involved. Thus, recommended dosage regimes should be based on temperature ranges appropriate to specific climatic conditions and also to the dominant insect species found in the commodities involved. Aspects of commodity moisture content (Navarro, 1978; Navarro et al, 1994; Adler et al, 2000; Donahaye et al, 2000), socioeconomically acceptable control levels, the time-frame within which the control must be accomplished, and the expected leak-rate standard under which the MA treatment will be performed will probably all play important roles in future recommendations.

In contrast to the conventional application of MAs at normal atmospheric pressure, the use of pressurized CO_2 in high-pressure chambers for control of *P. interpunctella* and *L. serricorne* was demonstrated (Gerard et al, 1988b). With this method, CO_2 is applied at pressures of 10–50 bar. Complete control of all stages of the tested insects was achieved after 20 min of exposure at 50 bar (Stahl et al, 1985). The special equipment needed to withstand the high CO_2 pressure is expensive and therefore limits the market for this method. However, food industry segments that process high-value products such as dried fruits, drugs, and spices have been taking advantage of this MA method (Adler et al, 2000; Nakakita et al, 2001; Prozell and Reichmuth, 2001; Riudavets et al, 2003). More research is needed to obtain additional biological data on storage pests.

TABLE 6
Suggested Provisional Dosage Regimes for Control of All Stages of the 12 Most Common Insect Species of Stored Grain, Using Modified Atmospheres at Temperatures Between 20 and 29°C[a,b]

Atmospheric Gas Concentration	Controls Most Common Grain Insects Including *Trogoderma granarium*	Exposure Period (days)
<1% O_2 (in nitrogen)	Yes	20
Constant % CO_2 in air		
40	No	17
60	No	11
80	No	8.5
80	Yes	16
CO_2 decay in air from >70 to 35%	No	15
Pressurized CO_2 at >20 bar	Yes	<0.35

[a] Source: Navarro and Donahaye (1990); used by permission.
[b] Data, except those on pressurized CO_2, compiled from Annis (1987).

Rate of supply. Because of the relatively long exposure time involved, one basic concept of MA application methods is the combination of two separate phases: an initial "purge" for the establishment of the desired atmospheric gas composition and a subsequent "maintenance" phase, in which the desired gas composition is maintained during the exposure period (Banks and Annis, 1977). This concept differs from the "single-shot" treatment suggested by Banks et al (1980). The single treatment is suitable for CO_2 when an initial concentration higher than 70% is established and the gastightness of the structure is sufficient to allow maintenance of a concentration above 35% for at least 10 days.

MA treatment must displace a large volume of the intergranular free space plus the headspace of the silo. The rate of gas supply is purely an economic aspect of the application of MAs, since a substantial portion of the expense involved consists of the cost of transporting the liquid CO_2 or N_2 and of the on-site purging, which is a time-consuming process (Guiffre and Segal, 1984). If on-site bulk gas tanks are not installed, truck demurrage charges must be added. With gas burners, the aspect of transportation is less critical, since the quantities of hydrocarbon gas used are considerably less.

The gas supply rates required for the application of selected MAs are listed in Table 7. The proposed supply time at "purge" phase for an MA of <1% O_2 is considerably shorter than for the other MAs applied at normal atmospheric pressure. This shorter purge time derives from the physical characteristics of N_2 (Banks and Annis, 1977). A method (not included in Table 7) that has been used by the present author in small bins of 50-t capacity consists of direct gas supply to the bin in a liquid state, thereby reducing the supply time considerably. This method is discussed in the section on gas supply in a liquid state.

The shortest supply time is achievable using a pressurized CO_2 system (Table 7). The natural pressure of liquid CO_2 is advantageous in creating high pressure in the exposure chamber.

TABLE 7
Rate of Gas Supply Requirements for Modified Atmosphere Application[a,b]

Selected Atmospheric Gas Concentration	Application Phase	Amount of Gas per Tonne Commodity	Supply Time (hr)
<1% O_2 in N_2	Purge	1–2 m^3 N_2	<12
	Maintenance	0.01–0.06 m^3 N_2	**[c]
>70% CO_2 in air	Purge	0.5–1.9 m^3 CO_2	<48
	Maintenance	0.02–0.04 m^3 CO_2	**
Gas burner <1% O_2	Purge	17–66 g C_3H_8	<48
With >14% CO_2	Maintenance	0.6–1.2 g C_3H_8	**
>70% CO_2 in air	Single-shot	0.5–1.0 m^3 CO_2	<48
Pressurized CO_2 at >20 bar	Single-shot	>18 kg CO_2	<0.5

[a] Source: Navarro and Donahaye (1990); used by permission.
[b] Compiled from Banks (1984a) (except data on pressurized CO_2). Only gas compositions supported by field experience are presented in this table. The basic assumptions for the above requirements are that the storage is filled with grain (minimum headspace) and that pressure decay time is >5 min for decay from 500 to 250 Pa.
[c] According to the dosage regime; see also Table 6.

Structural requirements. Storage structures designed specifically for the application of MAs are practically nonexistent, apart from those in Australia (Ripp et al, 1984). Banks and Ripp (1984) 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. Large-scale application of sealed storage by the food industry has recently received attention because of the approaching phase-out of methyl bromide (Casada and Noyes, 2001; UNEP, 2002). Therefore, before deciding on the method of 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 (Banks, 1984b; Navarro, 1997a, 1999; Casada and Noyes, 2001; Navarro and Zettler, 2001). For application of pressurized CO_2, specially designed chambers and installations to withstand the high pressures are needed (Prozell and Reichmuth, 2001).

Application of MA in a gaseous state. For application of N_2 or CO_2 into upright storages, simple inlet systems fitted into the bin wall can be used for gas introduction. The design of the system should be such as to prevent excessive pressure buildup over weak areas of the silo bin wall, especially around the inlet pipe. For purge rates of 6 m^3/min, an inlet pipe with an 8-cm diameter has proven convenient (Banks and Annis, 1980). However, in bins equipped with a grain aeration system, it is advantageous to use the inlet duct system as the gas introduction point to obtain improved purging efficiency.

When purging upward, high CO_2 levels tend to remain in the lower layers of large bins, which may result in uneven and sometimes inadequate CO_2 concentrations for insect control, especially in the upper layers of bins (Wilson et al, 1980). To overcome this, especially in the single-shot CO_2 application method, where no maintenance phase is used, it is important to introduce an air injector into the CO_2 stream, so as to produce a CO_2-air premix at the designed concentration, or to recirculate the CO_2-air mixture until the desired CO_2 concentration is attained in all regions of the bin (Navarro et al, 1986).

For the application of CO_2, Jay (1971, 1980) proposed three methods. These, together with the recirculation and blending methods (Navarro et al, 1979; Wilson et al, 1984) compose the five basic application methods suitable for MAs at normal atmospheric pressure. They are summarized in Table 8 and presented schematically in Figure 12. Recirculation gives the most uniform concentration, and it can be applied by moving the gases inside the bins upward or downward (Navarro et al, 1986). The main gain in using downward flow is with application by burner gas. It takes advantage of the long path of the external gas delivery pipe to cool and thereby dehumidify the hot gases after the burner (Navarro et al, 1979). The pressurized CO_2 method is most advantageous when an extremely short exposure time is a condition (Gerard et al, 1988b) (Table 8, method 6).

Application of MA in liquid or solid state. For small silos and MA treatment chambers of up to 100 m^3, a direct supply of CO_2 from cylinders equipped with a siphon was tested by Navarro and Donahaye (1990). By this means, CO_2 is released in a liquid state from the pressurized cylinder.

TABLE 8
Methods of Application of Modified Atmospheres[a]

Methods of Application	Applicable Modified Atmosphere	Main Advantages	Main Disadvantages	References[b]
1. Purge full silo from the top	CO_2	Requires only one application. Labor requirements are minimal.	Purging time is long. Some CO_2 is lost in outflow with air mix.	4,5
2. Apply CO_2 in the grain stream (snow, dry ice)	CO_2	Method is fast. No vaporization equipment is needed.	Danger of explosion (static electricity). Constant supervision during application.	4
3. Lift the atmosphere out (air displacement method). Continuous purge from bottom	CO_2, N_2, GB[c]	Labor requirements are low. No loss of gas in mixing. Works best with N_2.	Gas purging region of silo should be leak-free. With CO_2 it creates high localized concentration so blending may be necessary.	1,2,4,7
4. Blending and purging	CO_2	Homogenous concentration is obtained. No loss of gas in mixing.	Air CO_2 mixing equipment is necessary.	8
5. Recirculation	CO_2, GB	Homogenous concentration is obtained. No loss of gas in mixing.	Recirculation equipment is necessary.	6,8
6. Pressurized CO_2 at >20 bar	CO_2	Short exposure time required for complete control.	Method is costly and needs special exposure chamber.	3

[a] Source: Navarro and Donahaye (1990); used by permission.
[b] 1 = Banks and Annis (1977); 2 = Fleurat Lessard and Le Torc'h (1987); 3 = Gerard et al (1988b); 4 = Jay (1980); 5 = Jay and Pearman (1973); 6 = Navarro et al (1979); 7 = Storey (1973); 8 = Wilson et al (1984).
[c] GB = Gas-burner atmosphere, consisting of <1% O_2, 15% CO_2, and 84% N_2.

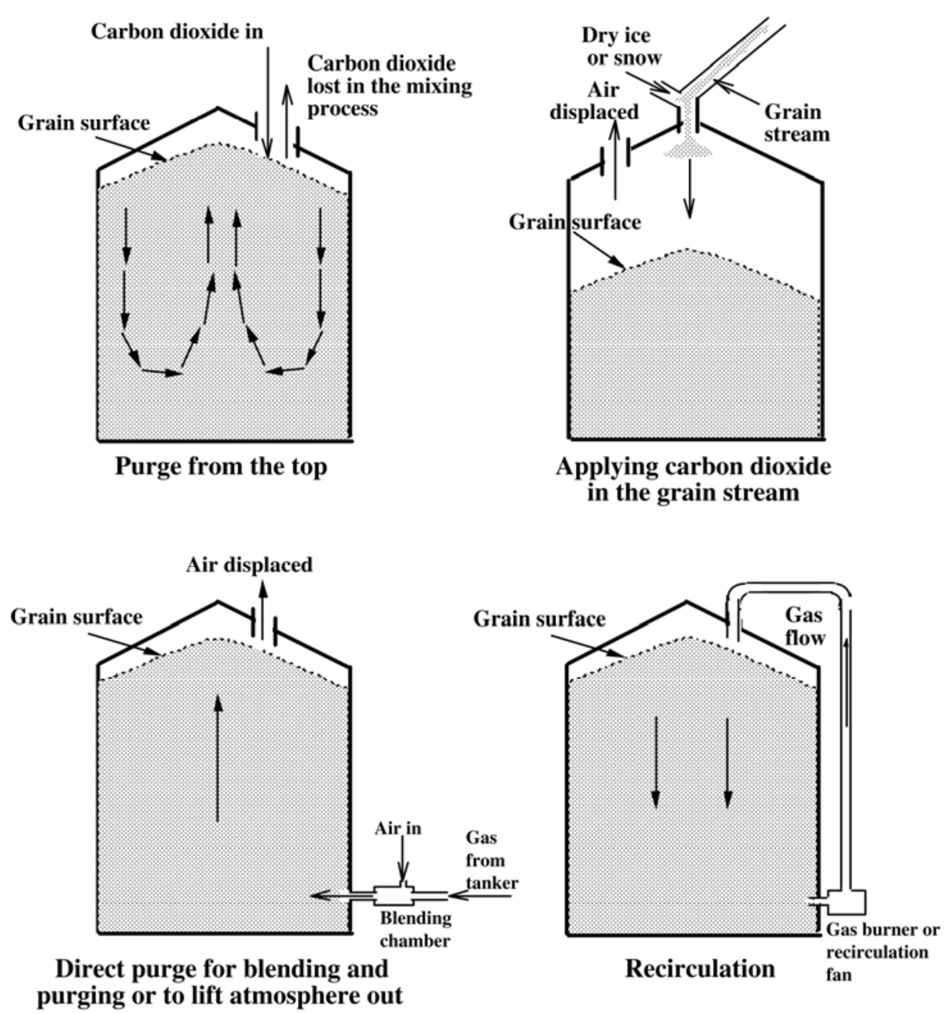

Fig. 12. Four methods of applying a modified atmosphere.

A large volume of CO_2 can thus be introduced into the treated enclosure in a relatively short time, thereby causing displacement by lift-out of a substantial portion of the atmosphere from the free space of the treated structure. Great care should therefore be taken to install a large enough vent pipe and to ensure that the structure can withstand the pressure buildup at the initial purging phase. In addition, it is strongly recommended that the pressure of the treated structure be monitored. Experience with this method of gas supply has shown that, at a rate of 4 m^3 of CO_2 per minute, the pressure buildup within a chamber of 110 m^3 was less than 60 Pa when the vent pipe's internal diameter was 75 mm (Navarro et al, 1998b).

Application of CO_2 in the form of dry ice to control insects infesting flour in hopper cars (Ronai and Jay, 1982) and in freight containers (Sharp and Banks, 1980) was investigated.

Hermetic Storage, Sealed Storage, and Airtight Storage

Factors affecting insect mortality in hermetic storage. The important role of low O_2 concentration, rather than high CO_2, in causing mortality of stored-product insects in hermetic storage was demonstrated by Bailey (1955, 1956, 1957, 1965). Only later was the importance of the synergistic effect of concomitant O_2 depletion and CO_2 accumulation for insect control clearly demonstrated (Calderon and Navarro, 1979, 1980; Donahaye et al, 1996). These synergistic and combined effects are essential for successful insect control, as shown by studies of the effects of incomplete airtightness upon insect populations (Oxley and Wickenden, 1963; Burrell, 1968). To demonstrate the effects of incomplete airtightness, Navarro et al (1994) modeled a fixed O_2 ingress rate equivalent to about 0.24% per day for a structure with a volume of 10 m^3. For these given values, changes in oxygen concentrations in response to different initial insect populations are illustrated in Figure 13. Accordingly, a cyclic change in concentrations is obtained as a result of O_2 ingress and the ability of insects to survive at low O_2 levels. These theoretical cyclic changes in O_2 concentrations are also observed in different laboratory and field studies (Navarro et al, 1984, 1994).

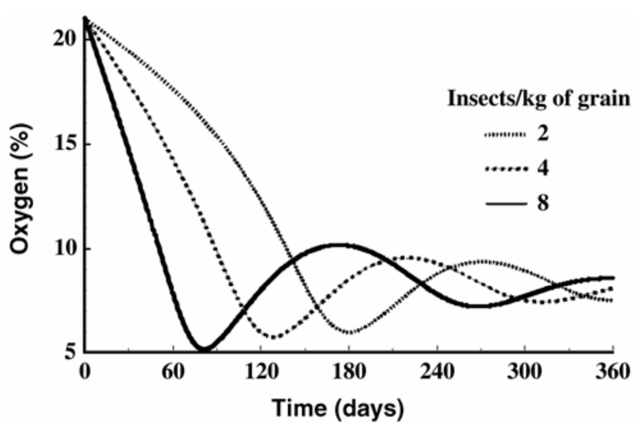

Fig. 13. Calculated oxygen concentrations in a 10-m^3 grain mass containing different infestation levels of insects and having an oxygen intake of 157 μL per insect per day, using a sealed liner with an oxygen ingress rate of 0.24% per day. (Redrawn, and used with permission, from Navarro et al, 1994)

The influence of temperature on insect respiration implies that, in warm climates, O_2 intake by insects is very intensive. Conversely, in temperate climates, insect metabolism is much slower; depletion of O_2 may be lower than its ingress; and insect control may not be achieved. This led Burrell (1980) to postulate that, for light infestations of cool grain, residual populations would provide an inoculum for reinfestation after the grain is removed from hermetic storage.

Modern hermetic storage. *In aboveground rigid structures.* Documented data on successful application of hermetic storage to aboveground constructions are largely lacking. Many existing silos and warehouses have been modified to provide a high degree of hermetic seal, especially in Australia (Delmenico, 1993). However, the objectives have been to convert these storages for MA treatments or improved fumigations and not for hermetic storage as such.

In contrast, the sealing of both bagged stacks and bulk grain in warehouses in China using plastic liners is part of a grain preservation regime termed "triple-low." This is an integrated approach to insect control consisting of obtaining reduced O_2 concentrations by metabolic activity within the grain bulk in combination with phosphine and low-temperature treatments (Wang et al, 1993; Xu and Wang, 1993). This procedure is claimed to provide effective protection.

In aboveground flexible structures. In the early 1970s, aboveground structures were designed in England for emergency storage using flexible plastic liners supported by a weld-mesh frame. These liners were made of butyl rubber, sometimes laminated with white ethylene-propylene-diene monomer, and consisted either of a wall-floor section, plus a roof section attached after loading, or both sections welded into a single unit. These silos were recommended for both conventional storage and hermetic storage of dry grain (Kenneford and O'Dowd, 1981). However, in tropical and subtropical climates, the liners were found to deteriorate, and gas permeability increased to a level at which the liners could no longer be used for hermetic storage (Navarro and Donahaye, 1976; O'Dowd and Kenneford, 1983).

In Israel, the manufacture of PVC liners that conform to prerequisite specifications of durability to climate, gas permeability, and physical properties has enabled the development of three storage systems based on the hermetic principle. These are 1) bunker storage for conservation of large bulks of 10,000–15,000 t (Navarro et al, 1984, 1993); 2) flexible silos supported by a weld-mesh frame of 50- to 1,000-t capacity for storage of grain in bulk or in bags (Calderon et al, 1989; Navarro et al, 1990); and 3) liners for enclosing stacks of 10- to 50-t capacity (termed "storage cubes") designed for storage at the farmer-cooperative and small-trader level (Donahaye et al, 1991b; Rindner et al, 2002). These structures are in current use for capacities of up to 300 t for bagged storage of cereals.

The problem of applying present-day technology to provide hermetic storage for subsistence farmers lies in the need to provide an easily sealable low-cost container with a capacity of 50–100 kg. The high ratio of surface area to volume necessitates a liner with very low permeability to gases. The most recent attempt to address this problem has been through the "Joseph bag," which is made of a plastic-metal foil laminate, sealable by means of a hot iron (Murray, 1990).

In underground flexible structures. The main approach to achieving lower levels of O_2 and higher accumulations of CO_2 has been by lining pits with plastic liners to improve the hermetic seal (Donahaye et al, 1967; Dunkel et al, 1987). With a similar approach, small-scale underground storages have been developed for farmer storage of corn and dry beans in Brazil (Sartori and Costa, 1975; Sartori, 1987).

Experience gained using flexible liners. The accumulated experience with hermetic storage using several types of flexible liners for aboveground storage and in-the-open storage, under tropical and subtropical conditions (Navarro et al, 1968, 1984, 1990, 1993; Calderon et al, 1989; Donahaye et al, 1991b; Navarro and Donahaye, 1993a; Silberstein et al, 1998) is summarized in the following observations.

Structural durability. The use of PVC-based sheeting without mesh reinforcement produces a material of suitable strength and elasticity for storing grain. This material was formulated to have a high resistance to solar UV irradiation. Rodent penetration has been recorded on only exceptional occasions involving minor damage. The hypothesis that rodents find it difficult to gain a toothhold on the smooth surface has been corroborated by laboratory studies using wild-caught roof rats and house mice (S. Navarro, S. Moran, R. Dias, and E. Donahaye, *unpublished data*).

Liners have been used continuously for over 10 years, and although they have lost some plasticity, permeability to gases decreases as the plasticizers evaporate. This characteristic renders the liners more effective in retaining gas concentrations with time, e.g., for 0.83-mm PVC, the initial permeability (expressed throughout as a measure given at a gradient from 21% O_2) decreased from 87 to 50 mL of O_2 per square meter per day after four years of exposure under a Mediterranean climate (S. Navarro, E. Donahaye, and A. Azrieli, *unpublished data*).

Insect control. At a liner thickness of 0.83 mm and an O_2 permeability level of 87 mL/m^2 per day, there is a possibility of insect survival close to the grain-liner interface. This is especially so at the top layer of the structures, where moisture content tends to be higher than in the remaining parts of the bulk. However, after the minimum O_2 concentration is reached, survival is usually well below one insect per kilogram and multiple samplings would be required to detect a single insect (Navarro et al, 1984, 1993). This residual infestation is more of a problem on return to aerobic conditions, so the commodity should be consumed without additional prolonged storage. The residual infestation is less serious than the danger of reinfestation by insects from the surroundings under storage by conventional methods. For grain destined for export and in other cases in which freedom from insects is mandatory, a final treatment using phosphine may be undertaken if necessary. In the future, this treatment may be superfluous if higher degrees of gas retention achieve complete elimination of residual infestation.

Moisture migration. Diurnal temperature fluctuations, accentuated by solar radiation on liners, followed by rapid cooling at night, cause successive moistening and drying cycles at the upper grain surface. This may result in gradual moisture accumulation, particularly during the transient seasons between summer and winter when temperature fluctuations are greatest, so that initially dry grain may rise above critical moisture levels, enabling limited microfloral spoilage to occur. This is particularly likely along the peaks of bunkers, where warm air rising on convection currents tends to concentrate the moisture condensation in confined areas. For bunkers with a capacity of 12,000–15,000 t and constructed in recent years, the condensation phenomenon has been alleviated and almost eliminated by leveling the peaked top (with a ridge of less than 2 m) to a slightly convex, wide apex of bunker cross section (with a ridge of more than 6 m) that is just sufficient to permit rainwater runoff. This configuration appears to enable the dispersal of moisture migration over a much larger area. Differences in the intensity of moisture increase have been demonstrated between bunkers with narrow ridges and peaked tops, and bunkers with tops having a broad ridge (Silberstein et al, 1998). Although comparative results for concurrent storages are not available, these results come from observations made over four intermittent years at the same storage site in Israel (Navarro et al, 1994).

For dry grain kept in "storage cubes" in subtropical climates, moisture migration is not a pronounced phenomenon. However, for corn or paddy stored in the tropics, moisture migration is intensified because the initial grain moisture is closer to its critical level. For this purpose, placement of an insulating layer between the liner and the upper layer of bagged grain provides a limited solution to moisture migration. This consists of a layer of bagged agricultural wastes, such as rice hulls, or straw; if these are not available, a "felt-fiber" layer with insulating properties is appropriate. This method suffers from several disadvantages. An alternative method of insulating the stack from diurnal temperature fluctuations was investigated. It consists of using a shade screen formed from aluminum-coated high-density polyethylene threads. After five months of storage, no perceptible increase in moisture content was found at the top of the stack, and the grain remained in good condition (Donahaye et al, 2000).

Hermetic storage as a future alternative. The need for alternative methods of prevention and control of insect infestations in stored products has become acute over the last few years. This is because conventional measures using insecticides are being questioned by environmental agencies and pressure groups, and the choice of available permissible materials is decreasing. Two fumigants remain in general use. However, the U.S. Environmental Protection Agency has made the decision to phase out methyl bromide by the year 2005 due to its destructive effect on ozone in the stratosphere (UNEP, 2002). This is coupled with mounting evidence of the development of insect resistance to phosphine (Zettler, 1993), indicating that even phosphine may not be economically effective in years to come. Modern and safer acceptable technologies such as aeration, refrigerated aeration, and MAs are still expensive and require adequate infrastructure. In sharp contrast to the use of chemicals, hermetic storage is an environmentally friendly technology, involving no hazard to the storage operators, consumers, or nontarget organisms, and as such, its application should enjoy a high level of consumer acceptance.

In developing countries. Hermetic storage may provide an answer to the need for a less costly method of secure food storage for rural populations (McFarlane, 1970; Navarro et al, 1999, 2000). This could be achieved by supplying a storage solution at the farmer level, thereby affording the farmer protection from seasonal fluctuations in grain prices. The basic advantage of hermetic storage in developing countries is its simplicity, obviating the need for insecticidal mixing procedures or fumigations, both of which require high levels of expertise not usually possessed by the small-scale farmer (Pattinson, 1970; Sakho, 1971). Furthermore, it is generally the only MA option since MA generators or gas cylinders are neither affordable nor obtainable.

In technologically advanced countries. In spite of the trend toward improved sealing of existing silos in some countries (Newman, 1990; Delmenico, 1993), the objective has been either to obtain increased fumigation efficiency or to convert structures for storage under MAs. The relatively slow rates of O_2 depletion in the hermetic-storage process, especially when the initial insect population is low, render it infeasible to apply this method to short-term storage systems. Also it is not practical for application in rigid horizontal storage structures, where the headspace is always relatively large. Clearly, hermetic storage is best applied to long-term storage projects, such as national grain reserves, buffer stocks, and grain surpluses.

The conversion of rigid structures to sealed storages should be considered for long-term large-scale storage projects. The evidence that this method is most effective at the high temperatures prevailing in tropical climates is best documented by De Lima (1990). In such climates, aeration for cooling of grain is not feasible; reinfestation is frequent; and the available contact insecticides degrade rapidly because of the high temperatures. Under these conditions, hermetic storage may provide an advantageous solution.

High-Pressure Carbon Dioxide Treatment (HPCT)

Carbon dioxide as a fumigant remains slower-acting than phosphine or methyl bromide. The stored-product pests laboratories at Bordeaux and Berlin investigated the use of carbon dioxide at high pressure (Fleurat-Lessard, 1990; Reichmuth and Wohlgemuth, 1994). After extensive testing in the laboratory, a high-pressure fumigation chamber was designed and built in collaboration with the French company MG SIAC. The chamber can hold the equivalent of the contents of one transport trailer. The unit is designed to recover at least 85% of the carbon dioxide used. The pressure rises to 19 atm in 90 min, is held there for 60 min, and takes about 30 min to be released. With loading, fumigation, and unloading, a full cycle takes approximately 4 hr.

The treatment requirements include pressure-proof autoclaves and gas-permeable packaging material for bags and boxes. The product to be treated is left in its original packaging and is transported on pallets into the autoclave. Liquid carbon dioxide is vaporized via a heat-exchange unit, introduced into the autoclave in gaseous form, and raised to the treatment pressure. After the treatment period, about 2 hr at 20 bar, the release of carbon dioxide follows, and the pressure drops back to ambient atmospheric pressure. The gas is exhausted into the atmosphere in a short time (10–15 min) with the use of a silencer, to avoid a loud noise during release. The rate of decompression of pressurized storages may also have an adverse impact on insect mortality (Ulrichs, 1994; Ulrichs, et al 1997a,b).

Gerard, et al (1990) described a high-pressure chamber (CARVEX) connected to a tank of liquid CO_2 placed on a balance (Fig. 14). This unit is commercially available and utilized in Germany.

Low-Pressure Vacuum Hermetic Fumigation

Finkelman et al (2002a, 2003a) reported on the introduction of flexible, transportable sealed chambers made of welded PVC liners that have opened new opportunities to implement low pressures (vacuum treatment) as a competitive and affordable treatment to control storage-insect pests. Under vacuum, these chambers shrink over the periphery of the commodity and hold it fast. The system is sealed by an airtight zipper and is able to retain the vacuum (Fig. 15). At the base of the chamber, an inlet hose enables connection to the vacuum pump that creates the prerequisite low pressure (Fig. 16).

Finkelman et al (2002a) showed that attempting to hold a pressure below 45 mm of Hg is not a practical approach because of the energy required for prolonged operation of

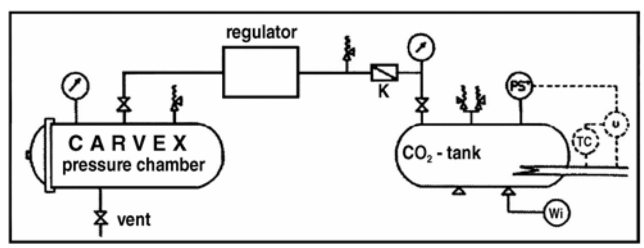

Fig. 14. Pressure chamber of about 3 m^3 designed to hold CO_2 at 20 and 37 bar, liquid CO_2 supply tank, and regulator unit. (Reprinted, with permission, from Gerard et al (1990)

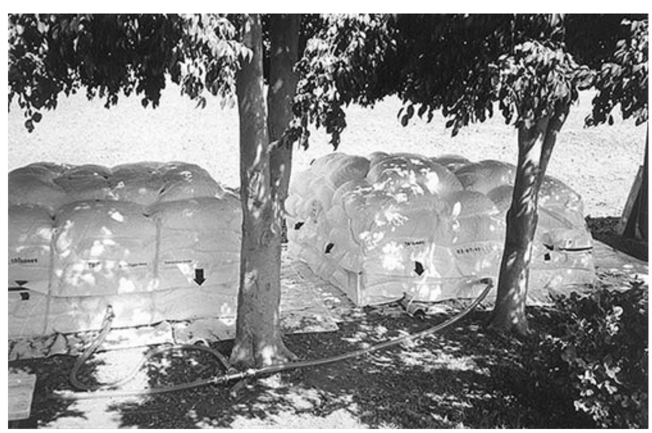

Fig. 15. Two vacuum hermetic fumigation cocoons under a pressure of 50 mm of Hg (three days of exposure on the right and seven days on the left) connected together to the pump at the trial site in Israel. (Reprinted, with permission, from Navarro et al, 2003a)

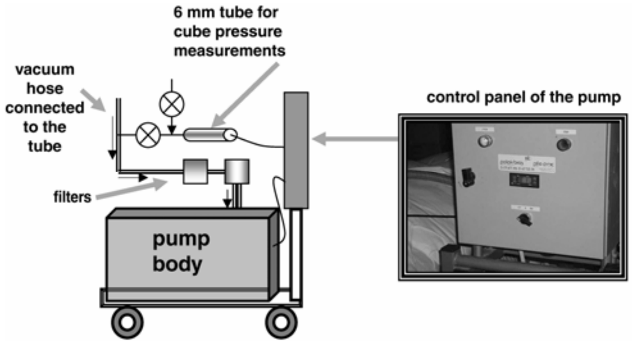

Fig. 16. Modifications installed to adapt the pump to the unique requirements of the treatment. (Redrawn, and used with permission, from Finkelman et al, 2002a)

the pump. Conversely, pressures above 55 mm of Hg prolong the time to achieve kill. In contrast to fumigations, for which schedules are provided by defining dosages to be applied for a predetermined time, low-pressure treatment schedules, for treatment at a set temperature range, must be presented as exposure times at both a temperature range and a relative humidity level in equilibrium with the commodity moisture content.

Tested commodities. Ten durable commodities (corn, corn chips, cocoa, garden peas, chick peas, wheat, wheat flour, rice, sunflower seeds, and semolina) were exposed to five days of vacuum treatment (Finkelman et al, 2003a,c). The commodities were packed in different ways. Commodities such as corn, garden peas, chick peas, and sunflower seeds were stored in big bags of 1-t capacity. Wheat, rice, and semolina were stored in 50-kg bags, and corn chips and wheat flour were stored in 25-kg bags loaded on wooden pallets. The temperatures of the stored commodities ranged from 26 to 33°C, and relative humidities ranged from 35 to 50%. In all tested commodities, the treated product was well preserved, and in cases where initial infestation was detected, complete mortality of insects was observed.

The advantage of this treatment is that no toxic chemicals are employed. Exposure times to provide kill are comparable to those of phosphine, and the exposure time of five days falls within a range suitable for quarantine treatments when rapid treatment is not essential. If the commodity can be placed in flexible liners and packed in a manner that can withstand the low pressure, vacuum treatment can provide a good solution (Navarro et al, 2001a, 2003a). As for fumigation, treatment schedules must be developed for low-pressure treatments by establishing a database on the relative susceptibilities of the different insect species (at all their stages) that are liable to infest the commodity.

Fumigants and MA

Carmi et al (1991), Leesch (1991), and Mueller (1994) suggested the use of phosphine and carbon dioxide together for stored-product protection. This combined use provides quick and even distribution of phosphine throughout large grain bulks and possibly other products, without additional mechanical circulation. Desmarchelier (1984) and Desmarchelier and Wohlgemuth (1984) demonstrated that this combination is not synergistic. The pupae of *S. granarius* require the same amount of phosphine irrespective of whether phosphine is presented alone or in a mixture. The developmental stages of insects (eggs and pupae) consume very little oxygen and appear to be little affected by the combined action of phosphine and carbon dioxide.

Sealing and Pressure Test for Gastightness

A fundamental requirement for the successful application of gaseous treatments to control stored-product insects is a well-sealed structure. In the sealed structure, the desired gas concentration should be maintained at a level sufficient to control insects; otherwise, the gas concentration would decrease rapidly without having an effect. The requirement for gastight storages for application of CAs and MAs appears to be more critical than for application of fumigants (Bond, 1984). In spite of the trend toward improved sealing of existing silos in some countries (Newman, 1990; Delmenico, 1993), the objective has been either to obtain increased fumigation efficiency or to convert structures for storage under CAs.

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. Gastightness has for years been a problem for the application of fumigants in storage. The consequences of poorly sealed storages under fumigation should be considered in view of the development of insect resistance to fumigants in poorly sealed structures (Banks and Desmarchelier, 1979; Banks, 1984b; Zettler, 1993, 1997).

Methods to determine gastightness have been investigated for different purposes. In the analysis of energy requirements of buildings, air infiltration is known to be a primary source of energy loss, and this infiltration can be measured experimentally. CO_2 has also been used experimentally as a tracer gas (Navarro, 1997a). Hunt (1980) reviewed some tracer-gas techniques to measure air infiltration into buildings and compared fan-pressurization-evacuation procedures to estimate the comparative tightness of those structures. The dynamic characteristics of air infiltration into buildings have been studied to predict the heating and cooling load of seasonal energy requirements (Hill and Kusuda, 1975). The *Handbook of Fundamentals* of the American Society of Heating, Refrigeration, and Air-Conditioning Engineers describes the air-change method and the crack method in predicting air infiltration rates (Anonymous, 1972). The crack method, or the constant-pressure test, is usually regarded as more accurate as long as the leak characteristics can be evaluated properly (Hill and Kusuda, 1975). With this method, airflow is expressed as:

$$Q = b \times \Delta P^n$$

where Q = the volumetric flow rate of air, b = the proportionate constant, n = the exponent, and ΔP = the pressure difference exerted on an enclosure.

Meiering (1982) investigated a constant-pressure test and a variable-pressure test for measuring specific silo permeability in silage systems, where the sealed shell was designed to limit entry of O_2 to minimize losses in quality. The effect of variations in environmental temperature and atmospheric pressure on O_2 intake of silos was simulated, and the permeability limits required for proper O_2 control in silos containing silage were defined. Gas interchange within freight containers and factors leading to gas interchange between containers and the external atmosphere were detailed by Banks et al (1975). They found that the relationship between applied pressure and gas leak rate gave a useful measure of gastightness. Banks and Annis (1977) developed a practical guide for storage of dry grain under MA and specified requirements for silo gastightness. Their specifications correspond to the pressure-decay times needed to maintain the atmospheric composition in the silos. Sharp (1982), using the constant-pressure test, measured the gastight level of sealed structures. These tests are designed to estimate the permissible limits for effectively maintaining the gas composition in the storages during the treatment.

Gas Permeation Through the Rigid Structural Membrane

Gas loss through the structural membrane during gaseous treatments is an important phenomenon. Membranes of concrete, plaster, and plastic permit gas permeation and gas exchange. Pressure tests, as described below are not capable of measuring the degree of such losses.

Comparative Results with the Variable-Pressure Test

In the variable-pressure test, the structure is pressurized to a value above atmospheric, using a fan. The air supply is then shut off, and the pressure is allowed to fall by natural leakage to a new value. The time taken to fall from the high (positive or negative) pressure serves as a measure of the degree of sealing. Time elapse to half the pressure is usually considered for comparisons of gastightness level. A constant-static-pressure test is more accurate than a variable-pressure-decay test and has the advantage of being independent of bin volume. However, the variable-pressure-decay test is quicker and therefore the most practical means of measuring gastightness in grain storage (Zahradnik, 1968; Annis and van S. Graver, 1991; Ball and van S. Graver, 1997; Navarro, 1997a; Navarro and Zettler, 2001).

To minimize the thermal influence, tests should be carried out preferably before sunrise and in still weather. A pressure of 250 Pa may be taken as an upper limit, but for some structures, even this pressure may cause poor seals to open. Welded steel cells and concrete silos may be able to stand 500 Pa, but higher pressures are usually unnecessary.

Comparisons of variable-pressure tests are scarce. Table 9 was prepared to provide provisional guidelines based on the best estimates available in the literature. The suggested times given in Table 9 were doubled for empty storages as an approximation of the intergranular airspace, since for barley, corn, rough rice, and wheat, this free space is in the range of 35–65% of the total volume (Trisviaskii, 1966).

For CA storage in Australia, with structures of 300- to 10,000-t capacity, a decay time of 5 min for an excess pressure drop from 2,500 to 1,500 Pa or from 1,500 to 750 Pa or from 500 to 250 Pa was regarded as satisfactory (Banks et al, 1980). According to Banks and Annis (1980), this range of pressures was chosen so that it would be the highest usable without unduly stressing the storage fabric of the store. They commented also that above 10,000-t capacity, pressure testing is difficult to carry out satisfactorily since it requires very stable atmospheric conditions. Analysis of the data presented by Banks et al (1980) suggests that, for storages with capacities in the range of 1,600–1,900 t in CAs with an initial CO_2 concentration of about 60–85% for an average decay time of 11 min, the daily decay rate was about 4% CO_2. With a similar range of initial CO_2 concentration in a structure of 150-m^3 capacity, daily gas loss was correlated with different levels of pressure-decay times (Navarro et al, 1998b). Their comparison resulted in a pressure-decay time of 3 min for a daily decay rate of about 4% CO_2.

The influence of hermetic storage on controlling insects was examined using small-scale sealed plastic structures with capacities of 15, 30, and 52 m^3 for outdoor storage of wheat, paddy, and corn (Navarro and Caliboso, 1996). Pressure-decay rates were compared with daily CO_2 decay rates. With these structures, successful insect controls were obtained with a daily decay rate of >1% CO_2, which was found to be equivalent to a 5-min half-life pressure-decay time. Similarly comparative data was obtained using hermetic bunker storages of about 19,000-m^3 capacity, where successful results were obtained when the half-life pressure decay was about 9 min (Navarro et al, 1984).

According to Banks and Annis (1984), daily ventilation rates tolerable in various insect-control processes are estimated as 2.6% for hermetic storage, 5% for N_2-based CA, 7% for CO_2-based CA, and 10% for phosphine fumigation. Based on the proportions of ventilation rates, this would account for the ventilation rates for fumigation using phosphine being twice those of N_2-based CA, and the latter being about twice those of hermetic storage. These values were also considered in extrapolating the different ranges given in Table 9.

Constant-Pressure Tests

For the constant-pressure test, a fan, a valve, hosing, a pressure sensor, and a gas flowmeter (e.g., a Rotameter) is required. The required capacity of the flowmeter varies with the size of the enclosure under test. It is often convenient to make up a series (battery) of flowmeters, which

TABLE 9
Provisional Recommended Ranges for the Variable-Pressure Test in Structures Destined for Gaseous Treatments to Control Storage Insects[a]

Type of Gaseous Treatment	Structure Volume (m^3)	Variable-Pressure Test Decay Time (min from 250 to 125 Pa): Empty Structure	95% Full
Fumigants	Up to 500	3	1.5
	500–2,000	4	2
	2,000–5,000	6	3
Controlled atmosphere	Up to 500	6	3
	500–2,000	7	4
	2,000–15,000	11	6
Modified atmosphere, including airtight storage	Up to 500	10	5
	500–2,000	12	6
	2,000–15,000	18	9

[a] Source: Navarro (1999); used by permission.

allows a range of flow measurements from 0.005 to 3 m^3/min, to accommodate widely varying requirements and give a wide range of test flows. Alternatively, an electronic thermoanemometer type of flowmeter to cover a wide range of flows may be used. An adjustable rheostat (e.g., Variac) is useful for regulating the speed of the fan if it has a brush motor.

In experimental laboratory work carried out by Navarro (1997a), a 665.7-L silo was used to demonstrate the dependence of the empirical parameter (n) values on orifice length. In a grain bin or silo, this orifice length may constitute the thickness of the wall. Accordingly, the longer the orifice was, the higher the value of n was. Based on experimental observations, it was also demonstrated that synoptic variations in barometric pressure played a significant role in air infiltration into the experimental silo. During the experiments, synoptic variations in barometric pressure ranging between 11 and 17 Pa/hr were observed in the test room. The measured CO_2 concentrations were compared with the calculated values based on equations that took into consideration initial CO_2 adsorption by the wheat, diffusion of CO_2 through the leak, and variations in temperature and barometric pressure. Under experimental conditions, close agreement between the measured and calculated values was obtained.

In view of the work involved in determining the n value, a rather simplified expression of using the airflow to maintain a constant pressure may be proposed as an alternative. With a similar approach, Banks et al (1975) used the flow rate at a constant pressure of 125 Pa to correlate with the daily gas-interchange rate in freight containers. Such comparative results with constant-pressure tests for larger structures and for different gaseous treatments are needed. Until such information is obtained, the provisional decay times for the variable-pressure test given in Table 9 may serve as guidelines for determining the suitability of specific storage structures for the successful gaseous control of storage insects.

Monitoring Gas Concentrations

Monitoring is the process of measuring the MA gas concentration inside a treated enclosure and in the area surrounding it.

Monitoring is done for two reasons: to be sure that workers and people near the MA-treated site are safe and there is no health hazard, and to measure the concentration and distribution of MA gas inside the enclosure to ensure the success of the MA.

Monitoring Equipment

A wide variety of equipment is available for measuring oxygen and carbon dioxide concentrations. The equipment used should be suitable for monitoring in 1) the concentration ranges involved in the workplace and 2) the insecticidal concentrations reached during MA treatments.

Instruments for monitoring are available that can measure oxygen and carbon dioxide gases at 1) low concentrations only—for workplace safety (threshold limit value) measurements, 2) high concentrations only—for checking the progress of MA exposures, and 3) low and high concentrations, from the low safety concentrations to the high insecticidal concentrations.

The range of instruments available extends from single-use gas detector tubes (for use either at safety or fumigation concentrations) to multipurpose electronic instruments.

Measuring Gas Concentrations in the Health and Safety Range

High-CO_2 and/or low-O_2 atmospheres can present safety hazards when used improperly. Humans exposed to such MAs can recover completely if removed quickly from the exposure and assisted in recovery. A concentration of 5% CO_2 is sufficient for persons to respond to the unpleasant atmosphere, whereas CO_2-free and O_2-deficient atmospheres with an O_2 concentration lower than 14% have no warning effects and cause unconsciousness.

The workspace around the treated enclosure should always be well ventilated to avoid accumulation of a high-CO_2 atmosphere because of the density and tendency of high concentrations of CO_2 to accumulate in lower areas of the enclosed regions around the treated enclosure. A potential safety hazard exists when using high-CO_2 atmospheres, particularly in leaky enclosures. High concentrations of N_2 to produce low-O_2 atmospheres are unlikely to present a similar type of hazard because they have a density similar to that of air.

SPECIFIC APPLICATIONS OF MA

Cereal-Grain Preservation

The pioneering research of recent modern times first concentrated on the possible application of MA technology to cereal grains (Jay, 1984a; Banks and Annis, 1990; Navarro et al, 1990; Adler et al, 2000). Work related to the preservation of cereal grains is also well documented by Calderon and Barkai-Golan (1990).

Jay and Pearman (1973) controlled a natural infestation of the rice weevil complex and the Angoumois grain moth, *Sitogtroga cerealella*, in 28,000 bu of corn in an upright concrete silo. In this test, a CO_2 concentration of about 60% (the balance was air) was successfully attained and maintained over a 96-hr period. Jay (1971) also published suggestions on how to use CO_2 to control insects in grain-storage facilities.

Australia began large-scale field tests with CO_2 in 1976 (Banks, 1979). In the first test, gaseous CO_2 was released at three points into the base of a 7,000-t welded metal bin containing wheat, and the pressure of the CO_2 eventually pushed the existing atmosphere out of the top of the bin. A second test was conducted in a similar manner except that an air pump was used to blend air with the CO_2. Thus, instead of a 100% concentration going into the bin, a 70% concentration was released, thereby saving CO_2. Since these tests, Australian researchers have conducted additional studies on large grain-storage facilities, including the sealing and subsequent treatment of a 16,000-t flat storage with CO_2 (Banks et al, 1979).

Interest in the use of N_2 for controlling stored-product insects began in Australia in the early 1970s and continued through 1976. These studies and Australian recommendations for the use of N_2 in grain storage are described by Banks and Annis (1990). Also, considerable research was conducted in Italy on the use of N_2 for long-term grain storage. This research lead to the development (by Assoreni, a member of the ENI [Italian Hydrocarbon National Concern] group of companies for scientific research) of a total marketable system that included metal silos, conveyors, and equipment for applying N_2 as well as the N_2 itself (Tranchino et al, 1980).

Preservation of Tree Nuts and Dried Fruits

The possibility of applying MAs for controlling insects in dried fruits and tree nuts has been reviewed by Soderstrom and Brandl (1984, 1990). Accordingly, the major volume treated with MAs consists of finished, packaged product. Bulk storages require extensive sealing before an MA can be applied, and the industry considers the process too slow and costly in comparison with other control methods. An effort is now being made to make the equipment for producing MAs more efficient and to improve the methods of sealing storages.

Jay et al (1970) were the first to attain and maintain effective CO_2 concentrations for two, four, and seven days in an upright concrete silo containing 68,000 bu of in-shell peanuts.

Ferizli and Emekci (2000) applied CO_2 to treat dried figs in a gastight flexible storage unit loaded with 3.5 t of dried figs in perforated plastic boxes. Test insects were *P. interpunctella* (larvae), *O. surinamensis* (adults and eggs), and *Carpoglyphus lactis* (driedfruit mite, mixed stages). Results showed that oxygen concentrations in the containers decreased to 0.8% and carbon dioxide concentrations increased to 96%. For the following five days, both O_2 and CO_2 concentrations remained stable. Exposure of eggs and adults of *O. surinamensis*, larvae of *P. interpunctella*, and the mixed stages of *C. lactis* to environments containing high carbon dioxide (96–98%) resulted in complete mortality.

Prozell et al (1997) exposed cocoa beans, hazelnuts, and tobacco to a quick disinfestation process by exposure to carbon dioxide under a pressure of 20–40 bar for a few hours. Experiments with caged pest insects (developmental stages and adults) of 12 species, (*L. serricorne, Oryzaephilus mercator, O. surinamensis, T. castaneum, T. confusum, Cryptolestes turcicus, Trogoderma granarium, Corcyra cephalonica, E. elutella, E. cautella, P. interpunctella,* and *Sitotroga cerealella*) were carried out on 1 t of bagged product in a 3-m^3 chamber. At about 10°C, under 20 bar of CO_2, the lethal treatment period was slightly longer (3 hr) than at 20°C. At 20°C and 30 and 37 bar, complete control was achieved within 1 hr and within 20 min, respectively.

Disinfestation of Dates

Dried fruits are subject to infestation by insect pests during and after harvest. Several species of nitidulid beetles are particularly associated with dried fruits because they are field and storage pests. Fumigation of dried fruits with MB upon arrival at the packing plant effectively controls infestation and also causes a high proportion of larvae and adults to emigrate from the fruit before they succumb (Navarro et al, 1989; Donahaye et al, 1991a).

The influence of different CAs in causing emigration of *Carpophilus* larvae from dates was compared with the effects of MB by Navarro et al (1989) and Donahaye et al (1991a). Recommended dosages for mortality of most stored-product pests using CA are >60% carbon dioxide for at least 11 days of exposure (Navarro and Donahaye, 1990). The influence of low-O_2 or high-CO_2 atmospheres as alternatives to fumigation of dried fruits has been investigated by Soderstrom and Brandl (1984), Soderstrom et al (1986), and Tarr et al (1994).

Laboratory experiments were carried out to investigate the influence of different MAs (20% carbon dioxide in air or 2.8% oxygen in nitrogen), low pressures alone, or MB alone in causing nitidulid beetles to emigrate from infested dried fruit, for which dates served as a model (Donahaye et al, 1992b; Navarro et al, 1998c). At 4 hr of exposure and 26°C, the treatments that had a marked influence in causing insects to abandon the infested fruit were low pressure of 100 mm of Hg and 2.8% oxygen in nitrogen, all of which caused more than 80% of the initial insect populations to emigrate from the fruit.

In another study (Navarro et al, 1989), 35% CO_2 was found to cause a similar emigration to O_2-depleted or low-pressure atmospheres. This method is currently in use to cause emigration of insects from dates processed in several packing houses in Israel.

In addition, CO_2 atmospheres were studied for the long-term preservation of dates. The conventional date preservation after harvest in Israel is cold storage at –18°C. This is the most suitable method for soft-fruit sensitive cultivars, but it is energy-consuming. Very limited work has been done to determine the influence of CAs on date quality. Under laboratory conditions (Navarro et al, 2001b) and in field tests at ambient temperatures (Navarro et al, 1992, 1998b), carbon dioxide significantly delayed browning and sugar formation in dates and extended shelf life as compared to storage at –18°C.

A CA of 60–80% carbon dioxide was used within a 151-m^3 plastic chamber partially filled with 30 t of dates stacked in crates on pallets (Navarro et al, 1998b). At the initial purge phase, the desired carbon dioxide concentration was obtained in the chamber within 1 hr by introducing the gas under high pressure. An intermittent maintenance phase was then applied for four to five months, using approximately 0.8 kg of carbon dioxide per day. At the end of storage, the quality of the treated dates was compared with that of controls stored at –18°C. No significant difference was found between the treated dates and the controls. The insect population was effectively controlled. This technology is proposed for the treatment of stored dried fruits to control pests and maintain quality.

Packaging of Food

Lang (1993) discussed the use of gases in the packaging of meat and fish, milk products, bakery products, fruits, vegetables, and nuts. The techniques involved include MA packaging (MAP), CA packaging (CAP), MA storage (MAS), and accelerated ripening. MAP, CAP, and MAS use combinations of CO_2, O_2, and N_2.

The effects of storage temperatures (8 and 36°C) and packaging atmospheres (air and N_2) on the quality of almonds were studied by Garcia-Pascual et al (2003). The quality of unshelled almonds remained high after nine months, even under storage at ambient temperature. No significant differences were observed for any of the measured parameters in nuts stored in air or N_2.

A number of packaged foods are purged with nitrogen or carbon dioxide atmospheres to prevent microbial activity and insect development (New and Rees, 1988). Guidelines for using MAs in packaged food, with special emphasis on microbiological and nutritional aspects, were published by the Council of Europe (Anonymous, 1999a).

Museum Artifacts

Nontoxic approaches to insect control in museum collections to provide safety to both the artifact and the museum professional were described by Rust and Kennedy (1993). This volume explains the research done to demonstrate how inert-gas atmospheres can be used to eliminate insect infestation in museum objects and collections. The application of MA technology in museums and the pioneering research of conservation scientists is well documented by Selwitz and Maekawa (1998). Also, the possibilities of controlling pests in artifacts using inert gases are reported by Frank (1991), Reichmuth et al (1991, 1993), Wudtke and Reichmuth (1994), and Sa-Fischer et al (1997).

Fresh Storage of Fruits and Vegetables

Fresh fruits and vegetables may be shipped or stored in CAs. This topic is covered in depth by Calderon and Barkai-Golan (1990).

Narcissus Bulb Treatments

In experimental procedures, Navarro et al (1997b) and Finkelman et al (2002b) found extremely rapid depletion of O_2 within the sealed gastight enclosure where newly harvested narcissus bulbs were stored due to the respiration of the bulbs (Navarro et al, 2002c; Rindner et al, 2003). Under these hermetically sealed conditions, a rapid depletion of O_2 to 0.1% took place within 18 hr, while the CO_2 concentration increased to 21%. The temperatures in the chamber ranged from 28 to 30°C, and the humidity rose steadily from 60 to 84% RH, while it reached 100% under the top liner within 18 hr. Complete mortality of the larger narcissus bulb larvae was observed in all 13 treatments made during the 2003 export season (Navarro et al, 2003a).

ECONOMICS OF TREATMENT

Cost of Sealing

It is essential to thoroughly seal the enclosure to be treated before the use of an MA. Sealing of silo bins or the MA-treated enclosure is not only to retain the intended gas composition but also to physically exclude insects from sealed bins. This is an important method for incorporation into an integrated pest management strategy. Mann et al (1997) reported a sealing method to retain and uniformly maintain CO_2 concentrations in welded-steel farm bins. The method is flexible and can be used for any bin opening.

Sealing techniques and costs are described in detail by Banks and Annis (1980), Newman (1990), Ripp (1984), and Ripp et al (1990). Only pressure chambers are sufficiently gastight; new concrete silos and welded-steel silo bins may be converted to gastight structures with an investment marginal to that of the entire storage complex. Cracks in concrete structures can be sealed with polyurethane foam, silicone adhesives, or other materials. A flexible paint may significantly reduce diffusion through concrete walls. Windows and doors may be sealed with gastight plastic liners and adhesive tape (Ripp et al, 1990). PVC has limited permeability to oxygen and to carbon dioxide, depending on the thickness of the liner (Navarro and Zettler, 2001). The starch in paper glue has proven to be a reasonably low-cost and environmentally friendly sealant for fumigations, but it is not recommended for MA treatment—the losses through the sealing are extremely high. The paper does not resist the pressure differences exerted on it, and permeability is extremely high, which cause rapid losses.

Bag stacks or flat storages may be sealed under a layer of plastic sheeting. Different sheets can be attached together by heat or adhesives. To avoid leaks, it is important to avoid folds where the foils overlap. On the floor, the covering sheets should overlap about 50 cm on each side, and they should be glued to the floor with silicone adhesive and held in place with wooden bars. The bars should be secured to the floor to ensure a gastight connection between the floor and the sheets. As an alternative, commercially available structures such as "GrainPro Cocoons" (described below) or "Volcani Cubes" offer a practical solution (Navarro et al, 2003a).

New Australian steel silos constructed from bolted or riveted steel sheeting are supposed to meet certain standards of gastightness—a pressure half-life of 3 min when full and 5 min when empty (Andrews et al, 1994). Ripp et al (1990) give a detailed description of Australian experiences regarding sealing methods and sealing costs, and they even mention a storage bin at the Kwinana Shipping Terminal that displayed a pressure half-life of 30 min.

Jayas (2000) reported on a recently developed method to calculate leakage areas in imperfectly sealed bins from pressure-decay times obtained from pressure-decay tests. Using the predicted leakage area and the planned initial CO_2 concentration, the CO_2 concentration profile can be projected over time, and the required length of treatment and amounts of CO_2 to be added can be calculated. A new concept, the cumulative lethality index, was developed to predict the mortality of rusty grain beetles (*Cryptolestes ferrugineus*) in bins with declining CO_2 concentrations. These relationships were considered important tools for successful control of insects in stored grain using CO_2 in Canada (Jayas, 2000).

According to Adler et al (2000), for a cost-effective treatment lasting four weeks, the daily rates of leakage from the treatment enclosure should not exceed 25% of the volume. This seems a very tolerant gastightness that causes rapid CO_2 loss. On the other hand, Navarro et al (1993) suggested a maximum infiltration rate for MAs as an increase of 0.5% O_2 per day.

In a study by Navarro and Zettler (2001) to test the infiltration rate of oxygen and carbon dioxide, a flexible empty structure of 7.5-m^3 capacity was used to demonstrate the critical limits of the degree of sealing using the variable-pressure test. This flexible structure is marketed as the "GrainPro Cocoon" and is used for outdoor storage of stacked commodities. Time, in minutes, for half-life pressure decay was correlated with daily ventilation rates of O_2 and CO_2. The ventilation rates were tested using different sizes of cross-section leak areas, with orifices of 1.6, 3.2, and 6.4 mm i.d. To evaluate the influence of temperature on the rate of gas exchange, the cube was tested when it was shaded to minimize the direct solar heating effect and when it was exposed to direct solar heating. The oxygen infiltration rate for MAs was 0.5% O_2 per day at a 5-min half-life pressure decay when the chamber was in the shade. For the same level of gastightness, gas loss was 0.8% O_2 per day when the cube was exposed (Fig. 17). The CO_2 loss rate was 2% per day at a 5-min half-life pressure decay when the cube was under shade and 3% per day when exposed (Fig. 18).

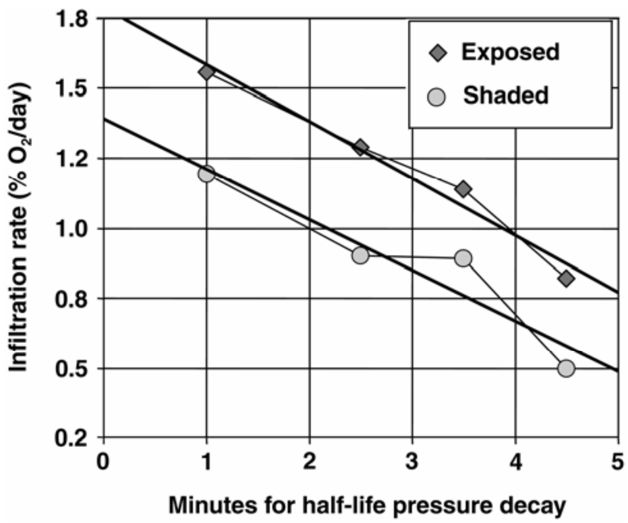

Fig. 17. Infiltration rate of oxygen into a nitrogen-treated empty structure of 7.5-m^3 capacity in relation to half-life pressure-decay time. The suggested maximum infiltration rate for modified atmospheres is an increase of 0.5% O_2 per day. (Data from Navarro and Zettler, 2001)

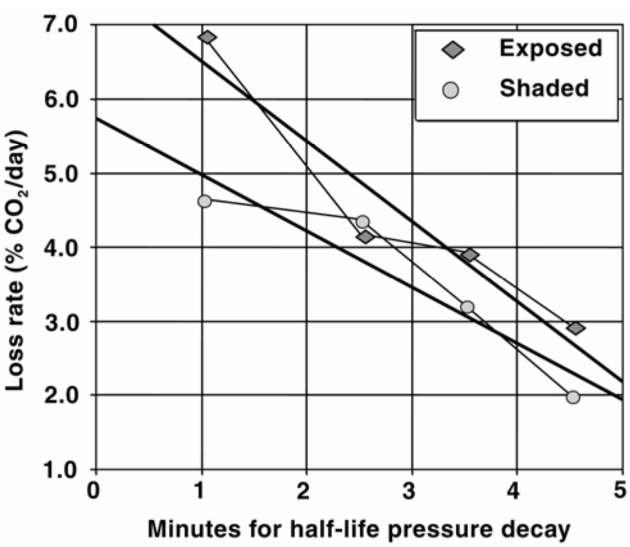

Fig. 18. Loss rate of carbon dioxide from a treated empty structure of 7.5-m^3 capacity in relation to half-life pressure-decay time. The suggested maximum loss rate is 2% CO_2 per day.(Data from Navarro and Zettler, 2001)

These data allow careful calculation of the sealing level required for successful application of an MA. Sealing costs could be compared to expected gas losses using the above-suggested gastightness criteria for achieving insect control.

According to Adler et al (2000), a reasonable sealing of a granary for subsequent treatments with carbon dioxide or nitrogen may cost about US$4.50/t. A flour mill treatment with carbon dioxide applied in combination with heat (Corinth and Reichmuth, 1995) was estimated to cost anywhere from US$5–7.50/m^3.

Cost of Gas

Several factors influence the cost of CO_2 or N_2 treatments. The largest of these are the costs of CO_2 or N_2, which are based on yearly usage, transportation costs, and competition among producers. Other costs include equipment rental (tank, vaporizer, etc.) and labor to apply the materials. Loss of gases through leaks, leading to the need to apply more gas to maintain the concentration, also contributes greatly to the cost. In Australia, where sealing is rigorously practiced before using MAs, an adequate treatment is considered to be 1 t of CO_2 per 1,000 t of wheat (Banks and Annis, 1980). At a cost of US$75/t for CO_2, this is equivalent to a treatment cost of less than US$0.076/t of wheat (Jay, 1984a). However, where sealing costs are high and CO_2 costs are low, as is the case in the United States, it may not be profitable to do extensive sealing of storage vessels before treatment.

There is an abundant supply of CO_2 in the United States. Carbon dioxide is a by-product of the production of ammonia, ethylene oxide, and alcohol and is also obtained from natural CO_2 wells. Nitrogen is generally produced by the energy-intensive process of separating it from the other components of air and is generally more expensive per unit than CO_2.

Jay (1980) estimated the cost of CO_2 to treat 28,000 bu (711 t) of corn in an upright concrete silo to be from US$0.75–1.33/t. However, this was based on a CO_2 cost of US$104–180/t. Recently, costs have been quoted as low as US$56/t, making the original estimates quite high. At US$56/t, those costs would range from US$0.36 to 0.42/t of corn for the CO_2, depending on the method of application.

Costs for CO_2 in the two previously described studies (Jay, 1980, 1984a) on wheat in the terminal elevators were estimated to be US$0.24–0.27/t of wheat, with the price for CO_2 at US$60/t. Treatment costs in the tests with milo and corn were estimated to be US$0.30 and 0.26/t of grain, respectively, for a 96-hr treatment. Most of these costs are slightly higher than (but compare favorably with) those quoted by one elevator for the fumigant "80-20" of US$0.21/t of grain (Jay, 1984a). For purposes of reference, 1995 chemical fumigation costs in the United States were cited as US$0.67/t of gain for large storage elevators (Hagstrum and Flinn, 1995).

The costs of MA treatment have been calculated for nitrogen and for carbon dioxide used in concrete silos in Berlin, Germany, where the gas is introduced slowly to the grain mass from the bottom, avoiding any turbulent flux (Adler et al, 2000). The initial costs were in the range of US$0.25/t for nitrogen and US$0.4/t for carbon dioxide. The costs for effective treatment may vary between US$1.5/t for moderately leaky structures to US$7.5/t for very leaky structures at a temperature of 15°C. At 20°C, the same costs range between US$1 and 3.8. These figures do not include the costs of sealing, which were provided by Love et al (1983) and Love (1984).

CURRENT USAGE OF MA

This section is an updated summary of the survey already published by Adler et al (2000).

Australia

Nitrogen treatment has been used commercially since 1992 for wheat, coarse grains, oilseeds, and pulses. A grain export terminal has integrated application of nitrogen for insect control before shipment into its procedures. A grain terminal in Newcastle treated about 262,678 t of grain with nitrogen between 1992 and 1999. In this terminal, the average usage of N_2 was about 1.5 m^3/t. Use of carbon dioxide is limited to organic-grade cereals. The typical average usage of CO_2 is about 1 kg/t of wheat. The total application of CO_2 on cereal grains is less than 100 t per year.

Bangladesh

Hermetic storage is used for paddy and wheat seeds in projects supported by nongovernmental organizations (Tom deBruin, GrainPro, *personal communication*, 2004).

Bostwana

The Ministry of Interior uses hermetic storage for about 3,000 t of food security stocks, mainly sorghum and maize (Tom deBruin, GrainPro, *personal communication*, 2004).

Cambodia

About 400 t of paddy and paddy seed is stored under hermetic conditions by Australian-supported projects (Tom deBruin, GrainPro, *personal communication*, 2004).

Canada

Carbon dioxide is registered as an insecticide on grain, but nitrogen is not. Most carbon dioxide fumigations have been experimental. The only commercial use is at one terminal elevator in Vancouver, where 15 concrete silo bins of 1,100 t are regularly treated with carbon dioxide. A few organic farmers use this gas. Grain treated with carbon dioxide annually amounts to less than 10,000 t.

China

A few thousand tonnes of wheat was stored in hermetic silos in Shandung and Henan provinces (Tom deBruin, GrainPro, *personal communication*, 2004).

Cyprus

Until 1998, about 20,000 t of barley was stored under hermetic conditions. The hermetic structures were above-ground bunker-type storages covered with plastic liner and destined for long-term storage.

France

Nitrogen generators are used experimentally to treat grain bins and grain-handling machinery to avoid risks of dust explosions in silos. On a limited commercial level, commodities such as coffee, cocoa, fruits, pistachio nuts, spices, dehydrated plants, seeds for drug extraction, and high-value-added products are treated using nitrogen.

Germany

A total of 5,239 t of carbon dioxide and nitrogen was sold as insecticides and acaricides for plant protection of durable stored commodities in 1998 (Anonymous, 1999b). Carbon dioxide was registered in 1989 and nitrogen in 1990. Less than 5% of the grain, and other food products, is treated with carbon dioxide. Some companies use carbon dioxide in combination with phosphine for faster distribution in grain. Herbs, spices, drugs, teas, dried fruits, and some other valuable products are commercially treated with carbon dioxide at high pressures. Nitrogen is used in packaged food items, for disinfestation of fruits, and to control pests of museum artifacts.

Indonesia

A few hundred tonnes of paddy seeds and paddy are stored under gastight conditions by the National Seed Company and some cooperatives in Java and Sumatra (Tom deBruin, GrainPro, *personal communication*, 2004).

Israel

The amount of grain in hermetic storage varies from about 20,000 to 60,000 t annually. Recently, on a semi-commercial basis, hermetic storage was used for 100 t of narcissus bulbs. Approximately 60 t per year of carbon dioxide is used for stored-product protection. This includes the use of the gas in combination with phosphine or MB for fumigation of approximately 30,000 t of grain. Additionally, about 4,000 t of dates (approximately 30% of the total) is treated with a mixture of MB and carbon dioxide. Another 300 t of dates is treated with carbon dioxide alone. A small quantity of organic wheat, seeds, herbs, and dates is treated with carbon dioxide.

Italy

About 5 t of carbon dioxide is used for treatment of stored grain. At present, the use of carbon dioxide does not require registration. Nitrogen is used for experimental purposes.

Philippines

The hermetic storage method is used by the National Food Authority for the preservation of paddy and milled rice. About 10,000 t of paddy and milled rice is stored using the commercially available “Volcani Cubes ” or the “GrainPro Cocoons” for bagged stored grain. In addition, seeds of Philrice (the Philippines Rice Research Institute) are stored under hermetic conditions (about 200 t). Some food and feed companies use hermetic storage to prevent infestation and moisture absorption. The International Rice Research Institute in Los Baños uses hermetic storage for seeds and paddy (an estimated 400 t) (Tom deBruin, GrainPro, *personal communication*, 2004).

Rwanda

The Ministry of Agriculture introduced hermetic storage for food security and seed stocks of maize and sorghum. A few thousand tonnes are stored in Kigali and some smaller amounts are scattered over the district (Tom deBruin, GrainPro, *personal communication*, 2004).

Spain

Carbon dioxide is used in packaged rice and dried fruits. High-pressure carbon dioxide is used by the spice and dry fruit industries for disinfestation purposes (Jordi Riudavets, IRTA, *personal communication*).

Sri Lanka

About 4,000 t of paddy is stored under hermetic conditions by the Ministry of Agriculture (Tom deBruin, GrainPro, *personal communication*, 2004).

Thailand

The Department of Agriculture has introduced hermetic storage for corn seeds, mung bean seeds, and paddy. It has introduced the use of CO_2 to the organic-rice sector (Tom deBruin, GrainPro, *personal communication*, 2004).

Turkey

Carbon dioxide is used on an experimental basis by the fig-processing companies as an alternative to MB fumigation. Also, a high-pressure carbon dioxide plant exists for the treatment of high-value commodities like spices and dried fruits.

United Kingdom

Carbon dioxide is used in mobile enclosures to control insect pests affecting museum artifacts. Carbon dioxide must be registered, and one company holds the label for the use of this gas. Nitrogen does not require registration, and its use may be limited to treating museum articles.

United States

Numerous MA studies were conducted by the largest cereal-grain processors using carbon dioxide, inert-atmosphere generators, pressure-swing adsorption systems, and gas-permeable-membrane systems. One company uses two inert-atmosphere generators for treatment of durum wheat. Carbon dioxide, nitrogen, and combustion-product gases are exempt from the requirement for a residue tolerance.

RESEARCH NEEDS

MAs have been shown to be a viable alternative to residue-producing conventional fumigants for controlling insects that attack stored commodities. Although most of the data are related to insect response and related topics, quality preservation of durable agricultural products under MA remains an aspect of interest. The following research needs are considered in two general categories: laboratory research and field research.

Laboratory Work

Laboratory studies that concentrate on elucidating various aspects of the responses of storage insects and the influence of MAs on treated commodities are still attracting the interest of researchers. In spite of numerous reports on the enhanced effectiveness of MA treatments at high-temperature and low-RH conditions, a contradicting report was submitted by Bell et al (2003) regarding the efficacy of heat in increasing the toxicity of carbon dioxide. Additional information that will contribute to clarifying this aspect of insect sensitivity is most relevant.

Most efficacy tests have been based on LT_{95} or LT_{99} values. To achieve Probit 9 (a criterion for insect treatments that defines 99.9968% mortality of the insect population), as required for quarantine, treatments could be based on extended exposure times, which would limit the application of MAs. In this regard, additional research on diapausing insects in temperate climates would be required, to determine a different exposure-time schedule than that for sensitive species. For this purpose, collaboration among different laboratories should lead to the development of standardized experimental protocols.

In high-pressure CO_2 treatments, systematic studies need to be made on the relevance to pest mortality of product temperature, as well as the influence of exposure time and pressure-release time. Data on the processes leading to insect mortality in ambient-pressure and in high-pressure treatments are needed to improve treatment methods and reduce exposure times.

Under low pressures, some insects show increased resistance. It may be possible that some sort of physiological mechanism protects these insects, as probably happened in the experiments of Cline and Highland (1987), when *Trogoderma variabile* adults survived for 12 weeks at 48 mm of Hg and 27°C. To elucidate this aspect, more work on insect response to low-pressure treatments is necessary, as well as research on the effect of temperature on insect metabolism at low pressures. Data on the effect of low pressures on some economically important insect species like *P. interpunctella* are not sufficiently documented in the literature. The accepted belief is that carbohydrate reserves in insects are utilized by the glycolytic pathway during anaerobic metabolism, but little information is available in the literature on lipid and carbohydrate metabolism under the stress of hypoxia and hypercarbia. These results add to the existing database, showing that low-pressure treatments can provide an effective alternative to fumigation within the framework of practical exposure times and commercially feasible equipment.

The review of Banks (1981) reveals that most studies on the effects of MAs have been done by testing grain quality. He concludes that for low-moisture-content grains, low O_2 and high CO_2 concentrations do not have a detrimental effect on the germination, milling, and baking properties of wheat or the organoleptic properties of rice, although for intermediate- and high-moisture-content grains, the quality is affected. Studies on long-term hermetic storage of wheat (Navarro et al, 1984, 1993) also clearly indicate that both the germination and baking properties of wheat are well preserved and the milling properties of dry paddy are not affected (Donahaye et al, 1991b). Detailed studies on the quality preservation of commodities sensitive to oxidation and on retention of aromatic components, as in the case of spices and beverages, remain to be made. Preliminary work on preservation of the organoleptic qualities of spices like chili pepper, coriander seeds, turmeric rhizomes, and cumin seeds showed excellent preservation under vacuum and better preservation under hermetic or carbon dioxide atmospheres than under exposure to normal chamber atmosphere (Navarro et al, 2001a, 2002b). Similar work to determine the beneficial effects of MA treatment on the quality of treated products is needed.

Field Work

Temperature and Humidity

Alterations in the temperature of large grain bulks to enhance the effectiveness of MA treatments are seldom carried out. Data on the use of heated air to increase the temperature of the commodity before MA treatment to modify the temperature of the commodity or of the MA-treated space would contribute to our knowledge. Similarly, the humidity of the interstitial space is dictated by the moisture content of the grain. Since it is a part of the system, it is difficult to modify. Additional information related to the control of humidity to enhance MA treatment is needed.

During high-CO_2 treatment, sorption of CO_2 causes significant reduction in the gas concentration. These changes must be taken into account when deciding on the required exposure time. Except in very tightly sealed small metal chambers, additional gas introductions are likely to be required, based on concentration readings taken during the exposure. To avoid structural damage due to daily temperature fluctuations, particularly in the headspace of metal structures, appropriate pressure-relief valves must be installed. Even in the best-sealed structures, a reduction in CO_2 concentration or increase in O_2 concentration occurs over time due to gas loss through pressure-relief valves or leaks in the structure.

MA treatments are not considered practical for treatment of mills, processing plants, or any other structures that are difficult to seal tightly.

If the investment costs for membrane or pressure-swing absorption units are markedly reduced, some durable products may even be stored continuously under MAs, to control pests and maintain product quality. Moreover, material protection and disinfestation of artifacts will most probably be carried out more widely by the use of humidified nitrogen. At least in museums, treatment time is seldom a problem.

Vacuum Technology

In the application of vacuum technology, fairly low pressures are required to obtain insect kill within reasonable periods. Additional systematic studies on insect response to low-pressure treatment are required. A process, named vacuum hermetic fumigation, was recently proposed that uses flexible liners to achieve low pressures in transportable structures. Low pressures (25–50 mm of Hg), can be attained and maintained for indefinite periods of time (Finkelman et al, 2002a, 2003a).

Special portable chambers made of flexible tarplike sheeting provide the benefit of treatment in any location where sufficient quantities of CO_2 or N_2 are available. These chambers enable low-cost treatment, utilizing nonrigid low-permeability envelopes. Use of these materials for museum artifacts seems to be most suitable.

High-Pressure Carbon Dioxide

CO_2 treatments can be significantly shortened to exposure times that may be measured in hours by applying increased pressure (10–37 bar) in specially designed metal chambers that can withstand these high pressures. Such high-pressure chamber treatments may be practical for high-value products, such as spices, nuts, dried fruits, medicinal herbs, and other special commodities.

If pressurized carbon dioxide is recycled in a double chamber, the moisture content of the gas may influence its efficacy.

Hermetic Storage

A type of MA that can be applied for the protection of grain is hermetic storage, also termed "sealed storage," "airtight storage," or "sacrificial sealed storage." In sufficiently sealed structures, insects and other aerobic organisms in the grain mass reduce the O_2 and increase the CO_2 concentrations through respiratory metabolism, to levels below those permitting insect development. Hermetic long-term storage, as used in Cyprus, Israel, and the Philippines, seems a feasible technique. More field data on the application of this technique would contribute to our know-how.

Degree of Sealing

Sealing methodologies have been well developed and published (Alexander, 1984; Lloyd, 1984; Sutherland and Thomas, 1984; Woodcock, 1984; Newman, 1990). However, in practice, sealing of rigid structures has been limited mainly to Australia, and elsewhere many new silos are being built to low standards of sealing that do not permit application of hermetic storage or MA treatments. Gas-tightness and temperature insulation factors are often underestimated and neglected when new storage facilities are constructed.

The future approach to silo construction should take a more professional attitude toward silo sealing. It should acknowledge that a high level of sealing is essential in the modern approach to fumigation, to reduce emissions (especially of MB because of its association with ozone depletion) and also to eliminate the possibility of development of phosphine-resistant insect populations. New environmental and food-quality regulations may force pest managers and fumigators to improve gastightness and to reduce the emission of toxic chemicals. These developments will improve not only the treatments with toxic fumigants but also the commercial competitiveness of MAs in the protection of stored products.

Headspace

A major problem in rigid structures is the volume of headspace above the grain bulk. This is not only a limiting factor in determining the rate at which O_2 concentration decreases, but it also accentuates pressure differences between the interior and exterior of the silo due to daily temperature and barometric fluctuations of the ambient atmosphere. Pressure-relief valves provide a satisfactory solution for MA storage. However, the use of breather-bags to unify internal and external pressures appears to be more desirable for hermetic storage since this system reduces the gas exchange. To the best of our knowledge, breather-bags have not been employed in silo bins, except for "Harvestores" used for silage. Experimental data on the use of such breather-bags under tropical and subtropical climates would be very useful when considering the application of hermetic storage to reduce the intensity of air ingress into the storage system.

Moisture Condensation

A phenomenon that discourages the use of hermetic storage in hot climates is moisture migration and condensation, which is especially intensified in metal silos. In conventional storages, engineers rely on designing well-ventilated headspaces to reduce the intensity of this phenomenon, and they even incorporate aeration systems with excessively high airflow rates, regardless of their efficacy in the tropics. In subtropical climates with a cool season, aeration systems were shown to effectively overcome this problem by equalizing grain temperatures (Navarro and Noyes, 2002). For this reason, Navarro and Calderon (1980) proposed the integration of aeration when MAs are used in metal structures. However, the efficacy of this approach was never adequately documented.

For metal silos in hot climates, moisture condensation is intensified when insect infestation causes grain heating. The most disturbing effect of moisture migration, especially in rigid constructions, is the difficulty in removing the damaged layer, usually at the top of the bulk.

So far, two approaches are known to reduce the intensity of this phenomenon: equalizing grain temperatures and insulation of the roof. Equalizing grain temperatures by aeration is limited to climates with a cool season. Comparative data is lacking on the efficacy of aeration and the effect of insulation in preventing moisture migration in metal silos in the tropics.

For small-scale applications using flexible liners, the influence of insulation materials in reducing the intensity of moisture migration in subtropical (Israel) and tropical (Philippines) climates has been investigated by Donahaye et al (2000). A model describing moisture migration by natural convection has been proposed (Nguyen, 1986). However, more experimental data are required to support the development of predictive models for hermetic storage.

Flexible Liners

One approach to hermetic storage has been through the use of flexible liners. All liners have the advantage that they follow the contours of the grain-bulk surface, and with no headspace, gas exchange is restricted to the intergranular air volume. However, there is still need for improvement in liner materials. Although liners with zero permeability to gases are available, other factors such as physical characteristics, durability, resistance to penetration by pests (rodents and insects), amenability to jointing and welding, and cost of manufacture are all critical to the choice of liner. Integration of all these characteristics into a single liner is still an objective of the future.

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