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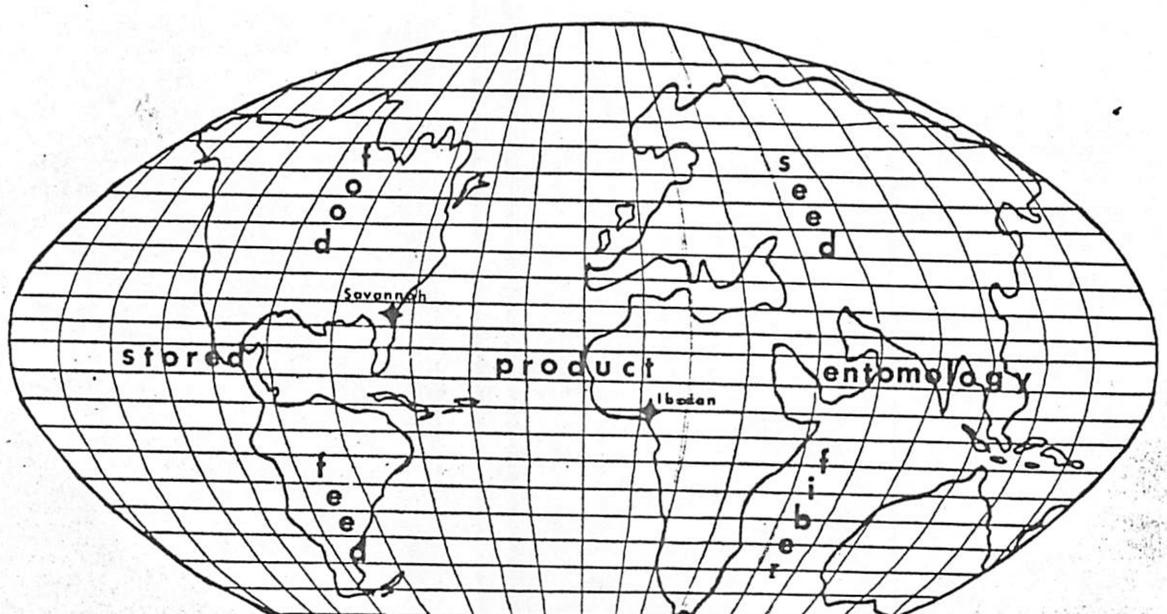

RECENT ADVANCES IN THE USE OF MODIFIED ATMOSPHERES FOR STORED PRODUCT PEST CONTROL

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Modified or controlled atmosphere (CA) storage of grain involves alteration of the proportions of the normal gaseous constituents of the storage environment, oxygen, nitrogen and carbon dioxide to provide an insecticidal atmosphere. This paper reviews experimental approaches made over the years directed towards the development of full commercial utilisation of this residue-free method of insect control in stored dry durable commodities, such as grain or pulses. Although working on a similar biological principle, hermetic and pit storage systems are specifically excluded from this review and only atmospheres created by external addition of gases to give the altered composition will be considered.

There are several questions which must be answered before controlled atmosphere storage can be used successfully, efficiently and routinely.

They can be summarised as:

(a) How well sealed must the storage be?

(b) What quantity of gas is required to create the desired atmosphere in this storage?

(c) How much gas will be required to maintain the correct gaseous composition for a set exposure period?

(d) What is the correct exposure period?

Two publications in 1971 (1) and 1977 (2) have set out 'state of the art' practical instructions for controlled atmosphere storage of grain. Both were based on field experience of the method and gave answers to these questions as then known. However, practical experience in the use of this technique is rapidly being amassed and it is time to review the information available and see where there are still problems to be solved.

REQUIREMENTS FOR APPLICATION OF MODIFIED ATMOSPHERES: The atmospheric composition and its biological effect - Two main types of modified atmospheres are insecticidal, both of which have been investigated under full scale field conditions: - low oxygen atmospheres and CO_2-rich atmospheres. The low oxygen atmospheres have been found to be insecticidal below about 2% oxygen to all species and stages of stored product pests so far tested (3). Some species are more susceptible and may be killed at 3% O_2 or more (e.g. *Cryptolestes ferrugineus* adults (4)). An atmosphere containing 1% oxygen appears to be the best compromise between increased speed of action at lower oxygen levels (5) and the increased difficulty of maintaining low O_2 levels.

The low oxygen atmospheres have been generated under field comditions either by purging the storage with nitrogen, supplied by tanker as a liquid, or by burning hydrocarbons in air and using the exhaust from the burner as a purge.

CO_2 rich atmospheres have been generated from CO_2 supplied in tanker as a liquid, as compressed gas in cylinders or as dry ice. It has long been recognized (6) that high levels of CO_2 give control in the presence of substantial quantities of oxygen and therefore that CO_2 acts as a toxic fumigant rather than merely displacing oxygen. The optimum level appears to be about 60% CO_2 in air, i.e. 8% O_2, 60% CO_2, balance N_2 and rare gases. Below 60%, the action of the gas mixture against several species (e.g. *S. granarius* declines (4) (7)). Between 60 and 98% it is approximately constant (8) and then declines slightly for still higher CO_2 levels.

The time taken to achieve a certain level of insect kill is dependent not only on the composition of the atmosphere but also on the temperature of the environment. With low oxygen atmospheres the temperature effect is substantial; the period required for complete insect kill at high storage temperatures, e.g. 35°C, is only a few days but may be many weeks below 15°C. Exposure times required for complete disinfestation at various temperatures have been published (2). Where only susceptible species such as *Oryzaephilus surinamensis* are present or a lower level of mortality is acceptable, these periods may be substantially reduced. There is insufficient data available to assess accurately the influence of temperature upon the effectiveness of carbon dioxide-rich atmospheres. However, it appears that there is a similar but less pronounced temperature dependence than for low oxygen systems. Generally, the speeds of action of the two systems are similar but there is some variation between species and stages.

The requirement for an exposure period of several days or even some weeks, to the modified atmosphere, means that the atmosphere of the correct composition must be maintained under conditions where some leakage is inevitable. During this period, the 'maintenance phase,' it has been found necessary with low oxygen atmospheres to add further gas to counteract leakage. This may also be the case with CO_2-rich atmospheres, although because they are effective over a wide range of CO_2 levels, this is not always necessary.

<u>The effects of sealing</u> - Before considering the results of the various field trials in detail it is necessary to explain the relevance of the sealing level of the enclosure to the general technique of modified atmosphere storage. It is obvious that given an inexhaustible supply of gas available at any rate on demand, it would be possible to create a specified atmosphere in almost any storage enclosure. Such a situation would clearly be uneconomic. Gas usage must thus be reduced by sealing. This

may sometimes be expensive, but the cost can be offset against the saving of gas.

There are a number of forces which cause gas losses from enclosures. These have been summarized for freight containers (9) and their various contributions investigated under practical conditions (10) (11). The influence of some forces, such as wind, may be reduced by increased level of sealing. Others, particularly those generated by temperature cycling in the headspace, are substantially decreased only by having very high levels of sealing. The optimal standard of sealing for storages for controlled atmosphere use is that where the gas loss caused by the forces in the former category is negligible compared with that caused by the latter. This standard can be predicted mathematically and has been published for storages of 300-10000 tonnes (2). The predicted gas interchange rate of such sealed stored with atmosphere is less than 5%/day. The setting of such a standard is important as it prevents the expenditure of unnecessary effort in attaining higher levels of sealing which do not result in reduced leakage. When because of practical difficulties, this sealing level cannot be achieved, a higher rate of gas usage can be expected to maintain a set atmospheric composition. For ISO 1C freight containers, a leakage standard of <20 m3/hr at 250 Pa excess pressure seems appropriate on present data (12).

The sealing of a storage also provides an insect-proof barrier. Thus, unless required for quality preservation, the maintenance of modified atmosphere is required only for as long as required for complete insect kill within the store.

It is unfortunate that an objective measurement of gastightness, such as a pressure test, is seldom given in reports of modified atmosphere trials. This prevents conclusive analysis of the reasons for the differing efficiencies attained in the different trials. However where a large maintenance rate is required, it is likely that the standard of sealing was low.

FIELD TRIALS: <u>Atmospheres generated with nitrogen</u> - There have been a number of field trials conducted with low oxygen atmospheres generated by introduction of nitrogen gas. Table I gives a summary of the trials carried out in Australia over the past few years. This table is an expanded version of that presented (3) to the First International Working Conference on Stored Product Entomology. Details of the pressure tests and duct work of the bins used are given in Table II.

Despite the wide range of gastightness, rates of gas input, configuration of gas introduction system and bin capacity and degree of filling, there was no difficulty in creating a sufficiently low oxygen atmosphere throughout the grain mass in the structures. In three of the trials, purging was terminated before the headspace was below 1.5% oxygen. In two of these, the addition of gas during the maintenance phase lowered the oxygen level below this. In the third, Cunnigar I, the oxygen level

TABLE I. Nitrogen usages and efficiencies for trials using liquid nitrogen carried out by CSIRO (13).

Trial (Site)	Date of Trial	Storage capacity m^3	Load (tonnes)	Filling ratio	Nitrogen used for purge (m^3)	Headspace % O_2 at termination	Efficiency (E_3 %)*
Bordertown I	1972	9049	7035 wheat	0.92	7306	1.1	76
Bordertown II	1972	9049	5220 wheat	0.74	9622	3.2	73
Cunningar I	1973	2930	2040 wheat	0.85	1990	3.9	84
Cunningar II	1973	2930	2040 wheat	0.85	2467	1.0	92
Sunshine	1974	3055	1809 barley	0.71	3300	1.2	89
Newcastle	1975	421	294 wheat	0.83	430	0.7	88
Balaklava	1976	9049	6474 wheat	0.91	7072	2.1	75
Bungunya	1976	2330	1780 wheat	0.91	2095	0.8	79

* Efficiency calculated according to formula in Appendix I.

TABLE II. Summary of purging and pressure tests for CSIRO nitrogen trials (13).

Trial	Inlet system	Average purge rate (m^3/min)	Calculated equivalent rise rate m/hr	Pressure test	
				Decay 250-125 Pa (filled) (secs)	Flow giving 250 Pa (l/sec)
Bordertown I	Annular perforated duct close to wall and central input point	4.9	1.8	100	-
Bordertown II	Shielded input on base on wall	9.4	3.5	1600	-
Cunningar I	Directly into base of cone	2.2	1.5	100	-
Cunningar II	Directly into base of cone	3.2	2.1	80	-
Sunshine	Y-shaped perforated aeration duct on floor	2.7	2.1	780	4
Newcastle	Perforated duct along diameter of bin	0.77	2.2	-	6
Balaklava	3 symmetrically placed shielded inputs around base of wall	1.7	0.6	960	1
Bungunya	Radial perforated duct	1.35	1.8	240	5

rose, as an inadequate level of sealing had been achieved and air was forced in by wind at a rate greater than could be counteracted by the gas input rate available.

In small scale trials in bins of 30-150 m^3 capacity by Shejbal et al. (5) (14) (15), nitrogen was introduced for purging directly into the headspace of the storage. Two to three times the interstitial volume was said (4) to reduce the oxygen tension in the bin to that of the purge nitrogen. Assuming a 95% full bin and an attained O_2 proportion of 0.5%, the usage of two and three nitrogen volumes (with 0.5% O_2 content) corresponds to efficiencies of 81 and 54% respectively (formulae for calculation of efficiencies are given in Appendix I). Because nitrogen is less dense than air, purging down through the grain may reduce density-related mixing and give a higher efficiency. There is no evidence for this as the efficiencies obtained by Shejbal et al. (14) are similar to those in the CSIRO trials (Table I) (13) where upward flow was used.

It can be seen that the creation of low oxygen atmospheres is a reasonably efficient process even in storages whose gasetightness is less than desirable to give an economic maintenance rate. The wide variety of systems found to be suitable show that the efficiency of creation of low oxygen atmospheres is not very sensitive to the ductwork design and introduction rates. It seems that the advantage of any increase effecting more complex introduction systems would be offset by their cost and inconvenience.

In most of the trials where maintenance of the atmosphere was attempted, the maintenance gas was added into the headspace. The minimum requirement was specifically investigated in three trials by progressively decreasing the input rate until maintenance was just achieved. Table III compares the observed

TABLE III. Minimum maintenance flow compared with air ingress due to head space treatment (12).

	Nitrogen flow to maintain low oxygen concentration m^3 day^{-1}	Maximum possible air ingress due to temperature cycle (E_1) m^3 day^{-1}	Calculated nitrogen requirement (E_2) dilute this ingress to 1% O_2 m^3 day^{-1}
Newcastle	15	4.3	13
Bungunya	37	8.4	26
Balaklava	69	55	169

and calculated requirements. It is assumed that there is direct displacement of the air coming in and free mixing with displacement of the diluted gas. In two cases the requirement was similar to that expected from free mixing. In the third it was close to that expected from displacement. This difference may partly be attributable to differences in the location of leaks, as well as to difference in total sealing level. Based on the experience obtained with these and other trials, a table of expected maintenance usages, with various bin sizes and fillings, has been published (2).

In two trials, Cunnigar I and II, the maintenance nitrogen was introduced at the base. In Cunningar II, there was a very high maintenance requirement of about 850 m^3/day. This high rate resulted partly from high general interchange rate as the storage was not sealed to a high standard, but also because the air, entering principally at the base of the bin, was forced into the grain mass, instead of, at least in part, being expelled through the leak where it entered. Admission of the nitrogen into the headspace would have substantially reduced the maintenance requirement by allowing this to occur.

In experimental treatments by Shejbal et al. (14) nitrogen was introduced at a continuous rate to give a perceptable outflow of gas at the base of the storage. The requirement ranged from 0.2 to 1.5 litres N_2/hr/100 kg wheat or barley. In commercial usage in Italy the requirement is 0.008-0.02 litres N_2/hr/100 kg grain (16). A demand system is used which maintains a slight positive pressure in the structure at all times. These rates correspond to usages of 96 to 720 and 29-72 m^3/day respectively. The latter rate is similar to that observed in the CSIRO trials (Table IV) using a continuous nitrogen input.

The biological effectiveness of the experimental trials using nitrogen has been high but not always complete. Shejbal et al. (5) (14) apparently obtained survival of eggs of *S. granarius* after 10 days at 22°C in their trials with 0.5% O_2 in nitrogen. In CSIRO trials, where short exposures have been used, some survival has been found. In the Sunshine trial, a mortality of 99.3% of mixed stages of *S. oryzae* was obtained, with only early stages and pupae surviving after 31 days at 14-20°C at <1% oxygen.

No infestation was recorded in the Bungunya and Balaklava trials, which remained sealed for a further five and eight months respectively after termination of maintenance of nitrogen despite infestations in the adjacent bins. The bins had been under low oxygen for more than 195 days. The grain from the Newcastle trial was also free of insects but had previously been disinfested with phosphine, and thus the effect of the nitrogen exposure could not be assessed. In these cases the insect proofing from the sealing was clearly demonstrated.

Recently, commercial pilot trials have been carried out in Victoria and Queensland with nitrogen-generated low oxygen

TABLE IV. Summary of trials conducted using CO_2 by CSIRO.

Trial	Enclosure	Load (tonnes)	Average CO_2 level achieved %	Initial charge	Purging efficiency E_1	E_2	Decay 250-125 Pa (filled) (secs)	Flow giving 250 Pa (l/sec)	Reference
Bordertown III	welded steel bin	6600 wheat	97†	10.4 tonnes	70†	-	450	-	[33]
Bordertown IV	welded steel bin	6600 wheat	73†	6.9 tonnes	79†	-	450	-	[33]
Sydney I	PVC sheet	2.7 wheat & rye	60	11 kg	-	60	-	2.5	[34]
Boggabri	freight container	18-22 wheat	45-70	20-40 kg	-	81-93	-	0.1-9.4	[35]
Sydney II	freight container	18 wheat	45	31 kg	-	71	-	2.5	[36]
Rabaul I	freight container	8 copra	74	75 kg	-	69	10	-	[35]
Rabaul II	LASH barge	135 cocoa*	53	851 kg	-	92	96†	-	[35]
Harden	grain storage	13600*	55†	33 tonnes	73†	-	470**	-	[33]

* Part load
** 125-62.5 Pa decay
† Provisional figures

atmospheres. These have been based on the trials reported here. No insects were present at outturn in either series, although infestation, largely *T. castaneum* and *R. dominica,* were present at the start. In both cases the exposure to low oxygen exceed two months.

Atmospheres generated by burning hydrocarbons - The use of liquid nitrogen as a source of atmosphere may often be expensive, particularly where long transport distances are involved between the production site and the storage. This can be avoided if a method of on-site generation of suitable atmosphere is available.

The first record (1918) (17) (18) of a process of this kind is of the treatment of 230 tonnes of "very weevilly" wheat enclosed in malthoid with the exhaust gases from burning coke. An atmosphere of 10-15% CO_2 and presumably low oxygen was generated and maintained by passing the combustion gases from a furnace into the enclosure. The exhaust was said to be 80% nitrogen, 20% CO_2 and "no free oxygen." No live weevils were found after treatment. The process was extensively used in 1918 and 1919 (18). The only on-site method tested to modern standards consists of burning hydrocarbons in air, thus producing a low oxygen exhaust, with conditioning to remove excess moisture. The technique is widely used (19) in apple stores and in the storage of oxygen sensitive commodities such as alfalfa pellets.

Storey (20) used gas from two exothermic generators, output capacity 850 m^3/hr each, for the purging of 543 tonnes of wheat in a cell of 937 m^3 capacity (assuming flat roof and floor). The gas produced was <1% O_2, 8.5-11.5% CO_2 1-2% CO and balance nitrogen. In one experiment the gas was introduced with the grain in situ. The inert atmosphere front rose through the bulk at 2.4 m/hr corresponding to an input rate of 22 m^3/hr (actual rate not given) with an atmosphere of <1.0% O_2 being achieved at all points after 48 hours of purging. In a second experiment, the empty bin was partly purged to 8.5% O_2, and then inloaded with 543 tonnes wheat. The oxygen level in the bin rose to an average of 14.1% O_2. It was then purged at a slower but unspecified rate than in the first test giving concentrations from 0.3-1.7% O_2 after 72 hours. In the third test, levels were 3.8% before loading, 9.0% after loading with 0.1-0.9% O_2 after 20 hours at a purge rate as in the first experiment. In the first trial, complete mortality of adult *T. confusum* test insects was observed after 72 and 96 hours respectively. In all trials, immature stages of *S. oryzae* survived. The survival of adult *T. confusum* in the third trial was attributed to air ingress from wind, demonstrating that the cells were not adequately sealed.

An exothermic generator has been used in the USSR (2) for experimental storage of high moisture paddy rice (20-21%) and suggested (22) for storage of flour and grain. No details of tests on dry commodities are available, but the system produces a gas mixture similar to that used by Storey (20) and thus could be useful for insect control. Sixteen and eight-tenths cubic meters of natural gas were used per day to maintain the atmosphere over 24,000 tonnes rice in four storages for four months.

A catalytic burner using propane was used to reduce oxygen levels in a 1268 tonne capacity metal bin in Israel. The results of operations over two seasons have been reported (23) (24). In the first season the level in the bin was reduced from 16% to 1.2% in 49.5 hours using propane at 1.2 kg/hr. In the next season, the burner reduced the oxygen level from 21% to 0.2% in 60.3 hours using 67 kg propane in a load of 1208 tonnes wheat. Subsequent intermittent operation of the burner for a total of 19.5 hours maintained the oxygen level below 2% for 20 days, using 23.6 kg propane. The usage corresponds to an air leakage rate of 16.4 m^3 or about 2.6%/day. The storage was tested by pressure testing in both seasons giving decay times of 80 and 115 secs for a 500-250 Pa pressure drop. The air interchange rate calculated from the published graphs (23) (24) were 2.0 and 1.2%/day respectively. These rates show a satisfactory standard of sealing for efficient operation although the pressure test results were lower than the proposed standard (2).

High but incomplete insect mortality was observed in all trials. Even when a low oxygen atmosphere (<2%) was maintained for 20 days, there was survival at the base of the bin (*R. dominica* adults (1%), *T. castaneum* larvae (8%) and adults (4%)) and on the grain surface (*T. castaneum* larvae (4%) and *O. surinamensis* larvae (15%)). Surprisingly, there was complete mortality of *S. oryzae* adults and larvae. This species is often regarded as that most tolerant to low oxygen atmospheres. The temperatures at the sites for the test cases were not given but may have been low as the experiment was carried out in January.

<u>Atmospheres generated with carbon dioxide</u> - The possibility of using CO_2 for control of stored product insects has been recognized for many years. In Australia, a dosage of 0.72 kg CO_2 per tonne of grain in an airtight silo was said in 1917 to be the "most effective" fumigant then available (25), when compared with CS_2 and HCN. Later (1921) (26), a rate equivalent to 1.4 kg/tonne was recommended for maize in galvanized iron tanks. Despite these early uses, CO_2 was either superseded by other fumigants or never became generally used.

Since then a number of trials have been carried out to develop the use of CO_2 for insect control in stored products. Oosthuizen and Schmidt (27) in 1942 tested the use of CO_2 against *Callosobruchus chinensis* in old and new galvanized tanks of 1.2 m^3 capacity. Carbon dioxide was introduced into the base of the tank filled with cowpeas, with 0.55-0.70 kg of CO_2 giving an atmosphere of about 70% (by extrapolation of observed concentration decay with time, no initial reading taken) in the new bins. The CO_2 levels in the new bins decayed slowly, averaging 41%, in one case, after 14 days. The old bins leaked badly and the effectiveness of the treatment was low.

Mansour in 1955 (28) treated 180 tonnes of wheat in a bin (capacity 240 m^3) with the quantity of dry ice, 160 kg or 0.89 kg/tonne, calculated to give 100% CO_2 by displacement in the full bin at a porosity of 30% and an apparent CO_2 density of 2 kg/m^3. The CO_2 level achieved was not given but when the cell was outloaded after 25 days the cell was the 90% mortality of the weevils present at a temperature of 11-15°C.

Le Du (29) stored 11 tonnes of wheat of 16% m.c. under CO_2 in a small metal bin for two years. Carbon dioxide was added automatically from cylinders to keep an internal pressure exess of 250 Pa. Actual usage rates were not given. The experiment was designed for quality preservation not insect control but it is relevant to note it here as the system could have been used for the latter purpose.

Jay et al. (30) purged a silo bin of 2262 m^3 capacity containing 2203 m^3 (~490 tonnes) of undercorticated groundnuts with 1.49, 1.54 and 1.50 tonnes CO_2 in three separate trials. The gas was admitted into the top of the bin and the purge was terminated when the CO_2 level rose to about 35%. Gas was circulated through a small blower from the bottom to the top of the bin during the trials. At a solid density of 1.1 tonnes/m^3, the total void space is 1810 m^3, equivalent to 1.17 tonnes CO_2 at 35% CO_2 (15°C, 100 kPa). For free mixing (E_2) to 35%, the theoretical requirement on this basis is 1.44 tonnes, which is close to the observed usage. Additional CO_2 was put in to maintain the 35% level for periods of 2, 4 and 7 days. This averaged 0.34, 0.45 and 0.47 tonnes/day or about 180, 240 and 250 m^3/day.

Jay and Pearman (31) treated 647 tonnes of maize in a concrete vertical cell of 1102 m^3 capacity with 1.15 tonnes of CO_2 applied into the headspace over 8 hours. This gave an atmosphere of >50% CO_2 at all but one point, where penetration was slowed by dust in the load. The atmosphere was then maintained between 55-65% CO_2 with an automatic injection system for 91 hours using an average maintenance rate of 425 m^3 CO_2/day. The theoretical requirement for 55% CO_2 is about 0.92 tonnes on the basis of free mixing. There was a low level of survival of immature stages of *Sitophilus* spp. but *Sitotroga cerealella* was eliminated.

Jay et al. (32) treated loads of comb honey in 12 insulated semi-trailer units, each of a size similar to a 1A ISO freight container (40x8x8 ft; 72.5m^3 capacity). The CO_2 was introduced through ductwork laid along the floor of the unit. After purging to give CO_2 >95%, the units were sealed. Carbon dioxide levels were maintained automatically by a servo system with CO_2 added whenever the level dropped below 96%. For an average total usage of 606 kg CO_2/unit, the atmosphere was maintained for 10-12 hours at an average of 98.6% CO_2. Since complete displacement of the gas with no allowance for the volume occupied by the load would take about 100 kg CO_2, the process was clearly an inefficient one, presumably because of a high rate of leakage from the unit.

There have been a number of trials carried out in Australia recently (33) (34) in partially sealed structures with carbon dioxide applied to give an insecticidal atmosphere. Two were also carried out (35) in Papua, New Guinea, in order to show that the method could be used under tropical conditions. The trials conducted by CSIRO are summarized in Table IV.

The large scale trials with wheat were carried out in structures exceeding the proposed gastightness standard (2). Bordertown III was carried out to demonstrate that a high CO_2 level could be easily achieved in a grain storage under commercial conditions in Australia. In this trial CO_2, supplied by tanker as a liquid, was vaporized and introduced through shielded inlets in the bin wall directly into the grain mass, with the displaced air vented through a hatch in the roof. A narrow and discrete CO_2 front was observed in the grain mass during purging. After usage of 10.4 tonnes of CO_2, the purge was terminated and the bin sealed. The natural decay of the CO_2 levels was observed. As had been found elsewhere (1), the headspace concentration dropped with air ingress, with the zone of low CO_2 concentration descending slowly through the bin. This tendency to 'drop out' was counteracted by mixing the bin atmosphere by recirculation from the base to the headspace by means of a small fan. A recirculation rate of 0.084 air change/day was almost sufficient to prevent this 'drop out.'

The test was repeated, but using a purge of 75% CO_2 rather than pure CO_2 since the available literature (e.g. 4, 7, 8) suggests that it is unnecessary to levels higher than this in order to achieve insect control. The atmosphere was recirculated as previously. The average CO_2 level decayed from 73 to 45% in the 10 days before venting of the bin.

In the most recent trial, Harden, a large grain storage shed was sealed and treated with 33 tonnes of pure CO_2, generated from tanker supplied liquid. The purge was terminated before the whole of the gas space was filled with CO_2 and the atmosphere was then recirculated through a small external fan at a rate of 0.11 air changes/day. The CO_2 level, initially ranging from 18-99% CO_2, ranged from 37-86% after 30 hours of recirculation and 51-66% (mean 52%), after 72 hours. At termination of the trial, after 20 days, the atmosphere was still at an average of 25% CO_2. Initially the insecticide-free wheat was substantially infested but no live insects were detected on detailed inspection 12 days after termination. However a resurgence of infestation was noted after 50 days in one area only where a 'hot spot' had developed before treatment. Grain temperatures ranged from 11-37°C after treatment.

To demonstrate small scale use of CO_2, a small load of bagged grain was treated in a PVC enclosure with CO_2 as dry ice (34). Because of leakage, several additions of dry ice were required during the 22 day exposure period in order to maintain the CO_2 concentration above 35%. A high mortality of the predominant pest species, *R. dominica*, was obtained with survival amongst the earlier developed stages only. Some of the parasitoids *Anisopteromalus calandrae* and *Choetospila elegans* present survived too, apparently as pupae. The grain temperature was from 10-14°C.

A series of trials (35) has been undertaken to develop the use of CO_2 as an in-transit treatment against insects in commodities in freight containers. In one experiment, the Boggabri trial, 10 freight containers selected for gastightness were loaded with wheat, and treated with 20-40 kg CO_2 as dry ice applied directly to the grain surface. In some containers, an additional charge of 30 kg CO_2 was added in an insulated box giving a controlled release of 3 kg CO_2/day. Caged mixed age cultures of *S. oryzae* were added as test insects to two containers. The container with the box maintained levels between 50-72% CO_2 over 16 days at 23°C. This gave complete mortality of the test insects. In the other, the CO_2 level fell from a maximum of 89% to 26% CO_2 at 10 days and a low level of survival of some early stages was observed (overall mortality >96%). A light natural infestation of *T. castaneum* was eliminated in both cases. In a further trial (36) bagged wheat in an ISO general purpose freight container with 30 kg of dry ice with 30 kg additionally in an insulated box was exported to West Germany after an 11 day holding period in Australia. During the holding period the CO_2 level remained at about 45% and was still 7.5% after the 6 week sea voyage. At outturn, the added test insects (all stages of *S. oryzae*, 36,000 insects in total) were all dead. A very light infestation of *R. dominica* present on stuffing was not detected at outturn.

Under static, tropical conditions in Rabaul, New Britain, a load of bagged copra in a container (35), selected to meet a gastightness standard, was treated with 75 kg CO_2 added into the headspace through the ventilators from cylinders. The initial CO_2 level of 74% fell to 45% after 6 days. After 9 days the exposure was terminated. There was no survival found in the heavy infestation of *Necrobia rufipes* and *Oryzaephilus mercator* originally present, either at outturn or upon incubation of the sievings taken during the inspection.

CO_2 has also been used (35) to treat cocoa beans in a LASH barge at Rabaul. CO_2 was added to the headspace of the barge from cylinders, over about 30 hours. The rate was limited by freezing of lines or cylinder contents. The increase in CO_2 concentration in the barge closely followed the trend expected from free mixing. The CO_2 level fell from a maximum of 53% to 28% at 9 1/2 days. The barge was then aired and shipped to the west coast of the U.S.A. No live specimens of the added test insects, largely *Tribolium castaneum* and *Oryzaephilus mercator*, were found at outturn but, on incubation, a small number of *T. castaneum* were detected.

The general usage rates and calculated purging efficiencies are given for the CSIRO trials in Table V. The efficiencies do not include allowances for sorption which may be substantial (37). The insect mortalities for those CO_2 trials in which detailed observations were made are summarized in Table V.

GENERAL OBSERVATIONS: From the field trials reviewed above it is clear that it is possible to generate and maintain an insecticidal concentration of oxygen or carbon dioxide under a variety of field conditions. Nitrogen purging efficiencies were high. In CSIRO trials they exceeded 70%, (Table I), giving a usage of not more than 40% more than expected theoretically. For CO_2, those enclosures purged from below gave efficiencies of greater than 70% on the basis of direct displacement. Where a calculation based on free mixing was appropriate, as in addition of CO_2 into the top of an enclosure, efficiencies ranged from 60-93% (Table IV). With CO_2, maintenance was often unnecessary as the natural leakage was sufficiently restricted by a feasible standard of sealing to give an adequate time of retention of high CO_2 levels for insect control. As this standard of sealing the interchange rate of the atmosphere with air in bulk storages was less than 5%, typically 2-3%. The expected requirement of maintenance gas, nitrogen, CO_2 or gases from propane burning, can be calculated directly from this. With this information, the total gas requirements of a particular treatment can be predicted and economic comparisons can then be made with other pest control measures.

While efficiencies have been found to be high there is scope for some improvement particularly with nitrogen. It may be that this can be achieved by altering the purging rate, but the effects of this have not been studied. In trials so far with nitrogen though widely different introduction rates have been used, the range of rates of travel of the purge front has been small (see Table II) and thus there is no firm evidence on the effect of varying this on efficiencies.

The extension of modified atmosphere technology into use in tropical conditions is important. Both carbon dioxide and nitrogen can be produced locally and are usually more easily procurable than the toxic fumigants such as methyl bromide. The two trials outlined here, Rabaul I and II, were carried out under conditions similar to that to be expected in commercial use. The trial in the LASH barge gave a high degree of insect control. The CO_2 level achieved in the barge was limited by available gas supplies, not by leakage. With a higher initial level it is likely that total insect control would have been attained. In the other trial, in a container selected for gastightness, there was complete insect mortality, to our level of assessment, in a shorter period than used in the barge. Presumably the insect species present were more susceptible to CO_2 than is *T. castaneum*. This disinfestation was carried out on bagged copra, a notoriously difficult commodity to disinfest by fumigation.

TABLE V. Summary of mortality data obtained in the course of field trials utilizing CO_2.

Test Insect	Exposure period (days)	Temp. °C	Concentration range % CO_2	Mortality	Reference
Callosobruchus chinensis all stages	14	26.7	70-41	98.8†	[27]
Galleria mellonella larvae	0.5	25-40	96	97.8	[32]
Necrobia rufipes all stages	6	25	74-45	100	[35]
Oryzaephilus mercator all stages	9.5	30	53-28	100	[35]
Oryzaephilus mercator all stages	6	25	74-45	100	[35]
Rhyzopertha dominica all stages	22	11-14	25-90*	97	[34]
Sitophilus oryzae all stages	16	23	50-72	100	[35]
Sitophilus oryzae all stages	10	23	59-26	96	[35]
Sitophilus oryzae eggs, young larvae and adults	10	31	73-47	100	[33]
Sitophilus oryzae old larvae, pupae and adults	10	31	73-47	99.2	[33]
Sitophilus oryzae eggs, young larvae and adults	10	13.5	68-47	77	[33]
Sitophilus oryzae old larvae, pupae and adults	10	13.5	68-47	79	[33]
Sitophilus oryzae all stages	11	26	80-45**	100	[36]
Sitophilus spp. immature stages	3.75	14-25	55-65	99.9	[31]
Tribolium castaneum adults	4	18-32	35	93	[30]
Tribolium castaneum all stages	9.5	30	52-38	98.6	[35]

† Detailed series of results given under different conditions (27).

* Concentration variable.

** With a further 4-1/2 weeks for decay from 45-8% CO_2.

It will be noted that many of the modified atmosphere trials reviewed here have achieved high (>95%) insect mortality of test insects or natural infestations but have fallen short of complete insect kill. The levels achieved under these circumstances may be higher than that required to meet various inspection standards, except when heavy infestations were present before treatment. The survival of early developmental stages means that infestation will not be detected if the commodity is presented soon after treatment. However, if long term storage is required without risk of resurgence of infestation, a mortality of 99.5% must be regarded as a control failure, since it will not take many generations of the pests before they again become noticeable and possibly damaging. The laboratory data available have given an over-optimistic view of the speed of action of modified atmospheres, particularly at lower CO_2 concentrations (e.g. 35%). A 7-day exposure at 35% CO_2 has been suggested for practical use (1), but with tropical conditions and under somewhat higher levels, as in the Rabaul I trial, some survival has been noted. It appears from the limited data in Table V that an initial level exceeding 70% and an exposure time of 10 days under high CO_2 (>35%) is an appropriate dosage regime for CO_2 for complete insect control at temperatures >20°C. This can clearly be modified if susceptible species only are present, e.g. *Galleria mellonella*. The limits of use at lower temperatures have not been determined but substantially larger exposures may be required.

OUTLOOK: The development of nitrogen and CO_2-generated controlled atmospheres is now at a stage where they are ready for commercial evaluation for disinfestation and storage of durable products. There are several remaining problems. On the scientific side much more data is needed on the response of stored product insects to differing atmospheric compositions and particularly on the effect of a varying concentration. The effectiveness of modified atmospheres at temperatures below 20°C is inadequately researched. Evaluation is required on the effect of a decaying concentration of CO_2 as would be observed in a partially sealed structure with an initial charge of gas which received no maintenance doses.

Despite these inadequacies in our basic knowledge, field trials have been sufficiently successful to show that the general technique has a wide range of possible application now. The problems in application are technical and economic. The provision of a suitably gastight system to give gas usages which are competitive in cost with other pest control methods, is a major problem. In Australia there are now techniques available for sealing of all our major types of grain storage. Clearly such sealing must be done at an acceptable cost. Various sealing systems are being investigated at present, but in welded steel structures, sealing costs will be one of the major components of the overall cost of the method. Many general purpose dry containers can be made adequately gastight with minimal effort and are thus suitable for use with modified atmospheres for insect control.

One important pest control system in widespread use, fumigation in stacks under gasproof sheets, has not yet been directly tested to determine whether modified atmosphere storage is economically possible in it. However, ethylene oxide is often added with CO_2 to commodities under sheets for fumigation. The high rate of CO_2 loss observed under such conditions (38) shows that some modification of the technique will be required before efficient CO_2 use will be possible in such circumstances.

Despite these current limitations, there are many advantages of modified atmosphere storage. These include absence of chemical residues from the treatment, absence of effects on germination and many other parameters important in the quality of different commodities and decreased hazard of leakage into workspace of toxic material. These advantages, together with cost competitiveness against many existing practices, ensure that modified atmospheres will be used on a routine basis as a method of insect control in stored products in the future.

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APPENDIX I.

Formulae used:

Efficiency of purging for displacement of gas (E_1)

$$E_1 = C_c \left[\frac{nV_B + V_{HS}}{V_G} \right]$$

Efficiency of purging assuming free mixing throughout the storage atmosphere (E_2)

$$E_2 = -100 \left[\frac{(nV_B + V_{HS}) \ln (1 - \frac{C_c}{100})}{V_G} \right]$$

$$\text{or} \quad = 100 \left[\frac{nV_B + V_{HS}}{V_G} \right] \ln \frac{(21)}{(C_o)}$$

Efficiency of purging assuming complete displacement in the loadspace with free mixing in the headspace of the storage (E_3)

$$E_3 = 100 \left[\frac{nV_B + V_{HS} \ln \frac{(21)}{(C_o)}}{V_G} \right]$$

C_o = Concentration of oxygen at termination (%).

C_c = Concentration of CO_2 at termination (%).

n = Commodity porosity.

V_B = Volume of stored commodity (loadspace).

V_{HS} = Volume of headspace.

V_G = Volume of purge gas added.