Recent Developments in Controlled Atmosphere Technology

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Abstract

Laboratory studies have shown that controlled atmospheres, applied in combination with elevated temperatures, can reduce the exposure time needed for control of stored-product insects. This relationship is demonstrated by the presentation of recently developed data on all life stages of *Lasioderma serricorne* (F.).

Field studies conducted in the United States (U.S.) on the use of carbon dioxide to control stored product insects are described. These range from the treatment of herbs and spices in a 45m^3 chamber to the application of carbon dioxide into a bunker containing 27,680 tonnes of maize, and include an account of the use of carbon dioxide in a tobacco warehouse, a bolted metal bin, and a river barge. The future of controlled atmospheres in the U.S. is discussed.

LITERATURE on the use of controlled or modified atmospheres (CAs, MAs) to control pests of raw and processed agricultural products is extremely abundant. The large amount of literature is caused by serious interest in the subject, interest which has come to a peak twice over the last decade when two symposia on the subject were held and their proceedings published (Ripp et al. 1984; Shejbal 1980).

Most of the work reported, however, has been of laboratory studies on the effects of various combinations of carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) on one or more species of insects. These studies have usually been conducted with adults only, and the gas mixtures used when the effects of CO_2 were being studied are generally not those found when a storage container is actually treated with this gas in the field.

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Large gaps therefore exist in our knowledge on the effects of actual field concentrations of O_2 on all life stages of several of the more important stored-product insects. Jay (1986) reported on the time × elevated temperature × CA concentration effects on all life stages of *Tribolium castaneum* (Herbst). Although this work was conducted at from 32 to 43°C and some exposure times to produce 100% mortality of larvae and adults were not included it did show, for example, that at a CO_2 concentration of 90% (balance air) it took 40 hours to achieve complete mortality of eggs of this species, while at 43°C it took only 16 hours to obtain this level of control.

Future laboratory studies should include all life stages of the test insects involved and be conducted at several realistic temperatures and over the range of CA concentrations that can be attained and maintained in the field. We cannot be confident of achieving high levels of control in field situations under less than perfect circumstances until such data are available for the economically important species of stored-product insects.

An important requirement for use of CAs is that the storage to be treated is sealed to a high standard of gastightness. A remarkable sealing program has been carried out in Western Aus-

tralia to render most of that State's large bulk storages gastight and suitable for CA and treatment with phosphine (Ripp 1984). This program showed what a potent combination of money, talent, research, and manpower can do toward making CAs a viable insect management tool. The rest of the world, however, is not so fortunate. Although research is funded in developed countries such as the Canada, France, Israel, Italy, the United Kingdom, and U.S., most countries still rely on residue-producing conventional fumigants or protectants to control insect pests in storage facilities.

Several reasons were outlined by Jay (1986) for the apparent lack of involvement in the use of CAs in the U.S. These are still generally applicable and include the general lack of commitment to spend the funds needed to seal storage structures and the extreme competition in the phosphine business, leading to greatly reduced prices for this fumigant. Another reason for the lack of commitment is the general reluctance of the producers of CO_2 and N_2 to make a clear decision as to whether or not a large market exists for their products and, subsequently, to attempt to enter this market in a dedicated manner. In the U.S., five large CO_2 producers have labels from the Environmental Protection Agency (EPA) permitting them to use their product as pesticides on all raw and processed agricultural products. Three of these five companies have recently seriously studied the use of CO_2 for health foods, including herbs and spices, for large quantities of maize and sorghum stored in bunkers or temporary storages, and for use in tobacco warehouses. However, this is only a small portion of the potential market.

Carbon dioxide can be competitive in cost with conventional fumigants in the U.S. if the source of supply or plant is close (< 80 km) to the treatment site. Bulk carbon dioxide can, in a situation such as this, cost as little as $66 U.S./tonne (541m^3), rising to $143 U.S./tonne when transported about 400 km. Treatment costs can therefore be more than doubled if the treatment site is far from the production site.

There has been little or no interest in the use of N_2, except in laboratory studies, in the U.S. in the 20 years of sporadic field testing with CO_2. The reason for this is that private corporations involved in the raw and processed agricultural food industry are reluctant to expend the funds to bring their storage vessels up to a high degree of gastightness. This sealing is necessary when N_2 is used for treatment but is not so critical when CO_2 is used and supply is cheap. When CO_2 is purchased at or near $66 U.S./tonne some leaks can be tolerated and the use of CO_2 can be economically feasible when compared with conventional fumigants. However, this is not true when the cost for CO_2 approaches $100 U.S./tonne.

There has been some recent interest in the U.S. on the use of CA or inert atmosphere generators and some government and commercial research has been conducted in this area. The information produced from these studies has not been published and is not available, so this paper will not consider this method of CA application.

This paper will present data on laboratory studies involving all life stages of *Lasioderma serricorne* (F.) at temperatures >27°C. and using several different CAs. It will also present data from field studies involving the use of CO_2 in treating: tobacco in a warehouse; barley in a river barge; herbs and spices in a small container; rice in a sealed bolted metal bin; and maize in a large bunker or temporary storage.

Laboratory Studies on *Lasioderma serricorne* (F.)

Eggs (0–3 days old), larvae (25–26 days old), pupae (2–4 days old as pupae), and adults (0–6 days old as adults) of *L. serricorne* were individually exposed to four CO_2 concentrations and to one N_2 concentration at either 32, 38, or 43°C at 55–60% relative humidity (RH) using laboratory equipment described by Jay and Cuff (1981).

Table 1 shows the results of this study. Eggs exposed at 32°C to 65 or 78% CO_2 were completely controlled in 72 hours while 120 hours were required to obtain this level of control at 90 or 98% CO_2. At 38°C, 72 hours were required to obtain 100% mortality at CO_2 levels of 65 or 90% while those exposed to 78 or 98% CO_2 or to 99% N_2 were completely controlled in 48 hours. Eggs exposed at 43°C were completely controlled in 6–48 hours depending on the CA composition and concentration. Larvae of this species were totally controlled in from 24–96 hours at 32°C with

98% CO_2 giving the fastest kill. At 38°C the time for 100% control ranged from 24–72 hours while at 43°C it ranged from 6–48 hours with the higher CO_2 atmosphere and the N_2 atmosphere generally giving more rapid control.

All pupae of *L. serricorne* were killed at 32°C in 48–96 hours, in 48–72 hours at 38°C, and in 6–24 hours at 43°C when exposed to the five different CAs. Adults of this species were completely controlled in 24–72 hours at 32°C with 65% CO_2 giving the fastest control. The time for 100% mortality was reduced to 16–24 hours at 38°C and to 6–16 hours at 43°C, with the CAs containing 90 and 98% CO_2 giving total control of adults after six hours of exposure.

When the data for all life stages are pooled, the longest exposure time required to produce 100% mortality of these stages at 32°C was 120 hours, at 38°C 72 hours, and at 43°C 48 hours. The CA containing 99% N_2 produced 100% mortality of all life stages at 32°C in 96 hours or less. At 38°C the N_2 atmosphere produced 100% mortality of eggs, larvae, and pupae in 48 hours or less, i.e. as fast as, or faster than any CA containing CO_2. At 43°C the N_2 atmosphere produced 100% mortality in 24 hours which was faster than all the CAs containing CO_2 except for the atmosphere containing 98% CO_2 which controlled all life stages in six hours at this temperature and the CA containing 90% CO_2 which controlled adults in six hours.

Carbon Dioxide Treatment of a Tobacco Warehouse

L. Ryan, D.L. Faustini, and R.M. Lehman of Philip Morris, Inc., U.S., were the first to successfully treat bulk (> 28 000 m^3) tobacco storages with CO_2 in 1987 (L. Ryan, pers. comm. 1988). They found that maintaining a 40% CO_2 concentration for 8 days killed all stages of *L. serricorne* at a commodity temperature of 26.7°C. Brown and Williamson, Lorillard, and American Tobacco Companies, U.S., have also investigated CO_2 as a means of disinfesting warehouses, small chambers and shipping containers.

Keever (1989) treated with CO_2 a 12 700 m^3 warehouse located in North Carolina, U.S., which contained 1005 tonnes (3320 m^3) of tobacco. All life stages of *L. serricorne* were used for bio-assay. They were placed at the centres of the two types of tobacco containers (hogsheads: 1.42 m^3; cases: 0.66 m^3) and in the warehouse free air space. Nine gas sampling lines were installed in the warehouse: four within the bulk of the tobacco containers adjacent to the caged insects and five in the free air space inside the building.

Liquid CO_2 from a road tanker was injected into the bottom of the building through 1.3 cm i.d. copper tubing and a liquid injection nozzle which converts liquid CO_2 to a powdery form of dry ice. The dry ice was changed to gas by the elevated temperature of the warehouse and the turbulence of injection. Pressure was relieved in this and subsequent applications by opening a door on the opposite end of the building.

The initial purge of the building required 3.75 hours and 18 tonnes of CO_2 were used to bring the concentration to about 60% throughout the warehouse. After the initial purge, daily applications were made in the late morning hours for the remainder of the test to bring the concentration back to about 60% CO_2. About 9 tonnes were used in the first five daily

Table 1. Time (h) required to obtain 100% mortality of *Lasioderma serricorne* when exposed to five controlled atmospheres at three elevated temperatures and 55 to 60% RH.

Atmosphere (%)[a]		Eggs			Larvae			Pupae			Adults		
CO_2	N_2	32°C	38°C	43°C	32°C	38°C	43°C	32°C	38°C	43°C	32°C	38°C	43°C
65	28	72	72	48	96	72	48	72	48	24	24	24	16
78	18	72	48	48	72	48	24	48	48	16	48	16	16
90	8	120	72	30	72	48	16	96	72	16	48	16	6
98	1	120	48	6	24	24	6	96	48	6	72	24	6
0.03	99	96	48	24	48	24	16	96	48	16	72	24	16

[a] Balance of concentration is O_2, argon and rare gases

applications which required about 1.5 to 2.0 hours to complete. About 7 tonnes were used on the day before aeration of the warehouse. A total of 71 tonnes CO_2 was used during the test, which lasted seven days. Two fans were placed on the floor of the warehouse to distribute the CO_2 and were run continuously during the test. The temperature of the tobacco was 23.3°C during the test, while free air temperature ranged from 22.2–26.7°C (maximum) and from 11.7–16.7°C (minimum). The mean free air temperature was 22.9°C The RH in the free air space ranged from 59–96% with a mean of 65.4%.

Table 2 presents the mean pre- and post-purge CO_2 concentrations in the free air space of the warehouse. The prepurge CO_2 concentrations ranged from 34–39% while the postpurge concentrations ranged from 60–62%.

Table 3 shows the CO_2 concentration in the cases and hogsheads prior to the daily purge. The mean CO_2 concentration from day 2 to 7 was 38–42% in the cases and 42–45% in the hogsheads. The hogsheads were retaining more CO_2 because of their larger size.

One-half of the cages containing *L. serricorne* were removed from the warehouse after 5 days of treatment and the remainder at the end of the test. All insects from both the 5- and 7-day exposures were killed and mortality of control eggs, larvae, and adults was very low. Mortality of control pupae reached 36%, possibly because of handling injury.

The test showed that CO_2 will control *L. serricorne* in field situations. However, it is obvious that the warehouse was very leaky and a large amount of CO_2 was required to obtain this result. At a CO_2 cost of U.S.$77/tonne, the cost for this treatment was $5.44/U.S. tonne of tobacco. This heavy use could probably have been reduced by using 0.3–0.4 mm polyvinyl chloride (PVC) sheeting to seal doors, windows, and vents as a substitute for the 0.15 mm polyethylene sheeting that was used in the test. Also, the practice of rapid addition of CO_2 to bring the concentration up to about 60% each day could have been replaced by a slow continuous addition of this gas into the upper regions of the warehouse to compensate for the leaks. This technique would have probably greatly reduced the amount of CO_2 applied daily after the initial purge.

Table 2. Mean pre- and post-purge CO_2 concentrations in the free air space of a warehouse containing tobacco

Day	CO_2 (%) Prepurge	Postpurge
1	0[a]	60
2	36	62
3	34	61
4	34	60
5	36	61
6	38	62
7	37	62
8	39	0[b]

[a] Prior to treatment

Table 3. Mean CO_2 concentrations in cases or hogsheads containing tobacco prior to daily addition of this gas to warehouse or about 24 hours after previous application.

Day	CO_2 (%) in Cases	Hogsheads
1[a]	32	15
2	41	42
3	39	44
4	38	45
5	42	45
6	41	45
7	42	45
8[b]	52	54

[a] Readings taken after initial purge
[b] Readings taken about 12 hours after previous application

Treatment of a River Barge Containing Barley

A river barge with two grain holds about 9 m wide, 15 m long, and 12 m deep was filled with 1415 tonnes of barley. During the 4-hour loading period 4.8 tonnes of CO_2 were applied from a road tanker through a hose and a liquid injection nozzle. After loading, an additional 4.5 tonnes of CO_2 was injected below the surface of the grain along the longitudinal axis of the barge during a 2.5 hour period. The barge was shipped the following day from its loading point, Clarkston, ID, U.S., to a terminal elevator at Portland, WA, U.S.

Before loading, gas sample lines and caged insects were suspended in the barge. Four cables, each having 9 cages of insects in groups of three each and three gas sampling lines attached to them at different depths were

used in the study. The gas sampling lines were located next to the cages and two cables held sampling lines and cages at depths of about 0, 5, and 10 m from the bottom of the holds. The second pair of cables was positioned so that the sampling lines and cages were located 0, 3, and 7 m from the bottom of the holds. Bioassay was conducted using a mixture of from 1–5-week-old *Rhyzopertha dominica* (F.). About 5 g of wheat containing these immatures was placed in each cage.

Table 4 shows that the initial concentration in the barge immediately after application ranged from 61–100%, with the higher concentrations located at the middle and bottom sample sites. Gas samples taken after the barge had been in transit for 2.8 days showed that there was little or no CO_2 at the top sampling points, from 22–70% CO_2 at the middle sites, and from 75–90% CO_2 at the bottom sampling points. The barge arrived in Portland about 4.7 days after the CO_2 application. At that time there was little or no CO_2 in the top of the barley from 0–41% in the middle of the bulk, and from 57–86% CO_2 in the bottom of the grain bulk. The grain temperature dropped from 38°C at the start of the test to 35°C after 2.8 days and to 23°C at the time the grain reached the terminal elevator.

Determination of the effectiveness of the treatment was made by comparing the number of live insects that had emerged from the treated grain to those that emerged from untreated grain and converting this to a percent reduction in emergence (PRE) after the 30-day post exposure examination. Table 5 shows that from a + 9.1 (increase over control emergence) to a 77.4 PRE occurred in those insects treated in the top layer of barley in the barge. A 92 to 100 PRE was observed in the insects in the middle of the grain bulk and a 100 PRE was recorded for the insects in the bottom of the barge. The mean number of control insects that emerged per cage from 10 cages was 20.8.

Table 4. Percent CO_2 at 3 depths/4 locations in a river barge containing barley

Sample	Days after CO_2 application		
Depth	0	2.8	4.7
Top	64–68	0–2	0–1
Middle	75–100	10–22	0–41
Bottom	61–97	75–90	57–86

The loss of CO_2 in the upper level of the barley and the consequent inadequate control of the insects in this area was initially attributed to poor sealing of the hatch covers. However, during a safety inspection prior to unloading, CO_2 concentrations of 40–50% were found in the access areas outside the holds and adjacent to the bearings for the unloading augers which were built in on this barge. This was possibly the major reason for the drop in concentration although the loose hatch covers were believed to be partially responsible.

Carbon Dioxide Treatment of a Chamber Containing Herbs and Spices

A 45.3m^3 truck body was used as a chamber for the CO_2 treatment of seven different herbs or spices in their original shipping containers. Descriptions of the commodities and their containers are given in Table 6. The truck body was 7.6 m long, 2.4 m wide, and 2.4 m high, and was supposedly well sealed for methyl bromide fumigation of herbs and spices. Five cages, each containing about 30 larvae of either *Tribolium confusum* (J. duVal), *Trogoderma glabrum* (Herbst), *Attagenus megatoma* (F.), *L. serricorne*, or *Plodia interpunctela* (Hübner) were placed within the bulk of the commodity at each of 10 locations within the chamber. Gas sampling lines and thermistors for temperature measurement were placed next to the cages in the bulk of the commodity and an additional set of 5 cages, a gas sampling line, and thermistor were placed in the free air space above the commodity.

A supply vessel containing 340 kg of liquid CO_2 was used to treat the chamber. The CO_2 was injected using a plastic pipe which ran from the vaporiser to the floor at the front of the chamber. A sheet of PVC was placed in front of the rear doors to prevent leaks in that

Table 5. Percent reduction in emergence (PRE) of *Rhyzopertha dominica* resulting from the CO_2 treatment of a river barge containing barley[a].

Depth	PRE at insect cage location 1	2	3	4
Top	76	75	77	+9.1
Middle	92	100	100	98
Bottom	100	100	100	100

[a] Locations 1 to 4 were forward to aft in the barge

area, which was partially opened when the chamber was being initially purged with CO_2. It was sealed during the remainder of the test and a small flow of CO_2 maintained to compensate for leaks.

Two large electric heaters were placed in the chamber to raise the temperature of the commodity and reduce the exposure time. These heaters maintained the temperature in the free air space from 32°–48°C and caused a gradual rise in temperature in the commodities (Table 7). The initial purge of CO_2 lasted 7.5 hours and brought the concentration in the free air space to ca. 96%. The CO_2 concentration was maintained for an additional 111.5 hours and during this period ranged from 68–100% with most readings showing a mean free air space concentration of over 90%. A total of 1134 kg of CO_2 was used for this treatment, or about 3 to 4 times the amount that would have been needed in a tightly sealed container of this size.

Table 8 shows the CO_2 concentrations in representative herbs and spices and in the free air space. The CO_2 readily penetrated through the container walls into all the commodities, except for the sealed cardboard drum containing lemon peel where the concentration slowly rose to resemble that of the free air space.

Only 5 of the 1608 larvae that were exposed in the chamber survived the CO_2 treatment. One *L. serricorne* larva in a cage in a box of camomile flowers was alive at the end of the test, while four *T. glabrum* larvae survived in the cage placed in a cardboard box with a plastic liner containing parsley flakes. Control mortality was 20% for *P. interpunctella* larvae, 6% for *T. glabrum* larvae, and 0% for *T. confusum*, *L. serricorne* and *A. megatoma* larvae.

Table 6. Herbs and spices treated with carbon dioxide in a 45.3 m^3 chamber

Commodity	Package	Weight (kg)
Chamomile flowers	Cardboard box	11.3
Peppercorn	Burlap	63.5
Sage leaf	Woven poly, heat sealed	11.3
Poppy seed	Multiwall paper	22.7
Parsley flakes	Cardboard box, plastic liner	6.4
Basil	Burlap	25.0
Lemon peel	Cardboard drum	45.4

Table 7. Temperature in representative herbs or spices or in free air space during CO_2 treatment of herbs and spices

Commodity	°C at hours after initiation of purge			
	24	48	72	101 h
Free air	42	40	44	38
Chamomile	28	30	31	31
Peppercorn	19	23	25	28
Lemon peel	27	29	31	32 (cracked lid)
Lemon peel	26	29	31	32 (sealed lid)

Table 8 Carbon dioxide concentrations in representative herbs or spices or in free air space during chamber treatment.

Commodity	CO_2 (%) at hours after start of purge[a]			
	24[b]	48	72	101 h
Free air	68	94	88	97
Chamomile	71	94	88	98
Peppercorn	69	95	88	99
Lemon peel	80	90	89	100 (cracked lid)
Lemon peel	48	69	72	94 (sealed lid)

[a] Balance of concentration is air
[b] CO_2 flow off for 4 hrs prior to this reading

The doors to the container were opened at the end of the treatment and the sheet of PVC removed. A large box-type window fan was placed at the rear of the container and run for about three hours. Carbon dioxide was not detected in the free air space after this aeration and no more than 1% was detected in the containers having sample lines in them.

Treatment of Rice in a Bolted Metal Bin

A bolted metal bin was sealed by spraying with a PVC formulation on the joints of the overlapping sheet metal to reduce gas loss. An attempt was made to seal the wall to cap joint with polyurethane foam. The bin was then filled with 544 tonnes of rough rice and all ventilators, access ports, and the shaft to the aeration fan were covered with plywood which was sealed to the metal with silicon rubber adhesive. Sealing costs for the bin were (all U.S.$): labour $1344; materials $900; and equipment $1200. Assuming that the equipment would be used to seal 20 bins at the facility, the costs for sealing this bin was about $4.00/tonne. An effort was made to pressure

test the bin before CO_2 application, but some regions were apparently not properly sealed and the attempt failed.

Seven gas sample lines were placed from the top to the bottom of the bin at intervals of 1.8 m. A thermistor was placed in the bin 1 m below the surface of the grain in the area where insects were exposed for bioassay. Immature *R. dominica* were used for bioassay as this is the main pest of rice in the South Florida, U.S. location where this trial took place. Laboratory cultures 1, 2, 3, 4, or 5 weeks old were blended and about 5 g of the grain containing the immatures was placed in each of 50 cages. These cages were put into groups of 5. Eight of these groups were placed about a metre below the surface of the rice to be treated. The other two groups were not treated but held in an adjacent building in a large bag of rice from the treated bin.

The bin was purged from the bottom, with the pressure relieved by opening an access port at the top. A 4-hour purge at a rate of 408 kg/hour produced a 94–97% CO_2 concentration throughout the bin. Flow was reduced at this time and was adjusted to compensate for leaks. The access port was closed and sealed. A mean of 35.9 kg/hour of CO_2 was applied into the bin headspace to compensate for leaks for an additional 4 days, making the total used 5760 kg. The total cost for CO_2 at U.S.$134 per tonne was U.S.$762, making the treatment cost per tonne of rice U.S.$1.40. During the four days the CO_2 was slowly introduced into the bin the concentration at the bottom of the bin ranged from 57–98%; in the middle of the bin it ranged from 52–86%; and in the top at the same depth as the caged insects it ranged from 49–90% with a mean of 66.2%. The mean temperature of the grain 1 m below the surface was 31.6°C during the test.

Bioassay was successful in that no *R. dominica* adults emerged from the grain that was treated with CO_2, while a mean of 20.5 adults per cage emerged from the controls held in untreated grain.

Carbon Dioxide for Control of a Natural Infestation in a Bunker Containing Maize

A well-sealed PVC-covered bunker containing 27 600 tonnes of shelled yellow maize was treated with CO_2. Before treatment it had become naturally infested with a wide range of insect pests. The bunker was approximately 137 m long, 46 m wide, and 12 m high at the peak. It was constructed on an asphalt base with reinforced concrete walls and had a 0.3 mm thick cover which was tightly attached to the walls. Grain temperatures, as recorded from 38 thermocouple cables installed in the bunker, ranged from −5 to 40°C. However, the temperature of most of the grain ranged from about 2–15°C. The high temperatures were caused by insect and mould activity, and associated heating and moisture migration. The mean moisture content of the grain was 14.7% and ranged from 12.2 –28.7%.

Sixteen gas sampling lines were probed into the maize. Three sets of four lines were placed at depths of 3, 5.5, and 8.5 m at the northern and southern ends and at the center of the bunker. An additional line was placed about 1 m deep in the maize at each of the four corners. Before CO_2 application, 87 maize samples, each of about 0.7 L, were taken by vacuum sampler at depths of either 0.3 and 1.5 m, or 0.3, 1.5 and 3 m, or 0.3, 1.5, 3, and 6 m at 5 locations across the width of the bunker. This pattern was repeated 5 times down the length of the bunker. Similar samples were taken at 0.3 and 1.5 m or 0.3, 1.5, and 3 m at the northern and southern ends of the bunker. Ninety-five samples were taken from the same sites in the same manner at the end of the test.

Before treatment, the bunker was pressure tested using a large vacuum cleaner. An initial vacuum of 0.62 cm of water was obtained. This dropped to 0.46 cm after five minutes and to 0.33 cm of water after 10 minutes, indicating that the bunker was well sealed.

Carbon dioxide was applied from a mobile vessel which was filled, from three road tankers, with 54.5 tonnes of liquid. The liquid was converted into gas by a vaporiser and then led into the aeration shaft running the entire length of the bunker. The purge took 66.25 hours and 39.9 tonnes were applied in this period. An additional 1.6 tonnes of CO_2 was applied four days after the initial purge was stopped, over a period of about four hours. A small aeration fan was modified for use as a recirculation fan and this was attached to a hole cut in the end of a piece of plywood which sealed off a second longitudinal aeration shaft. A 10-cm i.d. hose was attached to the fan and

the hose was run to the top of the bunker and under the fabric. The system was designed to gently recirculate CO_2, carrying it from the bottom of the bunker to the top to ensure that no areas of low CO_2 concentration formed. The fan was generally run for about 10 hours each day for the 15 days the test was in progress.

Table 9 presents the mean and range of CO_2 concentrations in the bin following the purge. The mean CO_2 concentration was 79.7% one day after application stopped and 72.2% after three days, indicating a 3.3% per day loss of CO_2. A cold front came through the area during this period, with winds of 40–50 km/hour. Two small aeration fans were placed in the grain bulk on top of the bin to pull the cover down tight over the maize as there was some possibility of it blowing loose. These fans were operated intermittently throughout the test whenever it was windy and it is believed that this contributed to the loss of CO_2 and also to the variability of this loss. The daily CO_2 loss rate varied from 6.6 to 7.3% during the third through the seventh day of the test; from 6.6 to 5.5% between the seventh and tenth days of the test; and from 4.5 to 5.5% between the tenth and twelfth days. Daily percentage losses were 4.6% from the twelfth to the fifteenth days. A total of 41.5 tonnes of CO_2 was used in the test and the total cost for treatment was U.S.$3206 or U.S.$0.116 per tonne when the delivered price for CO_2 was $77 U.S. per tonne.

Table 9. Mean and range of CO_2 concentrations in a bunker containing maize

Days after start of test	CO_2 (%) Mean	Range
1	79.7	58-90
3	72.2	43-78
7	65.1	16-82
10	54.7	37-69
12	44.5	15-60
15	43.1	24-50

The following insects were collected from the bunker: *Oryzaephilus surinamensis* (L.), *Cryptolestes pusillus* (Schoenherr), *Ahasverus advena* (Waltl), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Sitophilus zeamais* Motschulsky, *Typhaea stercorea* (L.) and Pteromalidae.

The presence of *T. stercorea* (fungal-feeding beetles) and parasitic wasps indicate that the maize was in poor condition with mouldy regions and was heating. Table 10 shows that, of the original 87 samples collected before the treatment, 28 were infested with live insects and these 28 samples contained a mean number of 31.7 insects at the seven-day examination. Ten of these pretreatment samples could not be examined because of heavy levels of fungus growth in them after a 30 or 60 day holding period. At the 30 day examination, 41 of the examined 77 pretreatment samples had a mean infestation rate of 78.2 insects per sample while at the 60 day examination the 40 samples contained a mean of 110.8 live insects per sample. The increase in the number of samples infested after 7 days (28 out of 87) to the number of samples infested after 30 days (41 out of 77) is due to the presence of undetected immatures or eggs in the maize at the 7 day examination.

Table 10 also presents data from the examination of the 95 posttreatment samples. Only one live insect was found at the 7 day examination while three live insects were found in two samples at the 30 day examination. A total of five live insects was in 3 samples at the 60 day examination. The number of insects per sample

Table 10 Number of samples infested out of 87 pretreatment or 95 posttreatment samples collected; total number of live insects, and percent reduction in emergence (PRE) resulting from a carbon dioxide treatment of a bunker containing 27,680 tonnes of maize[a]

Days after sampling	Samples taken: Pretreatment Infested	Pretreatment Insects	Posttreatment Infested	Posttreatment Insects	PRE (%)
7	28	856	1	1	>99.99
30	41	3,207	2	3	>99.99
60	40	4,430	3	5	>99.99

[a] 10 pretreatment samples were heavily infested with fungus and were not examined after 30 and 60 days

in the pre-treatment samples (either 77 or 88) was raised to the equivalent of the 95 posttreatment samples and the number of insects in the pretreatment samples was divided into the number in the posttreatment samples. The result was converted to percent reduction in emergence (PRE) for the test. The PREs presented in Table 10 are all well over 99.99% for all three examinations for the samples indicating a very high level of control of the insects in the bunker. About two months after the test was complete the bunker was opened and 450 tonnes of grain damaged by moisture and fungus were removed. Live insects were not found during this operation.

Discussion

The laboratory study reported here on *L. serricorne*, together with the information provided by Childs and Overby (1983), provides some guidance for attempts to control this insect in the field. This is substantiated by results of the bioassay in the field study in a tobacco warehouse. If someone has not already done so, perhaps a computer program could be developed to analyse the vast amount of available data on CAs. Such a program would include time × temperature × CA concentration × insect species and life stage information which could be correlated into readily usable parameters for field use of these atmospheres.

Five field tests were reported on in this paper and only one, the use of CO_2 in a bunker containing maize, can be considered successful from an economic point of view since the CO_2 cost per tonne of maize in this study was $0.116/U.S. tonne. Three of the other four studies had costs for CO_2 ranging from $0.58 U.S./tonne in the barge study to $1.40 U.S./tonne in the rice study (plus sealing costs), and went up to $5.44/U.S. tonne in the tobacco study. These costs are not acceptable even by U.S. standards. Jay and D'Orazio (1984) reported CO_2 costs of from $0.23 to $0.39 U.S./tonne for the treatment of wheat, sorghum, rice, or maize in upright concrete, fiberglass-lined steel, or welded steel bins.

These tests, with the exception of the barge test, were highly successful in that they all produced insect mortality approaching or attaining 100% of the caged or natural infestations and, in two cases (tobacco, herbs and spices), provided new user groups the opportunity to witness this alternative, residue-free method of control.

Industry has acted on these studies. One small pest control company treated over 750,000 tonnes of maize and sorghum in bunkers in 1988 and has had no subsequent reports of insect activity in this grain. Similarly, in 1988 a large CO_2 processor successfully treated tobacco warehouses with a volume of over 285,000 m^3. The rice processor is continuing to use CO_2 because his product is for health food stores whose customers see residues as undesirable. Similarly, U.S. herb and spice trade organisations are very interested in CAs because a large portion of their product is destined for the health food market.

Perhaps this trend represents the direction CA use will take in the near future in the U.S. Several commodities studied in the tests described in this paper have a high unit (tonne, kg, etc.) value and their end users often object to pesticide residues. The trend may carry through into commodities such as tree nuts, dried fruit, and groundnuts—which have a high value per unit—and from there to general grain.

Economics is generally the driving force in any decision to change from conventional pesticides or fumigants to CAs. The economics is not necessarily directly related to costs of treatment and/or sealing but can be concerned with worker safety, residues, the emission of conventional fumigants into the atmosphere, the time needed for treatment, the regulatory removal of a pesticide or fumigant from use, or other factors. We are observing a gradual change to CAs in some areas of the raw and processed agricultural food industries in the U.S. Time and regulatory pressure will possibly be the main factors bringing about changes in this area.

Acknowledgments

The authors would like to acknowledge the able assistance of G.C. Pearman, Jr. of the USDA Stored-Product Insects Research and Development Laboratory, Savannah, Georgia 31403, U.S., in many phases of several of the laboratory and field studies reported here. J.E. Overby, USDA Tobacco Research Laboratory, Oxford, NC 27565, U.S., contributed greatly to the tobacco warehouse study. The authors appreciate the generous spirit of cooperation shown by R. D'Orazio of Ameri Gas, Inc., Carbon Dioxide

Division, Dallas, TX 75233, U.S. in the bunker study and J. Manner of this company in the river barge study. G. Baskin, T. Lane, and C. Briceland of Liquid Carbonic Corp., Chicago, IL 60629, U.S. were closely involved in the studies on the herbs and spices, and rice.

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