

# CARBON DIOXIDE AS A FUMIGANT TO REPLACE METHYL BROMIDE IN THE CONTROL OF INSECTS AND MITES DAMAGING STORED PRODUCTS AND ARTEFACTS

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**Abstract**—Mixed stage cultures of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Liposcelis bostrychophila* Badonnel, *Tyrophagus putrescentiae* (Schrank) and *Acarus siro* L. were placed inside, between and under 32kg sacks in a one tonne stack of bagged flour and exposed to 60% carbon dioxide in a fumigation "bubble" for 4, 7 or 14 days at about 20°C.

*T. putrescentiae* survived 14 days exposure. Complete control of *T. castaneum* required 7 days exposure; *S. oryzae*, *L. bostrychophila* and *A. siro* required 14 days. Burial within the flour stack apparently did not significantly impede the penetration of carbon dioxide to the buried cultures.

The flour stack was preheated by circulating warm air (22-29°C) around it. The temperature inside a bag in the lower part of the stack took 120 hours to rise from 11°C to 20°C. Free air space temperature near the stack centre responded similarly.

The use of carbon dioxide to control insects and mites infesting commodities (other than bulk grain) and artefacts, and its limitations as a structural fumigant, are discussed.

## INTRODUCTION

Because of methyl bromide's alleged role in ozone depletion it appears that its uses will become increasingly restricted (Anon 1992b). Carbon dioxide can replace methyl bromide as a fumigant in some applications. High concentrations of carbon dioxide retained in well-sealed enclosures have been used to control stored product insects in food commodities, particularly grain (Jay 1986) and insects infesting museum artefacts (Burke 1993).

Previous work at this laboratory has shown that several species of stored product insect and mite may be controlled by exposure for 14 days or less to atmospheres containing 60% carbon dioxide at 23 or 25°C (Newton 1991). However those tests were conducted on arthropods held in aliquots of no more than 200 grams of culture medium, which was unlikely to present any significant barrier to carbon dioxide penetration. Under field conditions some arthropods are likely to be buried in larger bulks of densely packed commodities such as flour.

Various means were considered of measuring carbon dioxide concentrations inside a bulk of flour. However, no simple means was available which would not be likely to accelerate the penetration of gas through the interstitial spaces by drawing off significant volumes of gas from the sampling point. The ideal means would be an infrared absorption cell buried in the commodity and linked to external instrumentation by a fibre optic cable. Such a system is theoretically feasible but not commercially available. Bioassay was therefore chosen as a crude means of determining whether carbon dioxide could penetrate flour reasonably rapidly. This study used a small scale, field setup at a typical UK warehouse temperature (20°C) to examine the ability of carbon dioxide to kill arthropods buried within a stack of bagged flour, and to investigate the practical problems of fumigation with carbon dioxide.

## METHODS AND MATERIALS

**Outline of experiment.** Mixed stage cultures of arthropods were placed in various positions within a one tonne stack of bagged flour inside a fumigation bubble (Smith 1988). The free air space within the bubble was maintained at about 20°C and 60% carbon dioxide. The arthropod cultures were exposed to carbon dioxide for 4, 7 or 14 days, and were then transferred to standard culturing conditions. There they were incubated for long enough for any surviving eggs and/or pupae to hatch and develop into late immature stages and/or adults which would be readily visible upon inspection.

**Scheduling test cultures.** Cultures of insects and mites were set up in pairs to a timetable such that at the start of exposure to carbon dioxide each pair of cultures would contain all stages of that

Table 1. Schedule of pre-exposure inoculation and post-exposure inspection of test cultures

Species	No. of days prior to start of exposure to carbon dioxide at which :-		No. of days between end of exposure and final inspection of cultures
	"M" cultures inoculated	"Y" cultures inoculated	
<i>S. oryzae</i>	57-58	13-15	62-63
<i>T. castaneum</i>	45	16-17	50
<i>L. bostrychophila</i>	34-35	8-9	37-39
<i>T. putrescentiae</i>	22-23	8-9	28
<i>A. siro</i>	13	8-9	18

Table 2. Rearing media and rearing conditions of test cultures of insects and mites

Species	Rearing medium (composition by weight)	Rearing conditions
<i>Sitophilus oryzae</i>	wheat	25 ± 1°C, 50 ± 5% r.h.
<i>Tribolium castaneum</i>	plain white flour : dried yeast powder (1:1)	25 ± 1°C, 50 ± 5% r.h.
<i>Liposcelis bostrychophila</i>	skimmed milk powder : wheatgerm : dried yeast powder : wholemeal flour (2:2:4:3)	27 ± 1°C, 60 ± 5% r.h.
<i>Tyrophagus putrescentiae</i>	dried yeast powder : wheatgerm (6:1)	22 ± 1°C, 80 ± 5% r.h.
<i>Acarus siro</i>	dried yeast powder : wheatgerm (6:1)	22 ± 1°C, 80 ± 5% r.h.

species (Table 1). Young ("Y") cultures of beetles (*S. oryzae*, *T. castaneum*) contained adults, eggs and young larvae; mature ("M") cultures contained older larvae, pupae and adults. "Y" cultures of booklice (*L. bostrychophila*) and mites (*T. putrescentiae*, *A. siro*) contained a high proportion of young stages; "M" cultures contained more balanced populations.

**Inoculating test cultures.** Standard rearing media and conditions were used (Table 2). For beetle cultures approximately 30 adults were inoculated into 200g or 84g of medium (*S. oryzae* "M" and "Y" respectively) and 172g or 60g of medium (*T. castaneum* "M" and "Y"). At least 50 individuals of *L. bostrychophila* were inoculated into 110g or 66g of medium ("M" and "Y"). For mite cultures a few millilitres of heavily populated culture medium were inoculated into 105g or 72g of medium ("M" and "Y"). Lower weights of medium (just sufficient to stock the bioassay tubes) were used for "Y" cultures to ensure a high density of immature stages.

**Setting up bioassay tubes.** These were clear plastic 30ml, 25mm diameter tubes, open at both ends. The ends were closed with 64 µ aperture mesh to allow free gas exchange. Two days before the start of exposure a sample of about 20ml of "M" or "Y" culture was placed in each tube.

**Stack placement and post-exposure assessment of bioassay tubes.** One day prior to the start of exposure the bioassay tubes were installed in their test positions. For each species a pair of tubes ("M" and "Y") was placed in each of four positions (B1 to B4, Figure 1). B1, B3 and B4 were buried in the centres of bags of flour; cuts in the bag walls were closed with adhesive PVC parcel tape. B2 was placed in the space between two sacks. At the end of the exposure period the tubes were removed from their test positions and incubated under standard rearing conditions (Table 2). Four days after the end of exposure an initial superficial check was made of the contents of each tube for signs of survival (ie movement). Approximately one generation time plus seven days after the end of exposure (Table 1) the bioassay cultures were subjected to a detailed final check. If no live arthropods could be seen the tube contents were spread out on a tray for further examination. A low power microscope was used to examine booklouse and mite bioassay cultures on both initial and final checks.

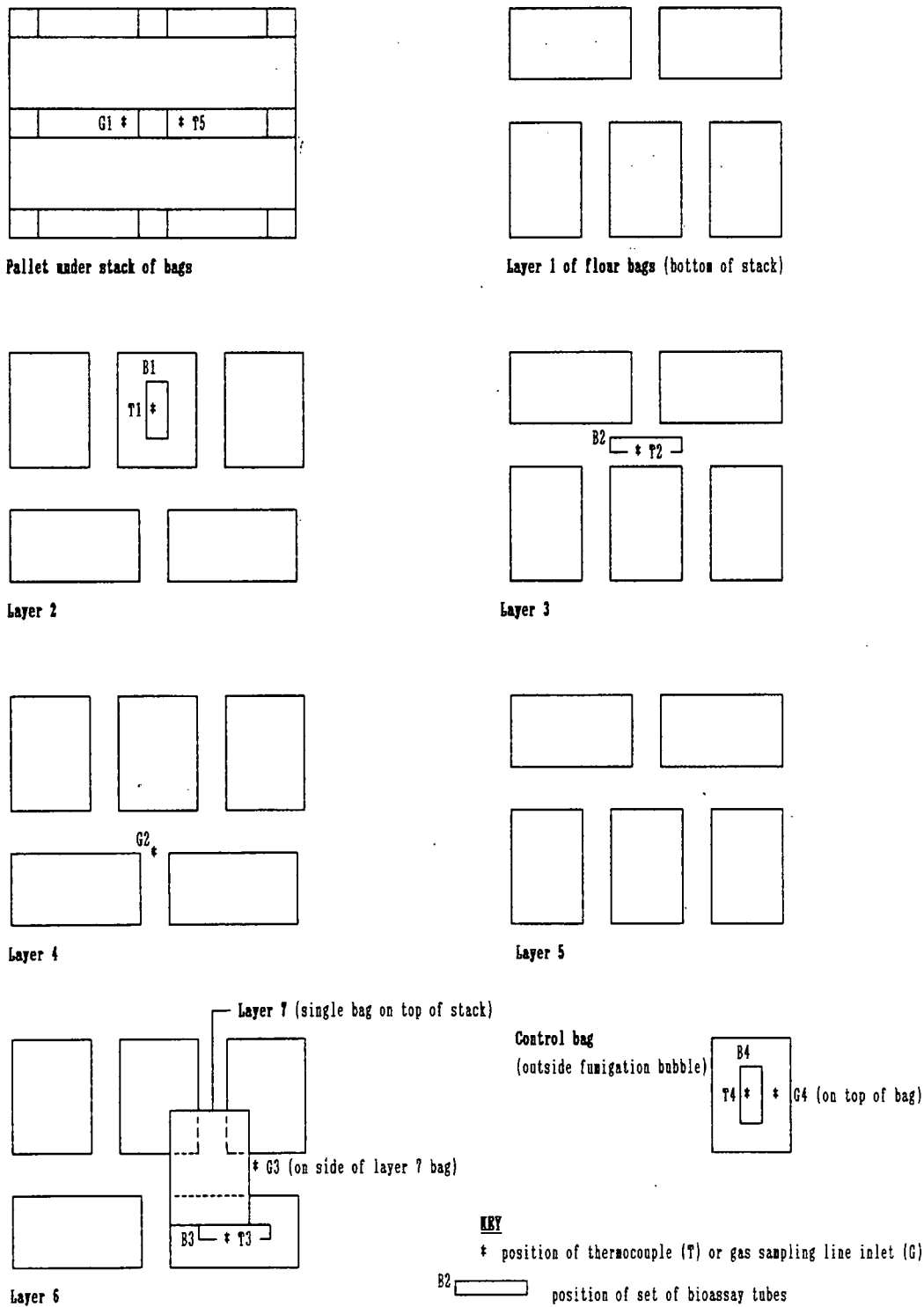
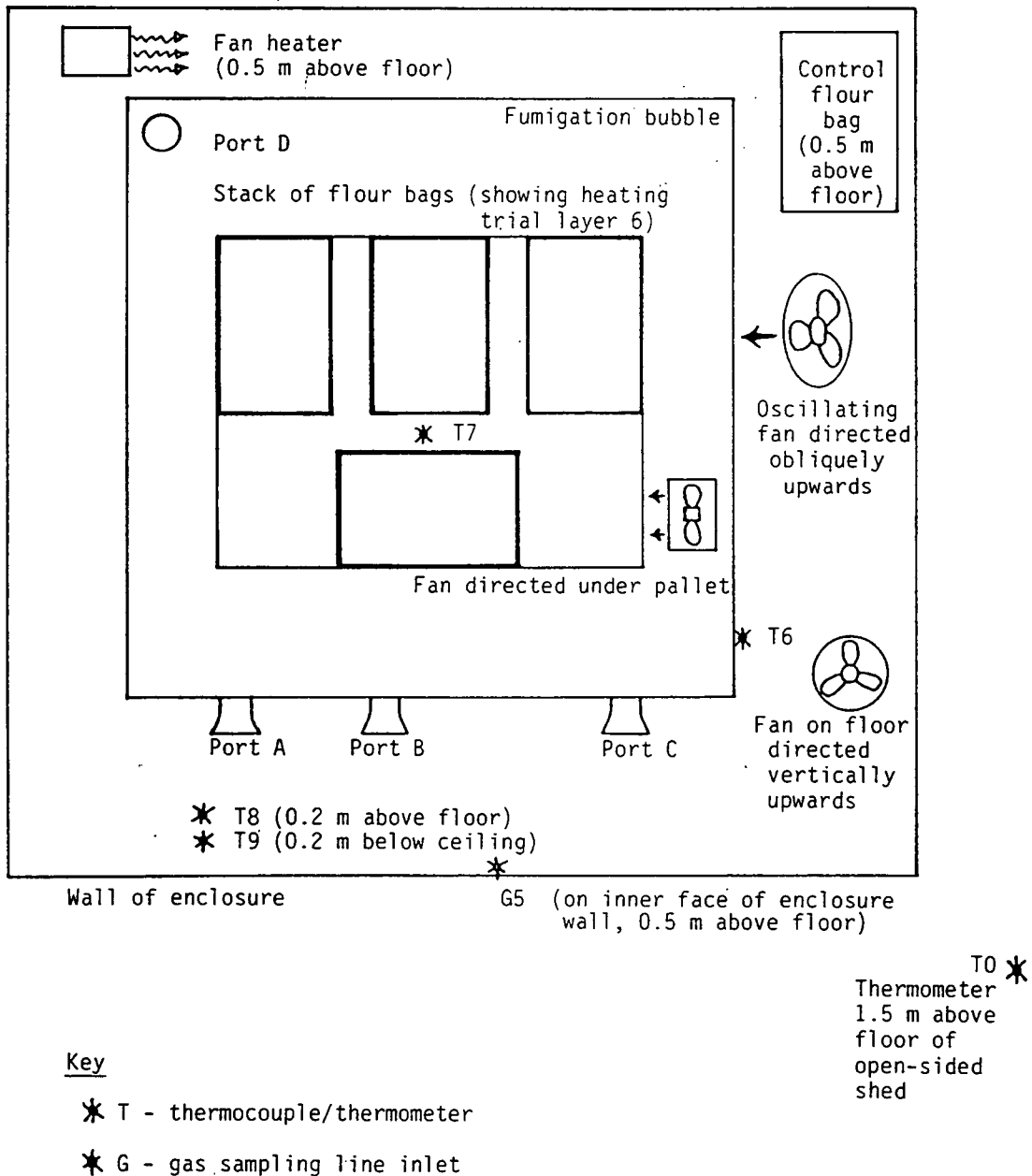


Figure 1. Positions of bioassay culture tubes, thermocouples and gas sampling line inlets amongst layers of bags in flour stack

**Setup and monitoring of fumigation bubble and flour stack.** A purpose-built fumigation bubble was used (2.0m × 2.0m × 1.7m high, inflated volume about 7m<sup>3</sup>). The bubble was a small version of the standard 30m<sup>3</sup> commercial bubble (Smith 1988). It was fabricated from standard materials (PVC-coated polyester fabric sheet with a synthetic rubber gas tight “zip” joining the base and top



Note - Inlet ports A, B and C in bubble base sheet  
 - Inlet port D in upper corner of bubble top sheet

Figure 2. Plan view of layout of insulated enclosure, stacked flour and fumigation bubble (fan positions as at later stage of heating trials)

sheets) and fitted with four inlet ports (Figure 2). The bubble was set up in a  $16\text{m}^3$  insulated enclosure in an unheated, open-sided shed (Figure 2). A tonne of low grade, white, unbleached flour was used as purchased, packed in  $32 \times 32\text{kg}$  multiwall brown paper bags (sacks) with stitched ends. Thirty-one bags were tightly stacked in a standard configuration (six layers of five bags, one bag on top) on a standard  $1.1\text{m} \times 1.3\text{m} \times 0.15\text{m}$  high softwood pallet. The stack (including pallet) measured  $1.15\text{m} \times 1.4\text{m} \times 1.2\text{m}$  high. The pallet stood on a sheet of  $13\text{mm}$  thick insulation board on the bubble base sheet, which was laid on  $6\text{mm}$  thick hardboard on the concrete floor. The remaining "control" bag of flour was held inside the enclosure but outside the bubble.

Bioassay tubes, gas sampling lines and thermocouples were placed in position during the rebuilding of the stack on the day before the start of each carbon dioxide exposure period. Gas sampling lines and thermocouple cables were fed into the bubble via ports "A" and "B" (Figure 2). Gas sampling line inlets G1 to G5 were secured in free air space (Figures 1 and 2). Thermocouples were placed under the stack (T5) and alongside the bioassay tubes, either between (T2) or buried inside (T1, T3, T4) flour bags (Figure 1). The bubble was then sealed. T6 was fixed to the outside of the bubble; thermometer T0 was placed outside the enclosure (Figure 2).

Between December 1991 and February 1992 when trials were conducted, daytime outdoor temperatures were frequently in the 0–10°C range. A thermostatically controlled 1.5kW electric fan heater was used to maintain the insulated enclosure at about 20°C. Temperatures inside and outside the bubble were recorded daily.

**Introduction of carbon dioxide into the bubble and measurement of gas concentrations.** To minimise free air space, the bubble was shrunk tightly around the flour stack by withdrawing air through port "C" using a standard commercial methyl bromide dispenser (vaporising/air mixing pump; Smith 1988). Commercial, food grade carbon dioxide (nominal 99.9% purity) was piped into the bubble from a vapour phase withdrawal cylinder, via port "C" or "D". At intervals during addition of gas the supply was disconnected and the atmosphere inside the bubble recirculated via the dispenser connected between ports "C" and "D". This was to avoid "layering" (stratification) of the cold gas. Addition of gas was alternated with recirculation until carbon dioxide concentrations of around the target 60% v/v had been achieved throughout the bubble. Carbon dioxide and oxygen concentrations in the bubble were measured at intervals no greater than 24 hours throughout each exposure period; if the carbon dioxide concentration dropped below about 57%, more gas was added via port "C" or "D". It was found not to be necessary to recirculate after these relatively minor additions of gas; concentrations throughout the bubble equilibrated and stabilised within about two hours. Carbon dioxide concentrations were measured using an Analytical Development Co. Ltd. PM3 Infrared Gas Analyser and oxygen concentrations using a Servomex 756A Oxygen Analyser (zirconia cell).

At the end of the exposure period the atmosphere inside the bubble was pumped well clear of the enclosure through an exhaust hose connected via the dispenser to port "C". The bubble was then partially inflated with air and again exhausted; this was repeated several times. Once the carbon dioxide concentrations throughout the bubble were below 1.5% (the UK short-term exposure limit; Anon 1992a) the bubble top sheet was removed and the bioassay tubes were retrieved.

**Flour stack heating trial.** Twenty-nine bags of flour were stacked on the pallet on the bubble base sheet as shown in Figure 1, except that layer 6 comprised only four bags and layer 7 was omitted (Figure 2). Thermocouples T1, T2 and T5 were positioned as before (Figure 1). T7 was placed between the sacks in layer 6; T8 and T9 were located in the enclosure (Figure 2). When the stack had cooled to about 10°C the bubble top sheet was replaced and sealed to the base, and the fan heater switched on. Temperatures were recorded at least once a day, and adjustments made to the heating regime at various stages when it became apparent that temperatures inside the stack were rising only slowly. The trial was terminated when stack temperatures reached 20°C.

## RESULTS

**Gas concentrations.** Carbon dioxide concentrations within the bubble were held between 56.9 and 64.8% v/v (Table 3, sampling lines G1, G2, G3). The exception to this was the 50.2% recorded during the final stages of the initial filling for the 4 day exposure. The gas was well mixed; there was little difference between the data from the three sampling lines in the bubble (the possibility of "layering" had caused concern). The wide range of gas concentrations recorded during initial filling, topping up and venting phases of each exposure are not included in Table 3.

The bubble held gas well; carbon dioxide concentrations dropped by 1.4 to 4.4% per day (calculated from successive readings from particular sampling lines). Topping up with carbon dioxide was carried out every one or two days, but this was necessary only to keep concentrations within the relatively narrow experimental limits. The relatively low loss rates were reflected in the low concentrations detected in the enclosure, no more than 0.4% compared with a normal atmospheric level of 0.033% (Table 3, sampling lines G4 and G5).

Table 3. Carbon dioxide and oxygen concentrations in bubble and surrounding enclosure during trials

Exposure period	Gas sampling line no.	Carbon dioxide concentration (% v/v)		Oxygen concentration (% v/v)	
		Mean (No. of readings)	Range	Mean (No. of readings)	Range
4 days	G 1	61.4 (6)	57.1–64.8	8.5 (5)	8.0– 9.1
	G 2	58.6 (6)	50.2–63.9	8.8 (5)	8.0– 9.9
	G 3	60.7 (6)	57.0–63.8	8.5 (5)	8.0– 9.1
	G 4	0.2 (6)	0.1– 0.3	20.5 (5)	20.5–20.5
	G 5	0.1 (6)	0.0– 0.4	20.5 (5)	20.5–20.5
7 days	G 1	59.1 (8)	57.0–61.5	8.8 (8)	8.2– 9.0
	G 2	59.1 (8)	57.0–61.1	8.8 (8)	8.2– 9.0
	G 3	59.0 (8)	56.9–61.2	8.8 (8)	8.2– 9.0
	G 4	0.2 (8)	0.1– 0.3	20.6 (8)	20.5–20.6
	G 5	0.01 (8)	0.0– 0.1	20.6 (8)	20.5–20.6
14 days	G 1	59.3 (15)	57.9–60.9	8.8 (15)	8.3– 9.2
	G 2	59.3 (15)	57.9–60.9	8.8 (15)	8.3– 9.2
	G 3	59.3 (15)	57.8–60.9	8.8 (15)	8.3– 9.2
	G 4	0.2 (15)	0.1– 0.3	20.5 (15)	20.5–20.6
	G 5	0.03 (15)	0– 0.1	20.5 (15)	20.5–20.6

Note: Data selected as follows:

- (i) measurements during filling or venting bubble excluded
- (ii) measurements first of day (prior to topping up)

Table 4. Temperatures in bubble, in surrounding enclosure and outdoors during trials

Exposure period	Thermocouple No.	Temperature ( $\pm$ C)		No. of readings
		Mean	Range	
4 days	T 1	16	16–16	6
	T 2	15.7	15–16	6
	T 3	19	18–20	6
	T 4	16	15–17	6
	T 5	13.7	13–15	6
	T 6	16.5	15–18	6
7 days	T 0	7.4	2–10	8
	T 1	20.1	20–21	8
	T 2	20.6	20–21	8
	T 3	22.1	22–23	8
	T 4	21	20–22	8
	T 5	17.1	16–18	8
14 days	T 6	22.9	21–24	8
	T 0	2.7	–3 to +6	15
	T 1	19.1	19–20	15
	T 2	19.8	19–20	15
	T 3	21.2	19–22	15
	T 4	20.5	16–21	15
	T 5	14.8	11–16	15
	T 6	21.3	19–23	15

Temperatures recorded at same time as gas concentrations in Table 3.

Oxygen concentrations inside the bubble varied between 8.0 and 9.9% v/v (Table 3); these levels are consistent with an atmosphere comprising 60% carbon dioxide and 40% air. In the insulated enclosure levels were around 20.5%; within the limit of accuracy of the analyser this was indiscernible from the normal atmospheric level (20.9%).

**Temperatures.** Insufficient time was allowed for warming of the flour during the cold weather prior to the start of the four day exposure; consequently the mean temperatures at which the bioassay cultures were fumigated were below the intended 20°C (Table 4; T1 to T4). The temperature below the stack (T5) was consistently low and the lower bioassay positions in the bubble (T1, T2) stayed cooler than the higher position (T3). During the seven and 14 day exposures the bioassay cultures were subjected to mean temperatures between 19.1 and 22.1°C (Table 4).

Table 5. Minimum periods of exposure to nominal 60% carbon dioxide at nominal 20°C which gave control of mixed stage cultures of insects and mites (exposures for 4, 7 and 14 days)

Species	Minimum exposure period giving 100% mortality *	Comments
<i>S. oryzae</i>	14 days	Two 7 day exposed cultures survived ("M", locations B1 and B2)
<i>T. castaneum</i>	7 days	—
<i>L. bostrychophilus</i>	14 days	All but one ("Y", location B2) of 7 day exposed cultures had survived at final check
<i>T. putrescentiae</i>	> 14 days	Only one of 14 day exposed cultures dead ("M", location B3)
<i>A. siro</i>	14 days	Two 7 day exposed cultures alive on initial check ("M" and "Y", location B3)

\* "100% mortality" means no discernible movement both at initial and at final check

Note – all unexposed "control" cultures (location B4) survived  
– all cultures survived four days exposure to carbon dioxide

**Bioassay.** Some of the *T. putrescentiae* cultures survived 14 days exposure (Table 5). Complete mortality of *T. castaneum* required seven days exposure; *S. oryzae*, *L. bostrychophila* and *A. siro* required 14 days. All cultures of all species survived four days exposure; no movement was seen on initial checks but all showed activity on final checks.

**Heating trial.** Initially the fan heater (thermostat set at 20°C) alone provided air movement. This led to temperature stratification within the enclosure (T9 reading 13 – 16°C higher than T8). Once a fan had been installed blowing upwards from the floor (Figure 2) the temperature difference decreased to no more than 5°C. In the ensuing 43 hours temperatures within and below the stack (T1, T2 and T5) remained at around 12°C (despite removal of the bubble top sheet 20 hours after the installation of the first fan). The fan was then redirected to blow air horizontally through the pallet, a second fan (oscillating) was installed to blow air obliquely upwards over the side of the stack (Figure 2), and the thermostat was turned up. Subsequently the temperature below the stack (T5) more closely approached that of the warm air circulating in the enclosure (T8 and T9; 22-29°C). The temperatures in the stack (T1 and T2; 11 and 12°C respectively) started steadily to increase, reaching the 20°C target temperature 120 hours after installation of the oscillating fan.

During the Christmas break when the thermostat was turned down, the stack cooled to 16°C (T1 and T2). Turning up the thermostat raised the circulating air temperature to 22–25°C; the stack warmed to 18°C in about 20 hours, and to 20°C within 100 hours.

## DISCUSSION

The carbon dioxide exposure periods found necessary for complete control of *S. oryzae* and *T. castaneum* in the present study are consistent with the published data as reviewed by Annis (1987), who tabulated the durations of exposure to 60% carbon dioxide required to obtain at least 95% mortality in these species at 20 to 29°C. However, Desmarchelier (1984) reported that when *T. castaneum* pupae, the most tolerant stage, were exposed to 75% carbon dioxide at 19°C, the time for 99% mortality was 12 days. The periods required to control *T. putrescentiae* and *A. siro* in the present study were significantly longer than those reported by Pagani and Ciampitti (1991) for complete mortality of the closely related *Tyrophagus longior* (Gervais) and *Acarus farris* (Oudemans) on salami at 11–15°C (eight days with a 40:40:20 mixture of carbon dioxide, nitrogen and oxygen; six days with 100% carbon dioxide).

When the results for *S. oryzae*, *T. castaneum* and *L. bostrychophila* in the present study are interpolated with those from this laboratory's previous studies using 60% carbon dioxide at 15, 23, 25 and 35°C (Newton 1991), they support the widely reported finding that control requires longer exposures to carbon dioxide at lower temperatures (Marzke *et al* 1970, Jay 1986, Banks *et al* 1991). In the earlier study control of *T. putrescentiae* at 23°C was achieved by a one day exposure, but

required 14 days at 35°C (Newton 1991). The present results indicate that the lethal exposure period for this species at 23°C was anomalously short.

The lack of initial activity seen in four day exposed cultures of all species suggests that the larger more visible stages were more affected by carbon dioxide than were other stages. With *S. oryzae*, only "M" cultures survived seven days exposure; this could be explained by greater carbon dioxide tolerance of older larvae and pupae. Annis (1991) reported that pupae of *S. oryzae* were more tolerant than other stages to 65% carbon dioxide at 25°C. Amongst the stored product insects *S. oryzae* is one of the species most tolerant of carbon dioxide treatment (Annis 1991).

Examination of the mortality data for buried and non-buried cultures in the present study gives no indication that burial of cultures within bags of flour significantly increased the time required for control. As far as could be determined from the times taken for equilibration and stabilisation of gas concentrations, there was no significant lag which might have indicated a slow interchange of gas between the free space in the bubble and the interstitial spaces in the flour. Thus it appears that carbon dioxide readily penetrates bagged flour (even when the flour is compacted by the weight of the sacks above, as in location 1).

However, some delay occurred in carbon dioxide penetration of cases of tobacco, particularly when the packaging (outer 2mm thick corrugated cardboard and inner 0.15mm thick polyethylene sheet) was left intact (Anon 1990a). During the first six hours of a fumigation at 25°C, the carbon dioxide concentration in the free space around cases of tobacco in a fumigation bubble fell from 83% to 65% (thereafter it was maintained at around 60% by adding extra gas). The concentrations measured from sampling lines probed into the centres of a 22kg, 0.12m<sup>3</sup> case (heat-sealed sheet) and a 200kg, 0.54m<sup>3</sup> case (non-sealed sheet) rose in the first six hours to 43% and 38% respectively. The six hour figures for partially unwrapped cases were 57% (22kg, 0.12m<sup>3</sup>) and 59% (225kg, 0.54m<sup>3</sup>). Only 1.5 hours into the fumigation the gas concentrations at the centres of the unwrapped cases had reached the six hour levels for the comparable wrapped cases. Mixed stage cultures of *Lasioderma serricorne* (F.) buried in the centres of all four cases survived the four day exposure to carbon dioxide. Keever (1989) obtained complete control of all stages of *L. serricorne* buried in the centres of 0.66m<sup>3</sup> and 1.42m<sup>3</sup> containers of tobacco at 23.3°C and exposed for five or seven days to carbon dioxide (free space concentration ranging between 35 and 62%).

Between 20 and 29°C the duration of exposure to 60% carbon dioxide required for the control of most species of insect infesting stored grain is about 11 days (Annis 1987); this is significantly longer than the time required for standard dosages of methyl bromide or phosphine to kill these insects (Graver 1990). Use of carbon dioxide at high and low pressures, and at slightly elevated temperatures shows promise in shortening exposure periods.

Le Torc'h and Fleurat-Lessard (1991) killed all stages of *Sitophilus granarius* (L.) at 20°C by exposure to carbon dioxide for 3.5 hours at 20 bar, 16 hours at 10 bar, 7 days at 5 bar (their extrapolation) or 18 days at atmospheric pressure (1 bar); *S. oryzae* appeared to be more difficult to kill. Prozell and Reichmuth (1991) subjected all stages of *S. granarius* to 99% carbon dioxide at 20 bar and achieved total control with three, two and one hour exposures at 10, 20, and 30°C respectively. Eggs were the most tolerant stage. A review by Reichmuth (1991) of published and field trial data indicated that at 20°C carbon dioxide was lethal to "stored product arthropods" after eight hours at 10 bar or three hours at 20 bar. Locatelli and Daolio (1993) used 98% carbon dioxide in a vacuum autoclave at low pressures (34.6 to 44.0 kPa; 1kPa 10<sup>-2</sup> bar) to achieve complete control of all stages of *Rhyzopertha dominica* (F.), *S. oryzae*, *Oryzaephilus surinamensis* (L.), and *Plodia interpunctella* (Hbn.) with exposures of 54, 30, 24, 18 and 12 hours at 20, 25, 30, 35 and 40°C respectively. The use of high or low pressure can thus significantly shorten lethal exposure periods. The capital cost of pressure chambers of a useful size is however, high.

In the present study it took a relatively long time to warm up the centre of a comparatively small stack (five days from 11–12°C to 20°C). Somewhat surprisingly, the free air space in the middle of the stack took as long to warm up as the centre of a bag of flour. Delays in warming the centres of commodities have been reported for a stack of 45kg drums of lemon peel (Jay *et al* 1990) and for a tobacco product (Benezet *et al* 1991). Long delays in warming a stack are commercially undesirable. But there is also a risk that slow warming of a resident insect population will reduce its susceptibility to carbon dioxide. Soderstrom *et al* (1992) demonstrated that preconditioning in air at 38°C led to



reduced mortality of *T. castaneum* larvae when subsequently they were exposed to carbon dioxide (60 to 98%) at the same temperature.

Even with the use of fans circulating warm air, it took five days for the small stack in the present study to reach a reasonable temperature for fumigation. A larger stack would certainly take longer to warm up, as would a stack enclosed in a fumigation sheet or bubble. Evidently it cannot be assumed that a stack of goods which has cooled down in an unheated container or warehouse will rapidly equilibrate with its surroundings when brought into a heated building. This has obvious implications for any fumigation carried out during the winter. The concentration of gas and duration of exposure will generally be selected by the fumigator as appropriate for the higher temperature of the heated building, and may be inappropriate for a stack which has not been given sufficient time to warm up. For those fumigations in which knowledge of the exposure temperature is critical to success, the core temperature of the stack should be determined.

Since carbon dioxide is more rapidly effective at higher temperatures (Jay 1986; Banks *et al* 1991) it will generally be desirable to fumigate at the highest temperature which the commodity (or artefact) will tolerate. For most cereal-based foodstuffs temperatures up to about 40°C should cause little or no damage. The precise nature of any particular museum artefact will determine the maximum permissible fumigation temperature and thus the minimum effective exposure period. However, the need for short exposures will generally be more pressing with foodstuffs because of the economic effects of delaying distribution and sale. Equipment which can heat small stacks relatively rapidly whilst dosing automatically with carbon dioxide is in commercial use in Europe. This heated recirculating fumigation bubble is currently used for carbon dioxide fumigation of foodstuffs and museum artefacts in Germany, Holland and Belgium. In the UK, carbon dioxide has only just been approved for use as a pesticide, although it has been used in "modified atmosphere packaging" of foodstuffs for some years (Smith *et al* 1988).

Entire buildings, if well sealed, can be fumigated successfully with carbon dioxide. Keever (1989) carried out a seven day, carbon dioxide fumigation of a sealed, 12,706m<sup>3</sup> tobacco warehouse. *L. serricornis* (all stages) held in the free space for five or seven days suffered 100% mortality. During the fumigation free space carbon dioxide concentrations ranged from 35 to 60% and free space temperature between 11.7 and 26.7°C. The initial gassing used 18 tonnes of carbon dioxide, daily topping up consuming a further 53 tonnes. This fumigation cost about nine times that of a conventional phosphine fumigation. However, Keever predicted a reduction in cost as techniques improved.

An attempt to fumigate a two storey house in Holland with 90% carbon dioxide at 40°C was not entirely successful (Anon 1990b). The external doors were sealed and the building was entirely sheeted in 0.2mm thick polyethylene, but despite the adoption of various gas introduction and air displacement strategies, it proved impossible to achieve the target gas concentration in the upper part of the building. A total of 25 tonnes of carbon dioxide was used in the course of two successive trials. During the second trial carbon dioxide concentrations of 90% and above were maintained for 24 hours in the lower part of the house. The main problem appeared to be rapid leakage of gas through the foundations of the house.

The classical fumigants, methyl bromide, phosphine, sulphuryl fluoride and ethylene oxide have all been associated with damage to fumigated articles (Brokerhof 1989). This is of particular concern to museum conservators, since the artefacts in their care have been fabricated from a huge variety of materials. Carbon dioxide shows promise as a non-damaging fumigant for use in this field (Story 1985; Brokerhof 1989). Studies by conservators have shown that blocks of wood, even when coated with a variety of finishes (resin, wax, oil-based), are "easily penetrated by carbon dioxide", although gas permeation may take two days. (Burke 1993). Fumigation with carbon dioxide is of interest to the "organic foods" industry, in which treatments with classical fumigants are considered undesirable. Repeated fumigations can be carried out, since, as far as is known, carbon dioxide leaves no significant residues (Leeson 1984; Keever 1989). Carbon dioxide may also be used on sensitive items such as electronic equipment where classical fumigants may cause damage. Since the gas used is a byproduct of industrial processes such as brewing, carbon dioxide fumigation does not *per se* add to the "greenhouse effect".

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