

CARBON DIOXIDE FUMIGATION IN A HEATED PORTABLE ENCLOSURE

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Abstract

Mixed stage cultures of Tyrophagus putrescentiae, Acarus siro, Periplaneta americana, Sitophilus oryzae, Oryzaephilus mercator, Tribolium castaneum, Lasioderma serricorne, Dermestes maculatus, Ptinus tectus, Ephestia cautella, Lepinotus patruelis and Liposcelis bostrychophilus, also larvae of Anthrenus verbasci, Trogoderma granarium and the wood borer Anobium punctatum, were exposed to atmospheres containing 60% CO₂ for periods between one and 56 days at temperatures between 15 and 35°C. Relative humidity was 75%.

Fourteen days exposure at 15°C controlled all species except S.oryzae which required 28 days. Exposure for four days or less at 23°C controlled all species except L.bostrychophilus which required 8 days, and S.oryzae which needed 14 days (at 25°C). At 35°C four days or less controlled all species except T.putrescentiae, T.granarium and A.punctatum which required 14 days. These results are in broad agreement with published data where available.

Carbon dioxide fumigation of infested stored commodities requires the maintenance of relatively high concentrations of CO₂ for relatively long periods. The heat retaining version of a special fumigation enclosure ("bubble") is capable of holding high concentrations of CO₂, which by means of a thermostatically controlled recirculating heater can be heated to reduce the exposure period required for disinfestation of commodities.

Introduction

Carbon dioxide (CO₂) has been used directly as a fumigant to control insects in stored grain for at least 70 years (Bailey, 1955). The earliest use was indirect; grain was stored in airtight pits, and grain respiration eventually raised CO₂ levels and depleted oxygen (O₂) levels to an extent which produced an atmosphere lethal to insects. More recently CO₂ from pressurised cylinders, from "dry ice" or from fuel burners has been added to grain bins. The use of CO₂ in conventional fumigation of sheeted commodities has been problematic because of the need to retain relatively high concentrations of CO₂ for relatively long periods (Banks, 1979).

However, successful fumigations have been carried out using carefully sealed sheets tailor-made for stacks of commodities (Annis and Graver, 1985). The fumigation "bubble" described by Smith (1988) is a portable enclosure in which a durable groundsheet is sealed by a gas-tight zip to a top cover. The relatively high initial cost (compared with a conventional fumigation sheet) is offset by the fact that the bubble may be re-used many times. The bubble permits the maintenance of high concentrations of CO₂ without the need for constant "topping up".

Atmospheres containing about 60% carbon dioxide are generally considered to be the most practical for control of stored product insects (Banks, 1979); higher concentrations do not confer any great advantage, and are more difficult to maintain. The duration of exposure to 60% CO₂ required for the control of most species of stored product insect in grain between 20 and 29°C 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). The period of exposure to CO₂ required to kill insects is known to decrease as temperature increases (Marzke et al, 1970; Jay 1986; Navarro and Jay, 1987). By heating the fumigation "bubble" it might be possible to reduce CO₂ exposure times so that they compare reasonably well with exposure times for methyl bromide and phosphine. This paper presents the results of tests in which insect and mite pests of stored food and other materials were exposed to 60% CO₂ at temperatures between 15 and 35°C.

Materials and Methods

Test cultures of insects were set up by inoculating 20 adults into 100g of standard rearing medium in a wide-mouthed half litre or one litre jar closed with filter paper or fine polyester mesh, and incubating under standard rearing conditions (Table I).

The cultures were set up to a timetable such that at the start of exposure to carbon dioxide there should be present in each culture eggs, young larvae, mature larvae, pupae and adults. On the day before start of exposure the cultures were inspected to confirm as far as possible the presence of the desired stages. In the case of S. oryzae X-radiography of the wheat culture medium was required to confirm the presence of larvae and pupae. Booklouse (Lepinotus and Liposcelis) cultures were inoculated with at least 10 individuals. Mite cultures were inoculated during the week before exposure with about 5ml of live culture medium. Groups of cockroaches (P.americana) were set up during the week before exposure (10 adult males, 10 adult females, 10 medium-sized nymphs and 10 oothecae). Cultures of A.verbasci and T.granarium were inoculated with 20 half to full-grown larvae 7 to 10 days and immediately before exposure, respectively. Laboratory cultures of A.punctatum larvae in pieces of hazel (Corylus avellana L.) branch wood were examined by X-radiography. Only pieces containing at least ten larvae were exposed to carbon dioxide.

The experimental cultures were stacked into 35 litre high density polyethylene drums (Figure 1). The drums were closed and, via a pair of spigots in the lid, flushed through with commercial (food) grade carbon dioxide from a vapour phase withdrawal cylinder. Flushing was continued until, after mixing by recirculation, the desired concentration of carbon dioxide (nominally 60%) was reached. At intervals thereafter, carbon dioxide levels were measured using an ADC PM3 Infrared Gas Analyser, and oxygen levels using a Servomex 756A Oxygen Analyser (zirconia cell). The analysed atmosphere was returned to the drum to reduce the need for topping up with carbon dioxide, which was added as necessary to maintain the concentration at around 60%. Any excess carbon dioxide was bled off. Untreated control cultures were stacked in a drum through which air was gently pumped.

TABLE I : List of species of insect and mite exposed to carbon dioxide, their rearing media and rearing conditions

SPECIES	REARING MEDIUM	(composition by weight)	REARING CONDITIONS
<i>Tyrophagus putrescentiae</i> (Schrank)	fishmeal : dried yeast powder	1:1	22 ± 1°C, 75 ± 5% r.h.
<i>Acarus siro</i> L.	plain white flour:dried yeast powder	12:1	" "
<i>Periplaneta americana</i> (L.)	rodent diet (cereal pellets) plus small pot of wet cotton wool*		27 ± 1°C, 60 ± 5% r.h.
<i>Trogoderma granarium</i> Everts	wheat : wheatfeed	1:1	" "
<i>Sitophilus oryzae</i> (L.)	wheat		25 ± 1°C, 50 ± 5% r.h.
<i>Anthrenus verbasci</i> (L.)	fishmeal : dried yeast powder : cholesterol	75:18:2	" "
<i>Oryzaephilus mercator</i> (Pauvel)	wheatfeed : rolled oats : dried yeast powder	5:5:1	" "
<i>Tribolium castaneum</i> (Herbst)	plain white flour : dried yeast powder	12:1	" "
<i>Lasioderma sericorne</i> (Fab.)	wheatfeed : dried yeast powder	10:1	" "
<i>Dermeestes maculatus</i> Degeer	fishmeal : dried yeast powder : minced bacon, above damp pad of cotton wool*	16:4:1	" "
<i>Pinus tectus</i> Boieldieu	fishmeal : dried yeast powder plus small pot of wet cotton wool*	4:1	" "
<i>Ephestia cautella</i> (Walker)	(i)** dates (organically grown, pitted) plus small pot of water with butter muslin wick (ii) wheatfeed : dried yeast powder : glycerol plus small pot of water with butter muslin wick	10:1:2	" "
<i>Lepinotus patruelis</i> Pearman	soya flour : wheatfeed : dried yeast powder : skimmed milk powder : plain white flour	1:1:1:1:1	22 ± 1°C, 75 ± 5% r.h.
<i>Liposcelis bostrychophilus</i> Badonnel	" " " " " "	" " " "	27 ± 1°C, 60 ± 5% r.h.
<i>Anobium punctatum</i> (Degeer)	pieces of hazel tree branch wood approx. 130-140mm long and 25-35mm diameter		22 ± 1°C, 75 ± 5% r.h.

* Cotton wool wetted or made damp with water

** (i) tests at 15°C and 23/25°C

(ii) tests at 35°C

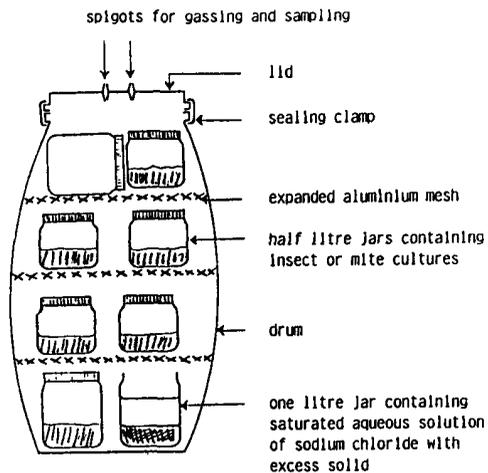


FIGURE 1 : Diagrammatic section through drum set up for carbon dioxide fumigation

Relative humidity was maintained at about 75% by placing an open dish of saturated aqueous solution of sodium chloride in each test drum (Winston and Bates, 1960), and by bubbling the air supply for the control drum through deionised water then a saturated aqueous solution of sodium chloride (Newton, 1981). Control and test drums were kept in a temperature-controlled room at 15, 23, 25 or 35°C. Cultures were exposed to carbon dioxide for periods between one and 56 days. One culture of each species was exposed to each treatment.

After removal from CO₂ cultures were held under standard rearing conditions (Table I). Mixed stage cultures were retained for a period of one generation time plus seven days, except for P.americana which were kept for the ootheca hatching period plus seven days. A.verbasci larvae were retained for 5 weeks, and T.granarium larvae for 8 weeks. Two days after removal from CO₂ and again at the end of the retention period, the rearing medium of each culture was tipped onto a tray and examined carefully for live insects. It was considered that complete mortality had occurred only if live mites or insects were absent on both inspections. Mite and booklouse cultures were examined through a low power microscope where survival was in doubt. A.punctatum infested hazel wood samples were split into small pieces and inspected carefully for live insects 9 to 12 weeks after removal from CO₂.

Results

Mean concentrations of CO₂ were generally within 5% of the nominal 60% (Table II). Oxygen concentrations in CO₂-filled drums were always lower than 11%. The lower mean O₂ concentrations associated with increasing exposure times up to eight days (Table II) were probably due to consumption of O₂ by the insect and mite cultures. The lowest oxygen concentration (0.1%) was recorded half way through a 14 day exposure in a particularly gastight drum. Even in the ventilated control drums some depression of O₂ concentrations below the normal atmospheric level (20.9%) was evident (Table II).

At 15°C, 14 days exposure to CO₂ was sufficient to control all species except S.oryzae, which succumbed to 28 days exposure (Table III). At 23°C all species were controlled by one to four days exposure to CO₂, except for S.oryzae and A.verbasci which were controlled by CO₂ after 14 days at 25°C. Exposure to CO₂ for four days at 35°C controlled most species. However T.putrescentiae, T.granarium and A.punctatum required 14 days under CO₂ to give complete control at 35°C.

The control of A.punctatum larvae by a one day exposure at 23°C may be due to a greater susceptibility of the five year old larvae used in that test. The larvae used at 35°C were 8-12 months old.

Cultures of insects and mites in ventilated "control" barrels all survived at 15, 23 and 25°C (for duration of exposures see Table II). At 35°C all species survived exposures up to 14 days, except for P.tectus which succumbed to 7 and 14 days exposure.

Discussion and conclusions

The periods of exposure to 60% CO₂ at 23 or 15°C which the present work found to be necessary to give complete mortality are broadly consistent with published data as reviewed by Annis (1987), who tabulated the durations of exposure to 60% CO₂ required to obtain at least 95% mortality of various stages of T.granarium, S.oryzae, T.castaneum, L.serricornis and E.cautella at 20 to 29°C. Much of the published work on CO₂ refers to atmospheres in which the non-CO₂

TABLE II. Carbon dioxide and oxygen concentrations in test drums held at various temperatures for various periods

Temperature	Nominal concentration of carbon dioxide (% v/v)	Exposure period (days)	Carbon dioxide concentration (% v/v)		Oxygen concentration (% v/v)	
			Mean (No. of measurements)	Range	Mean (No. of measurements)	Range
15°C	60%	14	59.7 (9)	55.0 - 68.3	7.0 (9)	5.7 - 7.8
		28	63.1 (15)	56.1 - 80.7	6.1 (15)	2.7 - 7.2
	Control (ventilated drum)	28	0.37 (15)	0.1 - 0.6	20.1 (15)	20.0 - 20.4
	Air (in test room)	28	0.15 (15)	0 - 0.4	20.4 (15)	20.2 - 20.6
23°C	60%	1	61.9 (2)	61.6 - 62.1	7.0 (2)	6.7 - 7.2
		2	68.4 (3)	67.0 - 69.7	5.7 (3)	4.7 - 6.6
		4	65.3 (5)	61.5 - 69.2	5.6 (5)	5.0 - 7.0
		8	65.2 (9)	60.9 - 69.6	4.9 (9)	4.2 - 6.8
	Control (ventilated drum)	8	1.3 (9)	0.8 - 1.7	19.5 (9)	19.2 - 20.0
Air (in test room)	8	0.04 (9)	0.0 - 0.1	20.8 (9)	20.7 - 21.0	
25°C	60%	14	59.8 (11) 59.8 (11) 60.1 (9)	40.6 - 66.7 53.4 - 65.0 50.5 - 70.0	6.3 (11) 1.2 (10) 7.8 (9)	5.1 - 9.6 0.1 - 5.4 5.8 - 9.6
		28	55.0 (20) 58.2 (19)	44.8 - 63.7 48.0 - 73.1	7.2 (20) 8.6 (19)	5.6 - 10.0 6.3 - 10.2
		56	58.7 (39)	49.7 - 69.1	6.5 (39)	4.2 - 9.6
		Control (ventilated drum)	56	1.0 (39)	0.2 - 3.4	19.6 (38)
	Air (in test room)	56	0.08 (39)	0.0 - 0.3	20.7 (38)	19.3 - 20.9
35°C	60%	1	62.8 (3)	62.0 - 63.6	6.3 (3)	4.1 - 7.8
		2	62.0 (5)	61.2 - 62.5	5.7 (5)	3.9 - 7.3
		4	64.5 (8)	62.8 - 67.4	4.8 (8)	1.2 - 7.2
		7	60.7 (9)	52.5 - 65.4	3.5 (9)	1.8 - 7.1
		14	62.1 (12)	58.2 - 67.1	1.7 (12)	0.2 - 7.2
	Control (ventilated drum)	1	0.9 (3)	0.6 - 1.2	19.8 (3)	19.5 - 20.0
		2	1.3 (5)	1.0 - 1.5	19.5 (5)	19.3 - 19.7
		4	1.1 (8)	0.4 - 1.5	19.6 (8)	19.3 - 20.3
		7	1.4 (9)	0.7 - 2.3	19.2 (9)	18.3 - 19.6
Air (in test room)	14	1.4 (12)	0.8 - 2.0	19.1 (12)	18.6 - 20.0	

Where treatments were repeated as various species became available, more than one set of data is presented above

residue is not air or equivalent to air, or where data are for mortalities less than 100%. Unfortunately the present data cannot be directly compared with those results. Work by Harein and Press (1968) on *T.castaneum*, Childs and Overby (1983) on *L.serricorne* and Marzke et al (1970) on *S.oryzae* yielded results with which the current data broadly agree. Jay et al (1990) found that complete mortality of all stages of *L.serricorne* required 4 days exposure to 65% CO₂ at 32°C, and 3 days at 38°C, significantly longer than in the current study.

A.punctatum larvae appear to be relatively tolerant of CO₂ (apart from the presumably anomalous result at 23°C). This might be expected of larvae which tunnel through wood for 2 to 5 years under natural conditions; CO₂ might be expected to accumulate in the tunnels, especially before flight holes have been produced in the surface of the timber by the first generation of emerging

TABLE III : Minimum number of days* exposure to atmospheres containing nominal 60% carbon dioxide which gave 100% mortality of mixed stage cultures of insects and mites at various temperatures

TEMPERATURE	15°C	23°C	25°C	35°C
EXPOSURE PERIODS (days)	14, 28	1, 2, 4, 8	14, 28, 56	1, 2, 4, 7, 14
<u>T.putrescentiae</u>	-	1	14	14
<u>A.siro</u>	14	1	14	1
<u>P.americana</u>	14	2	14	4
<u>T.granarium</u> (larvae)	-	-	-	14
<u>S.oryzae</u>	28	S	14	4
<u>A.verbasci</u> (larvae)	14	S	14	2
<u>O.mercator</u>	14	4	14	1
<u>T.castaneum</u>	14	4	14	4
<u>L.serricorne</u>	14	4	14	1
<u>D.maculatus</u>	14	4	14	-
<u>P.tectus</u>	14	1	14	4
<u>E.cautella</u>	14	4	14	2
<u>L.patruelis</u>	-	-	14	-
<u>L.bostrychophilus</u>	-	8	-	-
<u>A.punctatum</u> (larvae)**	14	1	14	14

S - some insects survived maximum exposure period

*For a given temperature, no insects or mites survived exposures longer than the minima tabulated above

**5 year old larvae used at 15 and 23°C, 8 to 12 month old larvae used at 25 and 35°C

adults. Paton and Creffield (1987) found that the larvae of the wood borer Hylotrupes bajulus (L.) would survive five days exposure to 60% CO₂ at 30°C.

Field work carried out with the fumigation "bubble" has shown that if properly maintained it will hold 60% CO₂ atmospheres for at least three weeks without the need for topping up. A recirculating heater has been developed (Figure 2) which will warm the atmosphere in the bubble to a preset temperature and maintain that temperature indefinitely. When used in cooler climates the bubble can be fitted with a heat-retaining jacket. The use of heated CO₂ should reduce the exposure times required to give control of infestations in commodities. However the thermal insulation properties of the particular commodity will need to be taken into account when specifying exposure periods; the time lag between the atmosphere around the commodity and the centre of the commodity reaching set temperature may be a few days (Jay et al, 1990). Further field work is needed to establish the exposure times required to control insects in a variety of commodities by use of CO₂ at elevated temperatures.



FIGURE 2 : Fumigation bubble fitted with recirculating thermostatically controlled heater

References

- Annis P.C. (1987) Towards rational controlled atmosphere dosage schedules : a review of current knowledge. Proc. 4th Int. Working Conf. on Stored-Product Protection, Tel Aviv, Israel 1986 (ed. Donahaye E. and Navarro S.) pp. 128-148
- Annis P.C. and Graver J van S. (1985) Use of carbon dioxide and sealed storage to control insects in bagged grain and similar commodities. Pesticides and humid tropical grain storage systems. Proceedings of an international seminar, Manila, Philippines 1985 (ed. Champ B.R. and Highley E.) pp. 313-321
- Bailey S.W. (1955) Air-tight storage of grain; its effects on insect pests. I. Calandra granaria L. (Coleoptera, Curculionidae) Aust. J. agric. Res. 6, 33-51
- Banks H.J. (1979) Recent advances in the use of modified atmospheres for stored product pest control. Proc. 2nd Int. Working Conf. Stored-Prod. Ent., Ibadan, Nigeria 1978, pp.198-217

Childs D.P. and Overby J.E. (1983) Mortality of the cigarette beetle in high carbon dioxide atmospheres. J. Econ Entomol. 76, 544-546

Graver J.E. van S. (1990) Fumigation and controlled atmospheres as components of integrated commodity management in the tropics. Fumigation and controlled atmosphere storage of grain. Proceedings of an international conference, Singapore 1989 (ed. Champ B.R., Highley E. and Banks H.J.) ACIAR Proceedings No.25, pp. 38-52

Harein P.K. and Press A.F. (1968) Mortality of stored-peanut insects exposed to mixtures of atmospheric gases at various temperatures. J. stored Prod. Res. 4, 77-82

Jay E.G. (1986) Factors effecting the use of carbon dioxide for treating raw and processed agricultural products. GASGA seminar on fumigation technology in developing countries, Slough, U.K. 1986, pp. 173-189

Jay E.G., Banks H.J. and Keever D.W. (1990) Recent developments in controlled atmosphere technology. Fumigation and controlled atmosphere storage of grain. Proceedings of an international conference, Singapore 1989 (ed. Champ B.R., Highley E. and Banks H.J.) ACIAR Proceedings No.25, pp.134-143

Marzke F.O., Press A.F. and Pearman G.C. (1970) Mortality of the rice weevil, the Indian-meal moth, and Trogoderma glabrum exposed to mixtures of atmospheric gases at various temperatures. J. Econ. Entomol. 63, 570 - 574

Navarro S. and Jay E.G. (1987) Application of modified atmospheres for controlling stored grain insects. Stored Products Pest Control. BCPC Monograph No.37. Proceedings of a symposium at Reading, U.K. 1987 (ed. Lawson T.J.), pp. 229-236

Newton J. (1981) A simple device for humidity control with ventilation. Int. J. Wood Pres. 1, 151-152

Paton R. and Creffield J.W. (1987) The tolerance of some timber pests to atmospheres of carbon dioxide and carbon dioxide in air. Int. Pest Control 29, 10-12

Smith C.P. (1988) Fumigation - a new concept. Proc. 8th Brit. Pest Control Conf., Stratford-upon-Avon, U.K. 1988, 2nd session, paper 3, 15 pp.

Winston P.W. and Bates D.H. (1960) Saturated solutions for the control of humidity in biological research. Ecology 41, pp. 232-237

LA FUMIGATION AU DIOXYDE DE CARBONE EN CHAMBRE PORTABLE ET CHAUFFEE

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RESUME

La fumigation de produits alimentaires au bromure de méthyle ou à la phosphine est de moins en moins bien acceptée. Ces gaz peuvent endommager certains articles non comestibles comme les pièces de musée. Des publications ont montré que des concentrations élevées de dioxyde de carbone sont efficaces contre les insectes des déprédateurs du grain. Des études en laboratoire ont été entreprises sur les effets des atmosphères contenant 60 % de CO₂, ou plus, sur les insectes et les acariens attaquant un éventail de produits divers comme les produits alimentaires, les pièces de musée et le bois. Des élevages à différents stades de *Tyrophagus putrescentiae*, *Acarus siro*, *Periplaneta americana*, *Sitophilus oryzae*, *Oryzaephilus mercator*, *Tribolium castaneum*, *Lasioderma serricorne*, *Dermestes maculatus*, *Ptinus tectus*, *Ephestia cautella* et *Lepinotus patruelis*, la larve de *Anthrenus verbasci* et la larve d'*Anobium punctatum*, ont été exposés à des atmosphères contenant 60 %, 80 % et 100 % de CO₂ à 15° et 25° C et à 60 % de CO₂ à 35° C. Le degré d'humidité relative général était de 75 %. A 15° C, un séjour de 14 jours dans 60 % de CO₂ a tué toutes les espèces sauf *S. oryzae* ; les résultats n'ont pas été concluants pour *T. putrescentiae* et *L. patruelis*. A 25° C, 4 jours dans 60 % de CO₂ ont tué toutes les espèces à l'exception de *A. verbasci* et *S. oryzae* pour lesquelles il a fallu 14 jours. Les résultats concernant *L. patruelis* n'ont pas été concluants. A 35° C, la plupart des espèces (y compris *S. oryzae* et *A. verbasci*) ont été tuées en 4 jours dans 60 % de CO₂. L'élimination de *T. putrescentiae* de la larve de *T. granarium* a demandé 14 jours ; *A. punctatum* a survécu à 14 jours d'exposition. Ces résultats sont semblables à ceux obtenus par d'autres auteurs et publiés antérieurement. Le dioxyde de carbone étant un gaz mobile, des fermetures bien hermétiques sont nécessaires pour maintenir une concentration de 60 %. La "bulle de fumigation" est un dispositif comprenant un tapis de sol résistant sur lequel est empilé le produit à traiter avant de le recouvrir d'une toile solidaire de celui-ci grâce à une fermeture éclair étanche. Le dioxyde de carbone intérieur peut y être chauffé jusqu'à obtention d'une température prééglée grâce à un chauffage de recirculation. Ceci réduit la période d'exposition. Des essais sur le terrain ont donné des résultats très prometteurs permettant d'affirmer qu'une telle technique constitue une alternative valable à la fumigation au bromure de méthyle et à la phosphine.