# EFFECTS OF OXYGEN ON THE TOXICITY OF CARBON DIOXIDE TO STORAGE INSECTS

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ABSTRACT

Atmosphere of 60-80% carbon dioxide ($CO_2$) in air often prove more lethal to insects than $CO_2$ alone. The toxicity of $CO_2$ can thus be enhanced by the presence of oxygen ($O_2$). The efficacies of gas mixtures containing up to 80% $CO_2$ and 20% $O_2$ with any balance as nitrogen, of similar $CO_2$ mixtures in air, and of $CO_2$ alone were compared. Eggs of *Ephestia cautella* and *E. kuehniella* aged 2, 20 and 44h were obtained by regulating oviposition in light/dark cycles and were tested at 15 and 25°C. At both temperatures, 2-h-old eggs were killed within 18 h by any mixture containing over 40% $CO_2$. Eggs aged 20 h or more were killed by any mixture containing over 30% $CO_2$ within 48 h at 25°C but required 5-7 days for control in mixtures containing 40% or more $CO_2$ at 15°C.

Increasing $O_2$ from 4 to 20% had little effect on the lethality of 80% $CO_2$ to eggs at 15 or 25°C, but an increase to 100% $CO_2$ shifted the most tolerant age group from 44 to 20 h. Implications for practical control are discussed.

## INTRODUCTION

The effects of modified or controlled atmospheres on pests of stored products have received much attention in recent years, but in some areas there is a lack of precise information because of difficulties in comparing data gathered in different ways by different authors regarding the influence of temperature, and the relative susceptibility of different species and life stages.

Early work by Bailey (1956; 1957;1965) included an examination of carbon dioxide ($CO_2$) concentrations of up to 60% in the presence of 21% oxygen ($O_2$). Working mostly with 14-day exposures at 29-32°C, he found that all insects died in $O_2$ concentrations less than 3-4%, or with 40-60% $CO_2$ (depending on the species) in the presence of 21% $O_2$ (Bailey, 1956; 1965). Subsequently, other workers concentrated largely upon the effect of atmospheres obtained by replacing air with $CO_2$, mostly at high temperatures. Hence Press and Harein (1967) describe the effects of purging peanut storage towers with nitrogen ($N_2$) or $CO_2$ to control *Tribolium castaneum* (Herbst), Pearman and Jay (1970) described the effects of a 46% $CO_2$ atmosphere in air at various humidities, and Marzke *et al.*, (1970) described the effects of various mixtures of $O_2$, $N_2$ and $CO_2$ (modelled on the replacement of air with either $N_2$ or $CO_2$) on three insect pests at various temperatures. Apart from the last mentioned paper, work on the effects of cool temperatures on the performance of atmospheric gas mixtures is scarce. Harein and Press (1968) working with *T. castaneum* and *Plodia interpunctella* (Hubner) included tests at 15.6°C in their examination of the effect of low $O_2$ concentrations in the presence of 36% or more $CO_2$, and Jay (1980) tested immature *Sitophilus oryzae* (L.) at 1.6, 4.7, 10.4 and 15.7°C in 60 and 98% $CO_2$ in air.

In looking at the replacement of air with $CO_2$ (or $N_2$) much emphasis has been placed on the effects of low $O_2$ concentrations. It is widely known, however that atmospheres of 60-80% $CO_2$ in air can be more lethal to some insects than near 100% $CO_2$ (Lindgren and Vincent, 1970; Jay and Cuff, 1981). Recently it has been shown that the toxic effects of low $O_2$ concentrations are much increased by the presence of relatively low levels (10-35%) of $CO_2$ (Calderon and Navarro, 1979; 1980; Navarro and Calderon, 1980). Similar results have been obtained in atmospheres generated by burning gas in air (Storey, 1977; 1980).

The effect of $O_2$ at concentrations up to 21% on the toxicity of $CO_2$ atmospheres has been looked at infrequently since the early work by Bailey over 20 years ago. AliNiazee (1971) tested 80/20% and 90/10% mixtures of $CO_2$ and $O_2$ at 26.7°C and found the latter mixture to be as lethal to *Tribolium confusum* J. duV. adults as a 98/2% mixture, and Lindgren and Vincent (1970) found the toxicity of an 80/20% mixture of $CO_2$ and $O_2$ to be comparable with that of 80% $CO_2$ in air against *Sitophilus oryzae* and *Sitophilus granarius* (L.) adults, again at 26.7°C. Both mixtures were much more toxic than 100% $CO_2$.

Bailey and Banks (1980) expressed the view that in contrast to low $O_2$ concentrations, there may be little temperature dependency for high $CO_2$ concentrations in the presence of $O_2$. The present contribution presents the early results of a programme studying the effects of $O_2$ on the toxicity of mainly high concentrations of $CO_2$ to storage pests at 15 and 25°C.

## MATERIALS AND METHODS

Experiments were performed on eggs of *Ephestia cautella* (Walker) and *Ephestia kuehniella* Zeller. Eggs were obtained by utilizing the ovipositional response in light/dark cycles whereby most eggs are laid in the first part of the dark period (Bell, 1981). It proved possible to obtain eggs aged to within one hour in sufficient numbers for experiments by collecting the yield after the first hour of darkness. Cultures were conditioned in a 15-h light, 9-h dark cycle at 25°C, 60% r.h. with the dark phase commencing at the hour chosen for the subsequent isolation of eggs for several days before setting up adults. During a photoperiod, between 100 and 150 adults were anaesthetised briefly with $CO_2$ and dropped into a nylon sieve. A PTFE-coated glass dish with a cotton wool drinking pad taped inside was then taped over the sieve to confine the adults. Eggs laid through the sieve were collected in a dish which was emptied immediately prior to the start of a dark period. After remaining in the dark for one hour the eggs obtained were counted on to watch glasses in lots of 100. Neither damaged eggs nor clumps were used. Glasses bearing eggs were then held at 25°C until reaching the age chosen for treatment. Eggs aged 2, 20 or 44 h were treated at 15 and 25°C. For treatment, each watch glass bearing eggs was placed on a platform inside a 15 cm diameter perspex dish at the experimental temperature. Each dish was fitted with inlet and outlet apertures for the flow of gas from a gas blending apparatus, and was sealed using vacuum grease by a perspex lid. Spratt (1979) described the apparatus used to provide a continuous, regulated flow of a mixture of gases. Before entering the sealed chamber, the mixture of $CO_2$ and $O_2$ or air was passed over a saturated solution of sodium and potassium chloride to bring it to about 70% r.h. After flushing for five minutes, the flow rate was adjusted to 50 ml/min. Eggs were exposed for up to 3 days at 25°C and 7 days at 15°C. Details of the measurement of gas concentrations using a Taylor Servomex oxygen analyser are given by Bell *et al.* (1980). Control eggs were exposed to turbulent air at 15 or 25°C and 70% r.h. in the constant temperature room containing the gas flow apparatus.

After the allotted period of exposure, the chamber was opened and the watch glasses were placed on petri dishes which contained food. After a few minutes in the exposure room these were returned to the rearing room at 25°C, 60% r.h. Starting three or four days later, the number of unhatched eggs was counted each day until no further hatch occurred. In some experiments the petri dishes were retained to observe development of larvae to adults.

## RESULTS

Eggs of both species were quite susceptible to exposure to $CO_2$ in the presence or absence of $O_2$. At 25°C, gas mixtures containing 40% or more $CO_2$ did not permit hatch of any age group of eggs after 48-h exposures. An 18-hr exposure to 60% $CO_2$, 20% $O_2$ and 20% $N_2$ killed all 2-h eggs of both species. The survivals observed after 24-h exposures are shown in Table 1 and hatch was delayed by about one day after these exposures. Eggs of *E. cautella* aged 2 h at the start of exposure were more susceptible to all mixtures tested than 20 or 44h old. Eggs aged 20h were the most tolerant when exposed to pure $CO_2$, or, in the case of *E. cautella*, to mixtures containing 30% or less $CO_2$ in the presence of 20% $O_2$. With 40–80% $CO_2$ in the presence of $O_2$, 44-h-old eggs of both species became the most tolerant age group. A few tests with $CO_2$ in the presence of $O_2$ on 68-h eggs revealed a fall in tolerance by this age in both species.

Table 1. Survival (%) of eggs of *Ephestia cautella* (E.c.) and *E. kuehniella* (E.k.) exposed to gas mixtures for 24 h at 25°C.

| Species | Age of eggs (h) | % Survival When Exposed To A Mixture Of - ($CO_2/O_2/N_2$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 20/60/20 | 30/50/20 | 40/40/20 | 60/20/20 | 60/32/8 | 80/0/20 | 100/0/0 |
| E.c. | 2 | 1 | 6 | 0 | 0 | 0 | 0 | 4 |
| | 20 | 98 | 87 | 0 | 9 | 0 | 37 | 78 |
| | 44 | 97 | 52 | * | 67 | 64 | 64 | 42 |
| E.k. | 20 | * | * | 60 | 1 | 33 | 31 | 98 |
| | 44 | * | * | * | 63 | * | 42 | 1 |

* Not tested

At 15°C, a shift was again seen in the age group most tolerant to $CO_2$ in the presence or absence of oxygen. Eggs of both species aged 2h were susceptible and none survived 18-h exposures to any mixture containing 40% or more $CO_2$. Table 2 gives the number of days required for less than 10% survival of eggs of *E. cautella* and *E. kuehniella* aged 20 or 44h. In general about a day longer was required for complete control and no eggs survived exposure for 7 days. When hatch was less than 10%, very few larvae developed to the adult stage, and with less than 5% hatch all larvae died before moulting. The most tolerant age group in pure $CO_2$ was 20h, and in $CO_2$ with $O_2$, 44h. At 15°C, eggs of *E. kuehniella* were as or more susceptible than those of *E. cautella*.

Table 2. Days for less than 10% survival of eggs of *Ephestia cautella* (E.c.) and *E. kuehniella* (E.k.) at 15°C.

| Species | Age of eggs (h) | Days For Less Than 10% Survival When Exposed To Indicated ($CO_2/O_2/N_2$) | | | | |
|---|---|---|---|---|---|---|
| | | 40/48/12 | 80/0/20 | 80/10/10 | 80/16/4 | 100/0/0 |
| E.c. | 20 | 5 | 4 | 4 | 4 | 5 |
| | 44 | 6 | 6 | 6 | 6 | 3 |
| E.k. | 20 | 5 | * | * | * | 4 |
| | 44 | 6 | 5 | 6 | 5 | 3 |

* Not tested

DISCUSSION

Eggs of *Ephestia* moths are easy to obtain in large numbers within an hour and provide a useful tool for physiological studies. As development proceeds, several changes occur in the response to $CO_2$. In the first hours after oviposition, eggs are highly susceptible to concentrations of 40% $CO_2$ or above in the presence or absence of $O_2$. A similar phase of high susceptibility is seen in eggs held in $N_2$ (Bell *et al.*, 1980). Eggs of Pyralid moths are fertilized as they descend the oviducts immediately prior to oviposition but it is unlikely that nuclear fusion occurs until several hours afterwards The sensitivity of the fertilizing sperm cell to anoxia or metabolic disruption caused by $CO_2$ may very well explain the extreme sensitivity of very young eggs to treatment. As embryogenesis proceeds, the susceptibility to $CO_2$ decreases at first (Bell *et al.*, 1980) and it continues to do so up to age 44h, in the presence of $O_2$ concentrations of 4-20%. However in the absence of $O_2$ it starts to fall again between 20 and 44h after oviposition. The results for *E. cautella* indicate that the presence of $O_2$ may in some circumstances enhance the toxic action of $CO_2$. During anoxia the development of the eggs virtually ceases (Price and Bell, 1981) and survival depends on the capacity of the embryo to accumulate glycolytic products and reduce its need for active metabolism. In the presence of $O_2$, however, although development may be delayed, there is no evidence that it ceases completely and death probably results from progressive $CO_2$ poisoning which either hampers the utilization of $O_2$ by inhibiting enzymes such as succinic dehydrogenase (Edwards, 1968) or causes oxidative metabolism to accumulate toxic products. The desiccating action of $CO_2$ at lower humidities can be disregarded for eggs because spiracles are absent. If development continues during exposure and different stages vary in tolerance, individuals will tend to develop through phases of tolerance and die when a susceptible stage is reached. A similar effect has been observed with the fumigant phosphine (Reynolds *et al.*, 1967).

A slightly longer exposure was required to kill all eggs with $CO_2$ in the presence of oxygen than in its absence when tests were conducted at 15°C, but the age group surviving best was one quite easily killed by anoxia. The age group most tolerant to anoxia was killed more quickly by $CO_2/O_2$ mixtures. This difference in the stages of development best fitted for survival necessitates further investigation of $CO_2/O_2$ mixtures. It is known that *Sitophilus* spp. are more tolerant to $CO_2$ exposure than most other pests and that 80% $CO_2$ in air or $O_2$ is much more toxic to these species at high temperatures than $CO_2$ alone (Lindgren and Vincent, 1970). In recent unpublished tests on *S. granarius* at Slough conducted by E. C. Spratt, an 80/20% mixture of $CO_2$ and $O_2$ killed adults faster at 15°C than 80% $CO_2$ in air, and very much faster than $CO_2$ alone. The period of exposure necessary for control of this species at 15°C by $CO_2$ in the presence of $O_2$ was in fact not so very much longer than at 25°C.

The time for action of $CO_2$ in the presence of air or oxygen at high temperatures in many species is little different than that for $CO_2$ alone, but results such as those described for *Sitophilus* spp. indicate that at low temperatures the difference may be more marked. If this is so then purging storage structures with $CO_2/O_2$ mixtures may give better results at low temperatures than purging with $CO_2$ alone. However, much work needs to be done on a wider range of species and stages. Up to this time much emphasis has been placed on adults and when compared with other life stages they tend to be fairly tolerant to anoxia. In the presence of $O_2$, however, the spectrum of tolerance to $CO_2$ among species and stages may be different than in the absence of oxygen, as indicated by the shift of the least susceptible age in the current tests on eggs.

Carbon dioxide, unlike nitrogen, does not rely solely on achieving anoxia to be lethal and 100% $CO_2$ is more toxic to moth eggs than 100% $N_2$ (Bell *et. al.*, 1980). In addition, $CO_2$ may be more toxic when $O_2$ is present although this aspect of increased toxicity requires further clarification. Hitherto, $CO_2$ has been used in a manner that more or less replaces air and hence $O_2$, although it has been recognised that $CO_2$ remains effective far longer than $N_2$ as air leaks back into the purged structure. The feasibility of purging structures with a mixture of ca. 10-20% $O_2$ in $CO_2$ will depend on the advantage gained in the initial stages of the treatment. This advantage may be small or localised when only 60-80% of the total air is replaced with pure $CO_2$. As the effects of anoxia in conjunction with $CO_2$ tend to disappear when oxygen levels rise above 4% (Calderon and Navarro, 1980), this period of potential advantage extends only until the $CO_2$ concentration drops to 80% at all points as the purged volume is replaced by air leaking back into the storage.

## REFERENCES

AliNiazee, M. T. (1971) Effect of carbon dioxide gas alone or in combinations on the mortality of *Tribolium castaneum* (Herbst) and *T. confusum* Du Val (Coleoptera, Tenebrionidae). *J. stored Prod. Res.* 7, 243-252.

Bailey, S. W. (1956) Airtight storage of grain; its effect on insect pests. II. *Calandra oryzae* (small strain). *Aust. J. agric. Res.* 7, 7-19.

Bailey, S. W. (1957) Airtight storage of grain; its effect on insect pests. III. *Calandra oryzae* (large strain). *Aust. J. agric. Res.* 8, 595-603.

Bailey, S. W. (1965) Airtight storage of grain; its effect on insect pests. IV. *Rhysopertha dominica* (F.) and some other Coleoptera that infest stored grain. *J. stored Prod. Res.* 1, 25-33.

Bailey, S. W. and Banks, H. J. (1980) A review of recent studies on the effects of controlled atmospheres on stored product pests, J. Shejbal (Ed.): Controlled atmosphere storage of grains, Elsevier, Amsterdam, pp. 101-118.

Bell, C. H. (1981) The influence of light cycle and circadian rhythm on oviposition in five pyralid moth pests of stored products. *Physiol. Ent.* 6, 231-239.

Bell, C. H., Spratt, E. C. and Mitchell, D. J. (1980) The effect of nitrogen and carbon dioxide on eggs of *Ephestia cautella* (Walker) and *E. kuehniella* Zeller (Lepidoptera:Pyralidae). *Bull. ent. Res.* 70, 293-298.

Calderon, M. and Navarro, S. (1979) Increased toxicity of low oxygen atmospheres supplemented with carbon dioxide on *Tribolium cataneum* adults. *Entomologia exp. appl.* 25, 39-44.

Calderon, M. and Navarro, S. (1980) Synergistic effect of $CO_2$ and $O_2$ mixtures on two stored grain insect pests. J. Shejbal (Ed.): Controlled atmosphere storage of grains, Elsevier, Amsterdam, pp. 79-84.

Edwards, L. J. (1968) Carbon dioxide anaesthesia and succinic dehydrogenase in the corn earworm, *Heliothis sea*. *J. Insect Physiol.* 14, 1045-1048.

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. (1980) Low temperatures: effects on control of *Sitophilus oryzae* (L.) with modified atmospheres. J. Shejbal (Ed.); Controlled atmosphere storage of grains, Elsevier, Amsterdam, pp. 65-71.

Jay, E. G. and Cuff, W. (1981) Weight loss and mortality of three life stages of *Tribolium castaneum* (Herbst) when exposed to four modified atmospheres. *J. stored Prod. Res.* 17, 117-124.

Lindgren, D. L. and Vincent, L. E. (1970) Effect of atmospheric gases alone or in combination on the mortality of granary and rice weevils. *J. econ. Ent.* 63, 1926–1929.

Merzke, F. O., Press, A. F. and Pearman, G. C. (1970) Mortality of rice weevil, Indian meal moth and *Trogoderma glabrum* exposed to mixtures of atmospheric gases at various temperatures. *J. econ. Ent.* 63, 570–574.

Navarro, S. and Calderon, M. (1980) Integrated approach to the use of controlled atmospheres for insect control in grain storage. J. Shejbal (Ed.): Controlled atmosphere storage of grains, Elsevier, Amsterdam, pp. 73–78.

Pearman, G. C. and Jay, E. G. (1970) Effect of relative humidity on the toxicity of carbon dioxide to *Tribolium castaneum* in peanuts. *J. Ga ent. Soc.* 5, 61–64.

Press, A. F. and Harein, P. K. (1967) Mortality of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) in simulated peanut storages purged with carbon dioxide and nitrogen. *J. stored Prod. Res.* 3, 91–96.

Price, N. R. and Bell, C. H. (1981) Structure and development of embryos of *Ephestia cautella* (Walker) during anoxia and phosphine treatment. *Int. J. invert. Reprod.* 3, 17-25.

Reynolds, E. M., Robinson, J. M. and Howells, C. (1967) The effect on *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) of exposure to low concentrations of phosphine. *J. stored Prod. Res.* 2, 177-186.

Spratt, E. C. (1979) Some effects of a mixture of oxygen, carbon dioxide and nitrogen in the ratio 1:1:8 on the oviposition and development of *Sitophilus zeamais* Mots. (Coleoptera, Curculionidae). *J. stored Prod. Res.* 15, 73–80.

Storey, C. L. (1977) Effect of low oxygen atmospheres on mortality of red and confused flour beetles. *J. econ. Ent.* 70, 253–255.

Storey, C. L. (1980) Mortality of various stored product insects in low oxygen atmospheres produced by an exothermic inert atmosphere generator. J. Shejbal (Ed.): Controlled atmosphere storage of grains, Elsevier, Amsterdam, pp. 85–92.