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CARBON DIOXIDE AND RELATIVE HUMIDITY: INTERRELATED FACTORS AFFECTING THE LOSS OF WATER AND MORTALITY OF *EPHESTIA CAUTELLA* (WALKER) (LEPIDOPTERA; PHYCITIDAE)[1]

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ABSTRACT

Ephestia cautella (Walker) pupae, 0-24 hr old, were exposed to atmosphere containing 4.3% and 18.6 - 19.0% carbon dioxide, in combination with relative humidities of 19.5-20.8%, 56.0-58.6% and 97.1 - 97.7%. Exposure times ranged from 1 to 6 days, and the tests were carried out at 26+1 C. Loss of water and mortality of the pupae were greater at the higher carbon dioxide concentration and lower relative humidities. The critical level of water loss was found to be about 30% of loss in weight. Dessication appears to be the main factor causing mortality in the test insects tinder the experimental conditions. The mode of action of the combined effect of carbon dioxide and relative humidity on loss of water is discussed.

(1) This study forms a part of a Ph. D. Thesis of the senior author under the supervision of Prof. I.Harpaz of the Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot.

INTRODUCTION

The use of hermetic storage to prevent insect infestation has been practised for a very long time. Insects present in grain stored in completely airtight containers will die as soon as the available oxygen is consumed. Bailey (1957) attributed the lethal effect of hermetic storage on insects to depletion of oxygen rather than to the rise in carbon dioxide concentration. Oxley and Wickenden (1963) established a level of about 2% oxygen as critical for the survival of insects in storage containers. However, in practice it is very difficult to obtain this low oxygen concentration in storage structures.

Harein and Press (1968), testing different mixtures of atmospheric gases, found that atmospheres containing up to 15% oxygen are lethal to insects provided 36% of carbon dioxide is also present. Jay *et al.* (1970) applied the findings of this study by introducing and circulating carbon dioxide in a groundnut silo for insect control. However, only in 1971 (Jay *et al.*, 1971) was the role of relative humidity in modified atmospheric gas compositions considered important for control of stored product insects. Discussing the results of their investigation the authors suggested that increased mortality at low humidities in the modified atmospheres was due to rapid loss of water from the insects caused by prolonged opening of the spiracles.

The aim of the present investigation is to provide quantitative information on the effect of the two interrelated factors; carbon dioxide and relative humidity, on pupae of *Ephestia cautella* (Walker). Furthermore, we attempt to express the effects of these treatments as indicated by a critical level of loss in water, at which mortality of the exposed test insect occurs.

MATERIALS AND METHODS

The test insect, *E. cautella,* was reared on ground wheat mixed with 12.5% glycerine (Navarro and Gonen, 1970). Insect cultures were kept at 26 ± 1°C and 70 ± 5% relative humidity. Pupae, 0-24 hr old (after pupation) were weighed on an analytical balance in groups of ten in small copper-mesh (no.80) cages (3 cm high, 1.5 cm diameter) and were then exposed to different atmospheres in 100 ml capacity test chambers (Erlenmayer flasks). After each exposure the insect groups were weighed and placed in 20 ml empty PVC tubes. Emergence of adults was recorded daily. Pupae from which adults had not emerged 15 days after emergence of adults from the untreated pupae were considered dead.

The different atmospheric gas concentrations were obtained by mixing ambient air supplied by a membrane pump with carbon dioxide released from a pressurized cylinder. The gas mixture was passed through gas washing bottles containing known concentrations of sulphuric acid solutions in order to obtain the desired relative humidity. Thus, the conditioned gas mixtures were continuously supplied to the test chambers containing insects after the relative humidity had been checked by passing the mixture through a 9800 ml capacity chamber containing a hair hygrometer. Flow rate in the test chamber ranged from 10 to 15 ml/min and the pressure measured on an inclined manometer was 2.4 to 4.4 mm water.

Gas samples of 100 µl were taken twice daily from the test chambers and their composition determined using a gas chromatographic method (Tadmor *et al.*,1971). The average values of the gas composition to which the insects were exposed are given in Table 1.

For each gas mixture 6 exposure times were examined (exposures from 1 to 6 days). Each experiment was replicated eight times and the results were analysed using a factorially designed test (Steel and Torrie, 1960).

Table 1: Average values of gas concentrations and relative humidities to which *E. cautella* pupae were exposed.

Treatments	% RH SE	% CO_2 SE	% O_2 SE
Group 1	*19.5 ± 0.43*	*4.3 ± 0.15*	*18.1 ± 0.85*
4.3% CO_2	*58.6 ± 0.79*	*4.3 ± 0.12*	*18.2 ± 0.31*
	97.1 ± 0.06	*4.3 ± 0.14*	*18.3 ± 0.77*
Group 2	*20.8 ± 0.92*	*18.6 ± 0.36*	*15.5 ± 0.35*
18.6-19.0%	*56.0 ± 2.11*	*18.8 ± 0.03*	*16.1 ± 0.12*
CO_2	*97.7 ± 0.12*	*19.0 ± 0.18*	*15.8 ± 0.11*

R E S U L T S

Effect on mortality

The effect of the two interrelated factors on the reduction in percentage of adult emergence can be seen in Fig.1. It is shown that the effect of carbon dioxide is strongly modified by the level of the relative humidity. Thus, at about 20% relative humidity mortality of *E. cautella* pupae was obtained in about 5 days, even at exposure to only 4.3% carbon dioxide. At 56.0 - 58.6 % relative humidity, the effect on mortality was obtained only in atmospheres containing 18.8% carbon dioxide after 4 days exposure. Air mixtures containing 97.1% relative humidity did not result in mortality at 4.3% carbon dioxide, while some reduction of adult emergence was recorded at the 19.0% carbon dioxide level during 6 days of exposure.

Effect on loss in weight

Fig. 2 shows the significant effect of gas mixtures tested on loss in weight of the pupae. Here also, the higher the carbon dioxide concentration and the lower the relative humidity the more pronounced is the effect on weight loss. A value of 56.7% loss in weight was obtained after 6 days exposure to 18.6% carbon dioxide and 20.8% relative humidity. It should be noted that mortality of the pupae exposed to the same treatment occurred at 4 days of exposure. It appears therefore, that loss in weight during the last two days of exposure occurred after death of the pupae. On the other hand, loss in weight was much smaller at about the same carbon dioxide concentration when 97.7% relative humidity was present. This treatment resulted in only 57% adult emergence (Fig. 1).

The critical loss in weight

Fig. 3 shows the correlation existing between loss in weight and reduction in adult emergence from the pupae. The regression lines in this figure were calculated from values obtained from the three treatments which produced mortality at the three relative humidity levels tested, namely: 1) 4.3% carbon dioxide at 5 days exposure, 2) 18.6 - 19.0% carbon dioxide at 2 days exposure, 3) 18.6 - 19.0% carbon dioxide at 3 days exposure. These lines indicate that mortality occurred when the pupae had lost about 30% of their weight.

FIG -1. THE EFFECT OF CO_2 AT DIFFERENT RELATIVE HUMIDITIES ON ADULT EMERGENCE FROM *E.cautella* PUPAE,

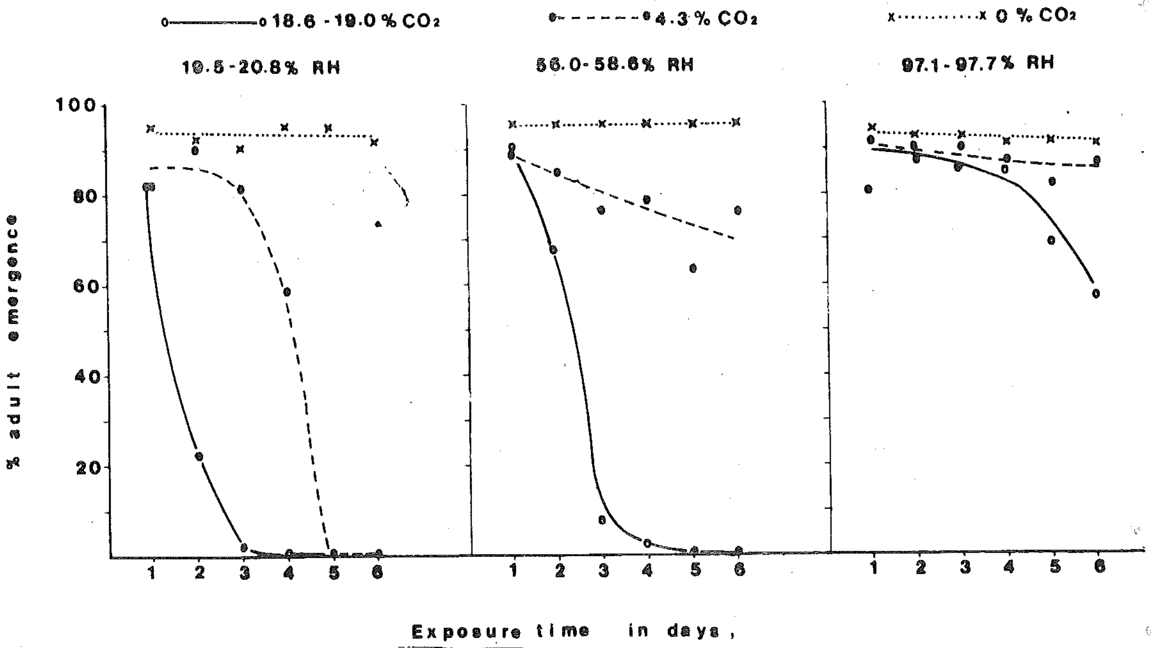


FIG - 2. THE EFFECT OF CO_2 AT DIFFERENT RELATIVE HUMIDITIES ON LOSS IN WEIGHT OF *E.cautella* PUPAE,

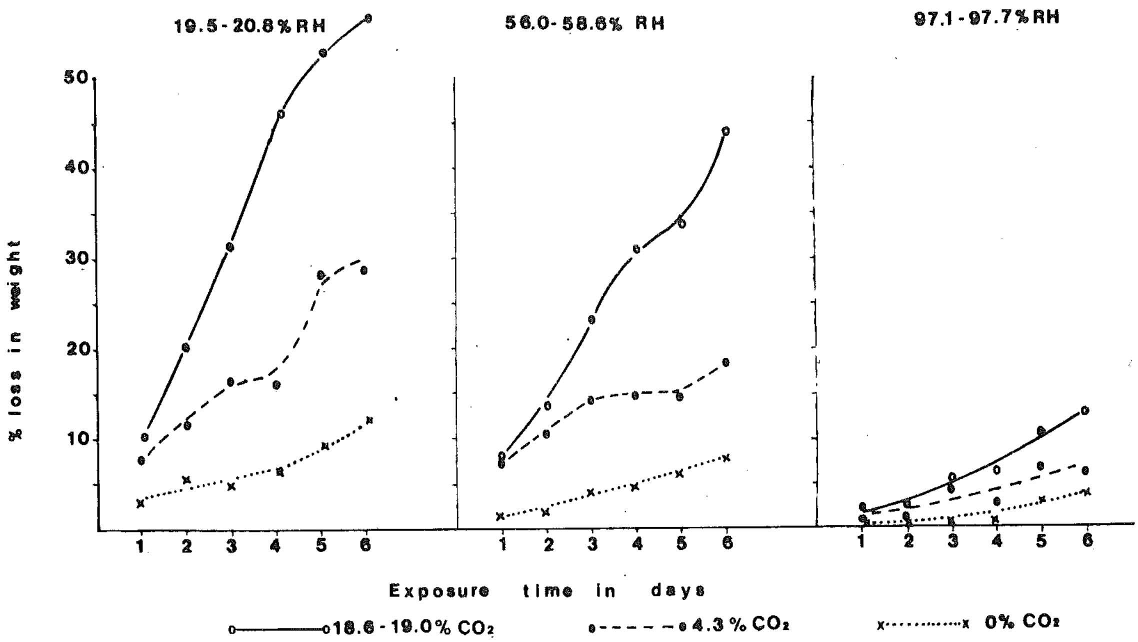
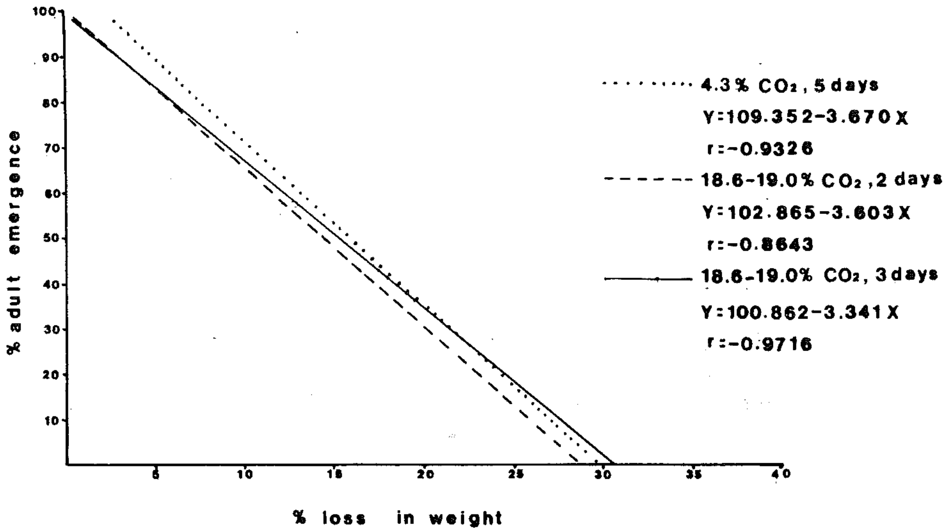


FIG-3. LOSS IN WEIGHT AND ADULT EMERGENCE FROM E. cautella PUPAE SUBJECTED TO DIFFERENT CO_2 CONCENTRATIONS AT DIFFERENT EXPOSURE TIMES, (range of RH 19.5 - 97.7 %)



D I S C U S S I O N

In this discussion the loss in weight by the insects will be considered for convenience sake as loss of water (Bursell, 1957).

It has been established that insects die when their water content is reduced to below certain limits (Bursell, 1964). This occurs under environment conditions in which water balance becomes critically upset, loss of water being greater than gain. Regulation of spiracular and excretory mechanisms may play an important role in loss of water by insects, when their integument is relatively waterproof. The function of the spiracles in regulating water balance is of prime importance for insects living in dry environments. This seems to be the case also for *E. cautella* and in particular at its pupal stage.

The effect of carbon dioxide, as a stimulant for keeping the spiracles open, has been examined by various investigators (Mellanby, 1934; Bursell, 1957). Wigglesworth (1935) demonstrated the effect of different concentrations of carbon dioxide on prolonged opening of the spiracles of *Xenopsylla cheopis* Rothschild : at about 1% carbon dioxide the period during which the spiracles were kept open was prolonged by 50%, while at 2% carbon dioxide the spiracles were kept permanently open.

The results of the present investigation demonstrated clearly the effect of carbon dioxide on water loss by insects; the higher the carbon dioxide concentration the greater the loss of water. This effect is more pronounced at low relative humidities, at which the water balance of the insects becomes more disturbed. Thus, dessication appears to be the main factor causing mortality in insects subjected to the treatments in these experiments. However, higher carbon dioxide concentrations may have an additional effect on insects, namely, an anesthetic effect (Whisenant and Eugene Brady,1967).

The critical level of water loss by insects was deduced from the three treatments in which complete mortality was obtained (Fig. 3). This value was found to be about 30% of loss in water. We presume however, that this critical value is not a constant value, and may vary according to the treatments provoking dessication and the time of exposure.

Further work is required for clarification of the factors involved in the above experimental treatments. All this information is of significance for application of environmental control methods in the control of stored product insects.

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