

SYNERGISTIC EFFECT OF CO<sub>2</sub> AND O<sub>2</sub> MIXTURES ON TWO STORED GRAIN INSECT PESTS\*

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## ABSTRACT

Tribolium castaneum and Rhyzopertha dominica adults and eggs were exposed to atmospheres containing 2%-8% O<sub>2</sub>, supplemented with 5%-30% CO<sub>2</sub> at 26°C and 55% r.h. for 24-144 h. Eggs of both species were more susceptible than their adult stage to low O<sub>2</sub> concentrations per se as well as to the O<sub>2</sub> and CO<sub>2</sub> mixtures tested. R. dominica eggs were more tolerant than T. castaneum eggs to the above treatments.

Addition of CO<sub>2</sub> to low O<sub>2</sub> atmospheres resulted in a synergistic effect on adult mortality of both species, while there was only an additive effect on the eggs exposed to the same treatments.

These results provide additional information to be considered in the use of controlled atmospheres for grain storage.

## INTRODUCTION

The effect of various combinations of atmospheric gases on stored product insects has been investigated extensively by numerous workers and interesting results have been recorded (Bailey, 1965; Harein and Press, 1968; Jay and Pearman, 1971; Navarro and Calderon, 1974). In many of these studies a clear indication of the enhancing effect of CO<sub>2</sub> on insect mortality was found. One of the most interesting and surprising findings was by Harein and Press, 1968, who showed that for T. castaneum and Plodia interpunctella adults, atmospheres containing up to 15% O<sub>2</sub> supplemented with 36% CO<sub>2</sub> were lethal after 10 days of exposure.

In a test of the effectiveness of atmospheric gas mixtures containing up to 5% O<sub>2</sub> and 5-35% CO<sub>2</sub>, a significant interaction was found between the levels of O<sub>2</sub> and CO<sub>2</sub> tested and the exposure period (Calderon and Navarro, 1979). These results indicated a clear synergistic effect of the two gases on the mortality of Tribolium castaneum adults.

The following reports an experiment to detect this interaction in gas mixtures containing up to 8% O<sub>2</sub>, and to study the effect of the mixtures on the adults and eggs of T. castaneum and R. dominica.

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## MATERIALS AND METHODS

Tribolium castaneum was reared on wheat feed mixed with 5% brewer's yeast (by weight), and Rhyzopertha dominica on whole wheat (11% moisture content), in a controlled temperature ( $26 \pm 1^{\circ}\text{C}$ ) and relative humidity ( $70 \pm 5\%$ ) room. Groups of 50 adults, 7-10 days old, were collected from cultures and each species was exposed, on 3 g of appropriate food, to different atmospheric gas mixtures in a chamber of 100 ml capacity (Navarro and Donahaye, 1972).

Eggs, 0-24 h old, were collected and confined in individual incubation cells devised for this purpose (Navarro and Gonen, 1970). Groups of 100 eggs confined in these cells were exposed in the same chambers to the same treatment as the adults.

In each experiment one group of insects was exposed to the given gas mixture for 24, 48, 72, 96, 120 and 144 h, at  $26 \pm 1^{\circ}\text{C}$  and an average r.h. of 55%. Adult mortality was determined as follows: after exposure, adults were transferred to vials of 100 ml capacity containing 3 g of appropriate food, and kept under controlled temperature and r.h. as in the insects' rearing room. Dead and live insects were counted 14 days after the end of each treatment. Egg mortality was determined by the failure to hatch 14 days after the hatch of the last egg of the same group. During the incubation period eggs were kept in the same exposure chambers - at normal atmosphere and ambient conditions - as the adults.

Mortality results were prepared for analysis by stepwise multiple regression analysis (Snedecor and Cochran, 1969). For determination of interaction among the variables, a factorial design was adopted using analysis of variance.

## RESULTS AND DISCUSSION

### Effect on adults

Data on the mortality of the two species exposed to different combinations of  $\text{O}_2$  and  $\text{CO}_2$  are shown in Fig. 1. Results obtained from tests with different exposure times were recorded, but for demonstration purposes only the results of the 96-h exposure are given. The curves in Fig. 1 show the enhanced toxicity of the gas mixture when  $\text{CO}_2$  was added to the given  $\text{O}_2$  atmosphere.

For T. castaneum, exposure to the different  $\text{O}_2$  concentrations alone, did not result in substantial mortality, except with 2%  $\text{O}_2$ . However, when 15%  $\text{CO}_2$  was added, the lethal effect of the reduced  $\text{O}_2$  concentrations was considerably increased. Even at 6%  $\text{O}_2$ , 90% mortality was achieved when 30%  $\text{CO}_2$  was added.

Similar results were obtained with R. dominica (Fig. 1), showing increased toxicity of reduced  $\text{O}_2$  concentrations supplemented by  $\text{CO}_2$ . The combination of the two gases seemed to affect R. dominica adults considerably more: at 2%  $\text{O}_2$ , 100% mortality was obtained by adding only 10%  $\text{CO}_2$ .

The data obtained for the adult stage of the two species tested indicate the existence of a strong interaction between the effects of the two gases. This was expressed in increased toxicity of the gas mixtures containing  $\text{CO}_2$  added to

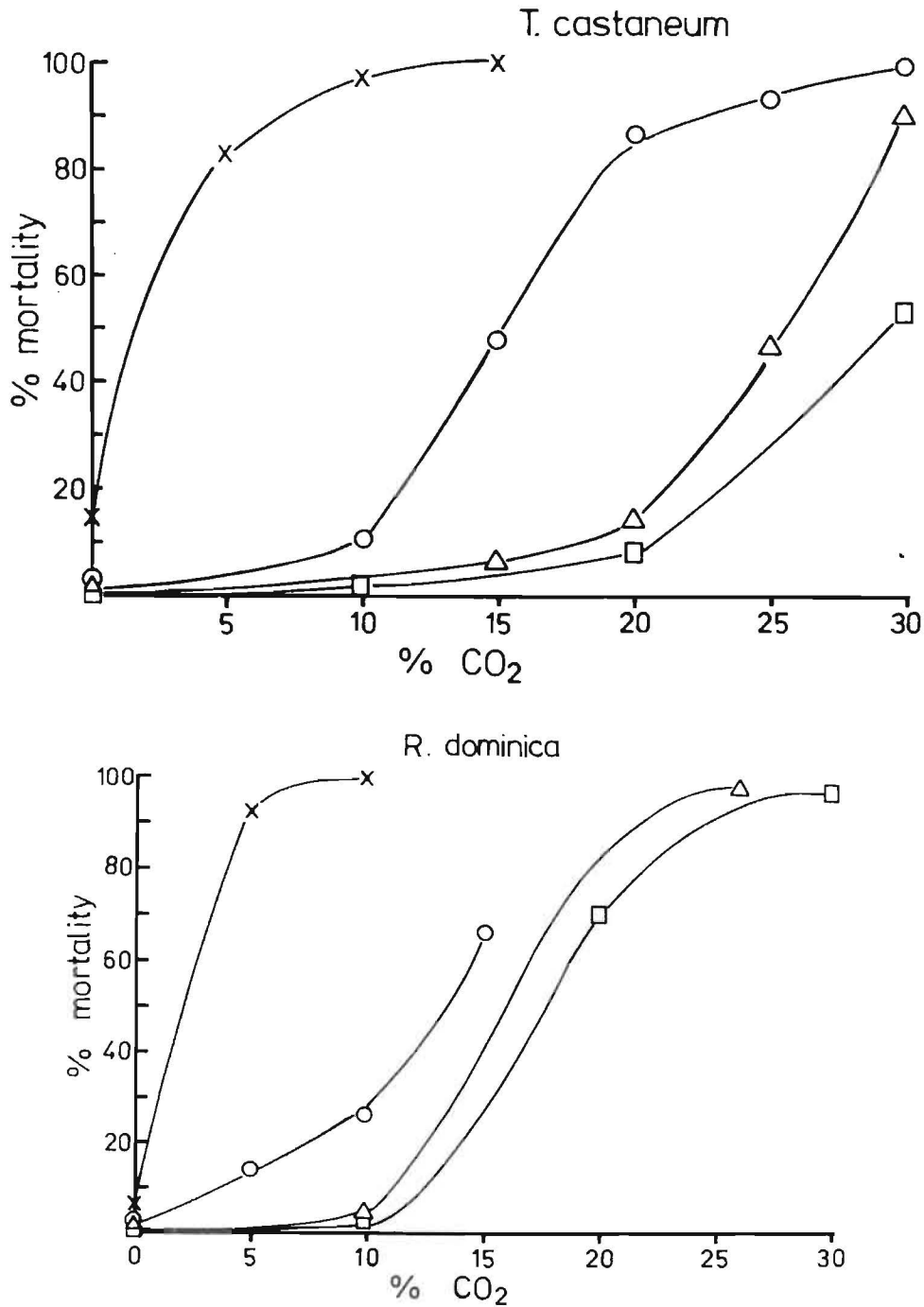


Fig. 1. Mortality of *T. castaneum* and *R. dominica* adults exposed for 96 h to different O<sub>2</sub> and CO<sub>2</sub> mixtures at 57% r.h. and 26°C (O<sub>2</sub> levels were: 2%—X, 4%—O, 6%—Δ and 8%—□.).

reduced O<sub>2</sub> atmospheres. Analysis of variance performed on the data revealed the significant level of this interaction, which explains the synergistic effect of the two gases.

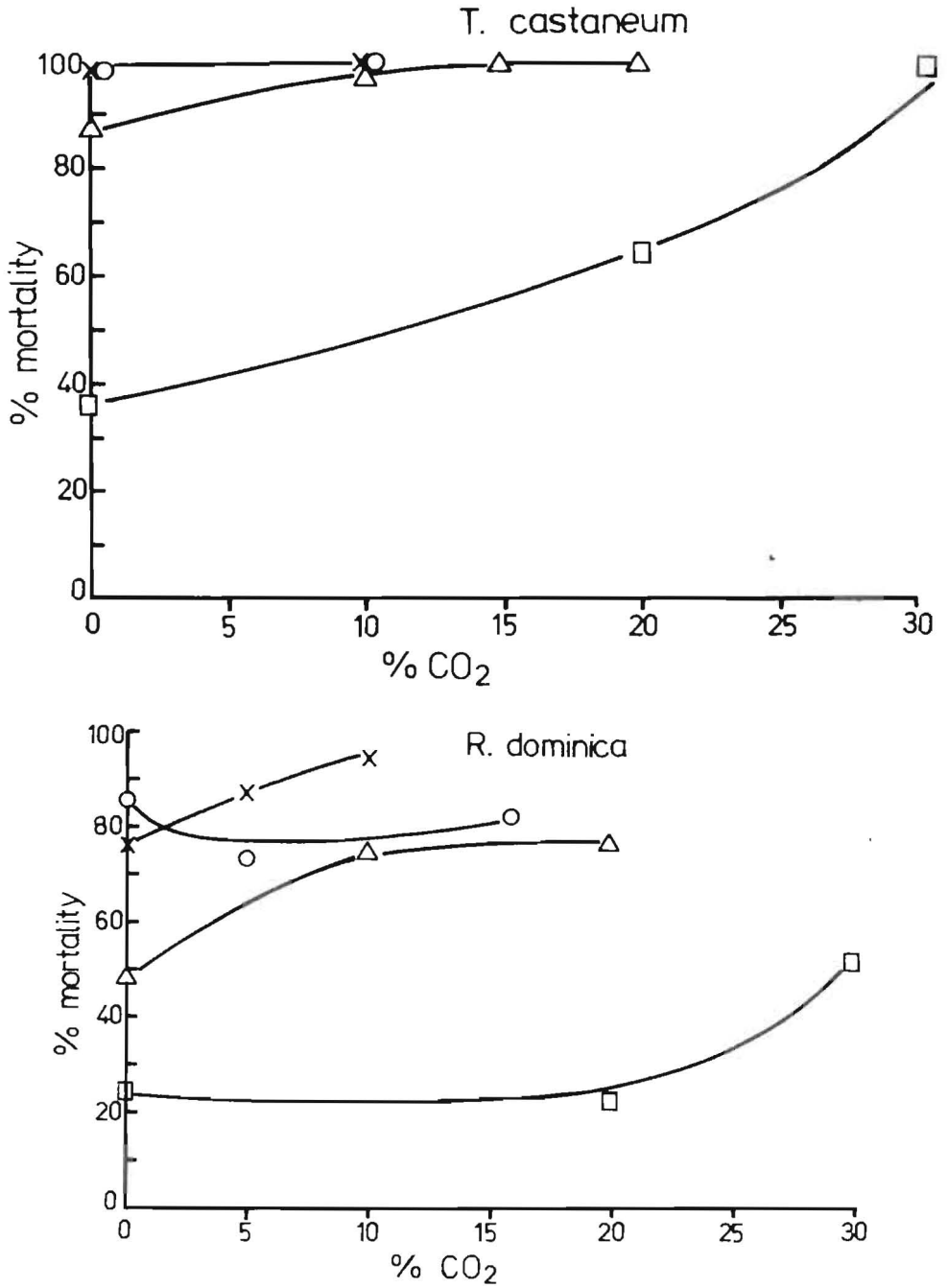


Fig. 2. Mortality of *T. castaneum* and *R. dominica* eggs exposed for 96 h to different O<sub>2</sub> and CO<sub>2</sub> mixtures at 57% r.h. and 26°C (O<sub>2</sub> levels were: 2%—X, 4%—O, 6%—Δ and 8%—□).

#### Effect on eggs

Figure 2. shows a different pattern of response of the eggs exposed to the tested atmospheric gas combinations. First, there was a marked difference in susceptibility to reduced O<sub>2</sub> atmospheres, compared with that of adults. For

T. castaneum eggs exposed for 96 h, 100% mortality was recorded at 2% and 4%  $O_2$  concentrations without  $CO_2$ . Exposure to 6%  $O_2$  resulted in about 87% egg mortality, and even at 8%  $O_2$  up to 47% of the exposed eggs failed to hatch. In contrast to the effect on adults (Fig. 1)  $O_2$  concentrations of 4% and higher, no adult mortality was obtained. Rhyzopertha dominica eggs (Fig. 2) were less susceptible to the above treatments than T. castaneum eggs, but the adult was again found to be more tolerant than the egg.

These findings appear to be in disagreement with conclusions reported by other authors (Ali Niaze, 1971; Lindgren and Vincent, 1970; Storey, 1977), whose results indicate relative tolerance of the egg as compared with the adult and the larva. However, those results cannot be compared with ours, since in the other investigations eggs were exposed to either very low  $O_2$  of 0.1-0.5% (Storey, 1977), or to pure  $N_2$ ,  $CO_2$  or He atmospheres (Ali Niaze, 1971; Lindgren and Vincent, 1970). Moreover, in most cases the above treatments were tested on 3-day-old eggs, while in our study the eggs were 0-24 h old. We consider our findings on the high susceptibility of the egg stage to low  $O_2$  atmospheres, interesting and of practical importance. More work on this subject is in progress.

Results on the effect on eggs of  $CO_2$  added to reduced  $O_2$  atmospheres show again a different pattern of response, to that of the adults. The addition of  $CO_2$  to the given  $O_2$  atmospheres had little effect on egg mortality. At the  $O_2$  concentrations tested, increasing  $CO_2$  concentrations did show an additive effect on egg mortality and did not indicate any synergism of these two gases on egg hatch.

We find it difficult to explain the above difference in response based on the physiology of insect eggs versus adults. However, we can speculate that the loss of water in adults which takes place at atmospheres of higher  $CO_2$  content, does not apparently occur at the same rate in the exposed eggs. Unpublished data have indicated that E. cautella and T. castaneum eggs are significantly less affected by low r.h. than are the adults. Further research is needed to elucidate this interesting point.

The findings described herein may have important practical implications in the use of controlled atmospheres for insect control in stored products.

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