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Physiological differences between strains of *Tribolium castaneum* selected for resistance to hypoxia and hypercarbia, and the unselected strain

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Abstract. The metabolic rates, as expressed by oxygen (O_2) consumption, carbon dioxide (CO_2) production, and losses in wet and dry weights, were examined for adults of three strains of the red flour beetle *Tribolium castaneum* (Herbst), during exposure to two modified atmospheres (MAs). Exposure of a strain selected for resistance over twenty-one generations to an atmosphere of 65% CO_2, 20% O_2 and the balance nitrogen (N_2), termed a high carbon dioxide concentration atmosphere (HCC) and exposure of an unselected strain to HCC, showed considerable levels of aerobic metabolism during exposure. For the unselected strain water loss and mobilization of energy reserves were rapid and mortality was followed by rapid desiccation. For the HCC-resistant strain water balance was maintained and energy reserves were utilized more slowly over a prolonged period. Exposure of a strain selected for resistance over twenty-one generations to a low oxygen concentration atmosphere (LOC) of 0.5% O_2 in N_2, and an unselected strain to LOC, revealed that even at 0.5% O_2, metabolism was largely aerobic in both strains. Maintenance of water balance was not a major factor causing mortality of either strain during exposure to LOC. In air, metabolic rates of both the resistant strains were lower than that of the unselected strain.

Key words. *Tribolium*, flour beetle, resistance, hypoxia, hypercarbia.

Introduction

Two strains of *Tribolium castaneum* that had been selected for resistance to modified atmospheres were used in this study. One was selected for resistance to a high carbon dioxide content (HCC) atmosphere of 65% CO_2, 20% O_2 and 15% N_2 at 95% relative humidity (r.h.) and another was selected for resistance to a low oxygen content atmosphere (LOC) of 0.5% O_2 in nitrogen at 95% r.h. A third strain, the original unselected laboratory strain, sensitive to both modified atmospheres (MAs), was also employed. Details of the selection process are given by Donahaye (1990a, b).

This study was undertaken to compare the levels and quality of respiratory metabolism of the three strains of *T.castaneum* before, during and after exposure to the respective MAs. The objective was to ascertain whether and if so, how, induced tolerance obtained at the twenty-first selection found expression in modification of the respiratory metabolism. Loss in weight during exposure was also examined as a further index of rate of metabolism of the three strains. In addition, mortality rates were examined for insects exposed to MAs without food medium in order to relate measurements of metabolism of groups of insects undergoing prolonged exposure (without food medium), to progressive mortality in the population. This was done because it had been found that mortality rates of insects exposed with or without food medium differed widely and the routine mortality analysis (with food medium) could not be used.

Several researchers have investigated the respiratory metabolism of *Tribolium* spp. Park (1936) studied O_2 consumption with reference to body weight, sex and food medium. Calderwood (1961) and Chaudhry & Kapoor (1967) studied the effect of temperature on O_2 consumption. AliNiazee (1971) showed that CO_2 at up to 8% lowered the rate of respiration in *T.confusum*, although beetles recovered and respiration returned to normal within the 24 h treatment period. Carlson (1968) measured O_2 consumption, CO_2 production and the respiratory

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quotient (RQ) at five different combinations of CO_2 and O_2 concentration. RQ was lowest at the lowest O_2 concentration tested and was below 1 for all except the normal CO_2:O_2 ratio for air. Carlson concluded that lack of O_2 rather than increased CO_2 content stimulated increased O_2 consumption. Kennington (1966), measuring O_2 uptake after total O_2 deprivation, found no CO_2 production during O_2 deficiency and no significantly greater O_2 use after the period of deficiency. These results differ from those of Thunberg (1905) for *Tenebrio* and Harnisch (1930) for *Chironomus* larvae, who found that at low O_2 tensions the RQ tends to rise owing to increased acidity driving off CO_2, and may even rise above unity. Then during the process of recovery, as the CO_2 capacity of the blood and tissues increases again, the RQ falls for a time below the normal level.

The action of CO_2 on the spiracular muscle has been demonstrated by Hoyle (1960) and the effect of high CO_2 tension or low O_2 tension on opening of the spiracles independently of the central nervous system has been shown (Hazelhoff, 1928; Burkett & Schneiderman, 1967). The extent of spiracular control during exposure to MAs may influence the insect's ability to resist desiccation, and this has been shown to be a contributing factor to mortality under MA treatments (Navarro & Calderon, 1973; Jay & Cuff, 1981). However, when *T.castaneum* adults were exposed to 96% r.h. at a 1.1% O_2 concentration in nitrogen, desiccation did not differ significantly from the control at 21% O_2 (Navarro, 1975). Selection of the resistant strains was carried out at 95% r.h. to minimize the desiccation effect though clearly water loss could still be a contributing factor to mortality even at exposure to this high humidity. Therefore the effect of the MAs on water balance was also investigated.

Materials and Methods

Sensitivity of the three strains when exposed to MAs with and without food medium. Sensitivities of the HCC-selected and the unselected strains to HCC, and the LOC-selected and the unselected strains to LOC, were examined by exposing insects at the twenty-first selection in exposure flasks over a range of times in an apparatus designed to deliver the appropriate MA (Donahaye, 1990a). In normal exposure procedure 100 adults were placed in each flask together with 1 g ground wheat. For comparison, identical treatments were done with omission of the food medium. Probit analysis was carried out using the program of Daum (1979) run on a VAX 11–750 computer.

Comparison of metabolic rates in air between selected and unselected strains. Measurement of metabolic rates as assessed by O_2 consumption and CO_2 output per unit time, was carried out by modification of the method developed by Carlson (1966). Three lots of 10–15-day-old adults were aspirated from pre-exposure culture jars from each of the three strains at the twenty-first selected generation. Each lot of 300 insects was held for 24 h without food, weighed to the nearest 0.1 mg and then transferred to one of three 50 ml Erlenmeyer flasks modified to form respirometers. Each flask was supplied with an upper and lower arm for circulation of the gas supply, that could be shut off by means of stopcocks, and the volume of each respirometer was carefully measured. Gas sampling was done with a 'Pressure-Lok' syringe through a rubber septum fitted to the flask. The flasks were held at 26°C in a thermostatically controlled water bath and humidified air (95% r.h.) was allowed to flow through the flasks for 1 h at a flow rate of 40 ml/min. At the end of this period, when insect activity had reverted to normal after the disturbance of transfer, the air composition within the flasks was sampled by withdrawal of a 2 ml sample and analysed by gas chromatography (Donahaye, 1990a). The stopcocks were then closed for 60 min, at the end of which time a gas sample was again withdrawn and analysed. Stopcocks were reopened for a 30 min period, and the process described above was repeated three times. Nine respiration measurements were made for each strain.

Gravimetric conversion of gas concentrations was calculated and O_2 consumption, and CO_2 output were expressed as $\mu mol \times 10^{-4}$/mg live-wt/min. Respiration quotients (CO_2/O_2) were calculated on a molar basis.

Measurement of metabolic rates before, during, and after a 24 h exposure to MAs. Measurement of metabolic rates was carried out as described previously. Batches of 300 insects were transferred directly after weighing, from pre-exposure jars to the respiration flasks. Each trial consisted of one selected and one unselected batch, and seven trials were carried out for each MA. Respiration rates in air were measured for 1 h prior to exposure and the flasks were then flushed for 1 h with the MA. The stopcocks were then closed for 1 h, with gas samples being taken at the beginning and end of this period. This was followed by up to seven 1 h flushing periods followed by 1 h measuring periods.

For both MAs, measurements were carried out within a 24 h period at the end of which the insects were removed from the respirometers and transferred to standard 100 ml Erlenmeyer flasks. These were kept open to the air for 1 h to permit the insects to recover. Then the mouths of the flasks were covered with septa and post-exposure respiration was measured for an additional hour. The insects were examined 24 h later under a strong light. Those that failed to move were considered dead, per cent survival was calculated and a correction made to post-exposure respiration rates.

Measurement of metabolic rates of the three strains to the MAs throughout exposure until mortality. Batches of 100 insects from each strain at the twenty-first selection were weighed and then transferred to standard exposure flasks, the volumes of which had been measured previously. The mouths of the flasks were covered with rubber septa and the pre-exposure respiration rates were determined. Then the flasks were attached to the appropriate MA by inserting the inlet and outlet syringe needles through the septa. Respiration rates were measured regularly throughout

exposure, until cessation of metabolic activity. Measurements were made by removing the needles from the septa to isolate the flask from the MA flow-system, and then gas concentration measurements were taken 1 h later. This was followed by re-attachment of the flasks to the MA flow system.

The syringe needles were of small diameter (no. 25) to minimize damage to the septa resulting from regular removal and reinsertion of the needles during exposure. However, as exposure progressed there were clear indications that the seal was no longer complete. This was expressed by a slight decrease in CO_2 concentration after 1 h of exposure to HCC instead of the anticipated increase, and a slight increase in O_2 concentration after 1 h of exposure to LOC instead of the anticipated decrease. Consequently, only the decrease in O_2 concentration for HCC exposures and the increase in CO_2 concentration for LOC exposures, were used as indices for measuring respiration rates, both gases being respectively closer to atmospheric conditions outside the flasks. The experiment was carried out with four repetitions for the HCC-selected and the unselected strain to HCC, and for the LOC-selected and unselected strain to LOC.

Loss in dry weight and water contents of the three strains during exposure to MAs. Since during exposure to HCC and LOC the insects do not feed (in the case of HCC they are immobilized, while at the LOC slow movement was occasionally noticed), loss in dry weight may be used as an index of metabolic rate during exposure. Coincidental to this, the water content of the insects was calculated, the interest in this being to verify whether the 95% r.h. during exposure did in fact nullify the possibility that the insects would be selected for resistance to desiccation or whether even at this high humidity, water loss was a significant factor in survival to the MAs.

Adults 10–15 days old were taken from each of the three different strains of *T. castaneum*. They were collected in groups of 100, weighed accurately, and placed in exposure flasks. Four groups from each strain were taken at random to determine base-line dry weights of populations of the three strains. This was done by weighing them in porcelain crucibles before and after desiccation in a drying oven held at 104°C for 2 h. the remaining groups were exposed without food medium in the flasks according to the described method (Donahaye, 1990a). For each exposure period ranging from 1 to 20 days, four groups of HCC-selected and unselected strains were exposed to HCC, and four groups of LOC-selected and unselected strains were exposed to LOC. At the end of each exposure period the insects removed from the exposure apparatus were reweighed, their water content was measured by the above method, and the loss in dry weight (dry weight basis) was extrapolated by subtraction from the initial dry weight of the population sample. Although in addition to water some volatile fatty acids may have been lost by heating, these were not considered to contribute significantly to the weight losses recorded.

Results and Discussion

Sensitivity of the three strains when exposed to MAs with and without food medium

Table 1 shows that on exposure to HCC, the LT_{50} of the HCC-selected strain without food was similar to that with food, although the slope of the probit-mortality line was much lower. For the unselected strain a large difference was recorded: the LT_{50} without food was 15 h less than that with food. A possible explanation for this lies in the unselected strain's difficulty in maintaining its water balance during exposure to HCC. Perhaps contact between insects and the food that rapidly absorbs moisture from the MA, lowers the initial rate of water loss and prolongs survival.

The opposite effect was recorded for exposure to LOC. Both strains survived far longer on exposure to LOC without food. It is interesting to note that the resistance factors of both selected strains at the LT_{50} level for exposure without food, were about twice those for exposure with food.

Metabolic rates of the three strains in air

Results of one-way analysis of variance on rates of O_2 consumption and CO_2 production using the Student multiple range test are given in Table 2.

These results show that both O_2 consumption and CO_2 production in air were significantly higher in the unselected strain than in the two selected strains. The respiration quotients were 0.79, for the unselected strain and 0.84 and 0.76 for the HCC- and LOC-selected strains respectively.

Metabolic reactions of HCC-selected strain

Measurement of metabolic rates before, during and after exposure to HCC. Fig. 1 shows the average O_2 consumption of the HCC-selected and the unselected strains before, during and after exposure to HCC. The figure shows that O_2 consumption during exposure dropped to about 50% of the normal rate and this level was maintained throughout the 24 h period. For the unselected strain, this drop in O_2 consumption continued after exposure had ended, whereas that of the HCC-selected strain rose immediately to above the original level.

Fig. 2 gives the CO_2 production of the two strains over the same period. It shows that the rate of CO_2 production fell to zero during exposure. The negative recordings indicate that not only did the high CO_2 levels of the HCC during exposure prevent the insects from releasing CO_2, but that CO_2 was actually sorped into the insects during this period. This phenomenon was checked by running tests with HCC in empty exposure chambers to verify that this was not due to leakage or sampling error, and also by exposing heat-killed insects to HCC in the chambers where the CO_2 sorption effect also occurred. In this con-

Table 1. Comparative mortalities and resistance factors (RF) for exposure (time in h) of the three strains of *Tribolium castaneum* at the twenty-first selection to HCC and LOC with and without food medium.

		Without food medium			With food medium		
	Strain	LT_{50}	LT_{99}	RF at LT_{50}	LT_{50}	LT_{99}	RF at LT_{50}
Exposure to HCC	HCC-selected	158	894	8.3	147	504	4.2
	Unselected	19	57	–	35	114	–
Exposure to LOC	LOC-selected	275	700	4.3	81	274	2.6
	Unselected	64	528	–	31	74	–

Table 2. Average O_2 consumption and CO_2 production (μmol × 10^{-4}/mg/min) by *Tribolium castaneum* adults at twenty-first selection for LOC and HCC strains in comparison to the unselected strain at 26°C and 95% r.h.

	(μmol $\times 10^{-4}$/mg/min)	
Strain	O_2 consumption	CO_2 production
Unselected	17.1 (a)*	13.6(a)
LOC selected	13.6 (b)	10.4 (b)
HCC selected	12.5 (b)	10.6 (b)

* Values assigned the same letter are not significantly different at the $P < 0.01$ level.

text the phenomenon of strong sorption of CO_2 into many plant products has been well documented (Mitsuda *et al.*, 1973).

The drop in O_2 consumption and CO_2 production of the unselected strain during the post-exposure period may be explained by the fact that 60.7% of the insects failed to recover. This was in contrast to the HCC-selected strain in which mortality averaged 5.8%.

The findings show that exposure to HCC did not produce an anoxia effect and O_2 consumption continued, albeit at a decreased rate, possibly due to inactivity caused by the anaesthetic effect of the CO_2. This is in agreement with the findings of AliNiazee & Lindgren (1969), AliNiazee (1971) and Carlson (1966, 1968). The high CO_2 tension outside the insect body would appear to prevent release of CO_2 which was retained within the insect body in addition to actual ingress of CO_2 from the atmosphere. In this context the importance of the toxic effects produced by CO_2 accumulation, as discussed by Brooks (1965), Edwards (1968), Navarro & Friedlander (1975) and Sillans & Biston (1979), must be considered.

The RQs are given in Fig. 3. Before exposure, RQs were 0.83 and 0.79 for the unselected and selected strains respectively; during exposure they dropped to zero; and

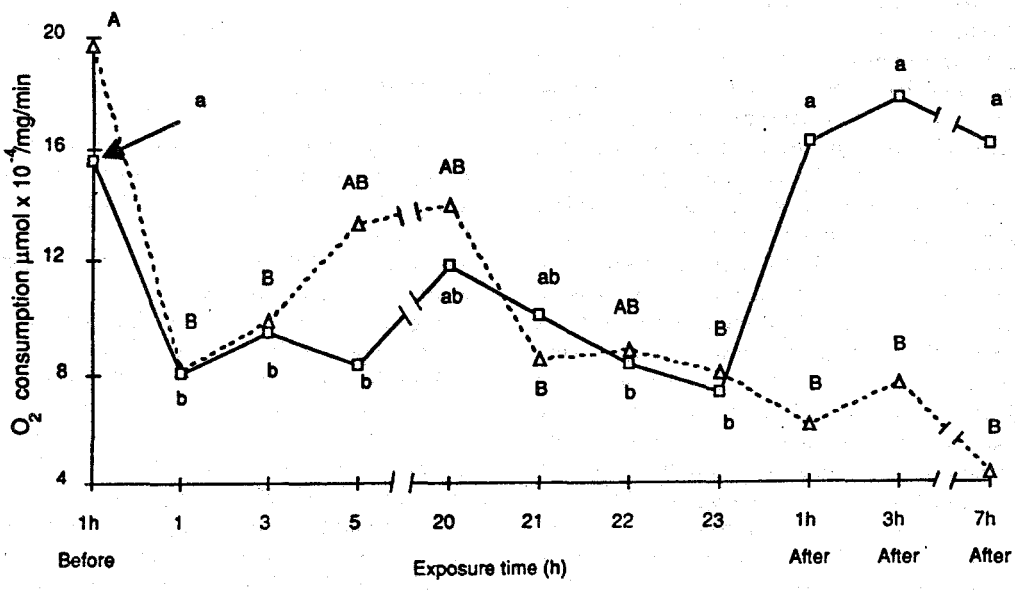

Fig. 1. Rate of O_2 consumption of the HCC-selected (LT_{99} = 894 h) (□) and unselected (LT_{99} = 57 h) (△) strains before, during and after 24 h of exposure to HCC. (Values assigned the same lower-case letter for the selected strain and the same upper-case letter for the unselected strain, do not differ significantly from each other at $P < 0.05$).

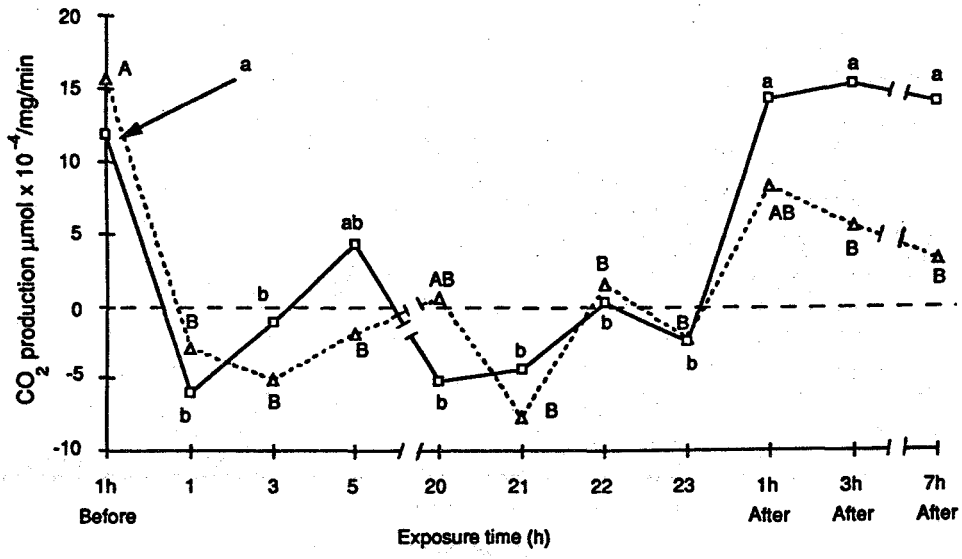

Fig. 2. Rate of CO_2 production of the HCC-selected (LT_{99} = 894 h) (□) and unselected (LT_{99} = 57 h) (△) strains before, during and after 24 h of exposure to HCC. (Indication of significance of differences in values as in Fig. 1).

within 1 h after exposure they had risen to their previous level. Clearly there was no indication that an O_2 debt had built up in either strain during exposure.

Measurement of metabolic rates of the HCC-selected strain throughout exposure to HCC until mortality. Exposure to HCC resulted in reduction in O_2 consumption in the unselected strain from 17.9 µmol $\times 10^{-4}$/mg/min, before exposure, to 0.26 µmol $\times 10^{-4}$/mg/min, after 48 h exposure (Fig. 4). Since the LT_{99} of the unselected strain without food occurs after 57 h it may be concluded that cessation of O_2 consumption after 4 days is due to physiological death of all the test insects though probit mortality analysis does not indicate exactly when the insects die but rather when they reach a stage beyond which they cannot recover. Oxygen consumption of the HCC-selected strain was very different. During the first 48 h there was a decrease in O_2 consumption as occurred in the previous experiment; however, this was followed by an 8 day period when consumption was *c.* 80% of consumption in air. The decrease in O_2 consumption, which signals the onset of mortality, began on the twelfth day and continued until the twenty-first day, when probit mortality analysis indicated a mortality of nearly 95%.

Loss in dry weight and water content (%) of the HCC-selected strain during exposure to HCC. Losses in dry weight and water content of the HCC-selected and the

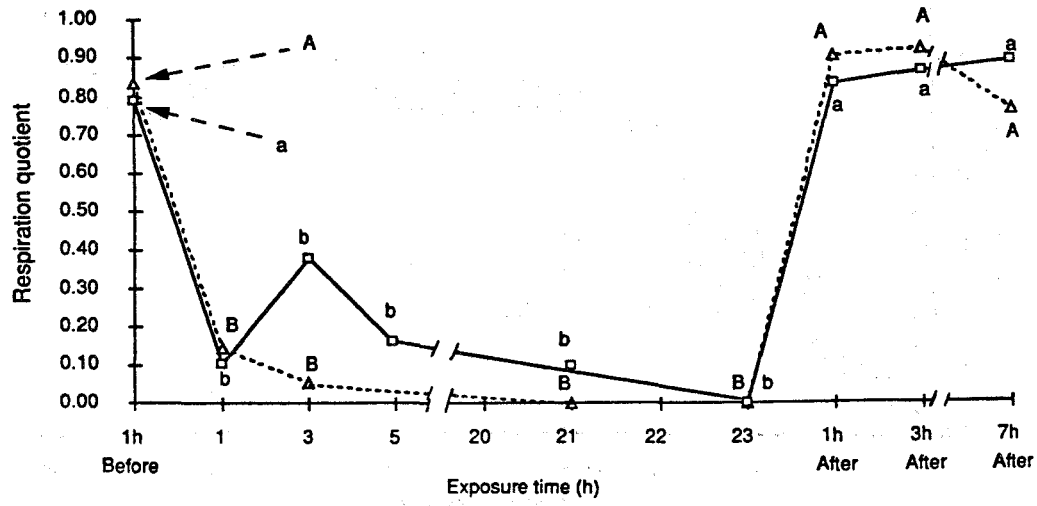

Fig. 3. Respiration quotients of the HCC-selected (LT_{99} = 894 h) (□) and unselected (LT_{99} = 57 h) (△) strains before, during and after 24 h of exposure to HCC. (Indication of significance of differences in values as in Fig. 1).

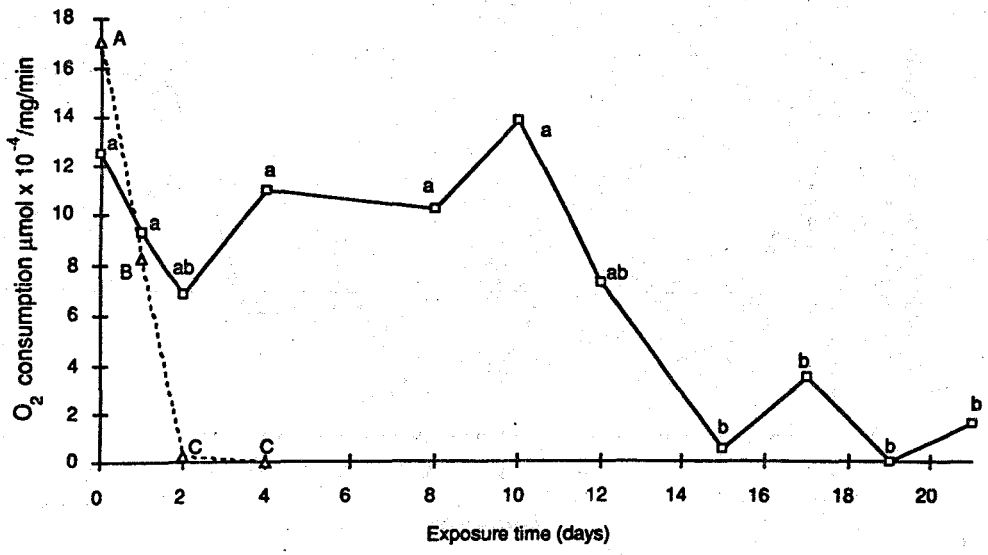

Fig. 4. Rate of O_2 consumption of the HCC-selected ($LT_{99} = 37.25$ days) (□) and unselected ($LT_{99} = 2.37$ days) (△) strains before and during prolonged exposure to HCC. (Indication of significance of differences in values as in Fig. 1).

unselected strains when exposed to the HCC atmosphere are given in Figs 5 and 6. For the HCC-selected strain, loss in dry weight was *c.* 3% per day, reaching a maximum of 30% on the tenth day when probit mortality experiments indicated a mortality of *c.* 72% (Fig. 5). Dry weight loss of the unselected strain was similar for the first 3 days, after which no significant further dry weight loss was recorded. Mortality analysis for this strain indicated that *c.* 95% had reached a state of non-recovery after 24 h. Fig. 6 reveals that the HCC-selected strain maintained its initial water content of *c.* 50% for the first 10 days of exposure. This was followed as mortality progressed by desiccation to *c.* 25% on the eighteenth day. In a separate experiment, this water content was shown in dead insects to be in equilibrium with a 95% r.h. in air at 26°C. Initial water content of the unselected strain was 55%. Loss in water content of this strain was *c.* 1% per day for the first 4 days, followed by increase in desiccation to 21.6% water content on the twelfth day. The differences in rate of mortality between the two strains as expressed in probit-mortality analyses, and the findings on O_2 consumption and CO_2 production during exposure, indicate a correlation between the incapacity of the unselected strain to conserve water and mortality. Although probit analysis revealed a mor-

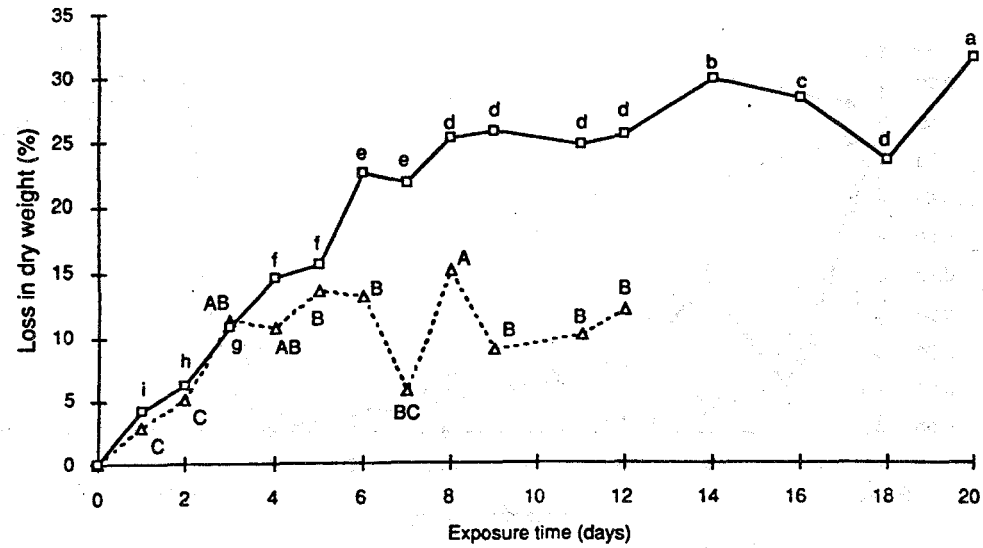

Fig. 5. Loss in dry weight (%) during exposure of HCC-selected (□) and unselected (△) strains to HCC. (Indication of significance of differences in values as in Fig. 1).

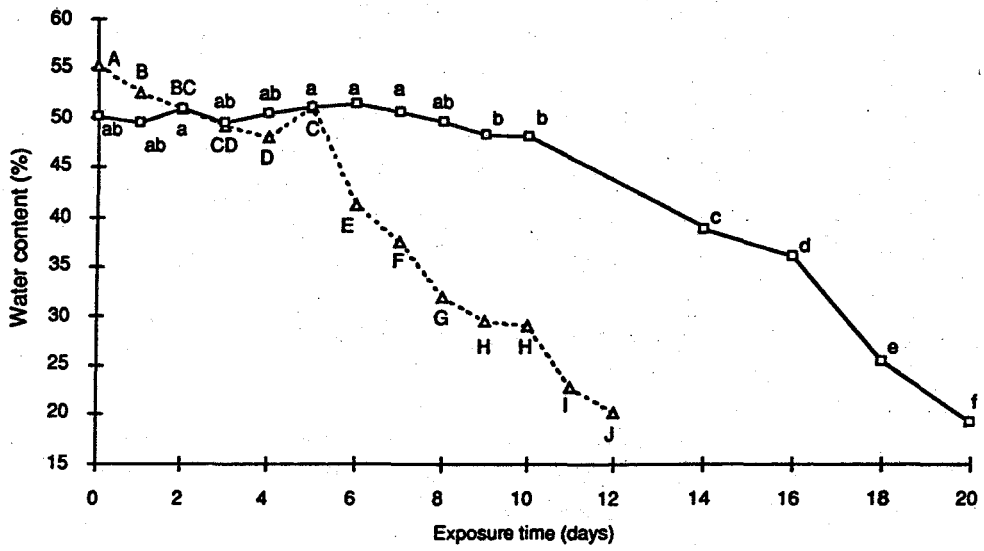

Fig. 6. Water content (%) during exposure of HCC-selected (□) and unselected (△) strains to HCC. (Indication of significance of differences in values as in Fig. 1).

tality (state of non-recovery) of 99% after 57 h exposure, respiration measurements showed that even after 48 h metabolic activity was still evident in surviving insects. The arrest in dry weight loss, and the increase in loss of water between the third and fourth days of exposure, may be attributed to the gradual dying process, after which water loss accelerated as water content of the dead bodies came into equilibrium with r.h. of the atmosphere.

In contrast, the HCC-selected strain maintained its water content over the first 8 days, possibly due to fat oxidation and release of metabolic water. Only after mortality started to increase (eighth to twentieth day) did the rate of dry weight loss decrease and the loss in water content increase. It is possible that the ability to control spiracular openings under the influence of CO_2 may have contributed towards the different behaviour of the two strains under exposure to HCC. The unselected strain was capable of maintaining a water balance for only a limited time in spite of the high ambient humidity, whereas the HCC-selected strain successfully conserved water during exposure, and was also able to generate more metabolic water through utilization of its much larger reserves of triglycerides (Donahaye, 1985).

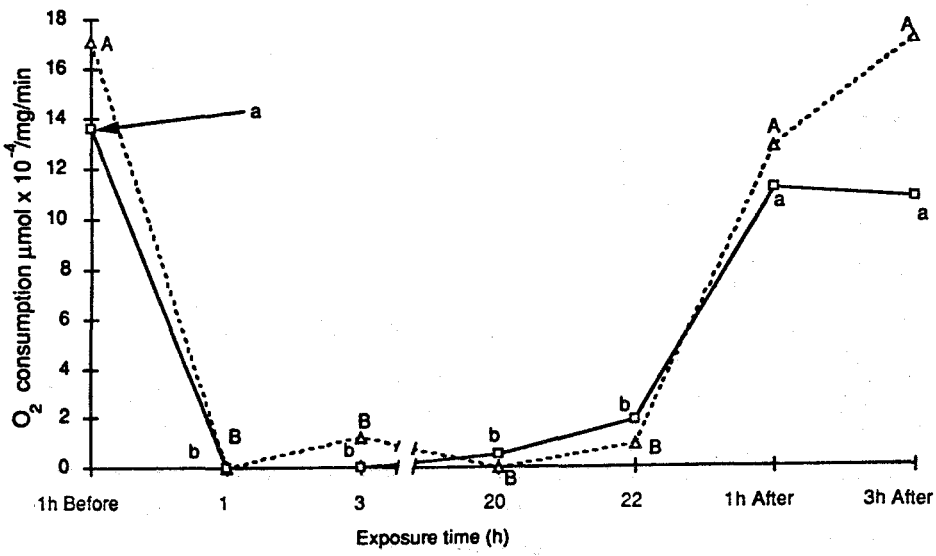

Fig. 7. Rate of O_2 consumption of the LOC-selected ($LT_{99} = 700$ h) (□) and unselected ($LT_{99} = 528$ h) (△) strains before, during and after 24 h of exposure to LOC. (Indication of significance of differences in values as in Fig. 1).

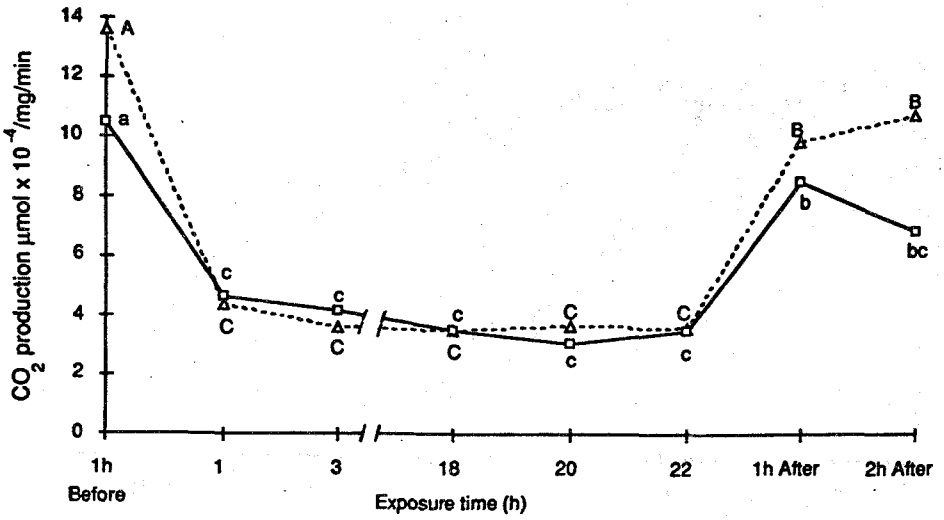

Fig. 8. Rate of CO_2 production of the LOC-selected ($LT_{99} = 700$ h) (□) and unselected ($LT_{99} = 528$ h) (△) strains before, during and after 24 h of exposure to LOC. (Indication of significance of differences in values as in Fig. 1).

Metabolic reactions of LOC-selected strain

Measurement of metabolic rates before, during and after 24 h exposure to LOC. Fig. 7 shows the average O_2 consumption rates of the LOC-selected and unselected strains before during and after exposure to LOC.

For both strains O_2 consumption dropped to close to zero throughout the exposure period. Upon restoration of normal atmosphere, O_2 consumption returned to its previous level within 3 h for the unselected strain, and for the LOC-selected strain it returned within 1 h to *c.* 80% of the pre-exposure level and then remained stable.

Carbon dioxide production, as presented in Fig. 8, was fairly uniform during the exposure period for both strains at *c.* 4 µmol $\times 10^{-4}$/mg/min, indicating either that increased acidity within the insect bodies was causing CO_2 to be driven off, or that even at this very low O_2 tension aerobic respiration was taking place, or that metabolic CO_2 was being produced by alternative pathways (Carlson, 1968; Hochachka & Somero, 1973).

However, RQs (Fig. 9) before and after exposure give no indication that an O_2 debt had been incurred for either strain. It would appear, therefore, that even at the low O_2 level of 0.5%, the insect metabolism continued to be mainly aerobic. This was borne out by analysis of metabolic end products (Donahaye, 1985). During exposure, the RQ rose above 1 for both strains. These results differ from those of Carlson (1968) but concur with those of Thunberg (1905) on *Tenebrio* and Harnisch (1930) on *Chironomus* larvae, who found that at low O_2 concentrations the RQ

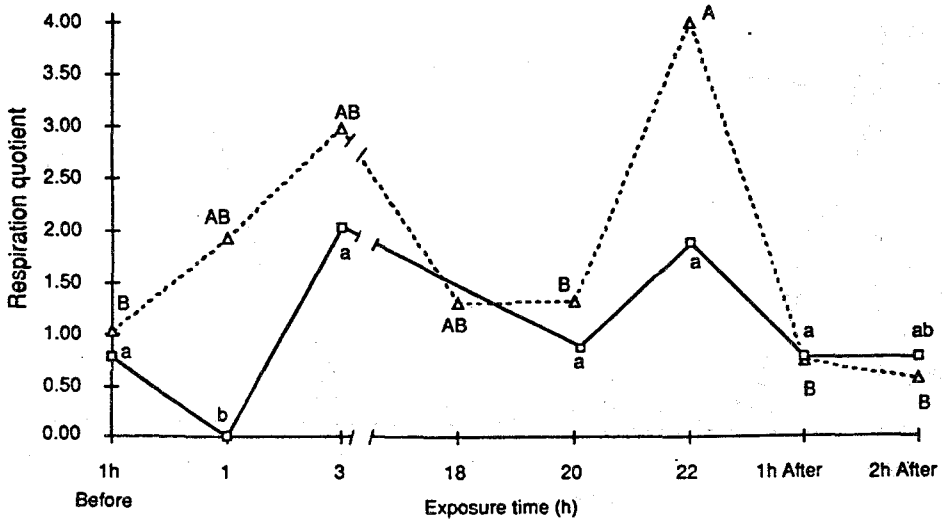

Fig. 9. Respiration quotients of the LOC-selected ($LT_{99} = 700$ h) (□) and unselected ($LT_{99} = 528$ h) (△) strains before, during and after 24 h of exposure to LOC. (Indication of significance of differences in values as in Fig. 1).

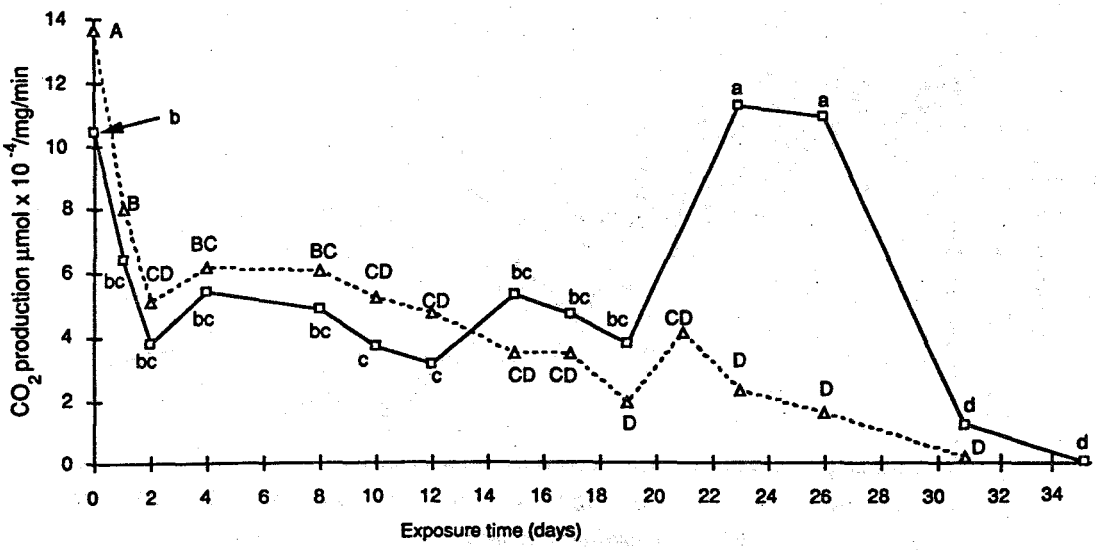

Fig. 10. Rate of CO_2 production of the LOC-selected (LT_{99} = 29.2 days) (□) and unselected (LT_{99} = 22 days) (△) strains before and during prolonged exposure to LOC. (Indication of significance of differences in values as in Fig. 1).

tended to rise due to increased body acidity and release of CO_2. This was followed by a recovery phase during which CO_2 is again sorped into the tissues and haemolymph, and the RQ drops below normal.

Measurement of metabolic rates of the LOC-selected strain throughout exposure to LOC until mortality. Measurement of CO_2 production of the two strains exposed to LOC (Fig. 10) show that respiration rates of both strains dropped to between 4 and 6 µmol $\times 10^{-4}$/mg/min, over the first few days of exposure. Initially, respiration of the unselected strain was consistently higher than that of the LOC-selected strain, and only as mortality ensued did CO_2 production decrease, reaching zero on the thirty-second day. For the LOC-selected strain there was an apparent increase in respiration between the twenty-third and twenty-sixth days of exposure followed by a similar decrease in respiration as mortality progressed, reaching zero on the thirty-fifth day.

Loss in dry weight and water content (%) of the LOC-selected strain during exposure to LOC. Losses in dry weight and in water content of the strain selected for resistance to LOC and the unselected strain are given in Figs 11 and 12. For both strains losses in dry weight were similar during exposure, finally reaching *c.* 20% of the total dry weight (Fig. 11). These losses are much lower than for exposure to HCC and indicate the lower level of

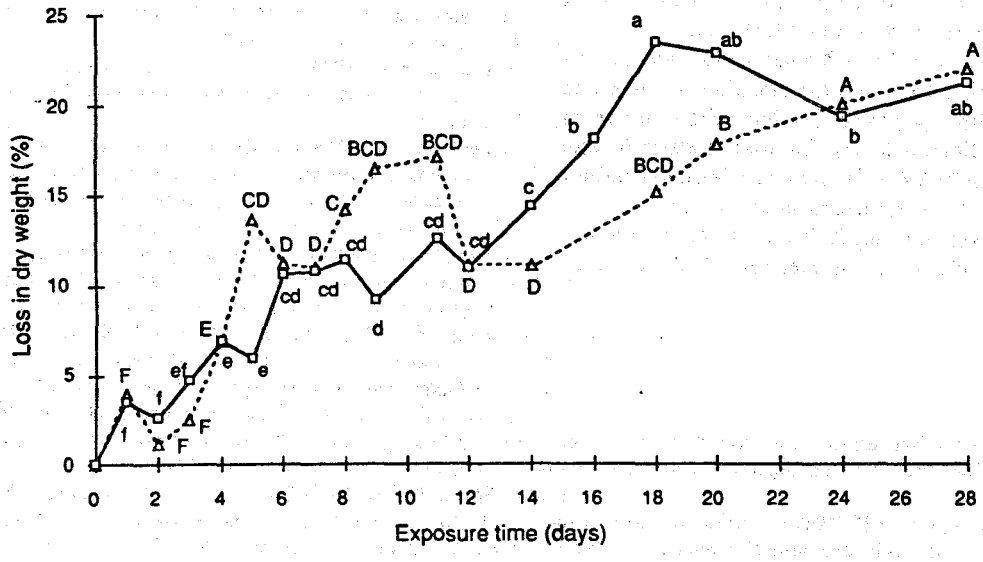

Fig. 11. Loss in dry weight (%) during exposure of LOC-selected (□) and unselected (△) strains to LOC. (Indication of significance of differences in values as in Fig. 1).

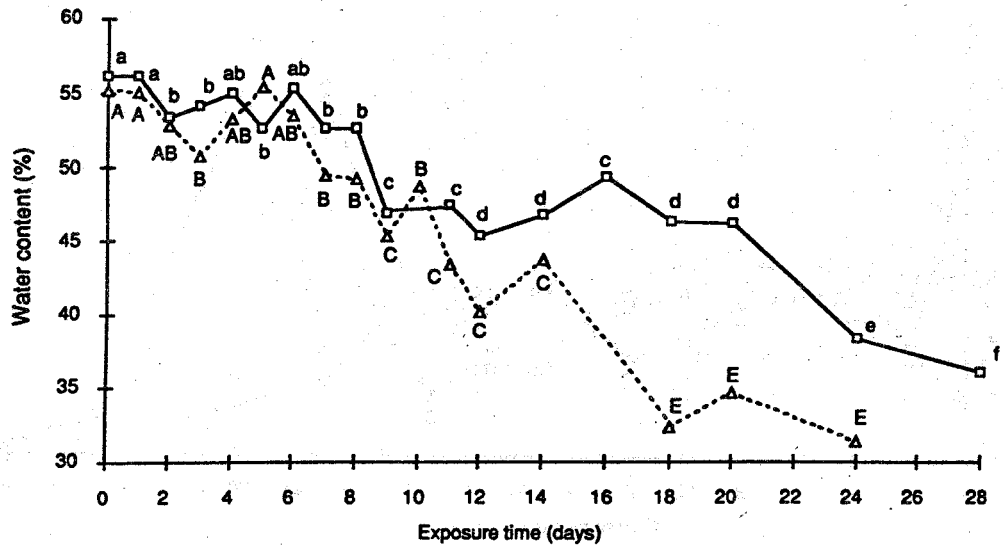

Fig. 12. Water content (%) during exposure of LOC-selected (□) and unselected (△) strains to LOC, (Indication of significance of differences in values as in Fig. 1).

metabolism during exposure to LOC, as revealed also in the respiration experiments Losses in water content were similar for both strains, although an acceleration in water loss after the sixth day (84% mortality by probit analysis) was recorded for the unselected strain, while for the LOC-selected strain the acceleration occurred after about the 10th day (37.5% mortality). Although water loss was fairly constant it was not greater than *c.* 5% during the survival periods of both strains, and cannot be considered a critical factor in causing mortality during exposure to LOC.

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References

AliNiazee, T.M. (1971) The effect of carbon dioxide gas on respiration of the confused flour beetle. *Journal of Economic Entomology*, **64**, 130.

AliNiazee, T.M. & Lindgren, D.L. (1969) Effect of carbon dioxide on toxicity of hydrocyanic acid and methyl bromide to adults of the confused flour beetle and granary weevil at two different temperatures. *Journal of Economic Entomology*, **62**, 904–906.

Brooks, M.A. (1965) The effects of repeated anesthesia on the biology of *Blattella germanica* (Linnaeus). *Entomologia Experimentalis et Applicata*, **8**, 39–48.

Burkett, B.N. & Schneiderman, H.A. (1967) Control of spiracles in silk moths by oxygen and carbon dioxide. *Science*, **156**, 1604–1606.

Calderwood, W.A. (1961) The metabolic rate of the flour beetle *Tribolium confusum*. *Transactions of Kansas Academy of Science*, **64**, 150–152.

Carlson, S.D. (1966) Respiration measurement of *Tribolium confusum* by gas chromatography. *Journal of Economic Entomology*, **59**, 335–338.

Carlson, S.D. (1968) Respiration of the confused flour beetle in five atmospheres of varying CO_2:O_2 ratios. *Journal of Economic Entomology*, **61**, 94–96.

Chaudhry, H.S. & Kapoor, R.P.D. (1967) Studies on the respiratory metabolism of the Red Flour Beetle. *Journal of Economic Entomology*, **60**, 1334–1336.

Daum, R.J. (1979) A revision of two computer programs for probit analysis. *Bulletin of the Entomological Society of America*, **16**, 10–15.

Donahaye, E. (1985) Studies on the development of resistance to modified atmospheres in the stored product insect *Tribolium castaneum* (Herbst), (red flour beetle). Ph.D. thesis, Hebrew University, Jerusalem, Israel [in Hebrew].

Donahaye, E. (1990a) Laboratory selection of resistance by the red flour beetle *Tribolium castaneum* (Herbst) to an atmosphere of low oxygen concentration *Phytoparasitica*, **18**, 189–202.

Donahaye, E. (1990b) Laboratory selection of resistance by the red flour beetle *Tribolium castaneum* (Herbst) to a carbon dioxide enriched atmosphere. *Phytoparasitica*, **18**, 299–308.

Edwards, L.J. (1968) Carbon dioxide anesthesia and succinic dehydrogenase in the corn earworm *Heliothis zea*. *Journal of Insect Physiology*, **14**, 1045–1048.

Harnisch, O. (1930) Effect of reduced oxygen tension on metabolism in fragments of *Chironomus* larvae. *Zeitschrift für Vergleichende Physiologie*, **12**, 504–523.

Hazelhoff, E.H. (1928) Carbon dioxide, a chemical accelerating the penetration of respiratory insecticides into the tracheal

system by keeping open the tracheal valves. *Journal of Economic Entomology*, **21**, 790.

Hochachka, P.W. & Somero, G.N. (1973) *Strategies of Biochemical Adaptation*. Saunders., Philadelphia.

Hoyle, G. (1960) The action of carbon dioxide gas on an insect spiracular muscle. *Journal of Insect Physiology*, **4**, 63–79.

Jay, E.G. & Cuff, W. (1981) Weight loss and mortality of three life stages of *Tribolium castaneum* when exposed to four modified atmospheres. *Journal of Stored Products Research*, **17**, 117–124.

Kennington, G.S. (1966) Quantitative aspects of maximum oxygen deficiency in *Tribolium confusum* (Duval.). *Physiological Zoology*, **39**, 77–80.

Mitsuda, H., Kawai, F., Kuga, M. & Yamamoto, A. (1973) Mechanisms of carbon dioxide adsorption by grains and its application to skin packaging. *Journal of Nutritional Science and Vitaminology*, **19**, 71–83.

Navarro, S. (1975) Effect of oxygen concentrations on *Tribolium castaneum* (Herbst.) adults exposed to different humidities. *Report Stored Products Research Laboratory, Israel Ministry of Agriculture*, 13–21 [in Hebrew with English summary].

Navarro, S. & Calderon, M. (1973) Carbon dioxide and relative humidity: interrelated factors affecting the loss of water and mortality of *Ephestia cautella* (Walker) (Lepidoptera; Phycitidae). *Israel Journal of Entomology*, **8**, 143–152.

Navarro, S. & Friedlander, A. (1975) The effect of carbon dioxide anesthesia on the lactate and pyruvate levels in the hemolymph of *Ephestia cautella* (Wlk.) pupae. *Comparative Biochemistry and Physiology*, **50B**, 187–189.

Park, T. (1936) Studies on population physiology. V. The oxygen consumption of the flour beetle *Tribolium confusum* Duval. *Journal of Cellular and Comparative Physiology*, **7**, 313–323.

Sillans, D. & Biston, J. (1979) Studies on the anesthetic mechanism of carbon dioxide by using *Bombyx mori* larvae. *Biochimie*, **61**, 153–156.

Thunberg, D. (1905) Oxygen tension and oxygen uptake in *Tenebrio* larvae. *Skandinavisches Archiv für Physiologie*, **17**, 133–195.

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