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RESPIRATION OF STORED PRODUCT PESTS IN HERMETIC CONDITIONS

M. EMEKCI,[1]* S. NAVARRO,[2] J.E. DONAHAYE,[2] MIRIAM RINDNER[2] AND A. AZRIELI[2]

[1]*Dept. of Plant Protection, Faculty of Agriculture, University of Ankara, 06110, Ankara, Turkey [e-mail: emekci@.agri.ankara.edu.tr]*
[2]*Dept. of Stored Products, Agricultural Research Organization, The Volcani Center, Bet-Dagan 50250, Israel*

ABSTRACT

Hermetic storage for the preservation of durables has been proven as an effective storage method. This method aims at taking advantage of interference with respiratory metabolism of the insects and minimizes their damage to the commodity. *Tribolium castaneum*, at its four developmental stages, was exposed to atmospheres containing 1 to 21% O_2 that may be experienced under airtight conditions. All tests were carried out at 30ºC and 70% r.h. Results showed that an initial acclimatization period of at least 5 h was required before each test to enable the insects to stabilize their response to the different CO_2 concentrations. At normal oxygen level, egg respiration was lower than that of other stages and young larvae had the highest respiration rate. For pre-adult stages at reduced oxygen levels respiration rates were proportional to the oxygen levels. However, adult respiration, was higher at 3 and 5% O_2 than at normal atmospheric air.

INTRODUCTION

Preservation of durable commodities by means of modified atmosphere (MAs) has been widely accepted as an effective method of storage (Bailey and Banks, 1975; Banks *et al.,* 1980; Navarro *et al.,* 1979; Navarro and Donahaye, 1990). Among the methods of generating MAs, the bio-accumulation of carbon dioxide (CO_2) and thus depletion of oxygen (O_2) in the intergranular space of the commodity through the respiration of pests and microorganisms is known as hermetic storage. This technique prevents arthropod pest development and minimizes their damage to the commodity (Navarro *et al.*, 1993; 1994).

Recent improvements in gas generation technology also serve to promote the use of MAs by increasing their availability and reducing the cost of gases. Thus, the use of MAs in the near future is expected to be more widespread (Annis and Dowsett, 1993).

The considerable research carried out on MAs has broadened our field of knowledge on the use of different gas compositions for arthropod pest control.

However gaps still remain in our understanding, particularly with regard to the effects of sub-lethal concentrations of MAs and their influence on pest biology. Cases in point are delayed development, impaired metamorphosis and altered fecundity (Bailey and Banks 1980), as well as the effect of MAs on insect respiration. The latter is of special importance in storage due to the critical effect of respiration on the heating processes of bulk-stored grain. Moreover, it is also a good index of the physiological responses of the insects to the environment in which they are exposed.

Most research carried out so far on respiratory mechanisms of stored-product insect pests has concentrated on respiration in normal atmospheric air with well known cosmopolitan pests. Thus, the respiration of such important species as *Tribolium castaneum*, *T. confusum Sitophilus oryzae*, *Rhyzopertha dominica, Tenebroides mauritanicus, Ephestia cautella, Cryptolestes ferrugineus, Oryzaephilus surinamensis,* and others, have been evaluated in terms of biotic parameters (body weight, sex, food medium, pest species, developmental stage, etc.) and abiotic parameters (low pressure, temperature, relative humidity, etc.). The main findings derived from these researches have centered on how insect respiration is affected by species, strain, and developmental stage (Birch, 1947; Campbell and Sinha, 1990; Donahaye, 1992; White and Sinha, 1981); by temperature (Calderwood, 1961; Chaudry and Kapoor (1967); by gas composition (AliNiaze, 1971; Carlson, 1966, 1968a, 1968b; Donahaye, 1992; Kennington 1957; Navarro 1978; Navarro and Calderon, 1979); and by low pressure (Dumas *et al*., 1969; Navarro and Calderon, 1979).

Where laboratory studies have been carried out on respiration under MAs, they have focused on the effectiveness of atmospheres containing between 0 and 1% O_2 in the control of the adult pests, whereas, atmospheres between 1% and 5% O_2 have not been studied in detail even though they are a much more realistic range of O_2 concentrations that can be obtained and maintained economically in sealed storages termed "hermetic storage" or even under the application of the nitrogen (N_2) purging MA technology (Annis and Dowsett, 1993). There is a lack of information on the effect of low O_2 concentrations particularly those higher than 1% on the different developmental stages of stored grain insects in terms of both lethal and sub-lethal effects. Such information can be particularly useful with regard to the development of simulation predictive models.

The objective of this investigation was to study the respiration rates of the developmental stages of *T. castaneum* at low levels of O_2 concentrations prevailing under gas tight conditions when atmospheric composition is modified by the metabolic activity of insects.

MATERIALS AND METHODS

Insect culture techniques

The *T. castaneum* cultures were maintained in 1-L glass jars. Pre-adult stages were fed on a mixture of 500 g of finely ground wheat and 5% yeast (by weight) till adult

emergence, while newly emerged adults were confined in similar jars containing a mixture of 250 g of flour and 5% yeast. Adults used for experiments were held for two weeks, while adults for oviposition were retained for up to one month. Cultures were started with ca. 2 000-3 000 three-day old eggs placed in jars, containing food media. The media were later sieved to obtain young larvae, mature larvae, pupae and adults (Donahaye, 1990).

Modified atmospheres

The respiration rates of *T. castaneum* were measured in atmospheres containing 1, 2, 3, 5, 10 or 15% O_2 in N_2 at 30°C. Normal air served as control. Gas mixtures were prepared as described by Donahaye (1990), and maintained at a flow rate of 15 mL/min at 70% r.h. (Navarro and Donahaye 1972). Table 1 shows the different developmental stages, number of individuals and age intervals of *T. castaneum* that were used for studying respiration rates at low O_2 concentrations.

Exposure flasks

The flasks consisted of 50-60 mL bottles with 2 cm wide necks, the volume of each flask having been accurately measured beforehand. Aluminium lids each containing a septum, were used to cap the flasks. Two No. 14 syringe needles (0.65 x 32 mm) were inserted through each septum, one of which served as an inlet connected to the gas mixture by appropriate tubing while the other served as an outlet.

Exposure Techniques

Test individuals (Table 1) were weighed and transferred to the experimental flasks containing 200 mg flour for all stages, except eggs and pupae, which did not require food. After capping the flasks, the lids were tightly sealed using a 'crimper' and the flasks were connected to the exposure apparatus. A soap-bubble flow meter was attached to the outlet needle of each flask to ensure gas flow and verify the flow rate. The flasks were held at 30ºC in a thermostatically controlled room. Following 24 h of acclimation to the gas mixture of all stages except eggs, the air composition within each flask was measured by withdrawing a sample through the silicon septum using a 1 mL "Pressure-lock" syringe. The inlet and outlet ports of the bottles were then clamped-off tightly for approximately 1 h (24 h for eggs). At the end of this period, another gas sample was taken as described previously. Gas concentrations were determined using a Tracer 565 gas chromatograph equipped with twin thermal conductivity cells, dual columns (each 4´ x 1/16´´ i.d.), and a sample loop thermostatically regulated at 50ºC. Column 1 was packed with "Porapak Q", while column 2 was packed with "molecular sieve 5a". Percent concentrations of the gas components were computed with a Spectra Physics SP 4100 integrator.

TABLE 1
Numbers and the ages of *Tribolium castaneum* (Herbst) according to the developmental stages used for testing their respiration rates

Developmental stage	Number of insects per treatment	Age of insects * (days)
Egg	300	0-1
Young larvae	100	7-10
Old larvae	100	18-22
Pupae	100	0-1
Adult	100	10-14

* Age for larval stages from egg stage; pupa from pupation; adults from emergence.

By preference, respiration figures were given as CO_2 production both in mg body weight per hour, and in mg per insect per hour, for each developmental stage, in order to provide more information on respiration. Such information is generally lacking in the literature. However, we also referred to measured O_2 intake figures when there was a need to make comparison with other studies.

RESULTS

Preliminary trials at normal atmospheric air showed that adults had acclimatized only after 5 hours of transfer to the exposure flasks in terms of steady respiration rates (Fig. 1). All stages except eggs were therefore held for a 24 h acclimatization period before initial gas sampling.

A positive correlation between the respiration rates of immature stages and O_2 levels were determined. Eggs and young larvae (Fig. 2) were the most susceptible stages to low O_2 concentrations in terms of respiration rate, whereas old larvae and pupae in particular were less affected (Fig. 2). During the course of development, respiration was suppressed mainly at the 1% O_2 level in immature stages (Fig. 2).

Adult respiration at 3-10% O_2 caused a higher CO_2 output than did immature stages (Fig. 2).

Respiration quotient (RQ) values at normal atmospheric air were above unity for active developmental stages, but declined to 0.72 at the pupal stage (Fig. 3).

DISCUSSION

Adult respiration at normal atmosphere

Most of the reported studies on respiration of *Tribolium* spp. were carried out on the adult stage in normal atmospheric air under different conditions of temperature, relative humidity (r.h.), and acclimatization periods. There is general agreement in the literature that the respiration rates of insects are positively correlated with

temperature (Table 2). Thus, Calderwood (1961) reported that O_2 consumption of *T.confusum* was 3.14 μL O_2/insect•h and 5.83 μL O_2/insect•h at the temperatures of 25ºC and 37ºC. The same phenomenon was revealed by Chaudry and Kapoor (1967), and by other researchers (Carlson, 1966; Carlson, 1968a; Donahaye, 1992).

TABLE 2
Comparative data on respiration rates of *Tribolium* spp. adults from literature

Species	Temperature (ºC)	Respiration rates*			Author
		μL O_2/insect•h	μL O_2/mg•h	μL CO_2/mg•h	
Tribolium confusum			1.79-2.31		Park, 1936
			1.6		Edwards, 1958
	29		1.6		Kennington, 1957
	25	3.14			Calderwood, 1961
	37	5.83			Calderwood, 1961
	30			2.64	Carlson, 1966
	30			2.69	Carlson, 1968a
Tribolium castaneum	30		1.09		Chaudry and Kapoor, 1967
	35		2.39		Chaudry and Kapoor, 1967
	26.6			4.26	AliNiazee, 1971
	26			2.15	Donahaye, 1992
	30	5.73	2.97	2.37	Present work

* Converted to μL where necessary.

Carlson (1966, 1968a) working with *T. confusum* at 30ºC, found an acclimatization period of a maximum of 1 h. In the present work, we found that respiration rates at normal atmospheric air only stabilized after 5 h of acclimatizing the insects to the experimental conditions (Fig. 1). Possibly, the difference between the findings of Carlson and ours can be attributed in part to differences in the test conditions.

Respiration of various developmental stages in normal atmospheric air

Information in the literature on respiration rates of various developmental stages of *T.castaneum* is very rare. Therefore, our discussion below is based on comparison with other stored product insects. Birch (1947), reported that the rate of O_2 consumption of *S. oryzae* at 30°C and 70% r.h. in normal atmospheric air increased from egg to pre-pupal stage, then fell during pupation. In the same work, O_2

consumption in *S. oryzae* populations composed of young larvae, old larvae, mainly pupae (84%) and 1 week old adults were given as 5.30, 26, 5.77, and 5.40 μL/insect•h (converted from mm^3/insect•h), respectively. In our experiment we observed a similar tendency in respiration rates in terms of life stages, except for larvae. We observed higher respiration rates in young larva than in old ones only in normal air (Figs. 3 and 4). In this respect our results can be more conveniently compared to those of Campbell and Sinha (1978). They reported a reduction in respiration rate of *C. ferrugineus* in terms of μL O_2/mg•h (dry weight of insects) from 149 in 1st instar larvae to 27.5 in the adult stage in normal atmospheric air at 30°C and 70% r.h. Similarly, White and Sinha (1981) stated that the respiration rate in *O. surinamensis* on the basis of μL O_2/insect•h at 30°C and 80% r.h reached a maximum of 108 μL O_2/insect•h, when the majority of insects were in the 2nd instar, then decreased to 0.4 μL O_2/insect•h during pupation and rose to 4.4 μL O_2/insect•h during the first days of the adult stage.

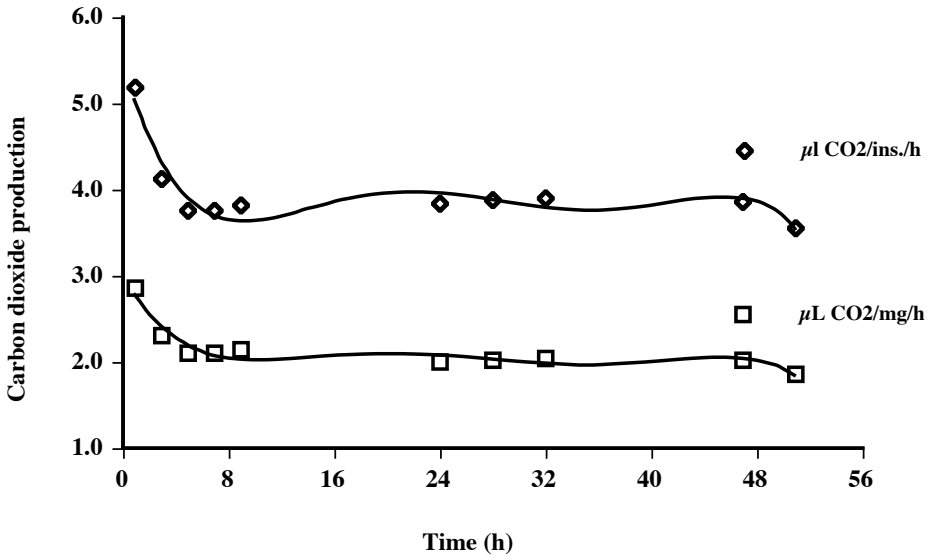

Fig. 1. Change in respiration rate of *Tribolium castaneum* adults during acclimatization in normal atmospheric air at 30ºC and 70% r.h.

Respiration at reduced O_2 levels

We had difficulty in comparing our findings to those of other workers due to lack of information on the respiration of *T. castaneum* at reduced O_2 concentrations, particularly for the developmental stages. Our results showed that respiration tended to increase as the O_2 concentration rose from 1% to 21% at all development stages

except adult. In eggs and young larvae the respiration rate was suppressed at O_2 levels less than 5% O_2 (Fig. 2). Low O_2 levels had an inhibiting effect on the respiration rate of young larvae in comparison with old ones when the rate was based on weight of insect. On the other hand CO_2 production per insect was higher in young larvae than in old larvae only in the control (21% O_2).

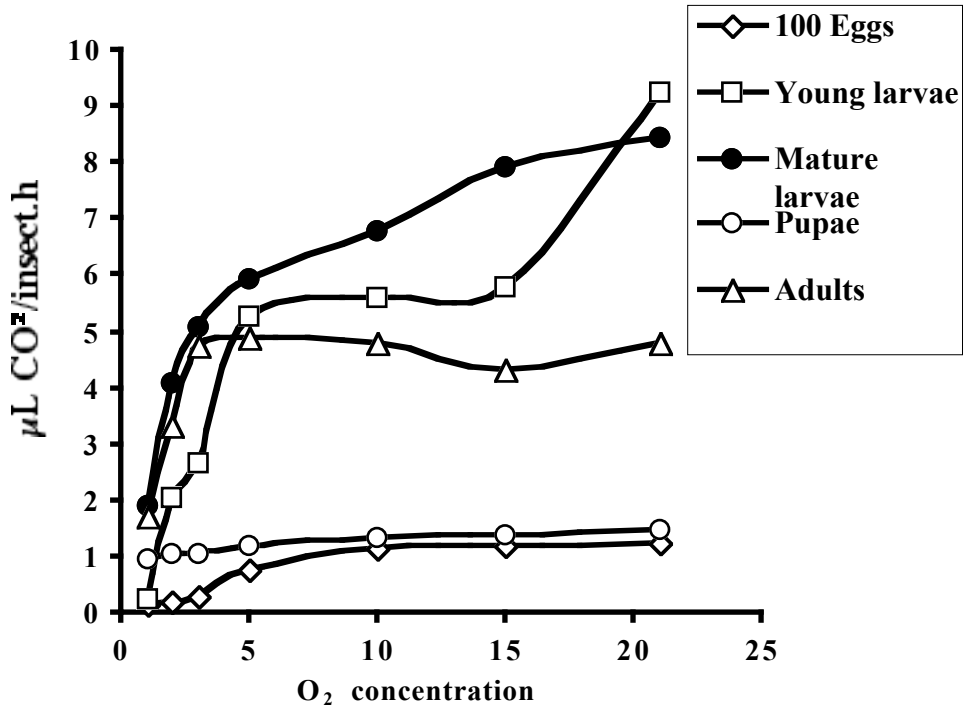

Fig. 2. Respiration rates of the development stages of *Tribolium castaneum* at different O_2 concentrations in nitrogen at 30ºC and 70% r.h.

Pupal respiration followed a similar respiration pattern to those of other immature stages, but was less marked at low O_2 levels (Fig. 2). Similarly, Navarro and Calderon, (1979) working with *Ephestia cautella* pupae at 26°C and 93-99% r.h. reported CO_2 production at approximately 0.1, 0.2 and 0.35 μL CO_2/100 mg·h at O_2 concentrations of 1%, 3% and 21%, respectively. These figures are in agreement with our results.

Adult respiration, on the other hand, showed a different pattern resulting in an increased respiration rate at 3% and 5% O_2 (Fig. 2) which can be considered as a compensation response due to the O_2 deficiency.

We calculated from the study of Donahaye (1992) that CO_2 production of *T. castaneum* at 0.5% O_2 in N_2 at 26°C and 95% r.h dropped from 1.88 to 1.34 μL CO_2/mg·h (converted from μmol/mg·min) before and after exposure for 24 h,

respectively. His results, though recorded at a very low O_2 are comparable with ours which resulted a respiration rate of 0.9 μL CO_2/mg•h after 24 h of exposure to 1% O_2. However**,** Carlson (1968a), who studied the respiration of *T. confusum* adults in atmospheres of 4.91, 10.15, and 14.90% O_2 at 30°C reported a lower CO_2 production than the control resulting between 1.74 - 1.88 μL CO_2/mg•h. These results were similar to ours and can be compared at 5, 10 and 15% O_2 which showed respiration rates of 2.2 to 2.6 μL CO_2/mg•h.

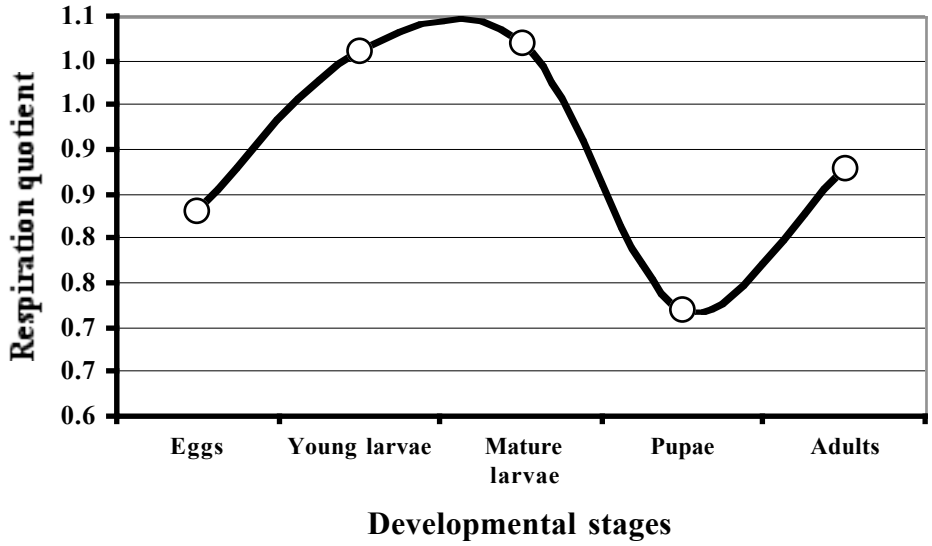

Fig. 3. Respiration Quotients of developmental stages of *Tribolium castaneum* at 30ºC and 70% r.h. in normal air (21 % O_2).

Respiration Quotients

RQ values are a good indicator to express insect metabolism. Edwards (1953) proposed that the RQ could be applied for determination of the nutritional activity of insects, and that RQ values of 1.0, 0.8 and 0.7 accounted for carbohydrate, protein and lipid oxidation, respectively. In our study, the RQ in normal air for developmental stages, (Fig. 3) refers to a carbohydrate metabolism for larvae, a lipid metabolism for pupae, and a carbohydrate-protein metabolism for adults. During the development of *O. surinamensis*, White and Sinha (1981) observed a similar fluctuation in which an RQ of 1.0 was obtained for larvae, 0.76 for pupae and 0.95 to 1.0 for adults. RQ values measured in normal air for adults were also reported as 1.1 in *S. oryzae* (Birch 1947), 1.02 for *T. confusum* (Carlson, 1966, 1968a) and 0.83 for *T. castaneum* (Donahaye, 1992). The values reported for adults in this paper in

normal atmospheric air were close to unity, and are in agreement with the literature (Table 2).

CONCLUSION

In the present work, exposure to O_2 levels of 3 and 5%, imparted a metabolic stress on adults in particular which was as expressed by their high CO_2 production after 24 h of exposure. This metabolic disturbance is possibly accompanied by other sub-lethal effects, that may cause mortality at prolonged exposures to low O_2 concentrations. This aspect deserves further investigation. Additional studies are needed on the metabolic effects of insects at the range of 3 to 5% O_2 concentrations, which usually prevail in the biogenerated atmospheres produced in sealed storages.

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