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Respiration of *Rhyzopertha dominica* (F.) at reduced oxygen concentrations

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Abstract

Developmental stages of *Rhyzopertha dominica* were exposed to atmospheres containing 1%, 2%, 3%, 5%, 10% or 15% oxygen (O_2) in nitrogen at 30°C and 70% r.h. Respiration rates were determined with a gas chromatograph. The O_2 intake and carbon dioxide (CO_2) output by insects were expressed in µl/insect h or µl/mg h. Respiration of eggs, young and old larvae, pupae, and adults at normal atmospheric air were at rates of 0.0029, 0.41, 2.52, 0.82, and 2.86 µl CO_2/insect h, respectively. Respiration rates of the same stages in terms of insect weight were 0.14, 4.83, 1.98, 0.64 and, 2.58 µl CO_2/mg h, respectively. At reduced O_2 levels respiration rates of eggs, larvae and pupae were proportional to the O_2 levels. Adult respiration rates were high at 3% and 5% O_2 levels almost reaching that of normal atmospheric air, and were 2.56 and 2.85 µl CO_2/insect h, respectively. In adults, respiration quotient values for the same O_2 levels were higher than at normal atmospheric O_2 and were 1.5 and 1.02, respectively.

Respiration of adults in normal air between 20°C and 35°C increased with temperature and gas values varied between 0.89 and 6.82 µl CO_2/insect h, respectively, or 0.93 and 5.63 µl O_2/insect h, respectively.

Keywords: *Rhyzopertha dominica*; Respiration; Reduced oxygen; Developmental stages

1. Introduction

Rhyzopertha dominica (F.) is an important pest of warm, dry cereal grain such as wheat, rice, paddy, millet, and maize. It is well adapted to dry conditions and hence, within a grain mass, it is generally found in the driest parts. Both larvae and adults, as internal feeders, cause serious damage to grain (Evans, 1981).

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Hermetic storage disrupts the respiratory process that produces energy for the maintenance and activity of the organism (Edwards, 1953). Thus, the respiration rate of insects in the storage system is important in that it may reflect the physiological state of insects under different conditions and provide us with a better understanding of the effects of prevailing environmental factors. Most of the respiratory studies that have dealt with stored product insects have been carried out under normal atmospheric air and generally restricted to the adult stages of storage species. Birch (1947), Campbell and Sinha (1978), Singh et al. (1976), and White and Sinha (1981) have all provided detailed information on the respiration of some important stored product insects during their growth. Other respiratory studies have dealt with restricted air supplies. Bailey (1965) showed that O_2 depletion rather than CO_2 accumulation was the main cause of death of insects under gas-tight atmospheres. He observed a decline in apparent respiratory quotient (RQ) (the ratio of the volume of CO_2 evolved to the volume of O_2 consumed by insects) values for some storage pests including *R. dominica* as the O_2 levels decreased with time. Oxley and Wickenden (1963) suggested an apparent RQ close to unity under gas-tight situations for a number of storage insects including *R. dominica*. They also demonstrated the effectiveness of hermetic storage even in leaky conditions. Kashi (1981) confined the adults of some important storage beetles including *R. dominica* under gas-tight conditions and measured their O_2 consumption over 5 days. He also evaluated the effectiveness of high CO_2 levels. There is no information in the literature which has dealt with respiration rates of different stages of *R. dominica* under varying or constant low O_2 concentrations. Navarro and Calderon (1979) reported respiratory suppression in *Ephestia cautella* (Walker) pupae by low O_2 levels. Information on respiration rates of storage insects in low O_2 atmospheres has an important role in understanding the controlling effects of sealed storage atmospheres on insect pests and in developing efficient control strategies based on predictive models. Therefore, this study was undertaken to investigate the respiratory response of *R. dominica* under reduced O_2 concentrations.

2. Materials and methods

2.1. Insect culture techniques

Egg, young and old larvae, pupae and adults of *R. dominica* at the ages given in Table 1 were investigated. To supply large numbers of development stages at the same age, whole meal flour (Howe, 1950) mixed with yeast (5% by weight) was found to be a satisfactory culture medium. To obtain adults, broken kernels supplemented by 5% yeast (by weight) were used. Adults were separated as they emerged from the medium by sieving cultures twice a week. They were then transferred to the oviposition jars containing wheat flour–yeast mixture. Eggs 0–24 h old were obtained daily by sieving the jars, which contained adults aged up to 1 month old.

2.2. Test atmospheres

To evaluate the respiration rate of *R. dominica*, 1%, 2%, 3%, 5%, 10% or 15% O_2 in nitrogen (N_2) were chosen as test atmospheres, and normal air served as a control. Gas supply was obtained from O_2 and N_2 cylinders and mixtures were prepared as described by Donahaye (1992).

Table 1
Numbers and the ages of *Rhyzopertha dominica* (F.) according to the development stages used for testing their respiration rates

Developmental stage	Number of insects	Age of insects (days)[a]
Egg	500–1500	0–1
Young larva	100	10–12
Old larva	100	20–24
Pupa	29–32	0–1
Adult	100	10–14

[a] Age for larval stages from egg stage; pupa from pupation; adults from emergence.

A flow rate of 15 ml/min was maintained to purge each exposure flask. The gases were humidified to obtain a continuous supply at 70% r.h. (Navarro and Donahaye, 1972).

2.3. Exposure flasks

The flasks were 50–60 ml bottles with 2 cm wide necks. Their volumes were measured carefully before the experiments. The bottles were made gas-tight by means of silicon septa and aluminum lids. A crimper was used to seal the bottles tightly. Two N.14 syringe needles of 0.65×32 mm were inserted through the septa. One served as an inlet for the gas mixture while the other served as an outlet.

2.4. Exposure techniques

Insects were counted to the numbers indicated in Table 1, and then were weighed before placing them in the exposure flasks containing 200 mg of flour supplemented by yeast. Flasks which contained eggs and pupae, on the other hand, were not supplied with food. The bottles were closed tightly and then exposed to the experimental gas concentrations. A soap-bubble flow meter was fitted to the outlet needle of each bottle to measure the flow rate through the bottle and to check that the gas flow was not blocked. The bottles were maintained in a thermostatically controlled room at 30°C. In addition, to enable comparison of respiration data with those in the literature, a series of experiments were carried out on adults at 20°C, 25°C and 35°C. Following 24 h of exposure for conditioning, the air composition within the bottle was measured by sampling with a 1 ml "pressure-lock" syringe through the silicon septum. The inlet and outlet ports of the bottles were then blocked tightly for approximately 1 h by means of metal clamps. At the end of this period another sample was taken as described previously. For eggs, 24 h of acclimatization period was not needed but 24 h was required to ensure accumulation of sufficient CO_2 for the tests. Gas concentrations were determined using a Tracer 565 gas chromatograph equipped with twin thermal conductivity cells, dual columns (each $4' \times 1/16''$i.d.), and a sample loop thermostatically regulated at 50°C. Column 1 was packed with Porapak Q, while column 2 was packed with "molecular sieve 5a". A Spectra Physics SP 4100 integrator computed percentage concentration of the gas components.

The CO_2 production was used to measure respiration. During the experiments a small leakage was observed in some flasks. This resulted from the gas sampling procedure adopted in the experiments and caused O_2 to increase slightly, especially in tests with the lowest O_2 concentrations, but it did not influence the CO_2 concentration at detectable levels. Therefore, O_2 consumption was not always as reliable a criterion as CO_2 production. In the preliminary studies aiming to evaluate the accuracy of the sampling method, two successive samples of 1 ml did not indicate a change in the CO_2 or O_2 readings at detectable levels.

Experiments with eggs and young larvae were repeated twice. Results obtained from old larvae and pupae were found to be satisfactory the first time. Adult exposures, on the other hand, were replicated three times to demonstrate clearly the increased respiration at 3% and 5% O_2 levels, in particular. Respiration results for adults were based on averages of three replicates.

3. Results

Respiration rates expressed in terms of µl CO_2/insect h (or 10 insect h for younger stages) and in terms of µl CO_2/mg h are given in Figs. 1–5. Presenting the results using these different units facilitates comparison with the literature. Respiration rates based on O_2 consumption in terms of µl O_2/insect h and in terms of µl O_2/mg h are given in Table 2.

Respiration of eggs (Fig. 1) at 1% O_2 was 0.003 µl CO_2/10 eggs h. It increased progressively and reached 0.029 µl CO_2/10 eggs h at 21% O_2. At 1% O_2, CO_2 production of young larvae was 0.5 µl CO_2/10 insect h (Fig. 2). Increase in O_2 concentration caused a progressive increase in respiration rate to 4.1 µl CO_2/10 insect h in normal air. The respiration rate of old larvae at 1% O_2 was 0.32 µl CO_2/insect h, which progressively increased to 2.52 µl CO_2/insect h at 21% O_2 (Fig. 3). The CO_2 production of pupae at 1% O_2 was

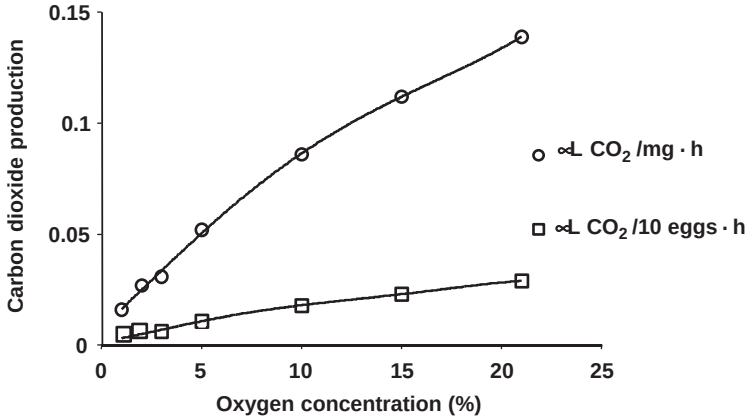

Fig. 1. Respiration rates of *Rhyzopertha dominica* (F.) eggs in response to low oxygen concentrations in nitrogen at 30°C and 70% r.h.

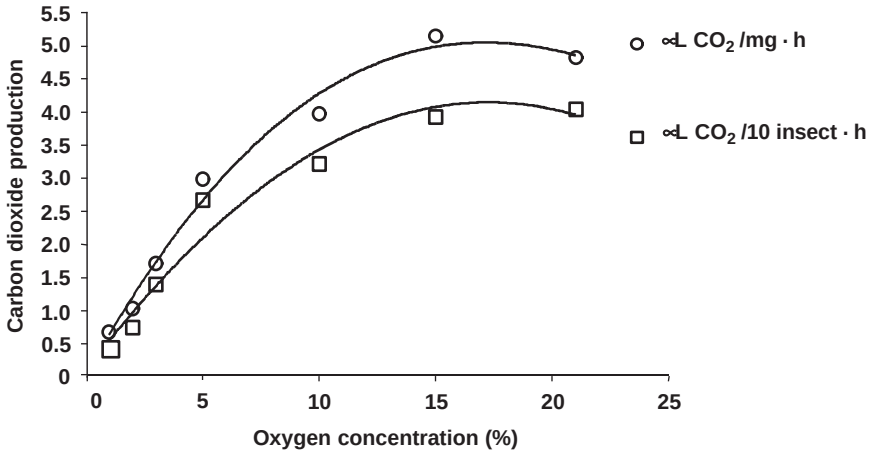

Fig. 2. Respiration rates of *Rhyzopertha dominica* (F.) young larvae in response to low oxygen concentrations in nitrogen at 30°C and 70% r.h.

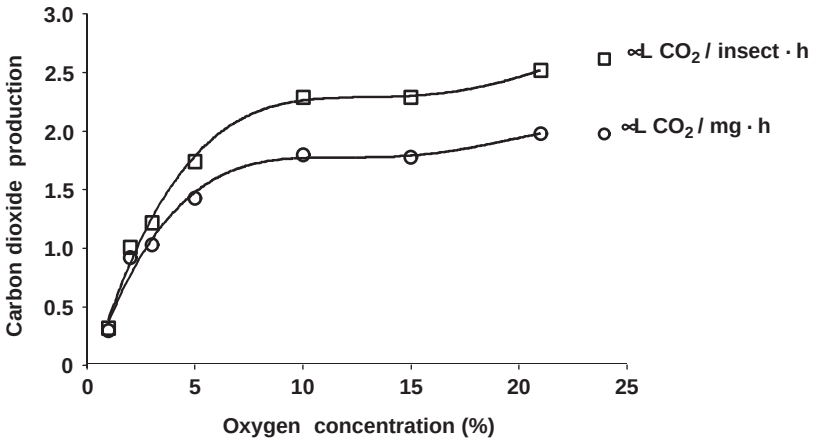

Fig. 3. Respiration rates of *Rhyzopertha dominica* (F.) old larvae in response to low oxygen concentrations in nitrogen at 30°C and 70% r.h.

calculated as 0.15 µl CO_2/insect h. It increased progressively with O_2 level and reached 0.82 µl CO_2/insect h at 21% O_2 (Fig. 4).

The CO_2 production of adults was 0.91 µl CO_2/insect h at 1% O_2 increasing to 2.85 µl CO_2/insect h at 3% O_2. At higher O_2 levels, CO_2 production declined and then rose again. At 21% O_2, it was measured as 2.86 µl CO_2/insect h (Fig. 5). RQ values generally tended to increase for all stages as the O_2 concentrations decreased (Table 3).

Respiration rates of adults at the temperatures of 20°C, 25°C and 35°C were in an increasing order and were 0.89, 2.33 and 6.82 µl CO_2/insect h, respectively, or 0.93, 2.37 and 5.63 µl O_2/insect h, respectively (Table 4).

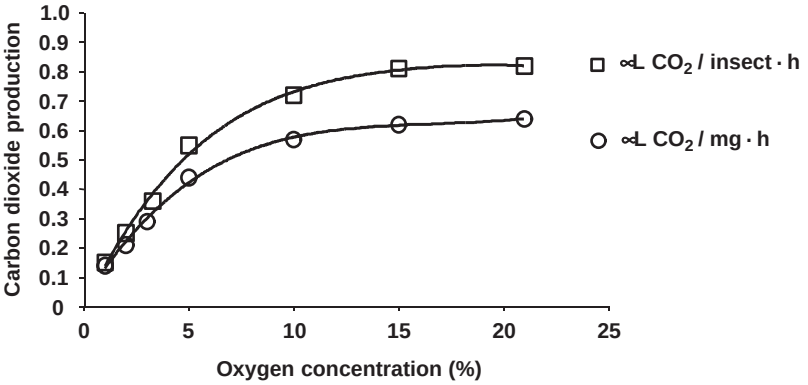

Fig. 4. Respiration rates of *Rhyzopertha dominica* (F.) pupae in response to low oxygen concentrations in nitrogen at 30°C and 70% r.h.

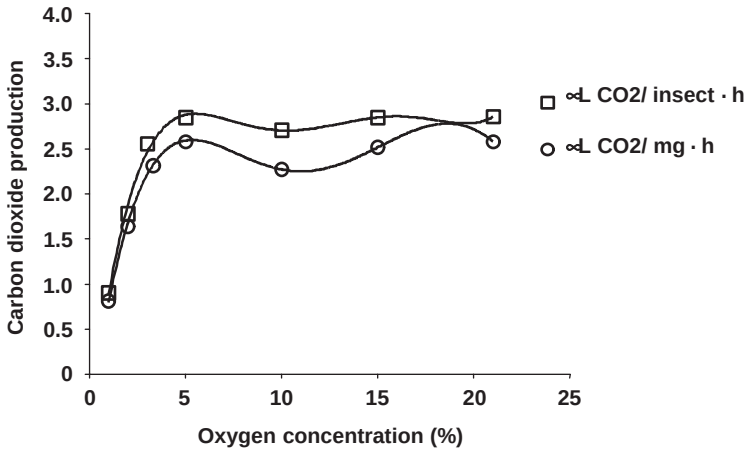

Fig. 5. Respiration rates of *Rhyzopertha dominica* (F.) adults in response to low oxygen concentrations in nitrogen at 30°C and 70% r.h.

4. Discussion

4.1. Respiration in normal atmospheric air

The literature on respiration of *R. dominica* in normal atmospheric air at different temperatures is summarized in Table 4. Results on O_2 consumption obtained in our study were used in comparison with results in the literature.

Birch (1947), who studied the respiration of *R. dominica* at 34°C and 55% r.h., reported that the O_2 consumption of developing larvae increased from 0.57 to 7.66 µl O_2/insect h (converted from mm^3/insect h). In the present study we also found that the O_2 uptake per insect increased during larval development (Table 2). Respiration of young and old larvae at 30°C was determined as 0.42 and 2.87 µl O_2/insect h, respectively (respiration rate for young larvae in Table 2 is given in units

Table 2
Respiration rates (μl O_2/insect h and μl O_2/mg h) of development stages of *Rhyzopertha dominica* (F.) at 30°C and 70% r.h. at various oxygen in nitrogen concentrations

O_2 concentration (%)	Development stages									
	Egg		Young larva		Old larva		Pupa		Adult	
	μl O_2/10 eggs h	μl O_2/mg h	μl O_2/10 insect h	μl O_2/mg h	μl O_2/insect h	μl O_2/mg h	μl O_2/insect h	μl O_2/mg h	μl O_2/insect h	μl O_2/mg h
1	—	—	0.63	1.17	0.19	0.18	—	—	0.69	0.61
2	—	—	—	—	1.16	1.05	—	—	1.55	1.41
3	—	—	1.90	2.36	1.20	1.02	—	—	1.68	1.57
5	—	—	1.93	2.14	1.66	1.36	0.40	0.33	2.77	2.50
10	0.015	0.07	2.73	3.43	2.35	1.85	0.83	0.66	2.85	2.34
15	0.033	0.16	3.27	4.36	2.36	1.83	1.05	0.79	2.68	2.36
21	0.043	0.21	4.18	4.89	2.87	2.26	1.05	0.82	3.38	3.05

Table 3
RQs of development stages of *Rhyzopertha dominica* (F.) at 30°C and 70% r.h. at various oxygen in nitrogen concentrations

O_2 concentration (%)	Development stages				
	Egg	Young larva	Old larva	Pupa	Adult
1	—	0.64	1.72	—	1.25
2	—	—	0.87	—	1.13
3	—	0.72	1.02	—	1.50
5	—	1.40	1.05	1.37	1.02
10	1.19	1.17	0.98	0.86	1.00
15	0.7	1.19	0.97	0.78	1.06
21	0.7	0.98	0.88	0.79	0.85

Table 4
Comparative data on respiration rates of *Rhyzopertha dominica* (F.) adults in normal air and at varying atmospheres from literature

Temp.(°C)	Respiration rates[a]				Author
	µl CO_2/insect h	µl CO_2/mg h	µl O_2/insect h	µl O_2/mg h	
20	0.89	0.82	0.93	0.86	Present work
22			2.02		Birch (1947)
25				0.67–1.19	Price (1983
25	2.33	2.15	2.37	2.19	Present work
25			3.47[b]		Kashi (1981)
26			3.17		Birch (1947)
30			3.96		Birch (1947)
30	2.86	2.58	3.38	3.05	Present work
34			5.05		Birch (1947)
35	6.82	6.10	5.63	5.04	Present work
25	3.04		0.96[c]		Kashi (1981)
25	3.54		0.5[d]		Kashi (1981)
25	32.17		1.67[e]		Kashi (1981)

[a] Converted to µl where necessary.
[b] Calculated from graph.
[c] Calculated from table; according to an exposure to 12.6% O_2 (40% CO_2 in air).
[d] Calculated from table; according to an exposure to 8.4% O_2 (60% CO_2 in air).
[e] Calculated from table; according to an exposure to 4.2% O_2 (80% CO_2 in air).

of µl O_2/10 insect h). Birch (1947) reported that 18-day-old larvae of *R. dominica* at 30°C and 55% r.h. respired 2.90 µl O_2/insect h. Our study included slightly older larvae, but the results can be regarded as in agreement with those of Birch (1947).

Campbell and Sinha (1978), on the other hand, reported that daily O_2 uptake based on dry body weight by *R. dominica* decreased from 29.5 to 16.85 µl O_2/mg h as the larval development proceeded from the first to fourth instars. The results of Campbell and Sinha (1978) are higher

than those presented in our work, but these differences may be due to their results being based on dry body weight, while our results were calculated using fresh body weight. In the present study, we observed O_2 uptake to decrease from 4.90 to 2.26 μl O_2/mg h as the larvae increased in size.

According to Birch (1947), respiration of *R. dominica* expressed on a per insect basis at 34°C decreased during pupation and increased in adults. He reported that respiration in a population composed largely of pupae (86% pupae, 6% larvae, 6% prepupae and 3% adults) and one composed of 1-week-old adults were, respectively, 1.71 and 5.05 μl O_2/insect h. In the present work, we observed a similar respiratory pattern in normal atmospheric air at 30°C, and pupal and adult respiration was measured as 1.05 and 3.38 μl O_2/insect h, respectively (Table 2).

Birch (1947) reported that O_2 uptake of 1-week-old adults per insect per hour at 22°C, 26°C, 30°C, and 34°C was in the increasing order of 2.02, 3.17, 3.96 and 5.05 μl (converted from mm^3), respectively. In the present study, the respiration of adults at the temperatures of 20°C, 25°C, 30°C and 35°C was measured as 0.93, 2.37, 3.38 and 5.63 μl O_2/insect h, respectively. Our results are in close agreement with those of Birch (1947). However, Price (1983) reported a very low level respiration in adults of *R. dominica* at 25°C, which was between 0.67 and 1.19 μl O_2/mg h (converted from μl O_2/g/30 min). Kashi (1981), on the other hand, reported that O_2 uptake in adults was 3.5 μl O_2/insect h (converted from μl O_2/insect/24 h) at 25°C, which was 50% higher than our data (Table 4).

4.2. Respiration at reduced O_2 levels

Respiration rates of immature stages of *R. dominica* increased proportionally as the O_2 concentration increased from 1% to 21%. Respiration of eggs was particularly suppressed at low O_2 levels of 3% or less. CO_2 production/h in eggs at 1% O_2 concentration was found to be 9.7 times less than that in 21% O_2. Calderon and Navarro (1980) reported that the egg stage was more sensitive than adults to low O_2 levels. In previous work, we obtained a similar respiratory response in *Tribolium castaneum* (Herbst) at the same experimental conditions: CO_2 production of eggs in 1% O_2 was reduced by nine times when compared to that in 21% O_2 (Emekci et al., 1998).

In the present study, young and old larvae produced a similar response to reduced O_2 as the eggs. However, older larvae responded to O_2 levels more predictably than young ones (Figs. 2 and 3). In young larvae, CO_2 production was adversely affected at 1–5% O_2 concentrations. Young larval respiration decreased by 8.2 times when the O_2 concentration decreased from 21% to 1%. When the respiration was described as production of CO_2 per unit of body weight, of the developmental stages, young larvae produced the highest level of respiration at all O_2 concentrations. This level then declined as development proceeded to the pupal stage. When the respiration rates were expressed in units per insect, the effect of size masked any meaningful trends. In young larvae of *T. castaneum* at the same O_2 conditions, respiration was suppressed more markedly than that of old larvae by the ratios of 42 and 4.5 times, respectively (Emekci et al., 1998).

Results obtained for pupae showed that respiration was mainly suppressed by low O_2 levels (Fig. 4). CO_2 production increased 5.5 times when the O_2 concentration increased from 1% to 21%. This suggested that the pupa is less disturbed by reduced O_2 concentrations in comparison with the other stages. In pupae of *T. castaneum*, we observed that respiration under the same

conditions was even more stable than in those of *R. dominica* and increased by only 1.5 times, when O_2 concentrations increased from 1% to 21% (Emekci et al., 1998).

In the present study, we found that at low O_2 levels such as 3% and 5% in particular, there was increased adult respiration based on CO_2 production (Fig. 5). We also observed a similar respiratory pattern in *T. castaneum* at the same conditions (Emekci et al., 1998). Kashi (1981), in a study with mixtures of CO_2 levels higher than 40% in air at 25°C, reported that CO_2 production of *R. dominica* adults at 4.2% O_2 was nearly 11 times higher than at 8.4% or 12.6% O_2 levels reaching 32.17 μl CO_2/insect h. These findings suggest that adults underwent respiratory stress when exposed to between 3% and 5% O_2, although the effect of 80% CO_2 in the atmosphere may have been dominant. They might have either increased their respiration to compensate for lack of O_2 or used alternative pathways such as anaerobiosis to maintain their energy balance. Further biochemical research is needed to clarify this aspect.

4.3. Lethal influence of low O_2 levels

Calderon and Navarro (1980) reported that mortality in *R. dominica* eggs at 2%, 4% and 6% O_2 in N_2 was 75%, 85% and 50% after 96 h of exposure, respectively, at 26°C and 55% r.h. They also reported that adult mortality was below 10% at the same conditions. Bailey (1965) reported no mortality of *R. dominica* adults at 32°C after exposure to O_2 levels higher than 6%. As the O_2 concentration gradually decreased, he reported progressively increasing mortality which reached 100% at 2% O_2 after 14 days exposure. Annis and Dowsett (1993) reported that in *R. dominica* high mortalities for all development stages could only be obtained with O_2 concentrations below 1%. These results would support the view that all development stages of *R. dominica* were alive when we measured their respiration rates. In the present study, adult mortality was 7% after 24 h of exposure to 1% O_2. For oxygen levels higher than 1%, no mortality was obtained after 24 h of exposure. Therefore, all the respiration rates measured were for live insects; except for those at 1% O_2 in which mortality was low.

4.4. Respiration quotients

Birch (1947) reported that the RQ for *R. dominica* adults in normal atmospheric air at 34°C was 0.94 for adults. RQs for larvae and adults of *R. dominica* in normal atmospheric air at 30°C were given by Campbell and Sinha (1978) as 1 and 0.8, respectively. In the present study, RQ values for the egg, young and old larva, pupa and adult at normal atmospheric air were calculated as 0.7, 0.98, 0.88, 0.79 and 0.85, respectively (Table 3). These results suggest that eggs and pupae had a lipid metabolism, whereas young larvae had a carbohydrate metabolism. In older larvae and adults a lipid-carbohydrate metabolism prevailed (Edwards, 1953; Beenakkers et al., 1981; Steele, 1981). In a previous study (Emekci et al., 2002), we observed a similar fluctuation in RQ values during the course of the development of *T. castaneum*. Our results are in agreement with the literature.

This type of metabolism was also reported in other insects. RQ values for other beetles, namely *Cryptolestes ferrugineus* (Stephens) (Campbell and Sinha, 1978), *Oryzaephilus surinamensis* (L.) (White and Sinha, 1981), *Sitophilus oryzae* (L.) (Birch, 1947), *T. confusum* (AliNiazee, 1971; Carlson, 1966, 1968), and *T. castaneum* (Donahaye, 1992) indicate a protein–carbohydrate

metabolism in adults. The RQ values obtained by these authors were between 0.83 and 1.1, overlapping largely with the results obtained here in normal atmospheric air (Table 4).

At reduced O_2 concentrations, the RQ tended to increase as the O_2 level decreased (Table 4). Kashi (1981), in a respiration study with the adults of *R. dominica* at various high CO_2 + air combinations at 25°C and 50% r.h., reported that CO_2/O_2 values increased from 3.17 to 19.30 as O_2 levels decreased from 12.6% (40% CO_2 + air) to 4.2% (80% CO_2 + air). Birch (1947) on the other hand reported a decrease in RQ values for *R. dominica* adults from 0.97 to 0.83 as O_2 levels decreased from 18% to 2%.

In the present work, the RQ value for adults in 3% O_2 was particularly high at 1.5. In our previous work with *T. castaneum* adults (Emekci et al., 2002), we found a similar trend in RQ resulting in an increase at 3% and 5% O_2 levels, when compared to controls. The possible reasons for increased RQ values at low O_2 atmospheres were discussed in detail in our previous work (Emekci et al., 2002). However, high CO_2 production or increased RQ values at low O_2 concentrations are good indices of metabolic stress.

This study in conjunction with our previous work with *T. castaneum* reveals that adults, in particular, undergo metabolic stress at low O_2 levels between 3% and 5% showing high CO_2 production and/or an increased RQ value. The implications for survival need to be clarified by further detailed physiological studies over extended exposure periods. Such results would provide additional data for sealed storage to help control insect pests more effectively in hermetic storage.

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