Respiration of the Confused Flour Beetle in Five Atmospheres of Varying CO_2:O_2 Ratios[1]

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ABSTRACT

The respiratory gas exchange of adult (2-week-old) confused flour beetles, *Tribolium confusum* Jacquelin duVal, was determined at 30°C in 5 different CO_2:O_2 ratios ranging from 1:0.74 to the "normal atmosphere" ratio of 1:717. The respiratory quotient (RQ) was less than 1.0 in all CO_2:O_2 ratios except 1:717. At the lowest O_2 concentration (4.9%) the RQ was lowest (0.60). Lack of O_2 rather than high CO_2 content appeared to stimulate increased O_2 consumption.

The typical atmosphere in infested stored grain usually contains more CO_2 than is present in open air. This difference may affect results of experiments on grain-storage insects in the field or in laboratory tests where insects may be taken from the typical atmosphere of the culture jar and fumigated in an atmosphere with the normal open-air ratio of CO_2 to O_2 and N_2.

The purpose of this study was to determine the respiratory gas exchange of adults of the confused flour beetle, *Tribolium confusum* Jacquelin duVal, in a range of CO_2:O_2 ratios that might be found in the interstitial air of grain recently stored in a bin or that might develop after a long infestation by insects had increased the CO_2 content. It was hoped that such data might indicate the atmospheres best tolerated by the confused flour beetle in stored grain and what variations in atmosphere are detrimental to the insect.

PROCEDURE.—Respiration of 2-week-old confused flour beetle adults was measured by gas chromatography (Carlson 1966). The gas mixture flowed at a rate of 100 cc/min through a modified 50-ml respirometer flask containing 1 lot of 350 beetles. The flask was partly submerged in a constant-temperature (30°C) water bath. The CO_2, O_2, and N_2 were separated and eluted in a gas chromatograph using a 2-column system (HMPA-Columpak and Columpak-molecular sieve) with twin thermal conductivity detector cells. The 5 atmospheres of CO_2, O_2, and N_2 used in the tests, as listed in Table 1, ranged in ratio of CO_2 to O_2 from 1:0.74 to 1:717. The average CO_2 production, O_2 consumption, and respiratory quotient (RQ) of 5 lots of beetles were determined for each atmosphere. Each lot of beetles was tested 5 times in a given atmosphere.

RESULTS.—Respiration of the insects in atmospheres of varying CO_2:O_2 ratios is shown in Fig. 1. Table 1 shows the average respiration in μmoles for 5 exposures of 5 lots of 350 insects each to the several atmospheres. In the following statements, the word "μmoles" refers to μmoles $\times 10^{-2}$/mg per min.

As the available O_2 in the atmosphere was decreased from 10.15 to 4.91%, and the CO_2 content also was decreased from 13.7 to 4.8%, the beetles' consumption of O_2 rose sharply from 0.18 to 0.21 μmoles (Fig. 1 and Table 1). As the environmental O_2 content more than doubled to 14.9%, the O_2 consumption by the insects declined to 0.17 μmoles. During this increase-decrease in O_2 uptake, the CO_2:O_2 ratio progressed from 1:0.74 to 1:1.02 to 1:3.29, with the peak O_2 uptake occurring at the ratio near 1:1. As the ratio of CO_2 to O_2 became more disparate (from 1:3.29 to 1:717), O_2 uptake rose only slightly from 0.17 to 0.19 μmoles.

CO_2 production by the beetles was, for the first 3 ratios, somewhat of a mirror image of the O_2 uptake at these values. As the CO_2:O_2 ratio went from 1:0.74 to 1:1.02 to 1:3.29, the CO_2 production first decreased, then increased. The low point of CO_2 output occurred when both O_2 and CO_2 in the atmosphere were at nearly 5%. When O_2 was increased to more than 21% by volume, nearly its usual atmospheric concentration, and CO_2 concentration was cut to nearly a tenth that of the previously mentioned atmosphere, CO_2 expenditure lowered to 0.13 μmoles. When the CO_2 content was reduced again to its usual atmospheric concentration (0.03%), CO_2 production sharply increased to 0.20 μmoles.

The RQs, based on the molar values, were considerably below 1.0 in all atmospheres except the one that represented "normal" air (0.03% CO_2:21.5% O_2). In this atmosphere, where the O_2 concentration was at its maximum and CO_2 at its minimum, the RQ reached its peak value of 1.02. In the atmosphere with the lowest O_2 concentration (4.91%), the RQ was depressed to 0.60.

In all atmospheres tested, the O_2 consumption by most of the insect lots decreased more-or-less uniformly throughout the 5 exposure periods. In every lot, total respiratory value in the 1st exposure exceeded that in the 5th. A progressive decrease in the CO_2 production from exposure 1 to exposure 5 was also evident in each lot; however, it was not as consistent as the progression in the O_2 data. The respiration of each lot was measured over approximately the same 3-hr period in the afternoon. No detrimental effects on the test insects were noted during their confinement in any of the atmospheres. The calculated standard deviations about each lot's CO_2 or O_2 average economy indicated homogeneity of data.

DISCUSSION.—To measure the respiration of confused flour beetles in a normal atmosphere, Park (1936) and Calderwood (1961) used manometric techniques which would tend to keep atmospheric CO_2 at low levels. Their respiratory data were similar to those of this study, in which gas chromatography was used to measure respiration of the insects in an atmosphere

[1] Accepted for publication September 22, 1967.

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[3] Loren I. Davidson of the Market Quality Research Division assisted by conducting chromatographic analyses of the respiratory gas samples.

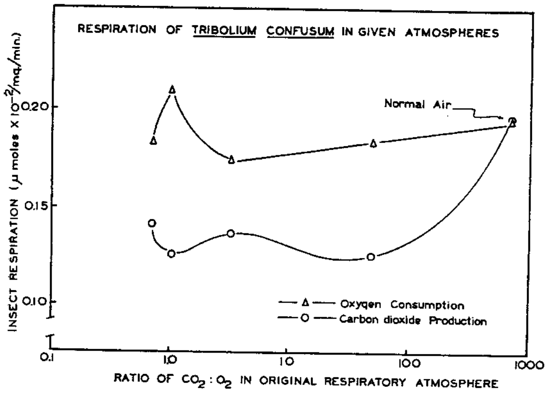

FIG. 1.—Respiration of the confused flour beetle adults in 5 atmospheres of differing CO_2:O_2 ratio. Each point represents an average of 25 respirometric exposures of 5 lots of test insects. Test temperature was 30°C.

of 0.03% CO_2 and 21.5% O_2. Kennington (1957) determined that a low concentration of O_2 caused an initial increase in O_2 consumption in confused flour beetles, followed by a steady but slow decline. Other studies show that many other insect species respire at a fairly constant rate over a wide range of O_2 concentrations (Edwards 1953, Wigglesworth 1947). Certain insects tolerate even anaerobic conditions (von Brand 1946). In a recent example, confused flour beetles had only 50% mortality after 36 hr in an atmosphere of N_2 (Knipling et al. 1961).

It is possible that results might have been different had the percent of O_2 and CO_2 in the atmospheres been changed while maintaining their ratio to each other, but the ratio of CO_2 to O_2 does not seem to be as important as the actual percent of each gas in the atmosphere.

The major objective of this study was fulfilled, that is, respiratory economy was determined for the confused flour beetle in 5 atmospheres differing in CO_2 and O_2 content. However, the point at which the excess or lack of these gases became detrimental was not ascertained.

At 4.8% CO_2:4.9% O_2 there was an unusual increase in O_2 consumption and concurrent fall in CO_2 output, making the RQ lowest (0.60) at this point. This low RQ may denote atmospheric unsuitability. Sizable deviations from 1.0 indicate an alteration in insect homeostasis which, if prolonged, could cause serious physiological problems for the insect. All RQ's, except those in the normal atmosphere, were low. It is not known if these low RQ's really represent a period of protein and fat metabolism. The insects were fasting only for the 3 hr of the experiment, and the carbohydrate endosperm eaten just before fasting would probably not be fully digested, assimilated, or oxidized during the experiment. These low RQ's may indicate respiratory stress, as similar RQ's recorded for this species, as part of O_2 debt repayment during recovery in a normal atmosphere after 2 hr of anoxia in CO_2 or N_2 atmospheres, are thought to indicate (Carlson 1968). This differs from the usual results with vertebrates, in which RQ's above 1.0 are common as the excess CO_2 is rapidly given off during O_2 debt repayment.

Which gas (CO_2 or O_2) is the chief factor in determining O_2 consumption in the confused flour beetle? These data indicate that low O_2 content stimulated O_2 uptake, but that increased CO_2 in the atmosphere probably did not do so. Consumption of O_2 was low in an atmosphere of 14.9% O_2 and 4.53% CO_2. When O_2 concentration increased from 4.9% to 10.15%, O_2 intake was lowered despite the fact that CO_2 increased from 4.8% to 13.7%.

In all atmospheres other than the "normal" one, CO_2 content was from 15 to nearly 450 times greater than 0.03%. In all these atmospheres with more CO_2 than normal, insect CO_2 output was from 28 to 36% less than that in an atmosphere with 0.03% CO_2. The physiological significance of this CO_2 retention is not known. CO_2 retention is usually thought of as a measure of the anaerobiosis undergone. However, even at the highest concentration of CO_2 used in the study (13.7%) the respiration did not suggest anoxia and, in fact, was well tolerated by the insect.

In summation, under the conditions of this experiment it appeared that O_2 lack (below 10%), rather than CO_2 excess, in the respiratory environment stimulated CO_2 production and O_2 intake. The confused flour beetles produced 70% more CO_2 in an atmosphere containing 0.03% CO_2 than they did in atmospheres from 0.46% to 13.70% CO_2. Low RQ values may indicate atmospheric unsuitability. By this criterion, the beetles were most adversely affected by a CO_2:O_2 ratio of about 1:1 (4.8% CO_2:4.91% O_2). In this atmosphere, O_2 intake was higher and CO_2 output was as low as in any other test. Future tests should evaluate beetle respiration in a dynamic situation in which the O_2 content could be lowered and CO_2 increased at periodic intervals without removing the insects from the test chambers.

Table 1.—Average respiration of the confused flour beetle with standard deviations in indicated atmospheres at 30° C.

Gas concentrations in atmosphere (% by volume) [a]			CO_2:O_2 ratio	CO_2 production[b] (μmoles $\times 10^{-2}$/min/mg)	O_2 consumption[b] (μmoles $\times 10^{-2}$/min/mg)	RQ[c]
CO_2	O_2	N_2				
13.70	10.15	76.15	1:0.74	0.14±0.03	0.18±0.03	0.78
4.80	4.91	90.29	1:1.02	.13± .03	.21± .04	.60
4.53	14.90	80.57	1:3.29	.14± .75	.17± .03	.79
0.46	21.10	77.44	1:48	.13± .03	.18± .04	.69
.03	21.50	78.47	1:717	.20± .05	.19± .04	1.02

[a] Analyses made by Matheson Co.
[b] Each value is the average from 5 lots of 350 insects, each replicated 5 times.
[c] Calculated on a molar basis.

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Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY
Volume 61, Number 1, pp. 94–96, February 1968