

# Respiration Measurement of *Tribolium confusum* by Gas Chromatography<sup>1</sup>

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## ABSTRACT

Respiration of 2-week-old adult confused flour beetles, *Tribolium confusum* Jacquelin du Val, was measured with a gas chromatograph. The use of a 2-column system enabled rapid and simultaneous measurement of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub>. Installation of a zero-suppression selector between voltage source and the recorder potentiometric system al-

lowed O<sub>2</sub> and N<sub>2</sub> to be measured at 100% detector cell sensitivity. At 30°C, the production of carbon dioxide and consumption of oxygen of 5 lots of 350 beetles, measured 5 times, averaged 0.086/μg per mg per minute, and 0.062/μg per mg per minute, respectively, with a resultant molar respiratory quotient of 1.02.

The application of gas chromatography in animal respirometry is quite recent. Initial respirometric studies involving this technique were designed for use with larger animals (Jay and Wilson 1960, Dressler et al. 1960, Hamilton and Kory 1960, Hamilton 1961). The only previous known report concerning measurement of insect respiration by this means was that of Whitney and Ortman (1962). These authors presented respirometric data on individual house flies, *Musca domestica* L.; granary weevils, *Sitophilus granarius* (L.); and observations on lots of 1 to several pea aphids, *Acyrtosiphon pisum* (Harris); and spotted alfalfa aphids, *Therioaphis maculata* (Buckton).

In the present study, respiration of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, was measured by gas chromatography. Recording of both O<sub>2</sub> and N<sub>2</sub> at 100% detector cell sensitivity was made possible by the zero-suppression selector built into the recorder. The CO<sub>2</sub> signals did not require voltage suppression to be measured at 100% detector cell sensitivity.

The data presented here are part of a more detailed study in this laboratory on the induction of fumigant susceptibility in stored-grain insects by the use of preconditioning agents. Metabolism and, specifically, oxygen consumption measured before and after preconditioning are indices to the stress and homeostasis of the tested insects. These give criteria in addition to fumigation mortality after preconditioning for judging a given preconditioning agent's efficacy.

The purpose of this study was to ascertain the respiratory limits of 2-week-old confused flour beetle adults at 30°C.

**METHODS AND MATERIALS.**—A Fisher Model 25V<sup>3</sup> gas chromatograph, stock equipped with twin thermal conductivity detector cells and 2 connecting columns, was used. Column 1 was 8 ft 6 in. by 0.25 in. ID, aluminum, and packed with 30% by weight HMPA (hexamethylphosphoramide) on 60- to 80-mesh Columnpack (Fisher Chromosorb P). Column 2 was 12 ft by 0.25 in. ID, aluminum, and packed with uncoated 60- to 80-mesh Columnpack over the 1st 5 ft of length. The remaining 7 ft were filled with 42- to 60-mesh activated molecular sieve 13X.

A 2-cc gas sample was aspirated from the respirometer flask and injected slowly into the entrance port of a 0.7-cc sample loop. This flushed and filled the loop completely. Excess sample gas returned to room atmosphere. The loop was connected to a 6-way gas sampling valve. Upon depression of the valve, the carrier gas (helium, flowing at 70 ml/min) flushed the contents of the 0.7-cc sample loop into column 1, where CO<sub>2</sub> was separated and retarded. Oxygen and nitrogen emerged together from column 1 and were recorded by detector filament no. 1 as a composite peak (Fig. 1). Shortly thereafter, CO<sub>2</sub> emerged from column 1 and was recorded. Oxygen and nitrogen entered column 2 and were separated and detected individually as they passed by filament no. 2. A 2-column system was necessary because no 1-column system gives satisfactory resolution to CO<sub>2</sub>, O<sub>2</sub>, and N<sub>2</sub>.

The column operating temperature was 30°C. To obtain this temperature, a thermostabilizer base was used and maintained at a constant 52.5°C. The laboratory was held at 28°C by thermostatically controlled heat fans and an air conditioner. A mercury thermometer, permanently installed on the chromatograph, was inserted into the center of the column coils. Actual heat loss from the column was enough to be exactly offset by the 52.5°C thermostabilizer base temperature, and a constant 30°C temperature was thus maintained in this critical area.

Two-week-old confused flour beetle adults were aspirated out of enriched wheat shorts cultures maintained at 26.7°C and 60% RH. Each lot of 350 insects was weighed to the nearest 0.05 mg on a Mettler Gramatic Balance. They were then placed in a 73.00-cc modified Erlenmeyer respirometer flask (Fig. 2). No food was present in the respirometer flask. The flask was suspended in a 30°C water bath, and humidified room air was allowed to circulate through the flask for 1 hr at a flow rate of 10 cc/min.

Following this acclimatization, the flask was purged thoroughly with preanalyzed reference air<sup>4</sup> at 200 cc/min for 10 min. The flask stopcocks were closed, and a 2-cc reference sample was taken with a syringe. The stopcocks were opened immediately after the reference sample was taken, and the purging continued at 200 cc/min for 5 min. The stopcocks were again closed for a 10-min stop-watch-timed exposure. The test sample was then taken with a syringe. A 10-min purge followed, then another reference sample was taken. The cycle was thus repeated for the 4 remaining replicates. Each lot of beetles remained in the

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<sup>3</sup> The use of trade names is for identification purposes only and does not constitute endorsement of the products by the USDA.

<sup>4</sup> Matheson Gas Corp. analysis of compressed air tank no. 1: CO<sub>2</sub> = 0.029 vol. %; O<sub>2</sub> = 21.500 vol. %; N<sub>2</sub> = 78.471 vol. %.

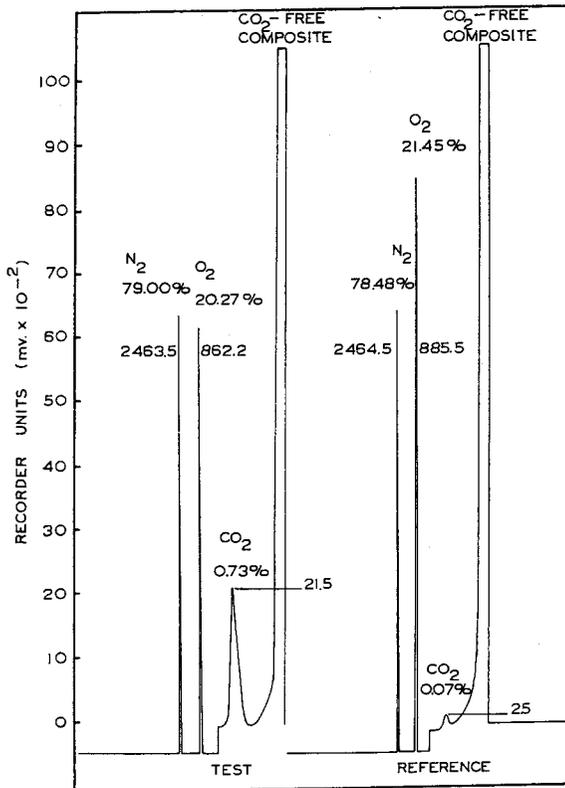


FIG. 1.—Reference and test gas chromatograms taken immediately before and after a 10-min insect-exposure period. The numbers beside each peak indicate the  $10^{-2}$  mv of thermal conductivity signal produced by that component in the gas sample.

flask without food for about 3 hr. The test insects were killed after the 5th exposure period.

A Brown Elektronik Recorder (1 mv full-scale deflection) was used and continuously registered the voltage output from the detector cells as each gas successively passed the sensing filaments. Oxygen and nitrogen peaks were drawn by the recorder pen when the signal voltage exceeded suppression input applied. Oxygen or nitrogen content was computed by adding the required zero suppression value (in millivolts, 1 mv equals 100 units) to the actual number of peak height units drawn. This value was multiplied by the calibration factor to derive the gravimetric units.

Carbon dioxide (at the low quantities available) did not require any suppression input for the peaks to be traced at 100% detector cell sensitivity. Only the actual peak height was used as the basis for the  $\text{CO}_2$  gravimetric transposition. In this way respiration rates could be expressed as  $\mu\text{g}/\text{mg}$  live wt/min. The gravimetric conversion was derived from calibration curves gained from analyzing pure gases and 3 Matheson preanalyzed  $\text{CO}_2 - \text{O}_2 - \text{N}_2$  compressed air mixtures. The chromatograph was routinely recalibrated before each lot's run with 1 Matheson gas mixture. Nitrogen values for each analysis were computed to adjust the volume total of the 3 gases to 100%. The molecular sieve column did not resolve argon from oxygen. No correction factor was required, as the inspired gas mixture was used for the reference (Hamilton and Kory 1960).

A Minneapolis-Honeywell custom-built zero-suppression selector was installed between the voltage source and the recorder's slidewire measuring circuit. A mercury cell powered the selector unit and eliminated a stray flux lines, which had previously interfered with the recorder zero balance. The selector controls consisted of 5 5-mv and 5 1-mv steps, permitting zero suppression voltages of from 1 to 30 mv to be applied against positive signal voltages.

**RESULTS AND DISCUSSION.**—Under the conditions and using the column types described, the chromatographic response in  $\mu\text{g}/0.7\text{-cc}$  sample for each peak height unit on the strip chart were:  $\text{CO}_2$ —0.446;  $\text{O}_2$ —0.232; and  $\text{N}_2$ —0.271.

All gases per sample were recorded within 4 min after the sample was injected. The corrected retention times are:  $\text{CO}_2 = 6$  sec;  $\text{O}_2 = 1$  min, 5 sec; and  $\text{N}_2 = 2$  min, 20 sec.

Table 1 presents the carbon dioxide production, oxygen consumption, and average respiratory quotient of each lot's test exposures. The average weight of each insect was 2.36 mg. This figure is somewhat higher than Park's (1936) confused flour beetle weight averages, which were 1.90 mg for females and 1.60 mg for males. The insects used by Park were cultured in flour, and only virgin females represented the female data. This comparatively deficient diet, the lack of developing eggs in the female population, and the advanced age of Park's insects (3 months) may explain the differences in weight.

I did not separate the beetles by sex and I made no attempt to determine the respiration rates of the different sexes. Park cites evidence that the heavier female respired slightly more than the male.

Carbon dioxide production trends slightly downward from the first exposure (replication) to the last, e.g., more gas was recovered in the 1st exposures than in the last. Oxygen consumption also declined from

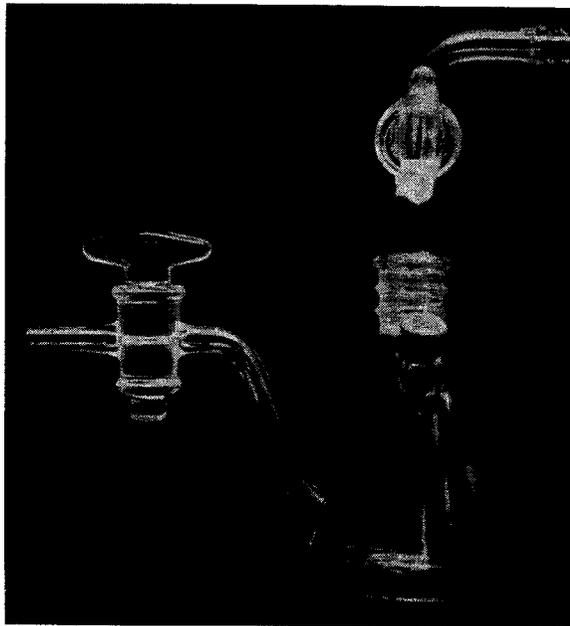


FIG. 2.—The insect respirometer chamber: a modified 50-ml Erlenmeyer flask with stopcock adapter, sidearm, and sampling port.

the 1st to the final exposure in all test lots. Observations of the insects in the test flask indicated that their locomotor activity decreased during the acclimatizing period (1st hr). Presumably this activity continued to decline (along with respiratory requirements) throughout the test period. The possibility of some diurnal rhythm interaction is not ruled out, as each lot was tested over nearly the same afternoon hours on consecutive days.

At 30°C, all replications of all lots averaged 0.0863  $\mu\text{g}/\text{mg}$  per min  $\text{CO}_2$  production, and 0.0617  $\mu\text{g}/\text{mg}$  per min  $\text{O}_2$  consumption. The resultant respiratory quotient, as calculated on a molar basis, was 1.02.

These figures are in general accord with those of previous workers. Park (1936) indicated that confused flour beetle oxygen consumption ranged from 0.046  $\mu\text{g}/\text{mg}$  per min to 0.0550  $\mu\text{g}/\text{mg}$  per min at 25°C (converted from  $\text{mm}^3 \text{O}_2/\text{mg}$  per hr). At the higher temperature of 29°C, Kennington (1957) listed an  $\text{O}_2$  consumption range of 0.0381–0.0957  $\mu\text{g}/\text{mg}$  per min in 48 individuals. These data were con-

verted from  $\text{cc O}_2/\text{mg}$  per hr. He gave no information as to sex composition or age of the insects.

Both Park (1936) and Kennington (1957) recorded  $\text{O}_2$  consumption by manometric means. For a discussion of this method's limitations, the reader is referred to Whitney and Ortman (1962).

The use of a zero-suppression selector greatly enhanced the accuracy of peak height measurements in the highly concentrated gases ( $\text{O}_2$  and  $\text{N}_2$ ) and was particularly useful in resolution of the small differences between reference and test oxygen peaks. Measurable differences in gas concentration could be detected within 10 min.

The extreme speed of the chromatographic responses disallowed suppression on the peak's upward ascent. This factor largely negated the use of the zero-suppression selector in the chromatographic analysis of unknown gas samples. In its normal operation 8 or 9 mv of suppression were dialed into the recorder after the  $\text{CO}_2$  peak was recorded. Fig. 1 depicts this point as the pen deflection on the strip chart's base mechanical stop. The spike  $\text{O}_2$  peak next appears, and the base line continues along the mechanical stop as 25 mv of suppression are entered to record the nitrogen peak. A failure by the operator of "guessing" to within approximately 0.5 volume % of  $\text{O}_2$  or  $\text{N}_2$  would result in over-suppression (no peak) or under-suppression (peak goes off the chart and is unreadable). After experimentation, it was determined that exactly 10 min was an optimum interval for respirometric exposure, as it required little or no additional suppression and allowed maximum  $\text{O}_2$  peak-height differentiation from reference-sample to test-sample analysis. The daily recalibrations indicated whether the same or 1 mv less suppression were required for the test oxygen analysis. Hamilton (1961) gives additional information concerning the design and principles of the zero-suppression selector.

Under the conditions of this experiment,  $\text{CO}_2$  was allowed to build up in the respirometer flask. This was in contrast to  $\text{CO}_2$  adsorption in the usual manometric technique. Both situations are problematical. It is well known that higher  $\text{CO}_2$  concentrations are normal in certain stored-grain insect environments. Whitney and Ortman (1962) found concentrations as high as 8.45%  $\text{CO}_2$  by volume in the bottom of a 1-quart, wheat-filled glass jar containing granary weevils. The retention and solubility of  $\text{CO}_2$  in test insects' tissue fluids would influence the respiratory centers and alter RQ values. However, reduction of the test exposure to 10 min or less and the use of confused flour beetles or other  $\text{CO}_2$ -tolerant species may tend to minimize the effect of  $\text{CO}_2$  buildup. In a more precise experiment, the reference gas could be compounded so that it would closely approximate that found in the interkernel spaces of a given grain mass.

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Table 1.—Gas chromatographic analysis of carbon dioxide production and oxygen consumption of 5 lots of 2-week-old confused flour beetle adults.

Lot <sup>a</sup> weight (mg)	Replicate no.	Carbon dioxide ( $\mu\text{g}/\text{mg}/\text{min}$ )	Oxygen ( $\mu\text{g}/\text{mg}/\text{min}$ )
819.21	1	0.114	0.150
	2	.088	.061
	3	.084	.059
	4	.081	.057
	5	.076	.055
		<sup>b</sup> .089 $\pm$ 0.015	<sup>b</sup> .076 $\pm$ 0.041
		RQ = 0.84 <sup>c</sup>	
842.67	1	.099	.063
	2	.089	.061
	3	.080	.062
	4	.077	.052
	5	.080	.052
		<sup>b</sup> .085 $\pm$ 0.009	<sup>b</sup> .058 $\pm$ 0.006
		RQ = 1.07 <sup>c</sup>	
823.25	1	.084	.065
	2	.079	.056
	3	.099	.054
	4	.087	.052
	5	.075	.049
		<sup>b</sup> .085 $\pm$ 0.009	<sup>b</sup> .055 $\pm$ 0.010
		RQ = 1.11 <sup>c</sup>	
828.51	1	.090	.063
	2	.085	.057
	3	.080	.058
	4	.081	.058
	5	.077	.055
		<sup>b</sup> .083 $\pm$ 0.002	<sup>b</sup> .058 $\pm$ 0.003
		RQ = 1.04 <sup>c</sup>	
812.55	1	.105	.067
	2	.103	.069
	3	.081	.058
	4	.081	.055
	5	.079	.054
		<sup>b</sup> .090 $\pm$ 0.013	<sup>b</sup> .061 $\pm$ 0.007
		RQ = 1.08 <sup>c</sup>	

<sup>a</sup> Each lot comprised 350 individuals. Exposures were replicated 5 times at 30°C in a closed 73.00-cc respirometer flask without  $\text{CO}_2$  absorbent. Each replication represents a 10-min exposure.

<sup>b</sup> Average and standard deviation.

<sup>c</sup> RQ values calculated on a molar basis.

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