

Respiration of Confused Flour Beetle Adults in CO₂ or N₂ and After Sublethal Fumigation¹

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ABSTRACT

Thirty-minute exposure of adults of the confused flour beetle, *Tribolium confusum* Jacquelin duVal, to CO₂ or N₂ reduced O₂ consumption to a very low level, where it remained for the rest of the 2-hour exposure to the gas. During recovery from CO₂ or N₂ anoxia, the respiration rate rose above normal. Peak O₂ consumption occurred at ½ hour after N₂ or 1 hour after CO₂ exposure. In neither case was the O₂ debt completely repaid.

During N₂ exposure, both O₂ consumption and CO₂ production declined. During CO₂ exposure, CO₂ production increased considerably for a short period.

Sublethal fumigation with CCl₄:CS₂ (80:20 by volume) at the peak of O₂ consumption after CO₂ or N₂ anoxia caused a prolonged respiratory depression. In beetles fumigated without previous exposure to CO₂ or N₂, respiration rate first increased, then declined below normal.

It has been thought that an increase in insect respiration rate would increase susceptibility to fumigants (Cotton 1932, Page and Lubatti 1963, Lindgren and Vincent 1962). However, respiratory data have seldom been recorded with subsequent fumigant mortality. In most of the experiments on this relationship, high temperature and/or CO₂ was used to increase the respiration rate. Studies by Carlson (1966a) indicated that confused flour beetles *Tribolium confusum* Jacquelin duVal, were more susceptible to CCl₄:CS₂ (80:20 by volume) fumigant at low respiration rates and that susceptibility was not increased at high respiration rates during oxygen debt repayment periods.

The objective of this study was to determine the respiratory metabolism of confused flour beetles before, during, and after CO₂ or N₂ preconditioning and CCl₄:CS₂ fumigation.

METHODS AND MATERIALS.—Preconditioning.—The procedure for preconditioning and respiration analysis has been detailed previously (Carlson 1966b) but is restated briefly. Lots of 350 adult confused flour beetles, 2 weeks old, were placed in respirometer flasks which were suspended in a water bath maintained at about 30°C. Humidified room air was circulated through the flasks for 1 hr at a flow rate of 10 cc/min. The flasks were then purged with preanalyzed air (0.03% CO₂, 21.5% O₂, and 78.47% N₂), followed by purging with the preconditioning gases N₂ or CO₂, at the rate of 200 cc/min. Atmosphere samples to determine respiration were taken from the flasks just before introducing the N₂ or CO₂ and at 10-min intervals for the next 2 hr. After 2 hr the flasks were again purged with the preanalyzed air, and samples were again taken periodically until respirometric exposures had been completed.

For respiratory gas analysis a Fisher³ 25V gas chro-

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³ Mention of trade names does not necessarily imply their endorsement by the U. S. Department of Agriculture.

matograph was used. A 2-cc gas sample was aspirated from the respiratory flask and injected into the entrance of a 0.7-cc sample loop. A Brown Electronik Recorder (1 mv full-scale deflection) registered the voltage output from the detector cells. All signals of

Table 1.—Respiration of 7 lots of 350 2-week-old confused flour beetle adults after CO₂ exposure or prior to, during, and after CO₂ exposure and after sublethal fumigation. CO₂ exposure was 2 hr. Test temperature was 30°C and respirometric exposure periods were 10 minutes each.

Lot no.	Lot weight (mg)	Respiratory environment composition ^a	Analysis time (min) after onset of given respiratory environment	CO ₂ production, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	O ₂ consumption, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	Respiratory quotient
1	881.7	PA/CO ₂	23	0.19	0.24	0.82
		PA/CO ₂	60	.28	.23	.87
		PA/CO ₂	91	.20	.24	.85
		PA/CO ₂	137	.14	.18	.80
		PA/CO ₂	171	.14	.20	.68
		PA/CO ₂	245	.13	.16	.86
		PA/CO ₂	273	.13	.17	.79
		PA/CO ₂	303	.14	.18	.76
2	825.8	PA	3	.19	.24	.76
		CO ₂	31	.69	.18	3.82
		CO ₂	58	^b	^b	
		CO ₂	84	.33	.08	4.44
		CO ₂	111	.23	.05	4.23
		PA/CO ₂	65	.23	.34	.67
		PA/CO ₂	95	.13	.23	.58
		PA/CO ₂	122	.08	.17	.48
3	864.6	PA	2	.16	.23	.68
		CO ₂	34	.36	.09	3.78
		CO ₂	62	^b	^b	
		CO ₂	89	.21	.08	2.59
		PA/CO ₂	50	.18	.28	.88
		PA/CO ₂	78	.14	.22	.65
		PA/CO ₂	106	.13	.18	.71
		PA/CO ₂	135	.11	.16	.67
4	873.4	PA	6	.11	.15	.75
		CO ₂	24	.83	.03	31.46
		CO ₂	53	.42	.10	4.37
		CO ₂	107	.34	.07	4.53
		PA/CO ₂	42	.17	.31	.54
		PA/CO ₂	71	.16	.25	.65
		PA/CO ₂	98	.12	.12	1.01
		PA/CO ₂	125	.03	.15	.22
5	919.7	PA	8	.14	.20	.67
		CO ₂	32	^b	^b	
		CO ₂	66	.24	.07	3.27
		CO ₂	88	.16	.05	3.48
		CO ₂	110	^b	^b	
		PA/CO ₂	33	.15	.24	.64
		PA/CO ₂	54	.21	.32	.65
		PA/F	11	.16	.21	.77
6	941.8	PA	7	.15	.19	.80
		CO ₂	70	.11	.03	3.12
		CO ₂	112	.33	.09	3.71
		PA/CO ₂	34	.18	.32	.56
		PA/CO ₂	56	.21	.32	.67
		PA/F	34	.13	.18	.70
		PA/F	60	.10	.16	.66

Table 1.—Respiration of 7 lots of 350 2-week-old confused flour beetle adults after CO₂ exposure or prior to, during, and after CO₂ exposure and after sublethal fumigation. CO₂ exposure was 2 hr. Test temperature was 30°C and respirometric exposure periods were 10 minutes each. (Continued.)

Lot no.	Lot weight (mg)	Respiratory environment composition ^a	Analysis time (min) after onset of given respiratory environment	CO ₂ production, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	O ₂ consumption, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	Respiratory quotient
7	941.8	PA	8	.11	.23	.47
		CO ₂	21	^b	^b	
		CO ₂	102	^b	.01	
		PA/CO ₂	23	.02	.18	.12
		PA/CO ₂	47	.24	.30	.80
		PA/F	11	.16	.19	.86
		PA/F	32	.14	.19	.73
		PA/F	63	.10	.18	.57
PA/F	96	.11	.14	.77		

^a PA = Preanalyzed air = 0.03% CO₂; 21.50% O₂; 78.47% N₂.
CO₂ = Pure, dry, flowing (100 cc/min) CO₂.
PA/CO₂ = Preanalyzed air provided for recovery after CO₂ exposure period.
PA/F = Preanalyzed air provided after sublethal fumigation.
^b No gas consumption or production noted.

greater than 1 mv were recorded when the signal voltage exceeded suppression input applied by a zero suppression unit.

Fumigation.—It was determined that at 80°F a concentration of 194.7 mg/liter of the 80:20 fumigant (CCl₄:CS₂ by volume) and an exposure of 10 min would kill less than 2% of normal unpreconditioned beetles. A Cheney adapter on the syringe used to apply the fumigant insured dosage reproducibility. The exposure period started when the syringe plunger had been completely depressed. At the end of the fumigation period the flasks were purged with air flowing at 100 cc/min for 10 min. The first respiratory gas sample was then withdrawn.

RESULTS.—The average rates of O₂ consumption and CO₂ production by confused flour beetles in normal air have been determined by Carlson (1966b). These rates were used as base lines for comparison with the respiration rates of the preconditioned and/or fumigated beetles in the present study (Fig. 1-3). To determine above- and below-average respiration rates, the area under each respiration curve was calculated by the Trapezoidal Rule (Kells 1950).

Preconditioning with CO₂.—Table 1 and Fig. 1 and 2 show the respiration of confused flour beetle adults during and after the entire sequence of preconditioning, recovery, and fumigation. Fumigation was performed at the peak of O₂ consumption, which occurred 1 hr after CO₂ preconditioning.

In lots 5 and 6 (Table 1), insect CO₂ production during CO₂ exposure is apparently not in accord with the rest of the data. Note unfilled circles as graph symbols in Fig. 1. Two readings are above and 2 are below the normal production rate. However, the extremely high respiratory quotients (RQ) (3.27, 3.48, 3.12, and 3.71) still indicate a transient period of high CO₂ production during CO₂ exposure.

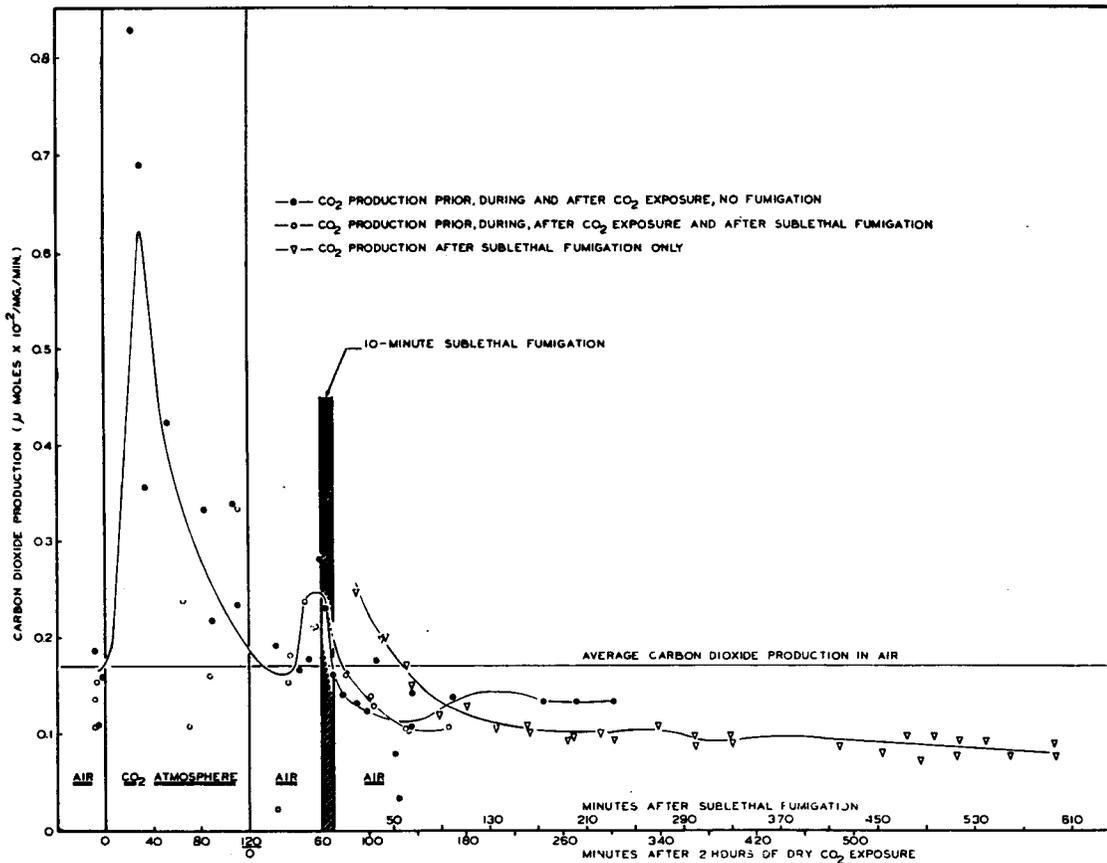


FIG. 1.—CO₂ production of 2-week-old confused flour beetle adults in dry flowing CO₂, then air, then a 10-min exposure to 194.7 mg/liter of 80:20 (CCl₄:CS₂) at 80°F. Air and CO₂ flow was 100-cc/min at 86° F.

During the recovery period of lot 7, CO₂ production rose from approximately normal rates to a high of 0.24 μ moles/mg of body weight per min after 47 min of recovery (Table 1). This rise appears to be compatible with earlier data, and the trend line indicates peak CO₂ production after 1 hr of recovery—coinciding with the insects' O₂ consumption peak (Fig. 1 and 2).

After this CO₂ production peak, if no fumigation occurred, insect CO₂ production declined during the following 2 hr to about 0.11 μ moles before leveling off at about 0.13 μ moles 5 hr after CO₂ exposure. This last value is about 25% below normal CO₂ production.

When the insects were fumigated at the point of maximum respiration, their CO₂ production did not initially decline as rapidly as that of the unfumigated insects. However, at 1 hr after fumigation, CO₂ production had dropped to 0.10 μ moles. The trend line leveled off at that point (40% below normal) for an additional 40 min without a perceptible inclination toward normal values.

O₂ consumption (Fig. 2) by confused flour beetles during CO₂ exposure appeared to be 62% less than normal. However, 1 hr after the CO₂ exposure ended, O₂ was being consumed at a rate of 0.33 μ moles/mg per min, which is 30% above normal.

When no fumigation occurred, O₂ consumption dropped rapidly from this peak for an hr, leveled off

at about 0.15 μ moles after 3 hr of recovery, and then slowly increased again to about 0.18 μ moles (21% below normal) during the next 2 hr. During the 2 hr of CO₂ preconditioning and the first 2 hr of recovery, 32% less O₂ than the normal amount was consumed, and it did not appear likely from the trend line that this O₂ debt would soon be paid.

When the insects were fumigated at the peak of O₂ consumption, the respiration rate immediately declined and reached about 0.19 μ moles 10 min after fumigation. Thereafter, it continued to decline but not so rapidly, reaching 0.14 μ moles 90 min after fumigation.

Insects that were fumigated after CO₂ preconditioning consumed 3.3% less O₂ than did those preconditioned but not fumigated.

Preconditioning with N₂.—Table 2 and Fig. 3 show results from this test. Dry flowing N₂ was used for the 2-hr preconditioning period. Sublethal fumigation was administered after a 1/2-hr recovery period, because at that time O₂ consumption was at its peak.

After the first 30-min exposure to N₂, CO₂ production was extremely low. It remained at this low level throughout the remaining 1 1/2 hr of N₂ exposure. In the recovery atmosphere, for insects that were not fumigated, CO₂ production rose sharply to about 0.20 μ moles at 45 min (15% above normal) and declined to 0.15 μ moles within the next 90 min. CO₂ production increased slightly during the next 30 min, then

declined again slowly during the last 150 min of observation. After the insects had 5½ hr of recovery from N₂ exposure, their CO₂ production was 26% below normal.

When the insects were exposed to sublethal fumigation after N₂ exposure and recovery, within 100 min their CO₂ production decreased steadily from 0.19 μmoles/mg per min to 0.10 μmoles. This was 23% less than the rate of CO₂ production by the preconditioned but unfumigated insects for a comparable period and about 40% less than normal CO₂ production values.

After 25 min of exposure to dry N₂, the insects' O₂ consumption had been reduced to 0.06 μmoles. This rate was succeeded by an apparent increase to nearly 0.13 μmoles, which was maintained for half an hour longer. O₂ consumption then dropped to 0.08 μmoles for the last 25 min of the 2-hr exposure to N₂.

If the insects were not fumigated during the recovery period, O₂ consumption increased rapidly from 0.08 μmoles at the end of exposure to N₂ to more than 0.25 μmoles after 30 min of recovery. Following this peak, consumption declined to 0.19 μmoles at 1 hr after preconditioning and remained relatively constant for another 30 min before declining to slightly more than 0.15 μmoles at the end of 5 hr in the recovery atmosphere.

A straightforward decline in O₂ consumption began immediately if insects were fumigated at the peak of O₂ consumption during recovery. This decline was from 0.25 μmoles to 0.13 μmoles within 110 min, at which time observations were ended. This level was about 21% less than the consumption rate of preconditioned but unfumigated insects for the same period, and 40% less than normal consumption of O₂.

Repayment of the O₂ debt during the first 2 hr after N₂ hypoxia was even less than that after CO₂ exposure. The total O₂ consumption was 46% less than normal during the 2-hr exposure to N₂. O₂ consumption during the next 2 hr in the recovery atmosphere was nearly 9% less than normal, even though

this period included the consumption peak when the rate was higher than normal. After 2 hr of recovery there was still no indication that the deficit in O₂ consumption would be made up.

Insects that had been both preconditioned and fumigated consumed 13.5% less O₂ during the 2-hr recovery period than those that had been preconditioned only.

Fumigation Only.—Respiration data for insects fumigated without prior preconditioning are listed in Table 3. Fig 1 shows CO₂ production and Fig. 2 the O₂ consumption.

The first measurement of CO₂ production was 0.25 μmoles/mg per min at 20 min after fumigation. This production was 32% more than normal. From this highest recording, production decreased to normal at 1 hr after fumigation and continued to decline throughout the entire 10-hr period recorded. At the end of that time, CO₂ output was about 0.08 μmoles or 47% less than normal.

The first O₂ consumption rate determined was 0.34 μmoles, 20% higher than normal, at 20 min after fumigation. It then decreased to normal values at 1 hr after fumigation and decreased more-or-less uniformly for 9 hr thereafter. Ten hr after fumigation, O₂ consumption was 0.12 μmoles, only 54% of normal.

In each of the 4 trials, the RQ remained within the rather narrow range of 0.62–0.75.

DISCUSSION AND CONCLUSIONS.—*CO₂-Respiration Series.*—The abundant CO₂ production by the insects during exposure to the CO₂ atmosphere remains the principal enigma of these data. In 5 of the 7 lots, CO₂ production values were high, and the RQ's during this period of the preconditioning were more than 3.

During CO₂ exposure, when O₂ consumption was depressed by 62%, the O₂ available to the insect was less than 0.3% by volume. This deficiency of O₂ would kill most animal life, but current studies on insect anaerobiosis and the α-glycerophosphate cycle (Winteringham 1965, Gilmour 1965) suggest that

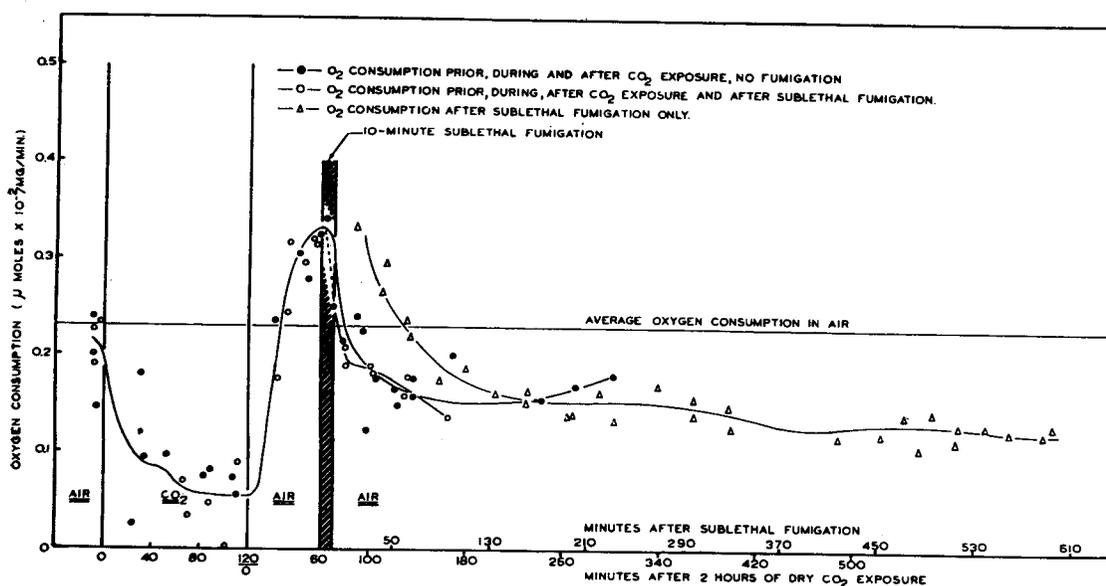


FIG. 2.—O₂ consumption of 2-week-old confused flour beetle adults in dry flowing CO₂, then air, then a 10-min exposure to 194.7 mg/liter of 80:20 (CCl₄:CS₂) at 80°F. Air and CO₂ flow was 100-cc/min at 86°F.

Table 2.—Respiration of 6 lots of 350 2-week-old confused flour beetle adults after N₂ exposure or prior to, during and after N₂ exposure and after sublethal fumigation. N₂ exposure was 2 hours. All other parameters are cited in Table 1.

Lot no.	Lot weight (mg)	Respiratory environment composition ^a	Analysis time (min) after onset of given respiratory environment	CO ₂ production, $\mu\text{m} \times 10^{-2}$ / min / mg	O ₂ consumption, $\mu\text{m} \times 10^{-2}$ / min / mg	Respiratory quotient
1	866.5	PA/N ₂	28	0.23	0.26	0.86
		PA/N ₂	53	.24	.25	.95
		PA/N ₂	81	.17	.19	.91
		PA/N ₂	109	.15	.18	.82
		PA/N ₂	136	.14	.17	.85
		PA/N ₂	170	.14	.17	.85
		PA/N ₂	254	.13	.15	.83
2	863.9	PA	3	.16	.23	.70
		N ₂	24	.01	.09	.04
		N ₂	50	^b	.14	
		N ₂	80	^b	.11	
		N ₂	118	^b	.09	
		PA/N ₂	31	.16	.22	.71
		PA/N ₂	95	.14	.19	.71
3	897.6	PA	6	.16	.23	.70
		N ₂	43	.01	.11	.07
		N ₂	70	.01	.14	.05
		N ₂	97	.01	.09	.04
		PA/N ₂	42	.18	.25	.70
		PA/N ₂	69	.12	.19	.64
		PA/N ₂	129	.15	.16	.94
4	918.1	PA	4	.19	.25	.77
		N ₂	22	.06	.06	1.01
		N ₂	96	.01	.10	.01
		N ₂	118	.04	^b	
		PA/N ₂	30	.18	.26	.68
		PA/F	16	.14	.18	.75
		PA/F	39	.13	.18	.72
5	904.1	PA	6	.22	.31	.72
		N ₂	20	.07	^b	
		N ₂	97	.01	.09	.13
		N ₂	111	.01	.05	.23
		PA/N ₂	20	.15	.21	.73
		PA/F	13	.14	.17	.80
		PA/F	35	.12	.16	.77
6	880.7	PA	8	.17	.23	.74
		N ₂	21	.08	^b	
		N ₂	90	.01	^b	
		N ₂	115	.01	^b	
		PA/N ₂	28	.16	.30	.55
		PA/F	13	.15	.20	.73
		PA/F	47	.12	.16	.77
		PA/F	81	.12	.18	.66
		PA/F	108	.10	.13	.76

^a PA = Preanalyzed air = 0.03% CO₂; 21.50% O₂; 78.47% N₂.
 PA/F = Preanalyzed air provided after sublethal fumigation.
 N₂ = Pure, dry flowing (100 cc/min) N₂.
 PA/N₂ = Preanalyzed air provided for recovery after N₂ exposure period.

^b No gas consumption or production noted.

many insects can survive an extreme deficiency of O₂. The considerable nonpayment of contracted O₂ debt indicates also the buildup of more benign anaerobic glycolytic end products.

Peaks of activity in both CO₂ and O₂ economy occurred after 1 hr in the recovery atmosphere. However, the RQ's were less than 1, revealing considerably more O₂ intake than CO₂ excretion.

When insects were preconditioned with CO₂, but not fumigated, the recovery period began with a rise from the respiration depression to a peak of O₂ consumption that was above normal. This peak was followed almost immediately by a decline that brought the rate below normal. After a few hours, a return to normal O₂ consumption appeared to begin. When the recovery period was interrupted by sublethal fumigation added to the preconditioning, the decline in the consumption of O₂ began more suddenly, although it was reduced to about the same level as for the non-fumigated insects. However, the rate of O₂ consumption did not show a tendency to return to normal after the initial peak and subsequent depression that began the recovery period. If the respiration curve were continued, it would indicate a profound depression.

The high endogenous CO₂ production during the first 30 min of CO₂ exposure seems to warrant further study. von Brand (1946) stated that "liberation of CO₂ can be used as a convenient index of the extent of the anaerobic metabolism." A few possible explanations are suggested.

(a) In a relatively anaerobic atmosphere consisting almost completely of CO₂, the hemolymph may increase in acidity caused by accumulated metabolites. This increase would tend to reduce its CO₂-carrying capacity and liberate CO₂ from the bicarbonates in the hemolymph and tissues (Wigglesworth 1953, von Brand 1946).

(b) Certain microorganisms of *Tribolium* may, under normal atmospheric conditions, fix CO₂ (von Brand 1946). During the initial 30 min of CO₂ anoxia, the microorganisms may release their fixed molecular CO₂, which is then excreted with the CO₂ from other cell processes.

(c) Carbonic anhydrase from *Tribolium* tissue may catalyze the removal of CO₂ from blood bicarbonate in the first 30 min of CO₂ exposure. After equilibrium is reached, the enzymatic reaction might reverse and facilitate the formation of carbonic acid from CO₂ and H₂O.

(d) The exclusion of most of the available O₂ by CO₂ would at least block oxidation of certain Krebs cycle intermediates and allow endogenous CO₂ to build up. Three of the reactions in the Krebs cycle release CO₂. If the enzyme of the immediately succeeding reaction were inhibited, CO₂ would accumulate along with unoxidized substrate. The low-level O₂ contamination in the CO₂ stream might have sustained some aerobic metabolism. This fact was indicated also by the consistent low-level oxygen consumption by the test insects during CO₂ exposure.

Recent research on the biochemistry of anoxia may aid in understanding endogenous CO₂ production. Price (1963) found that the concentration of pyruvate increased rapidly in house flies during the first 30 min of anoxia and then decreased. Also, during this period ATP and AP declined rapidly to very low values (Heslop et al. 1963, Ray and Heslop 1963). These facts suggest that some early Krebs cycle activ-

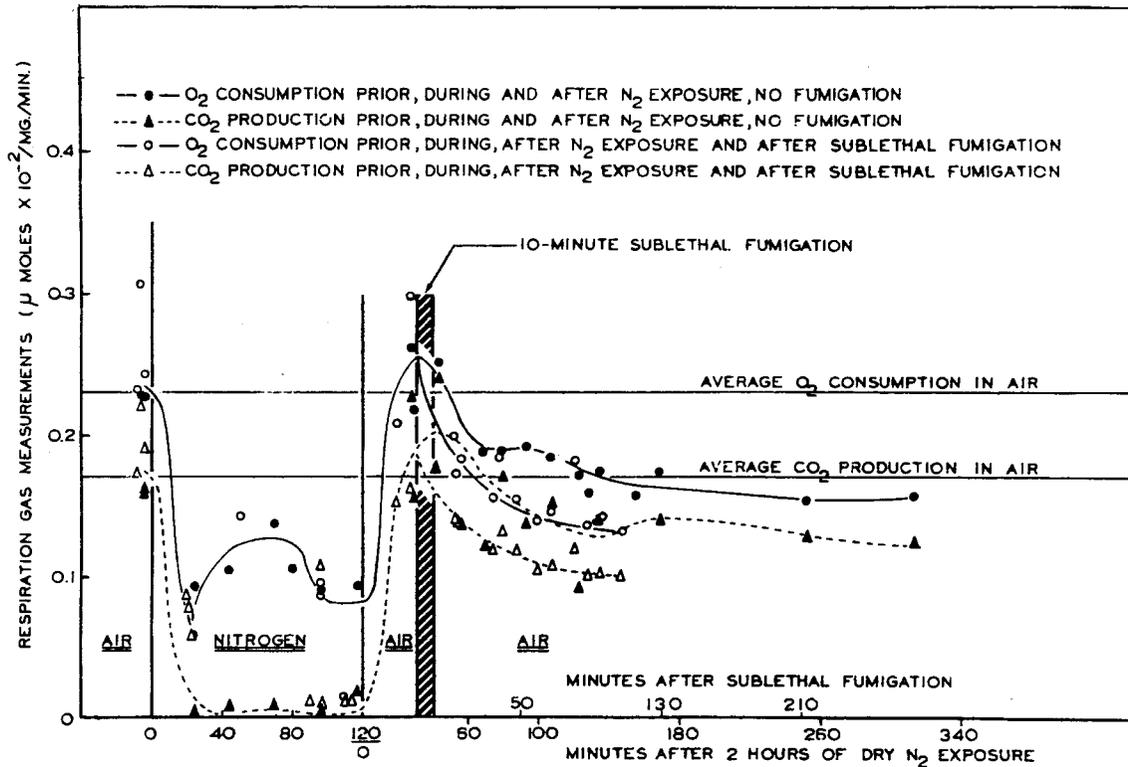


FIG. 3.—Respiration of 2-week-old confused flour beetle adults in dry flowing N_2 , then air, then a 10-min exposure to 194.7 mg/liter of 80:20 ($CCl_4:CS_2$) at 80° F. Air and N_2 flow was 100-cc/min at 30°C.

ity may have been blocked by the depletion of ATP. The lack of phosphate-bond energy might have inhibited the necessary enzymes from catalyzing the acetylation of Krebs cycle intermediates such as oxaloacetic acid to citric acid. If this inhibition occurred, acetyl coenzyme A would accumulate as well as CO_2 . Both latter compounds are derived from decarboxylation of the pyruvate, which is increasing during the first 30 min of anoxia.

Price (1963) also demonstrated α -alanine concentration in anoxic flies and suggested that at least some of this accumulation was derived from aspartate by β -decarboxylation. This reaction would also evolve CO_2 . The increase in α -alanine during early anoxia was somewhat balanced by a decrease in aspartate. The α -alanine increase may also, in part, account for the initial increase and later decrease in pyruvate through a reversible transaminase-catalyzed reaction.

These explanations may suggest reasons for the involvement of CO_2 during the first 30 min of anoxia, but they do not explain why insect CO_2 production dropped sharply after this time. Price (1963) indicated that after 30 min, concentration of pyruvate fell "indicating that it was being further metabolized." He did not suggest any explanation for this renewed metabolism. It is possible that "renewed" metabolism may mean the diversion of a previously established pathway through a type of "negative feedback" (Harper 1961).

In a similar manner, as CO_2 and H_2O accumulate, carbonic anhydrase may reverse its catalysis to form carbonic acid, thus taking increasing amounts of free

CO_2 out of circulation. Other reactions may be involved in the CO_2 production described in this report.

N_2 -Respiration Series.—Compared with CO_2 in its influence on respiration, N_2 appeared to exert a stronger and more direct effect on the test insect.

During N_2 exposure, CO_2 production was negligible. The decrease-increase-decrease recorded in O_2 consumption during this period was more difficult to explain. This description was based on the trend line (Fig. 3), which was drawn in an attempt to make all successive points on the graph somehow express continuity. If CO_2 and O_2 economy operate in a nearly parallel fashion, as they seem to in respect to respiration in normal atmospheres, we might expect O_2 consumption during N_2 exposure to be uniformly depressed. Additional replications did not iron out this slight increase in O_2 consumption, but, rather, accentuated it.

The shorter duration of high rates of respiration and the less complete O_2 debt repayment indicate that lower levels of aerobically oxidizable toxic metabolites may build up during 2 hr of N_2 anoxia than during a comparable CO_2 exposure. The fact that insects exhibited a higher respiration rate in recovery to oxidize the CO_2 -influenced metabolite buildup might mean that N_2 -induced anaerobiosis caused less physiological stress than that produced by CO_2 . However, previous findings by Carlson (1966a) indicated that N_2 was 3 times more synergistic with a fumigant than was CO_2 in $1/2$ -hr preconditioning before fumigation.

From these respiratory data we may infer that CO_2 and N_2 hypoxia each produced a different biochemical

Table 3.—Respiration of 4 lots of 350 2-week-old confused flour beetle adults after a 10-minute exposure to 194.7 mg. per liter of 80:20 (CCl₄:CS₂). Insects were respiring in a 73.0-cc flask without CO₂ absorbent in preanalyzed air*. Test temperature was 30°C. Respiratory exposures were 10 minutes each.

Lot no.	Lot weight (mg)	Analysis time following fumigation (min)	CO ₂ production, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	O ₂ consumption, $\mu\text{m} \times 10^{-2}/\text{min}/\text{mg}$	Respiratory quotient
1	873.0	20	0.25	0.34	0.74
		42	.20	.27	.75
		65	.15	.22	.68
		89	.12	.18	.69
		111	.13	.19	.68
		136	.11	.16	.66
		163	.10	.16	.62
2	900.8	45	.20	.30	.67
		62	.17	.24	.73
		162	.11	.15	.72
		195	.09	.14	.69
		222	.10	.16	.63
		269	.11	.17	.64
		301	.10	.16	.63
		329	.10	.15	.66
		3	904.4	199	.10
234	.09			.13	.71
475	.10			.14	.70
497	.10			.14	.68
518	.09			.13	.73
540	.09			.13	.73
596	.09			.13	.70
4	894.7	300	.09	.14	.64
		331	.09	.13	.72
		420	.09	.12	.75
		454	.08	.12	.67
		485	.07	.11	.69
		516	.08	.11	.68
		560	.08	.12	.62
		588	.08	.12	.64

* 0.03% CO₂, 21.5% O₂, and 78.47% N₂.

lesion or, at least, induced a differential in metabolite buildup. Such a hypothesis could be tested by studies involving radioisotope-labeled intermediates.

Fumigation-Respiration Series.—Respiration after sublethal fumigation illustrated the narcotic action of CCl₄:CS₂, i.e., initial stimulation followed by depression. Brown (1951) categorized CCl₄ and CS₂ as liposoluble narcotics—implying permeability into the lipid sheaths of nerve axons. Harper (1961) stated that "narcotics prevent the reduction of the cytochromes . . ." thus interfering with terminal respiration. Further literature on mode of action was reviewed by Carlson (1965).⁴

Fumigation increased the rate of respiration only in previously untreated insects. Insects that had been preconditioned and allowed to recover were not stimulated to higher respiration rates by subsequent fumigation. Instead, respiration was immediately depressed.

Respiration rates were not measured during the short fumigant exposure, because the fumigant vapors would have impaired the gas chromatographic columns used to elute CO₂, N₂, and O₂. The flask was purged with air for 10 min, then sampled. The insects' respiration during the next 10 min was measured by comparison with another sample taken 20 min after fumigation. If the insects were respiring at normal rates just before fumigation, the rate of respiration must have increased considerably when the fumigant vapors first reached the test insects. By the use of more refined techniques it should be possible to determine the respiration during or immediately after sublethal fumigation. The first respiration rate, measured 20 min after the end of fumigation, is by far the highest recorded in this study, but it is quite possible that it was not the apogee of gas exchange. The highest rate may have occurred during fumigation or shortly thereafter. Knowledge of the insect's first physiological response to fumigant vapor might assist in the search for fumigant additives that could biochemically counteract the insect's first defensive or detoxifying measures.

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⁴ S. D. Carlson. 1965. Effect of carbon dioxide and nitrogen preconditioning on the mortality of *Tribolium confusum* in subsequent fumigation. Ph.D. Thesis, Kansas State University, Manhattan. 195 p.