Fumigation of the Confused Flour Beetle with Methyl Bromide at High and Low Rates of Respiration[1]

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ABSTRACT

Adults of the confused flour beetle, *Tribolium confusum* Jacquelin duVal, were exposed to a sublethal concentration (2.9 mg per liter) of methyl bromide at low and high rates of respiration induced by CO_2 or N_2 preconditioning. Susceptibility to methyl bromide was noted at the low respiratory level. Fumigation of CO_2-and N_2-preconditioned beetles at respiratory depression produced 30% and 44% greater joint action, respectively, than that which could be predicted on the basis of additive treatments. These results suggest that detoxification rates are slowed at low metabolic levels. Certain antagonism to joint action was noted when insects were fumigated at the peak of O_2 debt repayment after preconditioning.

Previous data (Carlson 1966) indicated that pretreatment of confused flour beetles, *Tribolium confusum* Jacquelin duVal, with a stream of CO_2 or N_2 induced this species to be more susceptible than untreated beetles to the 80:20 fumigant (CCl_4:CS_2, by volume). Fumigation was performed when CO_2 or N_2 had depressed respiratory levels. This finding allowed a hypothesis to be formed asserting that, in the case of 80:20, fumigant susceptibility appeared to be an inverse function of insect respiration. This theory is somewhat in variance with the usual belief that high respiration rates allow more fumigant intake and greater susceptibility results (Page and Lubatti 1963). The purpose of the present studies was to determine the further validity of this hypothesis, using another fumigant, methyl bromide.

An analysis of these data and substantiation of this hypothesis require correlation of the biochemical mode of action of methyl bromide and anoxia. It is interesting that both methyl bromide and anoxic agents cause a depletion of ATP and other energy-supplying phosphate compounds (Winteringham et al. 1958, Price 1963). This causation appears to mesh with the observed behavioral and mortality effects recorded under the conditions of this experiment.

PROCEDURE.—Two-week-old adult confused flour beetles were exposed to CO_2 or N_2 for various durations with or without a recovery period to induce high or

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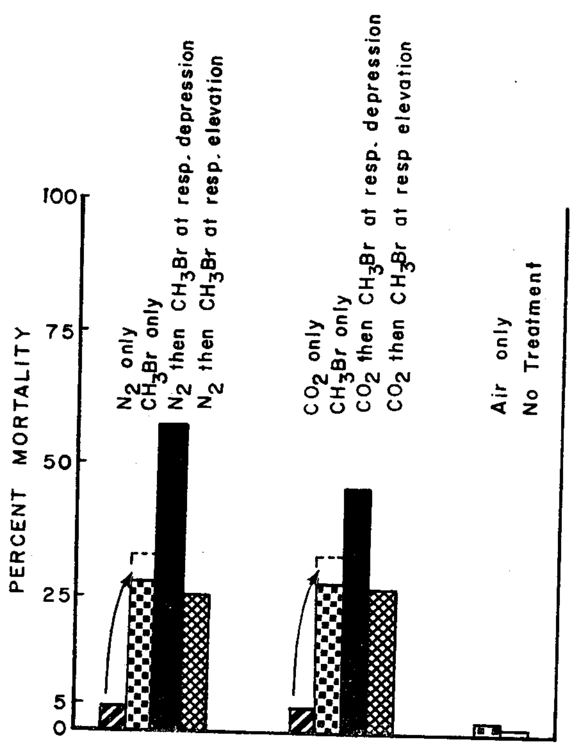

FIG. 1.—Mortality of *Tribolium confusum* following exposure to CO_2 or N_2 singly or in combination with methyl bromide. The arrows project the given preconditioning mortality to that of fumigation mortality for comparison with that of the combination treatments.

low levels of respiration. When the beetles were brought to a given respiratory state, sublethal fumigation with methyl bromide was performed. The preconditioning gases at a flow rate of 100 cc/min were perfused in usual fashion (Carlson 1966) through modified 50-ml respirometer flasks suspended in a 30°C water bath. Each flask contained 100 beetles.

When beetles were to be fumigated at respiration depression they were exposed to dry flowing CO_2 or N_2 for ½ hr. The beetles were then quickly aspirated out, placed in cages, suspended in the fumatorium, and treated with methyl bromide. Those insects which were fumigated at the peak of O_2 debt repayment were first preconditioned for 2 hr. Then they were allowed a ½-hr or 1-hr recovery period (for N_2- or CO_2-treated insects, respectively) in dry, preanalyzed air (0.03% CO_2, 20.5% O_2 and 78.47% N_2) flowing at 100 cc/min before fumigation. Beetles treated only with CO_2 or N_2 were also processed similarly relative to air recovery but were not fumigated. Lots treated with only air were handled simultaneously with all other lots. In this case, air flowed at the same rate and for the maximum duration (3 hr) of flowing gas any lot endured. Insects were also fumigated without preconditioning.

Fumigation was performed as follows: A saturated methyl bromide atmosphere was produced within a 2-liter compressed-air tank. A 2-way brass valve on the tank allowed coupling with a 3-way stopcock fitted to a gas-tight glass syringe. After fumigant intake by syringe the syringe stopcock was closed. A 20-gage needle was inserted on the stopcock assembly and methyl bromide was then injected via needle entry through a rubber septum on the fumatorium cap. Glass 20-liter carboys with stopcock plug assemblies were used as fumatoria. Each fumatorium contained 2 steel-mesh cages each containing 100 insects, suspended in the center of the carboy. After treatment the insects were placed in holding cages and mortality counts were made 10 days later. Lack of visible movement after mechanical and thermal stimuli was the criterion for mortality.

RESULTS.—Average mortalities are illustrated in Fig. 1. Table 1 gives the mortality data for the series. The data indicate that under the conditions of this experiment confused flour beetles were more susceptible to methyl bromide at respiratory depression. The additive mortalities of methyl bromide alone plus CO_2 alone were 32.1%. When the beetles were exposed to methyl bromide at elevated respiration rates mortality was 26.4%. At respiration depression methyl bromide fumigation produced 45.9% mortality, or a 30% increase over additive effects.

When insects were preconditioned with N_2 the additive mortalities of methyl bromide alone plus N_2 alone equalled 32.1%. When both treatments were combined and fumigation occurred at respiratory elevation, mortality was only 25.6%. At the time fumigation occurred at respiratory depression, percent mortality rose to 57.4, a 44% increase over the additive mortalities.

The passage of dry air over the insects appeared more stressful (2.5% mortality) than that of no treatment (1.3%). The 2-hr exposure of the beetles to either CO_2 or N_2 produced identical mortalities: 4.8%.

At the low methyl bromide concentrations used, little if any inhibition of movement was noted immediately after the 24-hr methyl bromide exposure. Paralysis was gradual and mortality (immobility) counts taken 8 days after fumigation were lower than those taken at 10 days.

DISCUSSION AND CONCLUSIONS.—The results of this experiment show that confused flour beetles are more susceptible to methyl bromide during a depressed state of respiratory metabolism. However, apart from recording the effects of preconditioning, a more important consideration—causation—exists. In assessing this data it should be remembered that CO_2 and N_2 exert characteristic modes of action which may enhance the biochemical lesion produced by methyl bromide. It is known that preconditioning gases possess pharmacologic activity beyond that of mere exclusion of respiratory O_2 (Brooks 1957). The importance of correlating the joint action of both preconditioning gas and fumigant gas was recently borne out in later studies, which demonstrated lack of interaction between CO_2 or N_2 and chloroform (Carlson 1965[a]).

The mode of action of methyl bromide in insects can be outlined. Winteringham et al. (1958) disproved Lewis' (1948) concept that methyl bromide methylated certain SH enzymes relative to in vivo situations. It was found that methyl bromide (depending on concentration-exposure time) immobilized the insects and that paralysis was due to a reversible breakdown of ATP. When larger concentrations of methyl bromide were used, the depletion of ATP was "marked and irreversible," and other phosphorous substrates diminished such as adenosine phosphoric acid and phosphoglyceric acid. These findings caused Winteringham et al. (1958) to conclude that

Table 1.—Ten-day mortality of *T. confusum* adults in lots of 100 after CO_2 or N_2 preconditioning induced elevated (RE) or depressed (RD) respiration[a], at which time sublethal fumigation with methyl bromide[b] (MB) followed, or these treatments were performed singly with air-treated and no-treatment (NT) control lots.

	% insect mortality per treatment (100 insects/lot)																	
	N_2 only, lots		CO_2 only, lots		CH_3Br, lots		N_2-MB-RD, lots		CO_2-MB-RD, lots		N_2-MB-RE, lots		CO_2-MB-RE, lots		Air, lots		NT,[c] lots	
Replicate	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	9	2	8	3	25	19	48	53	46	33	15	8	18	26	2	2	1	2
2	2	5	1	2	49	33	51	60	56	26	21	30	13	22	2	1	1	1
3	3	2	11	4	17	16	48	57	46	45	15	11	24	16	2	2	3	0
4	9	6	3	6	23	36	53	89	59	56	49	57	39	53	4	5	0	2
Mean	4.8		4.8		27.3		57.4		45.9		25.6		26.4		2.5		1.3	

[a] Respiration at peak following ½-hr and 1-hr recovery in air after 2 hr of exposure to N_2 and CO_2, respectively. Respiration depression after ½ hr of CO_2 or N_2 exposure.
[b] 2.9 mg/liter of methyl bromide for 24 hr at 26.7°C in 20-liter glass fumatoria.
[c] No treatment.

such poisoning symptoms as reversibility of effects, delayed collapse, and lack of change in respiration rate were not symptoms indicative of a sulfhydryl enzyme inhibitor.

Gross symptoms of methyl bromide poisoning in the flour beetles correlate with Winteringham's findings. Bond (1956) fumigated *T. confusum* with the LD_{99} of methyl bromide and noted a gradual cessation of activity in the poisoned beetles while the respiratory rate was largely unaffected. When Bond fumigated the cadelle, *Tenebroides mauritanicus* (L.), with methyl bromide, irreversible paralysis occurred before respiration appreciably declined.

He also indicated that this initial lack of paralysis (even to observing some hyperactivity) may be due to methyl bromide's inhibition of succinic dehydrogenase. This inhibition would decrease oxidative metabolism which in turn may stimulate glycolysis "so that the glycolytic processes could supply the extra energy required for hyperactivity."

Carlson (1965[3]) reviewed literature concerning the biochemistry of anoxia as related to that state produced by CO_2 or N_2 exposure. The research of Heslop et al. (1963), Price (1963), and Ray and Heslop (1963) shows that ATP and insect phosphagen (AP) were depleted during anoxia, and that paralysis and recovery probably paralleled depletion and resynthesis of these energy sources. In methyl bromide poisoning, ATP was also found to be degraded, and this fact may be due to acute anoxia in the presence of a high concentration of this toxin (Winteringham et al. 1958). The condition that constitutes a "high concentration" was not defined, and it is not clear if "high" means so high as to exclude usable tensions of O_2 or merely high in the sense of induction of acute toxicological symptoms.

In the present experiment, the gravimetric ratio of methyl bromide to air was 1:445. This ratio is certainly not anoxic from the point of appreciably lowering the partial pressure of O_2. However, anoxia is merely the "failure of the tissues, for any reason, to receive an adequate supply of O_2" (Best and Taylor 1945). This definition does not imply that O_2 atmosphere must be replaced by methyl bromide, but merely that the animal tissues are rendered incapable of obtaining available O_2. Thus, the fumatorium contained adequate O_2 but there was likely an anoxic situation because of the presence of methyl bromide.

It may be that the anoxia of the preconditioning atmosphere caused an initial depletion of high-energy phosphate bonds, and when methyl bromide in air succeeded CO_2 or N_2 (at respiration depression), the methyl bromide continued to exert an inhibitory action on ATP and AP for an additional 24-hr period (the fumigation exposure period). Thus, confused flour beetles were under a 24½-hr siege of anoxia, which had direct consequences not only on detoxification rates but also on all metabolic pathways requiring phosphate bond energy.

Conversely, when methyl bromide was introduced at that point when the insects were at a high level of respiration, ATP and AP may have been restored to normal, or possibly, above normal concentrations so that ample energy for degradation of methyl bromide would be possible. If it could be proved that above-normal concentrations of phosphorous compounds were available during above-normal respiration (at O_2 debt repayment) this hypothesis might explain why greater detoxification occurred in these insects than in those treated only with methyl bromide. This greater energy for detoxification would have negated the effect of any increased intake of fumigant in those insects which experienced fumigation while respiring at a high level.

[3] S. D. Carlson. 1965. Effects of carbon dioxide and nitrogen preconditioning on the mortality of *Tribolium confusum* in subsequent fumigation. Ph.D. dissertation, Kansas State Univ., Manhattan. 195 p.

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