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Fumigation of *Tribolium confusum* Adults with 80:20 (CCl_4:CS_2) During Carbon Dioxide- Or Nitrogen-Induced Respiration Elevation or Depression[1]

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ABSTRACT

Individual lots of 100 2-week-old adults of the confused flour beetle, *Tribolium confusum* (Jacquelin duVal), were exposed to dry, flowing (100 cc per min) carbon dioxide or nitrogen and then fumigated for 24 hours in an LC_{50} atmosphere of 80:20 mixture (CCl_4:CS_2 by volume) at 30°C. Previous studies indicated that ½-hour exposures to either CO_2 or N_2 produced extreme respiration depression. Two-hour exposures to CO_2 or N_2 followed by 1-hour and ½-hour recovery periods, respectively, induced maximum respiration rates. When insects were fumigated at minimum respiration (no recovery period permitted) the resulting mortality was greater than the additive effect of the individual preconditioning and fumigated treatment mortalities. Nitrogen exerted a greater synergistic effect than did carbon dioxide. No increased susceptibility to fumigant was noted when the insects were fumigated during peak respiration in the recovery period following CO_2 or N_2 anoxia.

In a control series, 4 replications of the following insect lots were evaluated: neither treated nor fumigated, fumigated but not treated, air-treated (100 cc per min, 2 hours) but not fumigated, and both air-treated and fumigated. Mortality of insects air-treated but not fumigated was more than 3% greater than natural mortality and was statistically significant. These data suggest that dry airflow produced stress in adult confused flour beetles.

From previous studies, the respiration of adult confused flour beetles, *Tribolium confusum* (Jacquelin duVal), had been determined during CO_2 or N_2 exposure as well as during subsequent recovery (Carlson 1963; Carlson, unpublished.) These data indicated that oxygen consumption was quickly depressed to a consistent low level after ½ hr of exposure to flowing CO_2 or N_2. Peak rates of oxygen debt repayment occurred at 1 hr and ½ hr into the recovery period immediately following 2-hr exposure to CO_2 or N_2, respectively. Accordingly, confused flour beetles were fumigated at respiration depression or elevation. The purpose of the present experiment was to determine which metabolic extreme enhanced susceptibility of 2-week-old confused flour beetle adults to fumigation with an 80:20 mixture of carbon tetrachloride and carbon disulfide.

Methods and Materials.—Two-week-old confused flour beetle adults in lots of 100 were aspirated from culture room stocks and placed in 73-ml modified Erlenmeyer flasks. These flasks were provided with inlet and outlet stopcocked sidearms which permitted continuous gas ventilation. All flasks were partially submerged in a constant-temperature (30°C) water bath.

Preconditioning-Fumigation Series.—Four replications of each fumigation at a given preconditioning interval were performed. Each replication consisted of near simultaneous treatments of 10 lots of 100 adult beetles each. Six lots received the dried preconditioning gas[3] at 100 cm^3/min for ½ hr or 2 hr. After preconditioning, 3 of these 6 lots were placed in plastic holding cages containing enriched shorts. These constituted the preconditioned-only treatment. The other 3 preconditioned lots were aspirated from their flasks and either (1) immediately fumigated (fumigation at respiration depression) or (2) permitted a recovery period of ½ hr for N_2 or 1 hr for CO_2 (when peak respiration rates occurred) before fumigation. In the recovery period a 100-cm^3/min flow of air[4] was maintained through the treatment flasks for ½ or 1 hr after 2 hr of N_2 or CO_2 exposure, respectively.

One lot of insects was exposed to flowing preanalyzed air for the same duration and at the same flow rate as that of the preconditioned lots (air-only lot). The remaining 3 lots of insects were aspirated from culture jars and fumigated (fumigated-only series).

Control Series.—Twelve lots (100 insects/lot) comprised each replication. Three lots were not treated except for aspiration from culture jars and placement in plastic holding cages. Three lots were taken from culture jars and immediately fumigated. Six lots were exposed to dry flowing (100 cm^3/min) preanalyzed air for 2 hr. Three of the lots so treated were immediately fumigated, while the remaining 3 lots, after air treatment, were housed in plastic holding cages without further treatment.

All lots of all series were permitted a food supply and held for 10 days following treatment, at which time the mortality count was made. The insects were adjudged alive if any movement was observed under a 3-power illuminated magnifier.

Fumigation.—Each 100-insect lot to be fumigated was placed in a steel mesh cage suspended in a 20-liter fumatorium. The fumatorium was sealed and a 20-in. (water) vacuum was drawn. The fumigant (35 mg/liter of 80:20 CCl_4:CS_2 by volume) was then introduced by syringe through a red rubber sampling septum on a glass stem which pierced the rubber plug at the carboy mouth. This tube projected into the carboy and was bent sharply toward the inside wall so that the liquid fumigant did not contact the test insects. A magnetic stirring bar aided in the distribution of the fumigant vapors. The fumigant exposure period was 24 hr.

Statistical Analysis.—Although not presented in numerical detail here, 3 statistical tests were applied to data of these tests. An analysis of variance determined the significance of date effect and treatment. The

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[3] Either CO_2 or N_2 of more than 99.5% purity.

[4] Matheson Gas Co. analysis: 0.03% CO_2, 21.5% O_2, and 78.47% N_2. This air was dried by passage through indicating calcium sulfate.

Table 1.—Ten-day mortalities of confused flour beetles following indicated treatments.

	Recovery time before fumigation (hr)	Percent mortality (mean of 12 lots of 4 replications)[a]					
		Air only	Fumigation only	Preconditioning only	Additive P + F[b]	Actual P + F[b]	No treatment
		Preconditioning—fumigation series					
		Respiration depression					
½-hr CO_2	0	10.3	62.4	13.8	76.2	82.7[c]	
½-hr N_2	0	16.0	60.8	17.1	77.9	98.4[c]	
		Respiration elevation					
2-hr CO_2	1	8.5	70.2	16.9	87.1	86.1	
2-hr N_2	½	17.8	67.4	36.3	100.+	96.1	
		Control series					
2-hr air	0	3.9	65.2	[d]	69.1	68.0	0.8

[a] Except for air-treatment mortalities (of the Preconditioning-Fumigation Series) which are averages of 4 lots of 4 replications.
[b] P, preconditioning; F, fumigation.
[c] Significantly different from additive effects of F and P at 0.05 level as indicated by Mann-Whitney nonparametric test.
[d] Considered air as preconditioning treatment.

Student-Newman-Keuls (SNK) multiple range test (Keuls 1952) ascertained significant differences among the individual treatments of a given series. For the determination of synergism, the Mann-Whitney test was used to evaluate the hypothesis that a combination of preconditioning and fumigation is more lethal than the additive effects of preconditioning (only) and fumigation (only). All references to significance relate to the 0.05 level of probability.

RESULTS.—Data in Table 1 indicate that ½-hr preconditioning periods followed by immediate fumigation (respiration depression) is the most lethal of the treatments investigated. In either of these short exposures to CO_2 or N_2 with subsequent fumigation without allowing a recovery period, the actual mortality exceeded its given predicted mortality, i.e., the additive effects of preconditioning and fumigation performed singly. On this basis, nitrogen appears to be more synergistic than carbon dioxide. Fumigation with the 80:20 mixture following the induction of high respiratory rates did not produce mortalities higher than those which might be predicted on an additive treatment basis.

The Mann-Whitney statistical analysis for synergism showed that potentiation occurred when preconditioning was followed immediately by fumigation.

The SNK tests indicated significant differences among the preconditioning (only), fumigation (only), and combination treatment of each series. In all cases but one, a given preconditioning treatment was not significantly more lethal than its air-treated counterpart. The exception occurred in the 2 hr N_2 series, and this duration of nitrogen flow was significantly more lethal than a like duration of flowing normal air.

Air-treated mortality averaged 13.2% in the preconditioning-fumigation series. This value ranged from a mean 8.5 to one of 17.8. These values are of particular interest when compared with the air-treated and natural (no treatment) mortality of the control series.

In the control series the SNK Multiple Comparisons test indicated that the mortality of air-treated insects was significantly greater than that of natural mortality. The 3.1% differential between air-treated and natural mortality would appear to be carried over to their respective fumigation series (68.0%–65.2% = 2.8%); however, this difference was not adjudged significantly different.

Analysis of variance tests of each series indicated that date differences were not significant but that all treatment differences were significantly different.

DISCUSSION AND CONCLUSIONS.—The principal finding of this study is that the confused flour beetle adults are more susceptible to fumigation with an 80:20 mixture of carbon tetrachloride and carbon disulfide after CO_2 or N_2 preconditioning if fumigation occurs at depression of respiration. This phenomenon may reflect on the test insect's differential ability to better detoxify the 2 fumigant compounds at a high rather than a low metabolic level. An increase in the effectiveness of a given amount of fumigant might well result in lowered fumigation costs.

It has long been held that the rate of respiration is directly related to the organism's active uptake of fumigant vapors. Also, of recent interest was the report of Brady and Sternburg (1964) that CO_2 anesthesia increased the toxicity of tepp and SD-3562 (Bidrin®, 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethyl phosphate) to American cockroaches, *Periplaneta americana* (L.). Knipling et al. (1961) cited the effects of 100% nitrogen atmospheres on the survival of confused flour beetles. There seems to be little doubt that these gases can increase insect susceptibility to certain types of subsequent intoxications.

Previous workers (Cotton 1932, Lindgren 1935) have indicated that fumigant mortality is directly correlated to respiration. However, under the conditions of this experiment, it would appear that the reverse is true, i.e., fumigant toxicity is inversely proportional to respiration rate. It is realized in this study that maximum ventilation may not have coincided with maximum gas exchange. The preconditioned insects became active when placed in the fumigation atmosphere directly after CO_2 or N_2 exposure. Thus considerable intake of fumigant vapors may have occurred when the insect was in the fumigation atmosphere.

It is known that CCl_4 will cause a loss of pyridine nucleotides from the mitochondria and thus greatly interfere with aerobic glycolysis in vertebrate in vitro systems (Christie and Judah 1954). It may be that this situation would effect a vital crisis when the change-over from largely Embden-Meyerhof glycolysis to Kreb cycle activity was necessitated.

Observations concerning the unexpected air-treatment mortality in both series suggest that flowing dry air may be a stressor on the following bases: (1) an observed immobility of the air-treated insects during treatment; (2) the inability of dry airflow to appreciably desiccate the insect over a 2-hr. period (Carlson, unpublished); (3) the lack of any compounds other than CO_2, N_2, and O_2 in the air stream (from a pressurized tank of constant composition); (4) an adequate oxygen level (21.5%) in the preanalyzed air precluding anoxia; (5) the presence of an unnatural and slippery footing in the glass flasks; and (6), the significant increase in mortality of air-treated insects over that of natural mortality.

This summary is merely evidence pointing to the likelihood of stress. Items 2 and 3 are negative testimony which would indicate that dry air per se did not react with insect tissue. The remaining points suggest the existence of a stresslike syndrome, i.e., immobility followed by an increased death rate. These effects may relate to the abnormal substrate and the buffeting action of airflow. If so, it is likely that the insect reacted to its altered environmental atmosphere by adaptive mechanisms involving neurosecretion and/or the elaboration of hemolymph-transported toxins.

A major difficulty remains in reconciling the Control Series air-treatment mortality (3.9%) to that of the 2-hr. Preconditioning-Fumigation Series (all air-only replicates of this series averaged 13.2%). The current weight of data would indicate that the Control Series air-treated insect mortality was abnormally low.

Certain preliminary studies performed in 1963 indicated somewhat less response of the test species to 80:20 fumigant (a mixture of carbon tetrachloride and carbon disulfide) and both preconditioning gases. The continuing trend in genetic homozygosity with no particular selection pressures may have led to a general increase in susceptibility of the insect culture.

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