Temperature Effect on Mortality of Confused Flour Beetles Treated with CO_2 or N_2 Before Fumigation[1]

STANLEY D. CARLSON[2]
Stored-Product Insects Research Branch, Market Quality Research Division, Agr. Res. Serv., USDA, Manhattan, Kansas

ABSTRACT

Adult *Tribolium confusum* Jacquelin duVal were fumigated at different temperatures with CCl_4:CS_2 (80:20 by volume) during metabolic depression caused by ½-hour exposure to flowing CO_2 or N_2. At 30°C, preconditioning increased the mortality of the insects to a greater degree than it did at 20° or 40°C.

Previous studies (Carlson 1966) indicated that adult confused flour beetles, *Tribolium confusum* Jacquelin duVal, immediately after a period of oxygen deprivation were more susceptible to CCl_4:CS_2 fumigant (80:20 by volume) than when oxygen was available. In other studies (Carlson unpublished) respiration analyses showed that oxygen consumption by the insects was sharply curtailed during exposure to the anoxic atmospheres of CO_2 or N_2. In both studies the test temperature was 30°C. The present study was made to determine whether variation in the temperature of the anoxic preconditioning environment would affect fumigation mortality when the fumigation temperature did not change. Under field conditions the temperatures for preconditioning and fumigation would probably be the same, as it would be very difficult to alter temperature for these treatment periods. However, it was desired to assess the effects of temperature variation separately for these 2 components to gain a better understanding of how such effects were accomplished.

METHODS AND MATERIALS.—Preconditioning was done at 3 temperatures, 20, 30, and 40°C. The procedure was similar to that of Carlson (1966). Flow rate of the preconditioning gases and air was 100 cc/min. This flow rate represented about 2 changes of atmosphere per minute in each flask. The 50-cc respiratory flasks in which the insects were preconditioned were held in a water bath of the required temperature. The preconditioning gas was warmed to the same temperature as the water bath. There were 4 replications at each temperature for the CO_2 and for the N_2 preconditioning. A replication consisted of 15 lots of 100 adult beetles. Six lots were preconditioned for ½ hr with N_2 or CO_2 at each temperature. The gases were dried with Drierite®[3]. Three lots of these preconditioned insects were held for 10 days in plastic cages containing wheat shorts enriched with B vitamins. The other 3 lots of preconditioned insects were immediately fumigated. Six lots were not preconditioned with CO_2 or N_2, but were held in respiratory flasks without air flow for ½ hr at the required temperature. Three of these lots were then fumigated. The other 3 lots not preconditioned were held in plastic cages as a no-treatment series. Lastly, 3 lots were exposed to a flow of dry air for ½ hr in the water bath. Composition of this gas was guaranteed by the supplier to contain 0.03% CO_2, 21.5% O_2 and 78.47% N_2. The test insects were taken directly from culture room temperature (26.7°C) to the test temperatures, no time was allowed for acclimatization.

Fumigation consisted of an exposure to 35 mg of CCl_4:CS_2 (80:20 by vol) per liter of air space at 26.7°C for exactly 24 hr. This concentration had previously been determined as LC_{50} for these insects. Less than 2 min elapsed between the end of a given preconditioning treatment and the introduction of the fumigant gas to the insects. During this short interval, the insects were aspirated from the flask and placed in the fumigation cage. The fumigation procedure was that of Carlson (1966). The cage was suspended in a 20-liter glass fumatorium, which was then sealed. A 20-in. (water) vacuum was drawn, and the fumigant was syringed in at an angle so that the liquid fumigant volatilized on the side of the carboy. A magnetic stirrer hastened vaporization and facilitated distribution of the fumigant. Because little or no recovery time was permitted, fumigation took place while the insects were still in a metabolic depression induced by the preconditioning.

RESULTS.—Average mortality recorded in 4 replications of each treatment at each preconditioning temperature is presented in Table 1 and Fig. 1.

Of the 5 treatments, air treatment and no treatment largely failed to indicate any trend in mortality as a result of temperature. The high mortality of the air-treated beetles at 30°C is unexplainable. This occurrence may possibly be discounted, as mortality was less than 3% in the air-treated lots at 20 and 40°C. With the exception of the combination of preconditioning and fumigation in the N_2 series, all combination, fumigated only, and preconditioned only treatments produced mortalities that appeared to have been influenced by altering the temperature.

For those insects that were fumigated only, there was little difference in mortality between those that had previously been held for ½ hr at 20°C and those that had been held at 30°C. Both had an average mortality of about 62%. However, when the temperature of the air during the previous ½ hr had been 40°C, the average mortality from fumigation rose to about 77%. The fumigation temperature for all these insects was 26.7°C.

Neither of the preconditioning gases was as effective at low as at higher temperatures. At 20°C the mortality was 4.4% for CO_2 and 4.8% for N_2. At 30°C average mortality increased to 13.8% for CO_2 and to 17.1% for N_2. An additional 10° elevation to 40°C slightly increased mortality of the CO_2 treatment (to 16.6%), while mortality of the N_2 treatment increased considerably (to 63.8%).

No simple correlation was evident between increased temperature and increased mortality in the lots that were both preconditioned and fumigated. The mortality differential between the combination treatment and the sum of the independent effects of preconditioning and fumigation was calculated. The

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[2] Present address: Department of Entomology, Virginia Polytechnic Institute, Blacksburg.
[3] The use of trade names is for identification purposes only and does not constitute endorsement of the products by the USDA.

Table 1.—A summary of the 10-day mortalities of confused flour beetle adults after exposure to ½ hr of dry, flowing CO_2 or N_2 at 100 cc/min followed by immediate sublethal fumigation with CCl_4:CS_2 (80:20 by volume) or these treatments performed singly or air treatment or no treatment controls. Lot size was 100 insects.

Gas	Temperature (°C)	Percent mortality (avg of 12 lots in 4 replicates)					
		Preconditioned only (A)	Fumigated only (B)	Preconditioned and fumigated (C)	Dry airflow	No treatment	Differential[a]
CO_2	40	16.6	77.7	87.8	2.2	1.1	− 6.5
N_2	40	63.8	75.9	91.7	2.3	0.5	−48.0
CO_2	30	13.8	62.4	82.7	10.3	[b]	+ 6.5
N_2	30	17.1	60.8	98.4	16.0	[b]	+20.5
CO_2	20	4.4	55.6	63.3	2.9	1.9	+ 3.3
N_2	20	4.8	68.1	91.4	2.0	1.3	+18.5

[a] C − (A + B).
[b] A no-treatment series was not included in the 30°C series.

positive differential was the largest for both gases at 30°C. There was a negative differential at 40°C for both gases, i.e., the sum of the mortalities from fumigation and preconditioning were more than the mortality from the combination treatment. The differentials at 20°C were very close to those at 30°C, although the N_2 differentials were considerably higher than the CO_2 differentials. The CO_2 differential was 6.5% at 30°C, but only 3.3% at 20°C. The N_2 differential was 20.5% at 30°C, and 18.5% at 20°C.

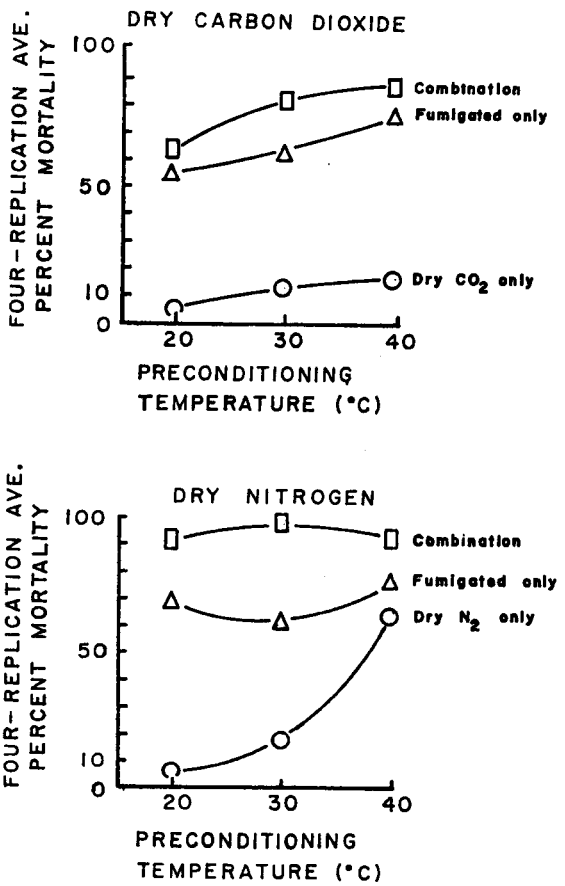

FIG. 1.—Mortality of confused flour beetles after exposure to preconditioning gases (CO_2 or N_2), fumigant (CCl_4:CS_2), or combination of preconditioning gas plus fumigant at various temperatures. Air-treated and no-treatment lots were run simultaneously but are not shown.

DISCUSSION.—Data in this study demonstrate that the temperature during anoxic preconditioning will influence the ultimate mortality of confused flour beetles. Additional evidences of thermal interaction will be sought in later studies.

There is a seemingly contradictory result in the fumigated-only series associated with the N_2 tests. The mortality at 30°C is lower than at 20°. More logical results are obtained if the fumigated-only mortalities are averaged for the series associated with both the N_2 and CO_2 tests. Averaging the 2 fumigated-only lots is a valid procedure, since these were identical treatments. When this is done, there is a pattern of increasing mortality with temperature rise.

The most striking increase in mortality with temperature increase was that of dry N_2 alone. Here, a 12-fold increase in mortality occurred with a 20°C rise in temperature. This was in contrast to only a 4-times mortality increase over the same temperature range for dry CO_2 alone. The underlying causes for this differential toxicity are not known. In both cases O_2 was virtually excluded and there appeared to be no appreciable difference in anesthesia induction time or recovery rate for the 2 gases at a given temperature, although the time required for payment of the O_2 debt following CO_2 or N_2 anoxia differed (Carlson, unpublished). It is well documented (Hoyle 1960, Edwards 1953) that both gases cause spiracle opening and attendant desiccation. Previous data (Carlson 1965) indicated a considerable difference in insect weight loss between similar-size lots of beetles treated with CO_2 and N_2 for 12 hr. The present studies represented only ½ hr, or $^1/_{24}$, of such an exposure duration, and it is unlikely that this period was sufficient to cause either an appreciable weight-loss differential or enough weight loss to jeopardize insect life. On a prorated basis, of a 12-hr weight loss of 14.9% and 15.9%, respectively, for dry-flowing CO_2 and N_2, as found in other studies, ½-hr exposure would result in less than 1% weight loss. Further, it was doubtful that 40°C represents a "transition temperature" as regards epicuticular integrity and desiccation via this route. The short time during which the beetles were exposed to 40°C would also lessen this possibility.

It is beyond the scope of this paper to consider the histopathology of heat-injured preconditioned insects. However, for more complete understanding of the

cellular mechanisms involved in heat injury to insects, Day and Oster (1963) should be consulted. The causes of temperature effects on preconditioning remain unknown, but they offer a potentially fruitful area of inquiry.

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