Comparison of the Sensitivity of the Developmental Stages of Three Strains of the Red Flour Beetle (Coleoptera: Tenebrionidae) to Modified Atmospheres

E. DONAHAYE, DALIAH ZALACH, AND MIRIAM RINDNER

Department of Stored Products, Agricultural Research Organisation, The Volcani Center, P.O.B. 6, Bet Dagan, Israel

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ABSTRACT All stages of a strain of *Tribolium castaneum* (Herbst) selected for resistance in the adult stage to a high-carbon-dioxide-content atmosphere and of an unselected strain were exposed to the high-carbon-dioxide-content atmosphere. Similarly, all stages of a strain selected for resistance to a low-oxygen-content-atmosphere in the adult stage, and all stages from an unselected strain, were exposed to the low-oxygen-content atmosphere. For both selected strains, partial resistance was displayed by the eggs and larvae.

KEY WORDS Insecta, *Tribolium castaneum*, controlled atmospheres, resistance

EXPERIMENTS ON laboratory-induced resistance to methyl bromide and phosphine in one life stage of stored-product insects have shown that such resistance is partially expressed in other stages (Upitis et al. 1973, Saxena & Bhatia 1980). The objective of this study was to determine whether the resistance acquired by the adult stage of two strains of *Tribolium castaneum* (Herbst) to a high-carbon-dioxide-content (HCC), and a low-oxygen-content (LOC) atmosphere, respectively, was also imparted to the developmental stages. Although, in practice, selection pressure would be applied to all life stages, expression of resistance acquired at one stage to all other life stages would be a major determinant of survival.

Materials and Methods

Resistant strains of *T. castaneum* were a strain selected for 20 generations to an atmosphere (HCC) containing 65% CO_2, 20% O_2, and 15% N_2 at 95% RH, and a strain selected to an atmosphere (LOC) containing 99.5% N_2 and 0.5% O_2 at 95% RH. All culture methods, the exposure apparatus, and the technique and method for determining sensitivity to the respective modified atmospheres were identical to those described previously (Donahaye 1990a, b) and are briefly as follows. At each generation, 10–15 adults (1 d old) of each strain were exposed to the respective MAs over a time range required to determine the levels of response by probit analysis. Then mass exposures were carried out at 50–70% selection pressure. The standard culture technique consisted of postexposure oviposition by surviving adults in oviposition jars containing flour followed by transfer of eggs to culture jars containing a 40:1 ratio of ground wheat and brewer's yeast. Emerging adults were later transferred to preexposure jars. The exposure apparatus consisted of a supply of each component gas delivered from cylinders by means of manometers and needle valves at a regulated flow rate in proportion to its composition in the mixture. Gases were mixed and the desired relative humidity was obtained by bubbling the gases through a sulfuric-acid solution. Finally the mixture was delivered via a distribution chamber to a series of exposure chambers consisting of capped 100-ml Erlenmeyer flasks. Gas entered each chamber through a syringe needle acting as inlet port with another needle to permit flow-through to the exterior.

In addition, the following experimental procedures were used.

Preparation of stages before exposure. Eggs for exposure to MAs were separated from flour in the oviposition jars by sieving daily, so that eggs at the time of collection were 0–1 d old. Larvae were separated from food medium in the culture jars on the 12th d and transferred, in groups of 100, together with 1 g of ground wheat, to exposure flasks. Pupae were removed from culture jars and held separately until exposure. From the day pupae were first observed, the culture jars were screened daily to enable collection of pupae of uniform age. Removal of adults from culture jars 5 d after the first adult appeared enabled collection of the adults in groups aged 0–5 d. These were held for 10 d before exposure.

Exposure Methods. Eggs were transferred with a camel's-hair brush into a group of 24 depressions set in an exposure device formed from a block of methyl methacrylate (Plexiglas) cov-

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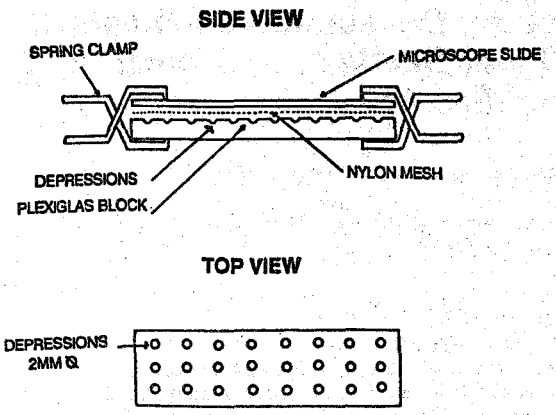

Fig. 1. Apparatus for exposure of insect eggs to modified atmospheres.

ered by nylon mesh and a glass slide (Fig. 1). This device prevented the eggs from rolling out of the depressions, but permitting ingress of the modified atmosphere. Exposure devices were placed inside exposure chambers. Mortality counts were made 10 d after exposure. At this stage, transparent hatched egg shells could easily be distinguished from dead eggs under a binocular microscope.

Larvae were exposed in 100-ml Erlenmeyer flasks with 1 g of ground wheat. Mortality counts were made 20 d after exposure by counting the number of emerging adults and subtracting from the number of larvae exposed.

Pupae were exposed in the same manner as larvae. Mortality counts were based on adult emergence 10 d after completion of exposure.

Adults were exposed as described above. Mortality was recorded 10 d after completion of exposure.

Determination of sensitivity of the different stages to MAs was carried out by probit analysis using a program written by Daum (1979). For each strain and life stage, chambers containing 50–100 insects were exposed for five exposure times and one chamber served as control. This was repeated to give a total of 30 exposures. At each repetition selected and unselected strains were exposed simultaneously to minimize differences in response resulting from experimental error.

Results and Discussion

Resistance to HCC. Sensitivities to the HCC atmosphere of all stages of the HCC-selected strain and the unselected strain are given in Table 1, together with resistance factors. Response at both the $LT_{99.9}$ and LT_{50} levels is given, the former to indicate the time required to produce complete control and the latter to provide a more reliable comparison between stages, because the 95% confidence bands at the LT_{50} are much narrower than at the $LT_{99.9}$.

The data in the tables show that the resistance acquired by the adults of the HCC selected strain was transferred in part to both the egg and larval stages. The considerable difference in sensitivities of the different stages of the unselected strain to the HCC should be noted, in which the pupal stage is by far the most tolerant at the LT_{50} level. Because of this natural tolerance the pupal stage still remains more tolerant than the egg and larval stages in the HCC-selected strain at the LT_{50} level. The low angles of slope of the probit mortality lines for the eggs of both the HCC-selected and the unselected strains indicate a wide range of exposure response by the eggs. The $LT_{99.9}$ for eggs of the HCC-selected strain showed this to be the most resistant of the life stages.

Resistance to LOC. Sensitivities of the stages of the LOC-resistant strain and the unselected strain to the LOC atmosphere, and RFs of each stage, are given in Table 2.

As with selection to HCC, selection of adults to LOC also imparted partial resistance to both the egg and larval stages, whereas sensitivity of the pupal stage remained unaffected. Again the natural high tolerance of the pupal stage, which is greater than that of all other stages at the LT_{50} level, signifies that, for the LOC-selected strain at the 21st generation, the pupal stage remained more resistant than the egg and larval stages. However, because of the low slopes of probit mortality lines of the eggs and larvae of both

Table 1. Sensitivity of two strains of *T. castaneum* to a CO_2-enriched atmosphere

Stage	Strain	Slope ± SE	LT_{50}, h	$LT_{99.9}$, h	RF_{50}	$RF_{99.9}$
Adult	Unselected	5.13 ± 0.49	41.5	166		
	Selected	5.63 ± 0.51	157	557	3.78	3.34
Egg	Unselected	1.87 ± 0.31	19.8	871		
	Selected	2.29 ± 0.23	42.6	954	2.16	1.09
Larva	Unselected	3.93 ± 0.63	25.9	159		
	Selected	3.27 ± 0.36	47.6	420	1.84	2.65
Pupa	Unselected	2.95 ± 0.85	79.4	882		
	Selected	4.17 ± 0.48	73.0	401	0.92	0.45

Lethal exposure times in hours.