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SPECIFICITY OF INDUCED RESISTANCE TO HYPOXIA AND HYPERCARBIA IN TWO STRAINS OF *Tribolium castaneum* (HERBST)

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ABSTRACT

Two strains of *Tribolium castaneum*, one selected for resistance to a low oxygen content atmosphere (LOC) and the other for resistance to a high carbon dioxide content atmosphere (HCC), were used to test the specificity of their laboratory-induced resistance. The LOC-selected strain was shown to be susceptible when exposed to HCC, while the HCC-selected strain was found to be susceptible to LOC. This indicates that survival under hypoxia and hypercarbia requires different adaptive mechanisms. Exposure of the LOC-selected strain to anoxia revealed greater tolerance than an unselected strain. Exposure of both strains to a 60:8:32 mixture of CO_2, O_2, and N_2 showed both strains to be more resistant than an unselected strain. Exposure of the selected strains to methyl bromide and phosphine failed to reveal any cross-resistance imparted to the insects against the fumigants.

INTRODUCTION

A strain of the red flour beetle, *Tribolium castaneum* (Herbst), had been selected previously in the adult stage during 40 generations for resistance to a low oxygen (O_2) content (LOC) atmosphere containing 99.5% nitrogen (N_2) and 0.5% O_2 at 95% relative humidity (R.H.). Similarly, another strain had been selected for resistance to a high carbon dioxide (CO_2) content (HCC) atmosphere containing 65% CO_2, 20% O_2, and 15% N_2 at 95% R.H.. Final resistance factors (RFs) at the LT_{50} level were 5.2 and 9.2, respectively (Donahaye, 1990a, b).

In this study, the HCC-selected strain was exposed at the 13th generation to LOC, and the LOC-selected strain was exposed to HCC to investigate whether the tolerance imparted during selection was specific to the modified atmosphere (MA) in question, or whether this was a general tolerance to the stress of MA, irrespective of whether hypoxia or hypercarbia was involved.

Also investigated in this study at the 22nd generation was whether tolerance imparted to the LOC strain by selection for hypoxia, would also be present when the LOC-selected and unselected strains were subjected to anoxia. This was done by exposing the insects to 100% N_2. Since commercial MA treatments using liquid CO_2 by the flushing and maintenance method (Jay, 1971) involve exposure to concentrations of both high CO_2 and low O_2, and these effects have been shown (Calderon and Navarro, 1980) to be synergistic, both selected strains were exposed at the 21st generation to a mixture composed of CO_2:O_2:N_2 at a ratio of 60:8:32 at 55% R.H. to determine what orders of resistance are obtained when the selected strains are subjected to less stressful concentrations of the gas to which they were selected, but in combinations of hypoxia with hypercarbia. Lastly, the possibility that MA resistance may impart increased tolerance to conventional fumigants was investigated by exposing all three strains to methyl bromide (MB) and phosphine.

MATERIALS AND METHODS

1. Comparison of sensitivities of the three strains to LOC and HCC

Adults of the three strains were exposed over a range of exposure times, to both LOC and HCC at the 13th generation in order to enable probit analysis of mortality against log-time to be carried out. The experimental techniques were the same as those used during the selection procedure (Donahaye, 1990a, b). Each experiment was carried out using five exposure times, with five exposure flasks of each strain employed for each exposure time. Probit analysis was conducted using the program of Daum (1979) run on a VAX 11-750 computer.

2. Exposure of the LOC-selected and non-selected strains to nitrogen

The exposure apparatus was adjusted to obtain a N_2 atmosphere with a humidity of 95%. Adults from the LOC-selected and non-selected strain of the 22nd generation were exposed for determination of mortality as described previously. Gas samples were withdrawn frequently during exposure and analysed by gas chromatography (Donahaye, 1990a) to ensure that no trace gases other than N_2 and water vapor were present in the system.

3. Sensitivity of the three strains to a 60:8:32 mixture of CO_2, O_2, and N_2 at 55% R.H.

The exposure apparatus was set up to deliver the above mixture to the exposure flasks. This mixture of 60% CO_2 in air was based on recommended rates by the purge and maintenance method with an atmospheric humidity

equivalent to treatment of wheat at 11% moisture content. All experimental techniques were as described previously.

4. Sensitivity of the three strains to methyl bromide and phosphine

Methyl bromide:

The sensitivities of the three strains to MB was compared by exposing 10-15-day old adults of the 28th generation to 7 different concentrations of MB for 2 hours. Fumigations were performed at 26±1°C and 60±5% R.H. in 3.5 l glass fumigation chambers made from flat-bottomed flasks, sealed by ground-glass stoppers, welded to the necks of the flasks. For each strain, eight groups of 50 adults were placed in brass-mesh cages. These were inserted into the fumigation chambers and suspended by nylon thread attached to hooks imbedded in the stoppers. Dosage calculations in mg/l were converted to the gaseous phase (Anon, 1981) and the required volumes of MB gas were removed from a 25-ml screw-cap septum vial using a "pressure-lok" syringe (Pierce). The doses were then injected into the chambers via a section of latex tube attached to a glass tap welded to the fumigation chamber stoppers and clamped at the distal end. The chambers equipped with teflon-coated stirring rods were then placed on magnetic stirrers for 30 min to ensure uniform gas concentrations. For each strain and each concentration, 4 sets of exposures were carried out.

Phosphine

Sensitivities of the three strains to phosphine were compared using the same apparatus, and under the conditions described above. Exposures for 17 hours were carried out using 8 different concentrations of phosphine. Three sets of exposures were performed for each strain. Phosphine was generated from phostoxin tablets using the F.A.O. recommended method (Anon, 1981).

Post-exposure procedure was the same for both treatments. At the end of the exposure periods, the cages were removed from the fumigation chambers and the exposed insects were transferred to 90-ml post-exposure jars containing 50 g wheat flour. Mortality counts were made after 10 days and sensitivities to the treatments were examined by probit analysis.

RESULTS AND DISCUSSION

1. Comparison of sensitivities of the three strains to LOC and HCC

Table 1 shows the results of exposure of the three strains to the two MAs. When exposed to HCC the LOC-selected strain was even more susceptible to HCC than the non-selected strain, at the LT_{50} level. The low

angle of slope of the regression line, and the broad confidence bands, indicate the heterogeneity of response of this strain to HCC.

The response of the HCC-selected strain to LOC shows a sensitivity similar to that of the non-selected strain at both the LT_{50} and LT_{95} levels, whereas the LOC-selected strain was nearly twice as resistant.

From the table it may be concluded that although the induced resistance to both MAs is probably poly-factorial, with a multiplicity of genes involved, the major mechanisms contributing to the development of resistance in each MA differ from the other, and strengthen the hypothesis that survival under hypoxia and hypercarbia requires different adaptive mechanisms.

Table 1: Comparison of sensitivities of the HCC-selected, LOC-selected and unselected strains of *Tribolium castaneum* to LOC and HCC atmospheres at the 13th generation.

MA	Strain	LT_{50}*	RF_{50}	LT_{95}*	RF_{95}	Slope**
	HCC-selected	73.4 (66-80)	1.72	158 (136-199)	1.57	4.94±0.59
HCC	LOC-selected	21.0 (9-30)	0.49	137 (93-339)	1.36	2.02±0.45
	Unselected	42.5 (34-49)	1	100 (83-135)	1	4.41±0.62
	LOC-selected	70.6 (60-86)	1.72	185 (130-460)	1.96	3.93±0.88
LOC	HCC-selected	42.3 (25-96)	1.03	119 (68-267)	1.26	3.66±1.47
	Unselected	40.9 (31-53)	1	94 (66-248)	1	4.55±1.31

* In hours; figures in parentheses are 95% confidence limits.
** Slope of regression line ± standard error.

2. Exposure of the LOC-selected and unselected strains to nitrogen

Results of probit analysis of mortality against log-time are given in Table 2.

Survival of both strains under anoxia was three to five times shorter than at 0.5% O_2. These results corroborate the finding that metabolism is mainly aerobic under LOC, as demonstrated in respiration experiments (Donahaye, 1992), and in an analysis of metabolic end-products (Donahaye,

1985). However, the longer survival of the LOC-selected strain indicates its enhanced ability to survive anoxia either by better utilisation of anaerobic metabolic pathways for energy production, or reduced sensitivity to toxification by the end products of glycolysis. It may also be attributed to the lower metabolic rate as indicated by lower respiratory rate in air (Donahaye, 1992), or in utilisation of energy reserves under LOC (Donahaye, 1985).

3. Sensitivity of the three strains to a 60:8:32 mixture of CO_2, O_2, and N_2 at 55% R.H.

Results of probit analysis of mortality against log-time are given in Table 3.

The resistance obtained by laboratory selection to HCC and LOC at the 21st generation (RF_{50}s of 3.68 and 2.61, respectively) was largely retained upon exposure to the commercial mixture. Furthermore, a recommended exposure of 4 days with this mixture, as based on the findings of Calderon and Navarro (1979, 1980) would have killed all adults of the LOC-selected and unselected strain, but would have failed to kill approximately 5% of the HCC-selected strain.

4. Sensitivity of the three strains to MB and phosphine

Comparison of sensitivities of the three strains to MB and phosphine at the LD_{50} and LD_{95} levels are given in Table 4. The results show that there were no significant differences in sensitivity between the three strains for either of the fumigants.

The importance of these findings lies in the potential future development of resistance to MAs, should this control method find wider application for stored-product insect control. The absence of cross-resistance between strains selected for resistance to hypoxia and hypercarbia, and their lack of resistance to phosphine and MB, suggest a possible control strategy using both fumigation and different MA techniques in order to retard the development of resistance to MAs.

Table 2: Mortality of LOC-selected and unselected strains of *Tribolium castaneum* exposed to N_2 and 95% R.H. at 26°C at the 22nd generation.

Strain	LT_{50}*	LT_{95}*	RF_{50}	RF_{95}	Slope**
LOC-selected	16.5 (13-22)	30.3 (22-240)	1.51	1.96	6.42±2.16
Unselected	10.9 (10-12)	15.5 (14-17)	1	1	10.95±1.29

* In hours; figures in parentheses are 95% confidence limits.
** Slope of regression line ± standard error.

Table 3: Mortality of the HCC-selected, LOC-selected and unselected strains of *Tribolium castaneum* exposed to a 60:8:32 mixture of CO_2 O_2 and N_2 at 55% R.H. and 26°C at the 21st generation.

Strain	LT_{50}*	LT_{95}*	RF_{50}	RF_{95}	Slope**
HCC-selected	46.5 (42-49)	147 (118-204)	2.3	4.5	4.57±0.41
LOC-selected	29.2 (27-31)	60.3 (54-77)	1.47	1.82	7.40±0.83
Unselected	19.8 (18-21)	33.1 (29-41)	1	1	10.45±1.46

* In hours; figures in parentheses are 95% confidence limits.
** Slope of regression line ± standard error.

Table 4: Comparison of sensitivities of the HCC-selected, LOC-selected and unselected strains of *Tribolium castaneum* to methyl bromide and phosphine at the 28th generation.

Fumigant	Methyl bromide (mg/l/2hr)		Phosphine (µg/l/17hr)	
Strain	LD_{50} *	LD_{95} *	LD_{50} *	LD_{95} *
Unselected	12.6 (11.6-13.5)	19.1 (17.2-23.0)	9.8 (6.5-12.5)	21.8 (16.5-41.6)
HCC-selected	13.1 (12.1-14.0)	18.1 (16.4-21.7)	12.5 (10.4-14.1)	27.3 (23.1-37.3)
LOC-selected	15.1 (14.1-16.1)	20.8 (18.8-25.4)	13.3 (12.1-14.2)	22.7 (20.5-26.3)

* 95% confidence limits in parentheses.

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