Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W. [Eds.] (2007) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia. 8-13th August 2004. FTIC Ltd. Publishing, Israel. pp. 55-67

EFFICACY OF CONTROLLED ATMOSPHERES AGAINST PHOSPHINE-RESISTANT STRAINS OF *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE) ORIGINATING FROM DIFFERENT COUNTRIES

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ABSTRACT

The efficacy of controlled atmospheres (CA) against phosphine-resistant strains of adult *Rhyzopertha dominica* originating from different countries viz., Bangladesh (BDRD), Malaysia (MLRD), Burkina Faso (BFRD) and the a laboratory PH_3 susceptible reference (RFRD) was studied. Three types of controlled atmospheres, viz. Gas I: 98% N_2, 2% O_2; Gas II: 32% N_2, 8% O_2, 60% CO_2 and Gas III: 8% N_2, 2% O_2, 90% CO_2 were considered for the present investigation. Results showed that there were no significant ($P>0.05$) differences in the mortality of PH_3-resistant adults of *R. dominica* when exposed to different CA's, though the exposure, gas type and their interactions exhibit highly significant differences ($P<0.001$) in adult mortality. Results showed that the Gas III type was found to be more effective followed by Gas II > Gas I, with lower LT values. Results also show that the tolerance indices decreased gradually as the exposure level increased for all the strains and gas types.

INTRODUCTION

Controlled atmospheres (CA) with elevated CO_2, reduced O_2 in N_2, or their combinations can be used to control stored-product insects (Ofuya and Reichmuth, 1994; Adler, 1998; Mbata *et al.*, 2000; Ding *et al.*, 2002; Shazali *et al.*, 2004). It has been considered as an alternative treatment to the use of methyl bromide for post-harvest insect control (Epenhuijsen, 2002). Moreover, the development of resistance to the most commonly used fumigants such as phosphine, methyl bromide and ethylene dibromide has been well documented (Bond, 1973; Reichmuth, 1975;

Champ and Dyte, 1976, Leong and Ho, 1994; Reichmuth, 1994). Resistance to phosphine has been detected in strains of *Rhyzopertha dominica, Tribolium castaneum, Sitophilus oryzae, Oryzaephilus surinamensis* and *Cryptolestes* spp. originating from south-east Asian countries (Mills, 1983; Tyler, 1983; Rajendran, 2001; Rajendran and Gunasekaran, 2002). Phosphine resistance is now known to be present in at least 11 species of stored-product insects in 45 countries and the list is growing (Chaudhry, 2000, Benhalima *et al.*, 2004). Several factors relating to storage, biotic and physical environment and insect physiological status affect the susceptibility of stored-product insects to CA (Fleural-Lessard, 1990; Reichmuth, 1990). However, extensive use of CA in insect control could lead to selection of insect populations resistant to hypercarbia and hypoxia (Donahaye, 1991). Several studies have demonstrated that stored-product insects have the genetic potential to develop resistance to CA (Bond and Buckland, 1979; Navarro *et al.*, 1985; Zhao and Zhang, 1993; Adler, 1997; Wang *et al.*, 2000). Although the build-up of tolerance to CA is difficult (Friedlander *et al.,* 1984), the proper way to prevent the development of resistance is to ensure adequate lengths of exposure in properly sealed bins so that all insects are killed (Annis, 1991). The purpose of this research was to determine the efficiency of CA's against the phosphine-resistant strains of *R. dominica* originating from different countries.

MATERIALS AND METHODS

Experimental insects

The four strains of R.dominica *were cultured on whole wheat and dried cassava at 25°C and 75%. The strains used in the tests comprised a susceptible (reference: RFRD), a laboratory selected* PH_3*-resistant* BDRD, MLRD *and* BFRD *which originated from a population sampled in Bangladesh, Malaysia and Burkina Faso respectively.*

Insects for CA fumigation test

Adults *R. dominica* (5-6 days old) were selected for a series of treaments with CA fumigation. They were fumigated in cylindrical steel gauze cages (5.0 x 1.5 cm). Treatments of each CA fumigation consisted of three replications having 25 insects in each.

CA fumigation procedure

Five Dressel flasks (2.5 l) were connected by polyethylene tubing and were then purged with one of the following gas mixtures (flow rate: 2000 ml/min):

Gas I: 98% N_2, 2% O_2 [exposure: 72, 120, 144, 168, 192 & 216 h]

Gas II: 32% N_2, 8% O_2, 60% CO_2 [exposure: 8, 16, 24, 32, 48 & 72 h]

Gas III: 8% N_2, 2% O_2, 90% CO_2 [exposure: 4, 8, 16, 18, 22 & 24 h]

The gas mixtures were produced manometrically from pure components in percentage by volume (Adler and Reichmuth, 1988). Before the introduction into the Dressel flasks each of the gas was cooled and humidified to 76% r.h. purging it through a saturated sodium chloride solution (Winston and Bates, 1960). The gas outlet of the last flask was connected to an oxymeter (Servomex 570 A) and purging was continued until the oxymeter showed the desired content of O_2 in the gas mixtures. After that, the flasks were kept in CT rooms controlled at 25°C and 75% r.h. until the end of desired exposure time. At the end of the desired exposure, the gas concentrations of the mixtures were also analysed by an oxygen analyzer (TORAY LF 750). A cage with an untreated control sample was kept under the similar conditions in the climatized chamber for each tested exposure time. This process of CA fumigation was repeated three times for each gas type and strain.

Post CA fumigation

After the end of desired exposure, the adults of all the strains were transferred into glass petri dishes containing food substrates and kept in an incubator at 25°C and 75 ± 5% r.h. for the mortality assessment. The adult mortality was recorded daily until all died.

Data processing

Probit analysis: Mortality data were corrected with Abbott's (1925) formula and analysed by probit analysis (Finney, 1971) to determine the lethal times. The value of chi-square was used to measure the goodness-of-fit of the probit regression. Statistical analysis of LT_{50} was based on non-overlap of 95% confidence intervals.

Mean Survival Times (MST): The mean survival time (MST) for *R. dominica* strains exposed to controlled atmosphere was calculated following the model developed by Cheng and Ducoff (1989):

$$MST = 1/n\ \Sigma(t \times Y_t)$$

Where, n = number of beetles in the group, t = hour t, and Y_t = number of beetles that die at hour t.

Tolerance Indices (TI)

The tolerance index expresses a time response to controlled atmosphere exposure in terms of a strain longevity was defined as follows:

$$TI = LT_n / MST$$

This parameter (TI), like LT, has the advantage of broad applicability for comparative purposes, predictive potential and statistical value. Factorial ANOVA for the mean survival times (MST) was calculated using a statistical software package (Minitab Inc.).

A curvilinear regression analysis for the tolerance indices was also fitted using Excel which describes a prediction curve given by the following third degree polynomial equation.

$$Y\ (TI) = a + bx + cx^2 + dx^3$$

where, a, b, c, d are the coefficients and x is the exposure level.

RESULTS AND DISCUSSION

The efficiency of CA on phosphine-resistant *R. dominica* strains are summerized in Table 1 and Figures 1-9. It shows insignificant results for the mean survival time (MST) of different strains of *R. dominica* when treated with various gas concentrations (Table 3). The factorial anova also shows that the exposure, gas mixture and their associated interaction varied significantly ($P<0.001$) (Table 3). It is interesting to note that Gas III (8% N_2, 2% O_2, 90% CO_2) was found to be more effective followed by Gas II and Gas I which had lower values of LT_{50} and LT_{99} (Table 2). Results show that 100 % mortality occurred within one day at 32, 72 and 216 h exposure of Gas III, Gas II and Gas I respectively (Fig. 1-3). The present findings also showed that the tolerance indices (TI) decreased as the gas exposure increased (Fig. 4-9). Figures 4-9 also show that the highest TI was observed for Gas III for all the strains while the lowest was for Gas I. Also these figures indicate that little variation of TI occurred among the strains of *R. dominica.* Results clearly indicate that Gas III containing enriched CO_2 was more toxic while Gas I containing less CO_2 was less effective against *R. dominica* than others. The present results are in agreement with the findings of Banks and Annis (1990) who reported that the rich CO_2 atmospheres were more effective controlling stored product insects than hypoxic atmospheres. Adler (1998) also observed the higher efficacy of CO_2 rich atmosphere against *P. interpunctella.* As

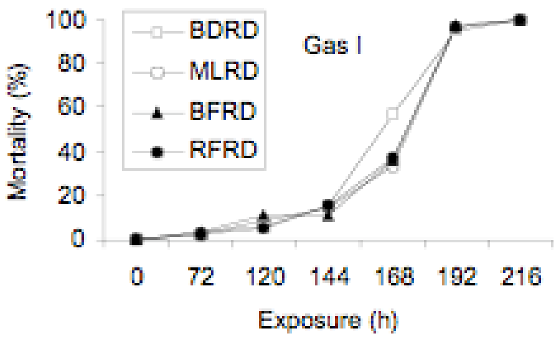

Figure 1: Mortality of the phosphine-resistant strains of *R. dominica* adults after one day following the treatment of gas I (98%N$_2$, 2%O$_2$) at a series of exposure.

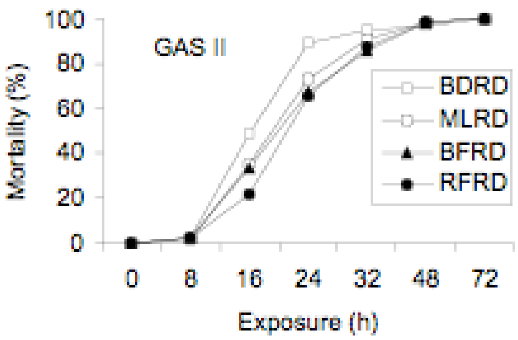

Figure 2: Mortality of the phosphine-resistant strains of *R. dominica* adults after one following the treatment of gas II (60%CO$_2$, 32%N$_2$, 2%O$_2$) at a series of exposure.

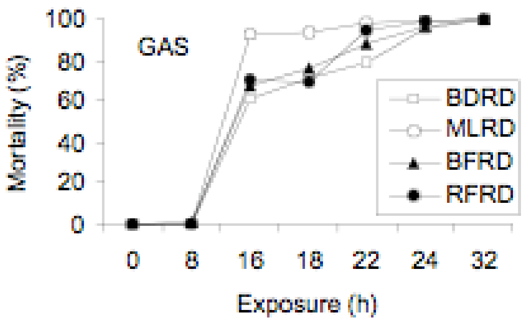

Figure 3: Mortality of the phosphine-resistant strains of *R. dominica* adults after one following the treatment of gas III (90%CO$_2$, 8%N$_2$, 2%O$_2$) at a series of exposure.

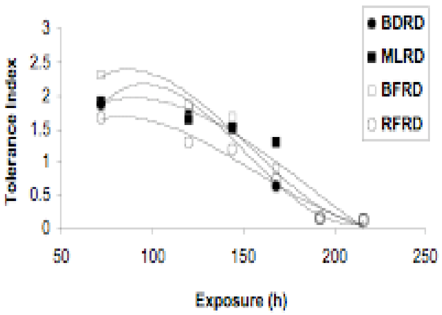

Fig. 4: Tolerance indices values relative to 50% kill for the PH_3 resistant strains of *R. dominica* treated with gas $98\%N_1$,$2\%O_1$.

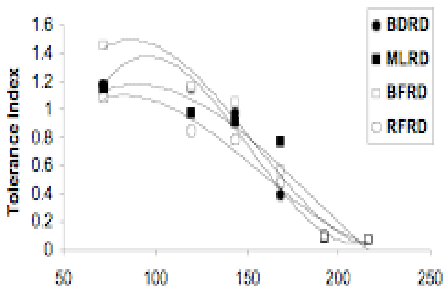

Fig.5: Tolerance indices values relative to 99% kill for the PH_3 resistant strains of *R. dominica* treated with gas $98\%N_1$,$2\%O_1$.

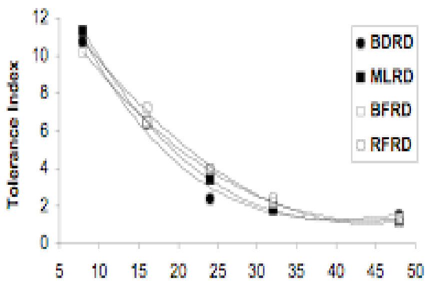

Fig. 6: Tolerance indices values relative to 50% mortality level for the PH_3 resistant strains of *R. dominica* treated with gas $60\%CO_1$,$32\%N_1$,$2\%O_1$. ¶

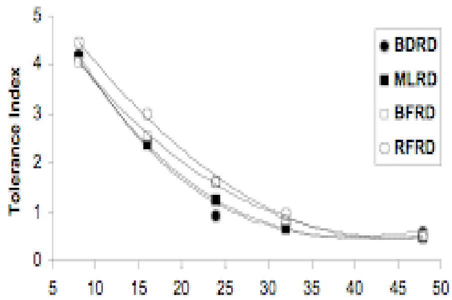

Fig. 7: Tolerance indices values relative to 99% mortality level for the PH_3 resistant strains of *R. dominica* treated with gas $60\%CO_1$,$32\%N_1$,$2\%O_1$. ¶

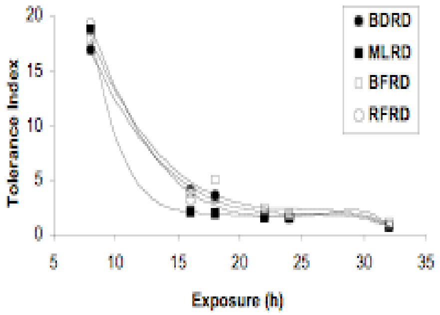

Fig. 8: Tolerance indices values relative to 50% mortality level for the PH_3 resistant strains of *R. dominica* treated with gas $90\%CO_1$,$8\%N_1$,$2\%O_1$. ¶

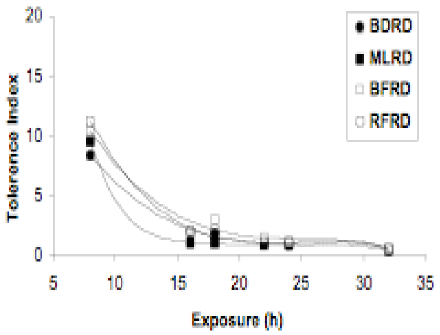

Fig. 9: Tolerance indices values relative to 99% mortality level for the PH_3 resistant strains of *R. dominica* treated with gas $90\%CO_1$,$8\%N_1$,$2\%O_1$. ¶

Banks and Annis (1990) demonstrated , many factors influence the efficacy of high CO_2 or low O_2 against stored product pests under certain practical conditions. To achieve 100% mortality of the insects in all stages of development, the CO_2 content in the interstitial spaces of the bulk-stored commodity must be at least 60% by volume (Adler *et al.*, 2000).

The effect of modified atmospheres on insect physiology, however, is complex, and there are differences between different insects groups (Bell et al., 2002). Low levels of CO_2 (<15%) have little effect on survival of stored-products species if humidity are high, indicating some acclimation within this group to fermenting or partly anaerobic conditions. The effect of spiracular opening on water loss is modified in typical small beetle pests of grain, because most spiracles open into the subelytral space which acts as a barrier to water loss. It has been reported that the high levels of CO_2 (>75%) have an anaesthetising effect on insects from which they usually make a complete recovery if restored to air quickly (Dawson, 1995), but if exposure to 95% CO_2 in air is prolonged, death by asphyxiation occurs in much the same way as would exposure to a nitrogen atmosphere. For some insects, exposure at such high levels of CO_2 is less effective than exposures at around 60% in air (Bell, 1984; Leong and Ho, 1995). The direct cause of mortality of insects exposed to modified atmospheres is not yet completely understood. The toxic effects are attributed to desiccation and a lack of triglycerides as substrates for energy metabolism (Donahaye, 1991).

The present findings clearly indicate that the phosphine resistant *R. dominica* adults did not show any cross tolerance towards different modified atmospheres (Figs. 1-3). Moreover, the enriched CO_2 atmosphere was found to be more effective against the phosphine resistant *R. dominica* than the others. The tolerance index values also clearly indicate the same trends, i.e., the susceptibility of the phosphine resistant *R. dominica* adults varies with the different modified atmospheres (Fig. 4-9). It has been reported that these resistant adults showed different degrees of resistance against phosphine, while they did not show this sort of trend against modified atmospheres used in the present investigation particularly at the 50% level of mortality (Fig. 4-6).

Controlled atmospheres based on high CO_2 concentrations have undergone considerable investigation, in the laboratory, as a means of insect control in cereal grains. Resistance to CO_2 has been induced under laboratory conditions (Bond and Buckland, 1979, Navarro *et al.*, 1985, Donahaye, 1991) and concern has recently been express that this could occur rapidly in commercial practice. Therefore it is important for potential users to realise that the treatment is not going to lose its efficacy in the short term due to the rapid development of resistance in the field. It is especially true as the perceived cost of preparing the storage, and creating and maintaining CO_2 rich atmospheres is high when compared with the equivalent cost of treating grain with the conventional fumigant phosphine. Some understanding of the natural tolerance of species to the treatment is essential to any discussion of resistance. Moreover, research needs to be directed to have a better understanding of the mode of action of CA's and the potential of arthropods to develop resistance to them. The data available today, however, do not indicate that the development of resistance is an immediate threat to the use of CA's for stored-product protection.

The results presented here and those from previous research clearly suggest that use of CA's against phosphine resistant stored product pests, particularly *R. dominica*, should not be treated with great caution with respect to potential efficacy. Moreover, additional research should be initiated to determine the consequences of variation in the efficacy of CA's against phosphine resistant *R. dominica.*

TABLE 1
Culturing details and specification of reference and phosphine-resistant strains of *R. dominica*.

Strains	**Origin**	**Collected & Tested Year**	**No of adults to seed culture**	**Adult wt. Mean ± SD* (mg)**	**Diet**	**Original Culture maintained**
Reference RFRD (S)	Not known	1978	150	1.447 ± 0.029	Whole wheat & dried cassava	Stored Product Protection Inst. BBA, Berlin
BDRD (R)	Bangladesh	1982	150	1.409 ± 0.033	Whole wheat & dried cassava	Central Science Laboratory York, UK
MLRD (R)	Malaysia	1993	150	1.305 ± 0.017	Whole wheat & dried cassava	Stored Product Protection Inst. BBA, Berlin
BFRD (R)	Burkina Faso	1993	150	1.238 ± 0.028	Whole wheat & dried cassava	Stored Product Protection Inst. BBA, Berlin

S- Susceptible; R- Resistant; * mean of four replicates each having 50 insects.

TABLE 2

Probit analyses, 95% confidence limits, slope and χ^2 values for the phosphine-resistant strain of *R. dominica* treated with different controlled atmospheres

Type of gases	Experiment No.	LT_{50} (h)	95% conf. limits lower	upper	LT_{99} (h)	95% conf. limits lower	upper	slope	χ^2 alues (4df
BDRD									
	1	156.69	79.98	306.97	240.83	45.52	1274.16	12.46	260.99
Gas I	2	159.38	44.62	569.24	258.46	8.36	7986.82	11.08	814.04
	3	155.55	57.04	424.17	241.73	19.95	2929.15	12.15	556.50
	1	16.97	15.69	18.19	39.20	34.50	46.82	6.39	1.00
Gas II	2	17.70	14.54	21.41	53.29	35.70	82.80	4.86	2.70
	3	14.32	8.53	24.04	34.22	12.68	92.76	6.15	21.68
	1	14.47	12.58	17.12	32.76	24.80	44.43	6.70	2.89
Gas III	2	16.34	14.49	18.21	29.44	23.55	38.21	9.098	2.38
	3	15.36	12.97	18.06	30.42	22.49	41.96	7.840	3.93
BFRD									
	1	155.47	110.52	218.67	302.19	105.36	868.85	8.06	35.72
Gas I	2	166.70	26.13	1063.42	266.71	1.43	49459.10	11.39	1849.34
	3	161.51	130.76	199.50	234.35	128.18	429.93	14.39	22.07
	1	17.86	16.39	19.31	52.33	44.95	64.28	4.98	1.00
Gas II	2	22.04	20.39	23.69	62.17	53.40	76.49	5.16	1.00
	3	18.52	17.13	19.87	45.75	39.95	55.19	5.92	1.00
	1	14.46	13.47	15.34	31.67	28.66	36.31	6.83	1.00
Gas III	2	15.02	14.01	15.82	26.59	24.50	30.06	9.38	1.00
	3	14.66	13.67	15.48	27.37	25.16	30.87	8.58	1.00
MLRD									
	1	162.03	84.03	312.41	26.60	42.40	1664.06	10.84	213.07
Gas I	2	161.20	26.07	996.72	24.02	2.32	26477.8	12.43	1967.98
	3	162.45	62.11	424.86	25.74	19.04	3543.84	11.41	491.98
	1	19.07	17.67	20.44	46.72	40.81	56.382	5.98	1.00
Gas II	2	21.15	19.63	22.64	53.59	46.55	65.117	5.76	1.00
	3	16.33	13.16	20.17	42.15	27.62	66.084	5.65	3.654
	1	12.33	10.28	14.68	21.54	17.16	27.402	9.59	3.580
Gas III	2	12.89	11.88	13.71	21.05	19.63	23.191	10.92	1.00
	3	12.17	11.31	12.94	21.24	19.66	23.492	9.61	1.00
RFRD									
	1	163.42	23.71	1126.21	252.64	1.59	40039.12	12.29	2201.047
Gas I	2	163.94	10.41	2580.34	243.39	0.24	242208.0	13.55	5090.802
	3	160.44	22.14	1162.62	245.25	1.63	36698.54	12.62	2361.71
	1	20.14	17.24	23.39	48.11	34.69	69.75	6.15	2.377
Gas II	2	24.92	14.67	42.32	61.48	15.07	252.46	5.93	24.497
	3	17.42	14.26	21.18	41.64	27.92	63.85	6.14	3.578
	1	13.50	11.16	16.22	25.21	19.39	33.23	8.57	4.149
Gas III	2	15.20	14.14	15.96	24.49	22.68	27.79	11.24	1.00
	3	15.06	14.00	15.84	25.04	23.16	28.35	10.53	1.00

Gas I: 98%N_2, 2%O_2; Gas II: 60%CO_2, 32%N_2, 2%O_2; Gas III: 90%CO_2, 8%N_2, 2%O_2.

TABLE 3
Factorial ANOVA for the mean survival time (MST) for the different strains of *R. dominica* treated with various gas concentrations at a series of exposure.

Sources	*DF*	*SS*	*MS*	*F*	*P*
Exposure (Exp)	5	1888.04	377.61	84.36	0.000
Replication (Rep)	2	23.76	11.88	2.65	0.074
Gas	2	617.88	308.94	69.02	0.000
Strain	3	29.57	9.86	2.20	0.090
Exp*Rep	10	46.39	4.639	1.04	0.416
Exp*Gas	10	775.79	77.58	17.33	0.000
Exp*Strain	15	162.75	10.85	2.42	0.003
Rep*Gas	4	20.33	5.08	1.14	0.34
Rep*Strain	6	4.38	0.73	0.16	0.986
Gas*Strain	6	39.47	6.58	1.47	0.192
Error	152	680.40	4.48		
Total	215	4288.76			

ACKNOWLEDGEMENTS

MMH would like to thank the Alexander von Humboldt Foundation, Bonn, for awarding the fellowship; Mr. G. Schmidt and Mrs. L. Misgaiski, Institute for Stored Product Protection, Federal Biological Research Centre for Agriculture and Forestry, Berlin, for their technical assistance. He is grateful to the University of Rajshahi, for extending his study leave.

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