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CONTROL OF *EPHESTIA CAUTELLA* WITH LOW LEVELS OF METHYL BROMIDE AND CARBON DIOXIDE GAS MIXTURES

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ABSTRACT

The potential application of low levels of methyl bromide (MB) in combination with CO_2 gas mixtures as a rapid disinfestation technique was evaluated under laboratory conditions at 30ºC and 70% relative humidity (r.h.), using the tropical warehouse moth, *Ephestia cautella* as test insect. CO_2 concentrations of 0, 10 and 20% in air were examined when mixed with different MB concentrations. Eggs, larvae, pupae and adults were exposed to the various gas mixtures for periods ranging from 4 to 32 hours. The influence of CO_2 on reducing the MB concentration required for control was assessed by calculating products of *concentration x time* (CtP) as expressed in mg.h□.l^{-1}. CtP_{99} values of MB in normal air for *E. cautella* eggs, larvae, pupae and adults were 25.4, 30.1, 50.2 and 16.6 mg.h□.l^{-1}, respectively. These values were markedly reduced as the CO_2 concentration was increased to 10 and to 20%. In the presence of 20% CO_2 the effective MB dose was reduced to 18.7, 20.3, 29.7 and 4.3 mg.h□.l^{-1} in eggs, larvae, pupae and adults, respectively. The resistance of the developmental stages of *E. cautella* to MB was (in decreasing order): pupae, larvae, eggs and adults. In the presence of 20% CO_2 the CtP_{99} of MB was decreased by 1.4 times in eggs, the least sensitive stage and by 3.8 times in adults, the most sensitive stage responding to the gas mixture. This work was carried out as part of a Ph.D. study of the first author, at the Hebrew University of Jerusalem, Israel.

INTRODUCTION

Methyl bromide (MB) is an essential fumigant for pre-shipment and quarantine disinfestation of postharvest products. The gas is effective within very short exposure periods, typically lasting between 2 and 48 h. It also airs rapidly enough from treated systems with minimal disruption to normal commercial practices (Chakrabarti, 1996). However, MB emissions were found to have a deleterious effect on the atmosphere, because of which, the parties to the Montreal Protocol decided to phase out its production and use by the year 2005 in industrialized countries and 2015 in developing countries. Its quarantine and pre-shipment uses and some agricultural uses yet to be identified remain exempted but further continuance of

these exemptions will be subjected to periodic examination (Bell *et al.,* 1998). During the continued usage of MB however, application methods that would minimize its emissions need to be adapted.

Carbon dioxide (CO_2) has long been known to enhance the lethal effects of fumigants on insects (Cotton, 1932). Its admixture with MB has manifested great potential as a rapid disinfestation technique. MB applied according to recommended effective dosages, when admixed with $C0_2$ levels ranging from 1-40% have been claimed to significantly shorten the lethal exposure time of some insects (Friedlander, 1977; Navarro *et al.,* 1997). Navarro *et al.* (1989) also observed that with 20% CO_2 and MB mixtures the dose rate of MB can be reduced by about 50%. Fumigant application systems based on this technology are now in commercial application for the disinfestation of dates in Israel.

This study was conducted with the objective of reducing MB dosages to minimum effective levels and consequently minimizing MB emissions. Specifically, it evaluated the effectiveness of MB dosages reduced to about 50% of its existing recommended dosage, mixed with low (up to 20%) concentrations of CO_2. The Tropical warehouse moth (almond moth) *Ephestia cautella*, one of the two most widespread and abundant moth-pests of stored-products in tropical and sub-tropical countries, was used as the test insect.

MATERIALS AND METHODS

MB and CO_2 gas mixtures

CO_2 concentrations of 0, 10 and 20% in air were used to evaluate which doses of MB would give complete kill within 24 h. The required volumes of CO_2 measured in absolute mm Hg, were supplied from a cylinder equipped with a pressure regulator. MB was withdrawn from the head-space above liquid MB sealed in a pressurized vial using a gastight syringe, and injected into the fumigation chamber.

Test Insects

Adults, eggs, larvae and pupae of *E. cautella* were obtained from cultures reared on a ground wheat diet in a controlled temperature (28±1°C) and relative humidity (65±5%) room at the Department of Stored Products, ARO, Israel. The adults, pupae and eggs were 1-2 days old at the time of exposure while the larvae were 14-15 days old.

Fumigation procedure

Batches of at least 50 insects were confined in steel-mesh cages and exposed to treatments in flat-bottom flasks (~ 3.7 L) equipped with ground-glass stoppers, that served as fumigation chambers. The relative humidity (r.h.) inside the chambers was maintained at 65 ±5% using saturated sodium nitrite salt solution (Solomon, 1951). For MB fumigations alone, the appropriate volume of gas was injected into the

chamber containing the insects via a septum set into the flask stopper. For MB and CO_2 mixtures, the chamber was first evacuated to a partial pressure calculated so that the desired volume of CO_2 when flushed into the flask would return it to atmospheric pressure. Following introduction of CO_2, the required dosage of MB was applied. Gases were mixed for about 30 min using a Teflon coated magnetic stirrer before samples were withdrawn and analyzed. MB was analyzed using a Tracor model 565 gas chromatograph, equipped with "Flame Ionization Detector (FID, 200ºC) and "chromosorb 101" filled column. CO_2 was measured with the SRI 8610C model gas chromatograph equipped with twin thermal conductivity cells and dual columns packed with "Porapak Q" and "molecular sieve 5A". Fumigation chambers were kept at 30ºC in a controlled temperature incubator at predetermined exposure periods of 4, 8 and 16 h.

After fumigation, the exposed pupae, adults and larvae were immediately transferred into small jars (50 mL), and maintained at 28±1ºC and 65±5% r.h. Larvae were provided with food medium. Eggs were transferred to watch glasses and incubated under the same conditions.

Mortality counts were made after 2 weeks for larvae, 1 week for pupae, 1 day for adults and 4-5 days for eggs. Mortality of larvae was based on those that failed to pupate, of pupae - those that failed to emerge as adults, and of eggs - those that failed to hatch completely. Moribund adults were also included with the dead. For all sets of treatments, batches of insects that served as control were handled as described above without undergoing fumigation. The mortality counts of each life stage of *E.□autella* were subjected to Probit analysis using a program written by Daum (1979).

RESULTS AND DISCUSSION

The calculated exposure times in hours of the MB+CO_2 dosage combinations tested, that were needed to obtain a 99% Ct product mortality (CtP_{99}) for eggs, larvae, pupae and adults, are presented in Table 1.

The results show that all life stages of *E. cautella* may be controlled in about 30.8 mg.h L^{-1} in a combination concentration of MB and 10% CO_2 in air. The most tolerant were the pupae, and the most susceptible were the adults. The sensitivity of eggs exposed to the MB + 10% CO_2 admixture, compared to MB alone was very similar (a ratio of 0.96). However, for larvae and adults, MB:MB + CO_2 sensitivity ratios increased to about 1.1 and 1.4 respectively. With admixtures of reduced MB + 20% CO_2, CtP_{99} was shortened by 1.4-fold for eggs, 1.5-fold for larvae and 1.9-fold for adults. Pupae showed a sensitivity of 1.91-fold and 1.82-fold to MB + 10% CO_2, and MB + 20% CO_2 admixtures, respectively.

These results reveal the potentials of a low MB concentration + CO_2 fumigant mixture as an effective rapid disinfestation method. This application technique not only reduces the amount of MB required but also reduces its emission that is hazardous to the environment.

TABLE 1

Concentration time product (CtP) expressed in mg·h L^{-1} of MB to obtain 99% mortality of *E. cautella* eggs, larvae, pupae and adults (numbers in brackets represent 95% confidence limits), at 30ºC, 65± 5% r.h.

Life stage	CO_2 (%)	MB (mg/L)	CtP_{99} mg h·L^{-1}	Ratio of MB to MB+CO_2
Egg	0	2.74	25.4 (23.61-28.46)	
	10	1.87	26.2 (21.28-59.65)	0.96
	20	1.52	18.7 (16.35-40.49)	1.35
Larva	0	2.98	30.1 (27.95-34.00)	
	10	2.37	28.2 (22.75-42.56)	1.06
	20	1.61	20.3 (17.82-26.88)	1.48
Pupa	0	3.8	59.1 (53.57-69.17)	
	10	4.1	30.8 (27.50-36.87)	1.91
	20	3.9	32.3 (29.50-36.83)	1.82
Adult	0	0.8	20.8 (18.09-25.86)	
	10	0.52	14.5 (11.88-19.77)	1.43
	20	0.39	11.1 (10.21-12.52)	1.87

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