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Sensitivity of the Greater Wax moth *Galleria mellonella* to carbon dioxide enriched modified atmospheres

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Abstract

Galleria mellonella (*L.*), is a cosmopolitan pest, causing serious economic damage to honey-bee combs during storage. Efforts are being made in Israel to replace existing control methods against this pest based on fumigation, with an environmentally friendly treatment that affords long-term protection. For this purpose, application of modified atmospheres (MAs) by single shot flushing with carbon dioxide (CO_2) has been developed using flexible treatment chambers, and modified transport containers. This laboratory study was made to provide information on the effect of exposure period on wax moth mortality over the range of MA concentrations and temperatures encountered in practice.

Three MAs, namely 80%, 70% and 60% CO_2 in air, were supplied at 75% r. h. to exposure chambers held at 26℃ and 30℃. Eggs (24－48 h), larvae (12 d), pupae (0－48 h) and adults (0－24 h) were exposed in the chambers for periods ranging from eight hours to twelve days. Mortalities were recorded and subjected to probit analysis except for eggs where exposure time till no-survival was recorded. For all treatments, adults were the most sensitive, and eggs the least sensitive, with sensitivity being consistently higher at 30℃. It was concluded that 10 days exposure to a range of 90－60% CO_2 will give complete control of all stages.

Introduction

The greater wax moth, Galleria mellonella (L.) is a cosmopolitan insect that causes considerable economic losses to beekeepers by damaging wax combs. Infestation can begin within the hive but is particularly evident over the period between the close of the bee-keeping (honey flow) season when supers are removed from the hive, and during storage, until the next season (Eckert, 1951, Smith, 1990, and Singh, 1962). In warm climates in particular, all combs should be treated and then protected during storage against wax moth damage. Failure to do so can result in rapid and complete destruction of the unprotected combs. Since the empty combs can only be built-up by the honeybees themselves, and a ready supply of empty combs during the brood-rearing and honey-flow season is essential for the well-being of the hive, wax moth infestations can turn bee-keeping businesses into losing ventures. The most usual practices by commercial beekeepers to control the wax moth are based either on fumigation－formerly with EDB and now with methyl bromide or phosphine, or by wrapping the empty combs with cellophane to prevent the entrance of the moth, as well as treating the empty combs with sulfur and/or naphthalene or paradichlorobenzine crystals (Burgess, 1978). Both methods are relatively ineffective while the former method involves a potential health hazard. Consequently the adverse effect of the wax moth on growth of the colony and honey production poses a serious threat to the economic viability of the bee-keeping industry.

There is therefore an urgent need to find alternative technologies for controlling the wax moth, with preference towards the use of non-toxic methods that pose no threat to health of operator or consumer, and which are environmentally friendly. The use of modified atmospheres (MAs) is one of the more promising methods. It has been developed as an alternative to fumigation of stored agricultural commodities in that it retains the special capacity of in-situ treatment to control storage pests (Navarro and Donahaye, 1990). It has been successfully applied in Israel, to wax moth control, using specially designed flexible treatment chambers and also modified transport containers (Yakobson et al., 1997). However, there is only scanty information on the effect of exposure period and MA concentrations on wax moth mortality (Cantwell et al., 1972, Greatti and D'Agaro, 1992). Therefore this study was undertaken to provide the data necessary to enable the preparation of dosage schedules for routine treatments of honey combs and supers against the ravages of the wax moth.

Materials and Methods

Modified atmospheres

In commercial scale trials (Yakobson et al., 1997) the

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treatment procedure we chose was by single-shot flushing of the storage chambers with carbon dioxide (CO_2) to initial concentrations ranging from 60% to 95%, after which concentrations dropped progressively over the storage period. Therefore, in the laboratory, we chose three MAs, namely 80%, 70% and 60% CO_2 in air, in order to provide data on wax moth mortality that would span the range of high CO_2 concentrations used in practice.

These gas compositions were obtained from supply cylinders of oxygen (O_2), nitrogen (N_2), and CO_2 using a gas-mixing apparatus described by Donahaye (1990). This consisted of the three component gases supplied in tubing at rates regulated by a series of valves and gas-flow meters, that enabled them to be mixed in the desired combinations. After the gas supplies converged, the gas mixtures in the common supply-lines were led to temperature controlled treatment chambers, and passed through wash-bottles containing sulphuric acid to obtain a constant relative humidity of 75%. Then the gas mixtures were delivered via distribution chambers to a series of 100ml Erlenmeyer flasks that served as exposure chambers arranged in-parallel. Gas supply was designed to provide a flow rate through the exposure chambers of 7.5 to 10 ml/min. To periodically check the MAs, gas samples were withdrawn from the exposure chambers and analysed using a gas chromatograph equipped with twin thermal conductivity cells and dual columns packed with 'Poropak Q' and 'Molecular sieve 5a'.

Temperatures

To cover the ambient temperatures prevalent under field conditions, the experiments were carried out in CT rooms maintained at 26℃ and 30℃.

Insects

Rearing technique: All stages of G. mellonella were obtained from cultures reared at 30℃ and 70% relative humidity (r.h.) in the dark on a diet of maize flour (22%), wheat flour (11%), ground wheat (11%), dried milk (11%), dried yeast (5.5%), beeswax (17.5%), honey (11%) and glycerin (11%) (Sehnal, 1965). The stock material was collected from a field population infesting bees-wax in the apiary of the ARO, Israel. Cultures were started from eggs obtained by placing 6 to 10 freshly emerged adults in a petri dish together with pieces of folded and dampened paper. About 500 eggs layed on the paper were then transferred to 500ml culture jars containing 150 ml of food medium. Larvae were retained in these culture jars until the 4th instar. Then they were transferred in groups of 50, to 500 ml jars containing 300 ml of culture medium which was changed every three to five days. Before pupation, a bag made from nylon mesh and containing sections of PE tubing, each 4mm i.d., and 25 mm long, was placed in the culture jars. Larvae that pupated in these tubes were then removed and placed in petri dishes, prior to use in experiments, or in order to obtain freshly emerged adults.

Preparation of stages before exposure to treatments: Eggs were obtained as described above. They were held until 24 – 48h old, and then rafts containing about 50 eggs were cut from the paper and placed in an exposure chamber for treatment. Larvae for experiments were removed from culture jars 12 days after the cultures had been set-up. Pupae were removed from culture jars every 2 days and exposed to treatments aged 0 – 48 h. Newly emerged adults were exposed to treatment aged 0 – 24 h.

Experimental procedure

For larvae, pupae and adults, groups of 25 – 30 insects were placed in sets of exposure chambers, and linked to the gas mixture apparatus. For eggs, pieces of paper containing a raft of eggs were placed in each exposure chamber. An additional flask containing insects held under atmospheric conditions served as control. Periodic removal of flasks was based on preliminary trials to cover the time ranges over which insect mortality was found to occur for each stage, gas-mixture and temperature. Due to heterogeneity of response, each set of exposures was repeated several times. For larvae, pupae and adults, mortality results were subjected to probit analysis (Daum, 1979).

At the end of each exposure time, one flask was removed from the apparatus and incubated at 30 ± 1℃ and 70 ± 5% r.h. until mortality was recorded. For larvae, food medium was added, and mortality of larvae was considered as comprising those insects that failed to pupate, while pupal mortality consisted of those that failed to emerge as adults. Adults that failed to recover after 24 h were considered as dead. For eggs, due to the great difficulty in counting egg hatch, and a delayed hatching effect caused by the treatment, the rafts of eggs were incubated with culture medium, and for exposures at which no larvae were recorded after 20 days, total kill of eggs was considered to have been obtained.

Results and Discussion

The exposure times required to produce 99% mortality for larvae pupae and adults, and 100% mortality for eggs at each of the three MAs and at the two temperatures are given in Figs. 1 and 2. For comparison of sensitivity between the insect stages to the different treatments, results at the LT95 and LT99 levels, including 95% confidence limits, are given in Table 1.

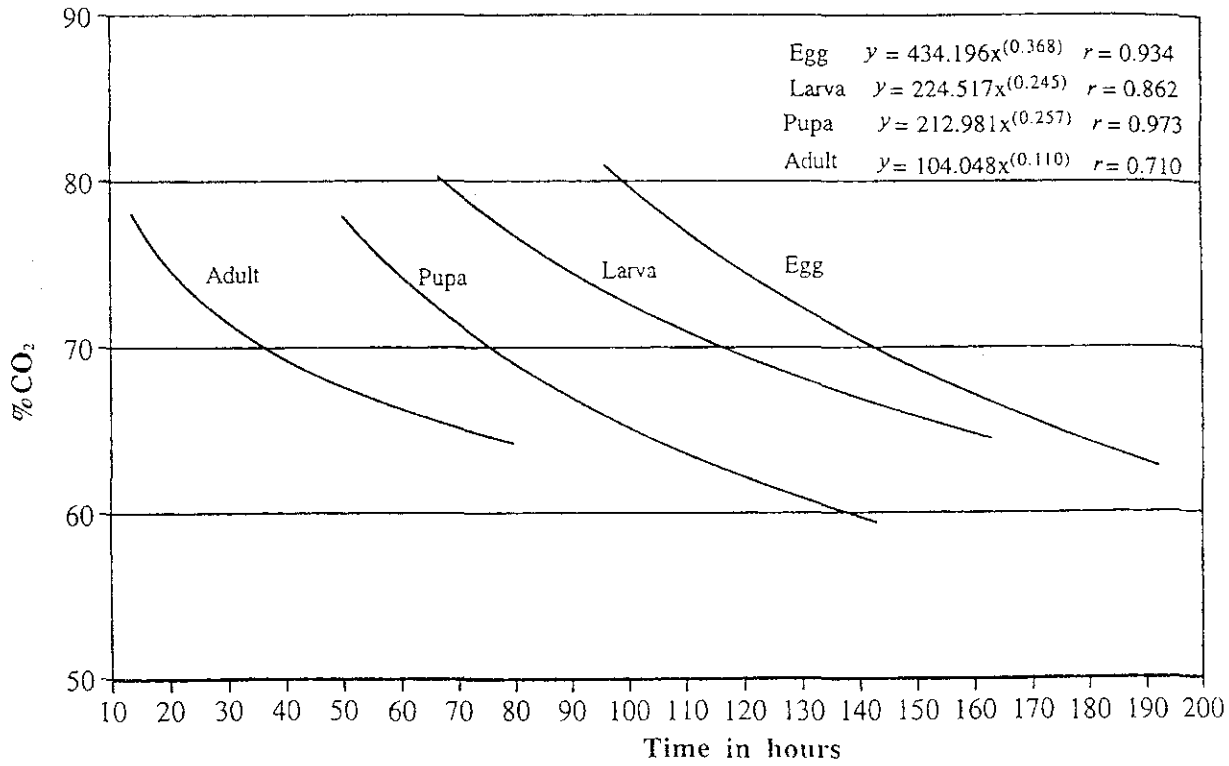

Fig. 1. Concentration/time mortality curves for the sensitivities of larvae, pupae and adults of *Galleria mellonella* to carbon dioxide at 26℃ at the LT99 level, and eggs at LT100.

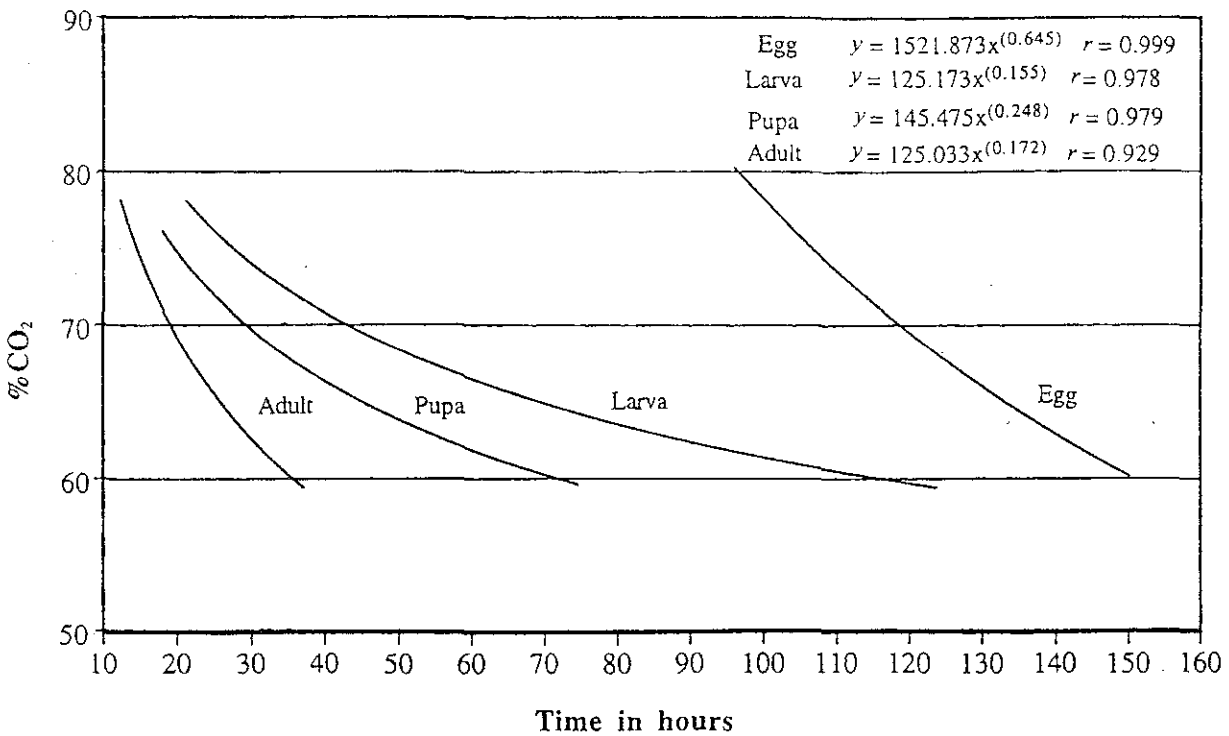

Fig. 2. Concentration/time mortality curves for the sensitivities of larvae, pupae and adults of *Galleria mellonella* to carbon dioxide at 30℃ at the LT99 level, and eggs at LT100.

Table 1. Summary of sensitivites at the LT95 and LT99 levels of three stages of *Galleria mellonella*, and time to total kill of the egg stage, at three modified atmopheres and two temperatures.

Modified Atmosphere	Temp.	Stage	Se	Slope	SE slope	LT95 (h)	95% Conf. limits	LT99 (h)	95% Conf. limits
60% CO_2	26 ℃	Egg				192*			
		Larva	1.00	4.38	0.48	114	96 – 114	163	130 – 226
		Pupa	1.00	3.05	0.79	85.5	62 – 171	143	92 – 464
		Adult	1.00	2.37	0.63	27.6	16 – 114	53.5	26 – 445
	30 ℃	Egg				150*			
		Larva	1.00	3.65	0.42	80.4	64 – 112	124	92 – 193
		Pupa	1.00	5.3	1.3	55.6	43 – 104	74.5	53 – 180
		Adult	1.00	3.71	0.89	24.3	17 – 53	37.1	23 – 117
70% CO_2	26 ℃	Egg				166*			
		Larva	1.70	3.65	0.98	103	71 – 339	158	96 – 950
		Pupa	1.00	7.76	1.81	53.5	45 – 78	65.5	53 – 111
		Adult	1.00	2.02	0.47	36.7	21 – 154	79.6	36 – 628
	30 ℃	Egg				120*			
		Larva	1.00	3.59	0.52	22.3	17 – 35	34.5	24 – 61.1
		Pupa	1.00	4.87	1.02	15.3	12 – 24	21.1	16 – 40.6
		Adult	1.00	6.57	0.9	13.2	11 – 17	16.8	14 – 23
80% CO_2	26 ℃	Egg				96*			
		Larva	1.67	4.62	1.07	47.5	34 – 144	66.7	43 – 331
		Pupa	1.00	10.2	1.95	43	38 – 54	50.1	43 – 68
		Adult	1.00	13.4	2.73	12.1	11 – 15	13.6	12 – 18
	30 ℃	Egg				96*			
		Larva	1.00	5.1	0.88	15.6	13 – 24	21.2	16 – 38
		Pupa	1.00	7.73	1.10	15.2	13 – 18	18.6	16 – 24
		Adult	1.00	8.03	1.73	10.1	9 – 14	12.3	10 – 19

* time to total kill.

From the figures it can be seen that for the three MAs and both temperatures, the egg was the most resistant stage. For all stages, both CO_2 concentration, and temperature had a marked influence on lethal times-the higher the CO_2 concentration and the higher the temperature, the shorter the lethal time. The 99% lethal times ranged from 15 h for adults at 80% CO_2 and 30℃, to 184 h (app. 8 days) for eggs at 60% CO_2 and 26℃.

Both figures also provide 99% mortalities at intermediate MAs, as well as more tentative extrapolation to lower CO_2 concentrations than those used in these experiments.

These findings may be compared with those of Cantwell et al (1972) who studied sensitivities of all stages of the greater wax moth to high CO_2 concentrations at temperatures ranging from 21 to 37.8℃. They concluded that the larval stage was the most resistant to high CO_2, followed by eggs, whereas our results showed that eggs were the most resistant. They found that at 37.8℃ (100℉) exposures to obtain complete kill were greatly shortened, requiring a maximum of 28 hours for larvae exposed to 73.4% CO_2 in air. However, this would require artificial heating of the treatment chamber, and because this is not avasilable to most apiculturalists the highest temperature used in our experiments was 30℃. Although their work was carried out under different temperatures and CO_2 regimes and most of the study was undertaken on larvae, which they considered as the most resistant stage, where their conditions were similar to ours, the results were also comparable though their larvae seemed less susceptible. For example at 27.8℃ and 60% CO_2 they obtained 42% mortality after 4 days exposure, while our results at 26℃ and 60% CO_2 gave 90% larval mortality after 4 days. Our results do not seem to conform with those of Greatti and D'Agaro (1992) who flushed CO_2 at a rate of 8L/min through a 0.49 m^3 chamber containing infested supers and obtained complete kill after 3 min.

Our findings support those of the field trials (Yakobson et al. 1997) in which complete kill was recorded during single-shot treatments in flexible liners and modified transport containers that were monitored for the first 10 days, during

which time initial high CO_2 concentrations (96 - 65%), dropped to near the 40% level. Jay et al. (1972) carried out CO_2 fumigations of comb honey in semi-trailer vans by flushing to a concentration of 98.6% CO_2 which was then held for 10 to 12 hours,. They obtained 92.7 - 100% kill of test larvae. However, this technology requires the use of a CO_2 supply vessel and other logistical back-up and is not widely applicable to the small scale bee keeper. Furthermore, the advantage of treating the supers in permanently installed modified containers or flexible enclosures, is that after the treatment, the wax combs continue to be protected from reinfestation within the enclosed space until required for use in the field.

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