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COMPARATIVE EFFICACY OF MODIFIED ATMOSPHERES AGAINST DIAPAUSING LARVAE OF *PLODIA INTERPUNCTELLA* AND OTHER INSECTS IN DURABLE PRODUCTS

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ABSTRACT

Modified and controlled atmospheres are an alternative to synthetic, residue building agents in stored product protection. They are safer in use than most other fumigants and could gain importance with the phase out of methyl bromide and the institution of stiffer regulations for the emission of toxic agents from treated structures. Modified atmospheres (MAs) require highly gas-tight storage structures in order to be economically feasible. In temperate climates MAs have the additional disadvantage that at temperatures around 20°C or less, treatment times are considerably longer than when a fumigant such as phosphine is used. On the other hand, the gas-tightness of a structure may prevent the re-infestation after a treatment, and in the absence of oxygen, product quality remains better than during storage in air. A difficulty of pest control in temperate climates is that with decreasing day length and temperature, arthropods may develop over-wintering stages that could be more tolerant to treatment than other individuals. Diapausing larvae of the Indianmeal moth *Plodia interpunctella* were tested for their tolerance to various mixtures of N_2, O_2 and CO_2. Whereas normal larvae from a culture at 25°C could be controlled at 10°C within 3 weeks with 60 vol.-% or 90 vol.-% of CO_2 in air, diapausing larvae from a culture, gradually adapted from 25°C to 10°C, required 6 or 4 weeks exposure to the respective MAs for 100% control. Exposure for 6 weeks was also sufficient to control all stages of the granary weevil *Sitophilus granarius*, one of the species most tolerant to MAs. A mixture of 98% N_2 and 2% O_2 controlled normal larvae within 4 weeks and diapausing larvae within 5 weeks of exposure, provided they were not older than 6 months. It became clear that the tolerance to this hypoxic atmosphere increased with the duration of diapause. Diapausing larvae kept for more than one year at 10°C were able to survive up to 7 weeks of exposure and developed into adults. After 9 weeks of treatment, 12 out of 30 larvae were found alive, of which one larva was still able to pupate but subsequently died. Thus, diapausing and quiescent stages of stored-product pests may be difficult to control particularly with a CO_2-free MA.

INTRODUCTION

Treatments with modified atmospheres (MAs), such as. by purging with carbon dioxide (CO_2) or nitrogen (N_2) are well-known, reliable and residue-free techniques

for the disinfestation of grain and other durable products. The technology of how to use modified or controlled atmospheres and an overview on this method have been given elsewhere (Banks 1983a, 1983b, Banks *et al.,* 1990, Calderon and Barkai-Golan 1990; Adler *et al.,* 2000). In comparison with phosphine (PH_3) fumigations, the high sealing standards required, the comparatively long treatment times, and the resulting higher treatment costs often render this technique less favoured by storage keepers and the food industry. On the other hand, consumer demands for organically produced food untouched by toxic chemicals, are growing in many industrial countries, while the stiffening of regulations on worker safety and emission control may also increase the costs of PH_3 fumigations in the years to come. Another factor that may increase the use of MAs in the future could be the development and spread of phosphine-resistant insect strains due to bad fumigation practices and the lack of alternative fumigants.

Numerous studies on the efficacy of MAs against stored-product insects have shown that lethal exposure times vary with temperature, moisture content, gas composition, species and developmental stage (Jay 1984; Annis 1987; Banks and Annis 1990). Among stored-product beetles, the pupal stage of the genus *Sitophilus* is among the most tolerant to treatments with atmospheres low in oxygen (O_2) content (hypoxic) and/or high in carbon dioxide (CO_2) content (hypercarbic) (Annis 1987, Adler 1993; Annis and Morton 1997). In stored-product moths, diapausing larvae that occur during winter seasons in temperate climates could also be quite tolerant due to their reduced metabolism. Diapausing larvae of the Mediterranean flour moth *Ephestia kuehniella* and the Indianmeal moth *Plodia interpunctella* were reported to be more tolerant to fumigations with MB and PH_3 than normal larvae (Sardesai 1972; Cox *et al*., 1984; Bell and Savvidou 1991). In the laboratory study described in this paper, diapausing larvae of *P. interpunctella* were exposed to various gas mixtures low in O_2 or high in CO_2 in order to determine the lethal exposure times. The results were compared to those obtained by treating normal larvae from a laboratory culture at 25°C in a similar way.

MATERIAL AND METHODS

Eggs of *P. interpunctella* were cultivated at 25±1°C and 70±5 % r.h.. in 2-L jars on wheat bran and broken almonds (Adler 1999). After three weeks, the jars with grown larvae were transferred to 15°C and artificial short light/dark-regime (8 h light:16 h dark) and after two more weeks to 10°C and similar light conditions. The transfer to 10°C was carried out because in earlier experiments (Adler 1999), untreated larvae had developed into adults after prolonged periods at 15°C. At 10°C, the larvae were kept for periods between 4 weeks and 71 weeks and were then placed in batches of 30 individuals into a wire mesh cage with some wheat bran. Cages were placed in 2.3-L Dressel flasks that were flushed at 10±1°C with one of the following gas mixtures (percent by volume):

- 98% N_2, 2% O_2 (Gas 1),
- 60% CO_2, 32% N_2, 8% O_2 (Gas 2),
- 90%CO_2, 8% N_2, 2% O_2 (Gas 3).

The gas mixtures were humidified to approx. 75% r.h. by bubbling them through a saturated sodium chloride solution at a flow rate of 2,000 mL/min before introduction into the flasks (Winston and Bates 1960). The Dressel flasks were flushed until the O_2 content measured with a Servomex Oxygen Analyser 570A at the gas outlet of the last bottle, attained that of the respective gas mixture (max. deviation: 0.1%). The gas inlet and outlet of each flask were then closed and the flasks were kept for periods of 3 d, 5 d, and at weekly intervals up to 10 weeks. After each exposure time was completed, O_2 contents were determined with a Bühler BA 4500 Oxygen Analyser. The insects were then transferred into petri dishes with food substrate and checked weekly for mortality and development. Survival was determined by pupation and hatching of adult moths, whereas, motionless, dried-out and darkened larvae were considered dead.

RESULTS

The results are summarised in Table 1 and Figure 1. With Gas 1, normal larvae from a culture at 25°C were controlled within 3-4 weeks, and diapausing larvae were controlled after 4-9 weeks. Gas 2 controlled normal larvae in 1-2 weeks, and diapausing larvae in 4-5 weeks. Gas 3 led to complete control of normal larvae after 1-2 weeks and of diapausing larvae after 2-3 weeks of exposure.

Diapausing larvae kept for more than one year at 10°C were able to survive up to 7 weeks of exposure to Gas 1. After 9 weeks of treatment 12 larvae out of 30 were found alive, and one larva was able to pupate but died subsequently. In the untreated control, 13 larvae were alive at the end of the experiment and 4 adults hatched after transfer to 25°C.

DISCUSSION

As mentioned in an earlier paper (Adler 1999), the contents of residual O_2 in the Dressel flasks were reduced to zero within a few weeks of insect treatment in the hypoxic mixture with a high nitrogen content (Gas 1) due to respiration. In the hypercarbic gas mixtures (Gas 2 and Gas 3) the amounts of O_2 present were only reduced by about one per cent by volume. This supports the statement that high levels of CO_2 can prevent the insects from undergoing aerobic respiration, using O_2 (Navarro 1975), and this may be due to anaesthesia caused by CO_2 (Adler 1993).

In normal larvae, there were small variations in lethal exposure times for each gas mixture. In diapausing larvae, variations in lethal exposure times seem to decrease with increasing contents of CO_2 (Table 1). Greatest variations in exposure times needed to control diapausing larvae were found in treatments with Gas 1. When the lethal times for diapausing larvae were plotted against their age, the data suggest that

an increase in the duration of diapause also increased the tolerance to this gas (Fig. 1). This effect could have been caused by the increasingly reduced metabolism of diapausing larvae kept for an unnaturally long time at low temperatures. However, long periods of diapause also markedly reduced the emergence of adults in untreated controls, and pupation, formerly taken as a sign of survival, was not necessarily followed by adult emergence.

TABLE 1
Exposure time in days needed for complete control of 30 *Plodia interpunctella* larvae at 10°C

Test No. (culture age in weeks)	Gas 1 normal larvae*	Gas 1 diapausing larvae*	Gas 2 normal larvae*	Gas 2 diapausing larvae*	Gas 3 normal larvae*	Gas 3 diapausing larvae*
1 (8)	-	-	-	28	-	14
2 (20)	28	-	7	28	7	21
3 (20)	28	28	7	35	7	21
4 (24)	21	28	7	35	14	21
5 (27)	28	28	7	28	7	14
6 (28)	28	42	7	35	7	21
7 (30)	35	42	14	28	14	14
8 (38)	28	56	14	28	5	14
9 (37)	21	28	7	14	7	14
10 (51)	21	28	7	21	-	-
11 (71)	-	63	-	-	-	-
Mean	26.4	38.1	8.6	28.0	8.5	17.1
SD	4.7	13.6	3.1	6.6	3.5	3.7

*Normal larvae came from a permanent laboratory culture at 25°C and were kept overnight at 10°C prior to treatment; diapausing larvae were kept for several weeks at 10°C prior to treatment. Where no figures are given, complete mortality was not achieved or the respective gas mixture was not tested.

If one compares the efficacy of Gas 2 against normal and diapausing larvae, it is quite striking that with a mean lethal time of 8.6 days this gas mixture with 60% CO_2 was more effective than Gas 1 with 98% N_2 (mean LT: 26.4 d, see Table 1) in controlling normal larvae, but was similar or less effective against young diapausing larvae (mean LT: 32.7 d as against 28 d for up to 20 week old cultures, see Fig. 1). This indicates some rather drastic changes in insect metabolism when the larva enters diapause, possibly the partial replacement of body water by glycerol that could reduce the detrimental effects of CO_2.

If the lethal exposure times of *P. interpunctella* are compared to those obtained with other stored-product pests at different temperatures, it becomes clear that while for stored-product moths, the diapausing larvae may be the most tolerant stage, they do not match the tolerance of pupae of the granary weevil *Sitophilus granarius* exposed to identical gas mixtures at 10°C using similar methodology (Fig. 2 and Fig. 3). Figure 3 shows the results of *S. granarius* pupae from a culture at 25°C exposed to 90% CO_2, but the results with 60% CO_2 were strikingly similar at all

tested temperatures (Adler 1994, 1998). This is in contrast to the findings in this study, where at 10°C diapausing larvae of *P. interpunctella* were more sensitive to 90% CO_2 than to 60% CO_2, showing how difficult it is to generalise from findings using one test species. It should be noted that for reasons of comparability, *S. granarius* pupae had not been adapted to the respective temperature, which may be the cause of shorter lethal times at temperatures below 10°C.

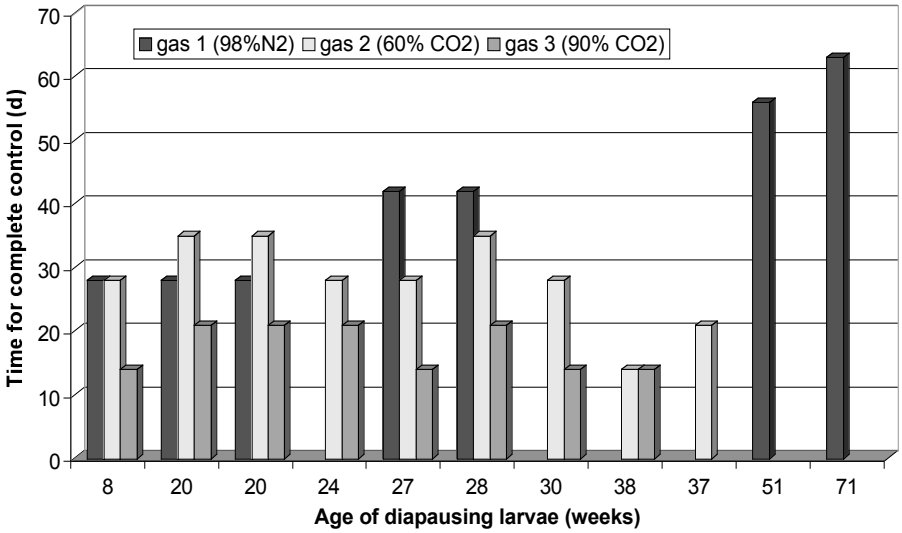

Fig. 1. Exposure times needed to control diapausing larvae of *Plodia interpunctella* kept for various periods of time at 10°C before treatment with different modified atmospheres (The age in weeks includes 3 weeks cultivation at 25°C and 2 weeks at 15°C).

From the results presented here, it may be concluded that diapausing larvae are more difficult to control with MAs than normal larvae. A treatment with N_2 relying on the absence of O_2 will take a longer treatment time to control all diapausing larvae and in late winter, exposure times needed for control may be even longer. If MAs were to be applied under such circumstances, a high content of CO_2 would be the best option to achieve control in a comparatively short time (less than three weeks with 90% CO_2 at 10°C).

In studying the effects of MAs on stored-product pests, one may still be surprised by the complexity and the variation of response of different species, developmental stages and strains. Post-treatment or end-point mortality seems to be another topic that needs closer attention in studies on hypoxic and hypercarbic atmospheres because of the post-treatment mortality of diapausing larvae noticed in this study and because of similar findings with adults of S. *oryzae* exposed to identical gas mixtures (Hasan *et al.*, unpublished data).

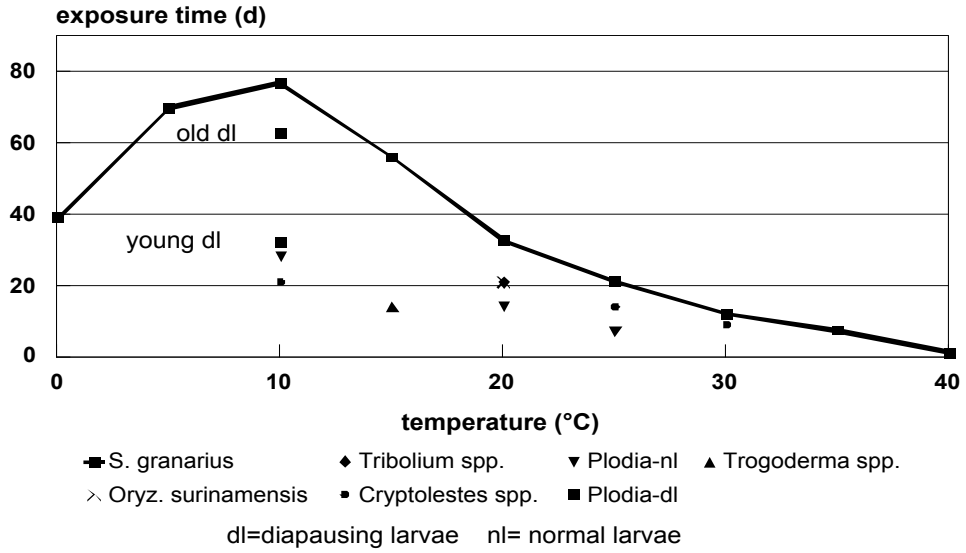

Fig. 2. Exposure times needed to control all stages of various stored product pests at a range of temperatures with 98% - 100% N_2 (rest: oxygen).

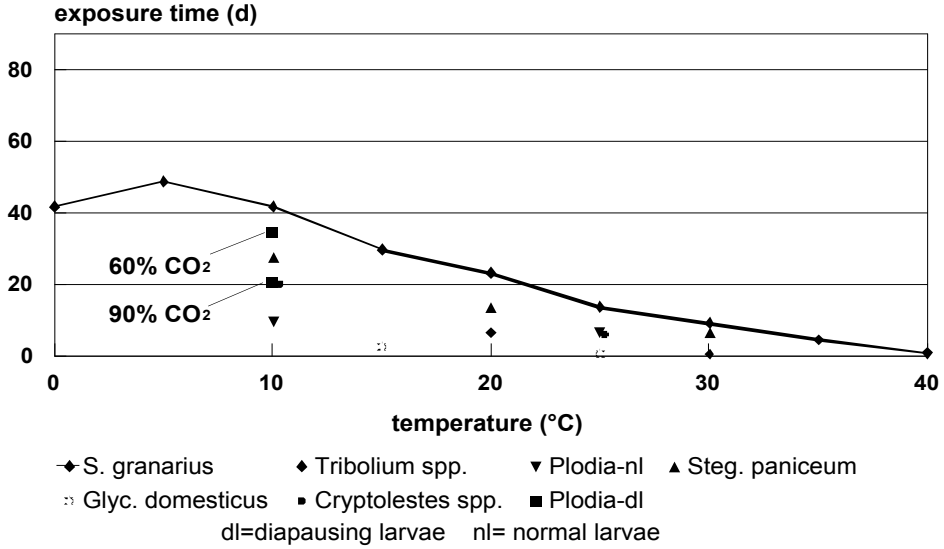

Fig. 3. Exposure times needed to control all stages of various stored stored-product pests at a range of temperatures with 60-100% CO_2 (rest: air). (*Sitophilus granarius* pupae: 90% CO_2, similar results with 60% CO_2, *Tribolium castaneum* and *T. confusum*, mixed stages: 70% CO_2, *Plodia interpunctella,* larvae : 90% CO_2, *Stegobium paniceum*, mixed stages: 90% CO_2, *Glycophagus domest*icus, mixed stages: 100% CO_2, *Cryptolestes pusillus* and *C. ferrugineus*: 60% CO_2, rest N_2 and O_2 in the ratio of 4:1).

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