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# Effects of carbon dioxide on *Sitotroga cerealella* (Olivier) larvae and their enzyme activity

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ABSTRACT

The susceptibility of 4th instar larvae of *Sitotroga cerealella* to modified atmospheres (MAs) containing 25, 40 and 60% $CO_2$ in air at 27 °C with different exposure periods was determined. Also, changes in the activity level of several enzymes were analyzed. Reduction in percentage adult emergence from the treated larvae tended to increase with $CO_2$ concentration and with exposure period. The reduction in emergence of adult from the 4th instar larvae reached 100% after 264 h for 25% $CO_2$, after 240 h for 40% $CO_2$ and after 168 h for 60% $CO_2$. The larvae showed the highest rates of escape for 25% $CO_2$. This could be due to the fact that at higher $CO_2$ contents the narcotic effect overrode the repellent effect. Trehalase, acid phosphatase, acetylcholinesterase, phenoloxidase and lactate dehydrogenase (LDH) enzyme concentrations were found to be higher in the treated larvae. Larvae exposed to MAs exhibited decreasing activity of amylase, alkaline phosphatase and adenosine triphosphatase (ATP'ase) enzymes. Additionally, MAs led to an increase in the total protein, triglyceride and lactate content.



## 1. Introduction

Insect damage is known as one of the most important problems faced during storage of grain cereals. The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), attacks the soft grains during pre-harvest, during harvest, and post-harvest. The damage by this insect comes from larval feeding on the endosperm, as well as an indirect damage to these grains from subsequent attack by certain secondary insects. Such damage leads to considerable quantitative and qualitative economic losses, e.g. in the weight and germination of grains (Weston et al., 1993).

Disinfestation using a controlled atmosphere represents an alternative to insecticide treatments for the control of pests attacking commodities (Bell, 2003; Ahmed and Hashem, 2012). Modified atmospheres (MAs) offer an effective, safe, and residue-free alternative to chemical fumigants and protectants for controlling insects and mites infesting stored grain and grain products (Fleurat-Lessard, 1990; Banks and Fields, 1995; Annis and Morton, 1997). MA treatment for stored-product protection involves methods of producing physiological and biochemical stress in the pest organisms. With this technique, conditions are created with reduced levels of oxygen ($O_2$), replacing it with other gases such as carbon dioxide ($CO_2$). Most stored-product insects are eventually killed under atmospheres of $<3\%$ $O_2$ or $>40\%$ $CO_2$ (Adler et al., 2000; Navarro, 2006). Data on the effects of different types of $CO_2$ treatments and dosages on key pests are available for many species and stages of stored-product pests under particular sets of conditions (Banks and Annis, 1990; White et al., 1995; Annis and Morton, 1997). The adults of *Sitophilus granarius* (L.), *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and *Rhyzopertha dominica* (F.) were exposed to MA containing low $O_2$ (Krishnamurthy et al., 1986). The life stages of *Callosobruchus maculatus* (F.) were exposed to MA at different levels of humidity (Ofuya and Reichmuth, 2002). Conyers and Bell (2007) carried out laboratory tests on five species of stored-product beetles, *C. ferrugineus*, *O. surinamensis*, *Sitophilus oryzae* (L.), *S. granarius* and *T. castaneum* to MAs. De Carli et al. (2010) studied the effect of MA on the mortality of *Sitophilus* spp. in organic maize grain. Hashem et al. (2012a) tested the susceptibility of the different life stages of *O. surinamensis* to different MAs containing various concentrations of $CO_2$.

Little research has been done to study the effect of carbon dioxide on the physiological aspects of insects. The physiological effect of carbon dioxide on spiracular muscles and nerve transmission has been described by Hoyle (1960). Carbon dioxide increases the concentration of certain blood proteins, stimulates

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oogenesis (Engels and Ramamaurty, 1976) and reduces the efficiency of food conversion to tissues (Brooks, 1965). There was a connection between carbon dioxide anesthesia and inhibition of gut succinic dehydrogenase of late instar larvae of *Heliothis zea* (Boddie), 30% $CO_2$ produced 72% inhibition while 15% produced 55% inhibition of the enzyme (Edwards, 1968).

The effect of MA on lactate and pyruvate levels in the hemolymph of *Ephestia cautella* (Walker) pupae was investigated by Navarro and Friedlander (1975). Pupae were exposed to 80% $CO_2$ to study the effect of $CO_2$ on triglyceride metabolism (Friedlander and Navarro, 1979b). Friedlander et al. (1984) also studied the effect of $CO_2$ on the main NADPH-producing systems in the same pupae.

The present investigation was carried out to clarify the effect of certain levels of carbon dioxide on the susceptibility of 4th larvae of *S. cerealella*, monitoring the larvae escaping from grains. Also, activity of amylase, trehalase, acid phosphatase, alkaline phosphatase, acetylcholinesterase, phenoloxidase, adenosine triphosphatase and lactate dehydrogenase (LDH) enzymes and total proteins, triglyceride contents and lactate were studied.

## 2. Materials and methods

This study was carried out in the laboratory of Modified Atmospheres at the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University. Wheat grains of Giza variety 168, with a moisture content of 12 ± 1% were used and all experiments were carried out under 27 ± 1 °C and 65 ± 5% r.h. To study the sensitivity of the 4th instar larvae of *S. cerealella* to modified atmospheres, certain biological information was essential, especially the duration of development. Based on the biological studies on this insect by El-Sherif et al. (2008), 4th instar larvae were used.

### 2.1. Preparation of gas treatment

As described by Desmarchelier (1984), the treatments with gas mixtures took place inside gastight sealed glass bottles of 550 $cm^3$ capacity (Dreshel flasks). Each flask was tightly plugged with a special glass stopper provided with two lateral valves (inlet and outlet) leading to two vertical glass tubes. One of these tubes was long and reached near the bottom of the flask and worked as a gas inlet. The other tube was short and reached the upper quarter of the flask and worked as a gas outlet. Valves were opened, at the beginning of the treatment, and left until the desired gas concentration as indicated by an oxygen analyzer (Hashem, 1990) was reached. A $CO_2$ cylinder was connected to the inlet tube with a short hose. The outlet tube was connected to the oxygen analyzer also with another short hose (Servomex 570).

### 2.2. The treatments

Based on knowledge on the rate of development of *S. cerealella*, 4th instar larvae were provided by keeping <24 h old eggs on grain for 22 days. The larvae were exposed to three modified atmospheres containing different concentrations of $CO_2$ in air: 1) 25% $CO_2$, 15% $O_2$ and 60% $N_2$, 2) 40% $CO_2$, 12% $O_2$ and 48% $N_2$, 3) 60% $CO_2$, 8% $O_2$ and 32% $N_2$. Five grams of grains with larvae were introduced into the Dreshel flasks and exposed to the specified MAs treatments. Exposure periods were of least 3 h or its multiple until 100% mortality was achieved. After treatment, Dreshel flasks were transferred to incubators adjusted to a constant temperature of 27 °C for different exposure periods. All tested MAs treatments were repeated three times and replicates were left untreated for control.

### 2.3. Reduction in moth emergence

By the end of the tested exposure periods the treated grains containing larvae, were taken out from the incubated Dreshel flasks and transferred into clean glass jars (50 mm diam. and 150 mm high depth), covered with muslin cloth and incubated under the same conditions. All jars were examined daily until the end of moth emergence. Due to the difficulty of detecting larvae inside the grains, % reduction in moth emergence was calculated according to the following formula:

$$\text{Reduction \%} = \frac{\text{No. of moths emerging in the control} - \text{No. of moths emerging in the treatment}}{\text{No. of moths emerging in the control}} \times 100$$

### 2.4. Monitoring of larval escaping

*Sitotroga cerealella* larvae treated with the three MAs for different exposure periods at 27 °C showed a tendency to escape the grains before completing their development (Fig. 1). These larvae were collected to calculate the percent of escaped larvae according to the following formula:

$$\text{Escaped larvae \%} = \frac{\text{No. of moths emerged in the control} - \text{No. of escaped larvae in the treatment}}{\text{No. of moths emerging in the control}} \times 100$$

Analysis of variance of all obtained data was conducted using SPSS (Anonymous, 2001). Mean separation was conducted using least significant difference (L.S.D.) test in the same program. Data on the effect of exposure period on the mortality of larval instars were subjected to probit analysis as described by Finney (1971). $LT_{50}$ and $LT_{95}$ values were also calculated using the computer program developed by Noack and Reichmuth (1978).

### 2.5. Determination of enzyme activity and contents

The 4th instar larvae were treated by exposing the infested wheat grains to 25% $CO_2$ in air at 27 °C for 48 h. An aliquot part from the grain sample with treated 4th instar larvae (0.5 g) and untreated ones was kept in a freezer until used. The samples were homogenized in distilled water using a Teflon homogenizer