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Comparative effects of different modified atmosphere exposures at 20 ° and 34 °C on the immature stages of angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae)

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ABSTRACT

This study aimed to determine the sensitivity of newly-laid eggs, 4th instar larvae and 3-day-old pupae of *Sitotroga cerealella* to four modified atmospheres (MAs) containing 30% (MA1), 45% (MA2), 65% (MA3) and 75% (MA4) CO_2 in air at 20 ° and 34 °C, and exposure periods between 2 h and 288 h. Results showed that egg mortality and adult emergence reduction from treated larvae or pupae increased gradually with the increase of either exposure period or CO_2 concentration in air at both 20 ° and 34 °C. Suppression of adult emergence from treated larvae reached 100% after 12 days at 20 °C for all MAs, but was achieved after 3 days for MA4 (75% CO_2) and 6 days for MA1, MA2 and MA3 at 34 °C. Suppression of adult emergence from treated pupae at 20 °C reached 100% after 11 days for MA1 and MA2 and after 9 days for MA3 and MA4, while at 34 °C it reached 100% after 5 days for MA1, and 4 days for MA2, MA3 and MA4. The order of sensitivity of *S. cerealella* stages to MAs at both 20 ° and 34 °C was eggs > pupae > larvae. The most effective MA treatment was that containing 75% CO_2 at 34 °C. This combination killed all eggs and larvae within 3 days and all pupae within 4 days.

1. Introduction

Modified atmospheres (MA) offer one of the most successful preservation techniques suitable for wide varieties of agricultural and food products (Jayas and Jeyamkondan, 2002). Low oxygen and high carbon dioxide (CO_2) levels retard respiration rate and extend the shelf life of fresh produce (Caleb et al., 2012). MAs have been used successfully to maintain grain quality (Navarro, 2012). The technique is also used commercially for preserving certain fruits and vegetables (Jayas and Jeyamkondan, 2002) and extending the shelf life of meat and many fishery products by inhibiting bacterial growth and oxidative reactions (McMillin, 2008; Sivertsvik et al., 2002). Storage under MA involves changing the proportions of the normal atmospheric components of the storage enclosure. Research on the effect of different proportions of these atmospheric components against insect pests is an important prerequisite for developing new control techniques based on this technology, thus avoiding use of chemical control methods. Application and distribution of CO_2 and N_2 in stored grain bulks or in products in containers or fumigation chambers can provide a solution to avoiding losses, not only those caused by insect pests.

The angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) has been recognized as a common pest of grains world-wide. Its larvae feed on corn grains causing direct damage (Weston et al., 1993). Additional damage to the grain can then occur through the attack of secondary insect pests (Sedlacek et al., 1998). Such damage leads to considerable quantitative and qualitative losses represented as weight loss, decrease of the nutritional value of the grains and failure of grain germination. Recently, Hashem et al. (2012b) investigated the sensitivity of newly-laid eggs (<24 h old), 4th instar larvae and 3-day-old pupae of *S. cerealella* to four modified atmospheres (MAs) containing 30%, 45%, 65% and 75% CO_2 in air at the optimum temperature of 27 °C and with exposure periods between 2 h and 264 h. They found that the percentage mortality of the newly-laid eggs, as well as the reduction of adult emergence from 4th instar larvae and 3-day-old pupae tended to increase with the increase of CO_2 concentrations in air and exposure period. The order of sensitivity for the three developmental stages of *S. cerealella* was: eggs > pupae > larvae. At the same temperature, Ahmed and Hashem (2012) studied the susceptibility of different life stages of two pyralid moths; the Indian meal moth *Plodia interpunctella* (Hubner) and almond moth *Ephestia* (*Cadra*) *cautella* (Walker) to different modified atmospheres (MAs)

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containing 40%, 60% and 80% CO_2 in air at different exposure times. They found that eggs and pupae of *P. interpunctella* were more sensitive to MAs than those of *Ephestia cautella* but the larvae of the latter were more sensitive than those of *P. interpunctella*.

There are many researches conducted on the efficacy of MAs against stored products insects. 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 oxygen (Krishnamurthy et al., 1986). They found that the mixtures containing 1–1.6% O_2 killed all the insects within 7 days if they also contained 10% or more CO_2. The life stages of *Callosobruchus maculatus* (F.) were exposed to MA at different levels of humidity (Ofuya and Reichmuth, 2002). They recorded that mortality of eggs and adults of the bruchid in 70% CO_2 in air and 1.0% O_2 in nitrogen (N_2) was higher at 10 $\pm$ 3% and 34 $\pm$ 2% than at 70 $\pm$ 2% and 90 $\pm$ 3% r.h. They also noted that mortality of larvae and pupae of the bruchid in the atmospheres was not affected by the r.h. in storage. Conyers and Bell (2007) carried out laboratory tests on five species of stored product beetles, *C. ferrugineus*, *O. surinamensis*, *S. granarius*, *Sitophilus oryzae* (L.), and *T. castaneum* to MAs. They showed that the O_2 content preventing population growth varied with species and temperature. De Carli et al. (2010) studied the effect of 20, 60 and 80% CO_2 on the mortality of *Sitophilus* spp. in organic maize grain. They observed that complete inhibition of the insects could be achieved with 30 days of exposure in CO_2 atmospheres. Hashem et al. (2012a) tested the susceptibility of the different life stages of *O. surinamensis* to different MAs containing various concentrations of CO_2. They stated that at 30 °C a two-day exposure period was adequate to kill all larvae and adults under all tested MAs, while eggs and pupae were killed after four days of exposure to the high-CO_2 atmospheres (75% and 85%).

Therefore, this study aimed to evaluate the effect of four different MAs; MA1 (30% CO_2 in air), MA2 (45% CO_2 in air), MA3 (65% CO_2 in air) and MA4 (75% CO_2 in air) at 20 ° and 34 °C on the susceptibility of newly-laid eggs, 4th instar larvae and 3-day-old pupae of *S. cerealella* to determine the most effective CO_2 concentration and exposure needed at each temperature in laboratory tests.

2. Materials and methods

The work was carried out at the Department of Economic Entomology and Pesticide Sciences, Faculty of Agriculture, Cairo University in 2005 and 2006.

2.1. Laboratory experiments

The susceptibility of three developmental stages of *S. cerealella*; eggs, 4th instar larvae and pupae, to different concentrations of CO_2 in air was investigated at 20 ° and 34 °C, 65 $\pm$ 5% r.h. and at different exposure periods. Eggs, larvae and pupae required for the MA experiments were obtained from stock cultures maintained at 27 °C and 65 $\pm$ 5% r.h. Based on the results obtained from the study of the life history of *S. cerealella*, newly-laid eggs were treated with MAs in glass tubes while larvae and pupae were treated by exposing maize grains 23–25 and 29–31 days after artificial infestation with eggs, respectively. After exposure to MAs, treated stages were maintained under the laboratory conditions of 20 ° or 34 °C and 65 $\pm$ 5% r.h. All tested MA treatments were repeated three times and for each, three similar replicates were left untreated for control purposes.

2.2. Gas treatment equipment

As described by Desmarchelier (1984), treatment with gas mixtures took place inside gastight sealed glass bottles of 550 cm^3 capacity (Dreshel flasks). Every flask was tightly plugged with a special glass stopper provided with two lateral valves (an inlet and an outlet valve) leading to two vertical glass tubes. One of these tubes reached to near the bottom of the flask while the other only reached down to the upper quarter. The long tube served as the gas inlet and the short one as the outlet. Valves were opened at the beginning of the treatment and left open until the desired gas concentration inside the flask was obtained as indicated by an oxygen analyzer. A CO_2 cylinder was connected to the inlet tube of the flask with a short hose to supply CO_2. The outlet tube of the Dreshel flask was connected to the oxygen analyzer with another short hose (Servomix 570 A) (Hashem et al., 2012b).

2.3. Insect material

Eggs were carefully transferred to Petri dishes then examined under a stereoscopic binocular to eliminate injured or abnormal ones (malformed or pale white in color). They were then carefully transferred to glass tubes (1 $\times$ 5 cm) at a rate of 50 eggs/tube and introduced into the Dreshel flasks which were then exposed to the chosen MA treatment. About 10 g of maize grains infested with 4th instar larvae or 3-day-old pupae obtained from stock cultures were prepared for each treatment. Grains, with larvae or pupae inside, were introduced into the Dreshel flasks and then exposed to the specified MA treatments. Eggs, 4th instar larvae and pupae of *S. cerealella* were exposed to four different concentrations of CO_2 in air as follows; MA1: 30% CO_2, 14% O_2 and 56% N_2; MA2: 45% CO_2, 11% O_2 and 44% N_2; MA3: 65% CO_2, 7% O_2 and 28% N_2 and MA4 : 75% CO_2, 5% O_2 and 20% N_2. Exposure periods to each MA treatment lasted for between 2 h and 264 h depending on how long was required for total kill.

2.4. Mortality percentages of the different developmental stages

After each MA exposure period, Dreshel flasks were aerated and eggs or grains containing larvae or pupae were taken out and incubated under the optimum rearing conditions of 27 °C and 65 $\pm$ 5% r.h. Eggs remained inside the tubes and were examined daily to record mortality percentages which were corrected according to Abbott's formula (Abbott, 1925). Treated grains infested with larvae or pupae were transferred to glass jars (5 cm in diameter and 15 cm in depth) covered with muslin cloth for incubation under the same optimum conditions and were examined daily until the emergence of adult moths stopped. Due to the difficulty of detecting larvae or pupae inside the grains, reduction in the percentage moth emergence was calculated according to the formula of Henderson and Tilton (1955).

3. Results

3.1. Effect of MAs on newly-laid eggs at 20 ° and 34 °C

Egg mortality increased gradually with the increase of either exposure period or CO_2 concentration in air at 20 ° and 34 °C (Table 1). Eggs were more susceptible to MAs at 34 °C than those at 20 °C. At 34 °C, the egg mortality was more than 60% for all tested MAs after a 12-h exposure, but it was less than 10% for all MAs at the same exposure period at 20 °C. Egg mortality reached 100% for all tested MAs after a 120-h exposure (5 days) at 20 °C, but at 34 °C 100% mortality was recorded after a 72-h exposure (3 days) for both MA3 and MA4 while over 99% mortality was recorded for both MA1 and MA2. Table 2 shows the calculated LT_{50} and LT_{95} values, together with their confidence limits, for MAs at 20 ° and 34 °C. These LT_{50} and LT_{95} values, together with the toxicity lines (Figs. 1