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LETHAL EFFECT OF CO_2 MODIFIED ATMOSPHERES ON EGGS OF DIFFERENT AGE OF TWO BRUCHID SPECIES

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ABSTRACT

Stored legumes are attacked by a diversity of insect pest species of the family of Bruchidae. The most economically important and widespread species are the cowpea seed beetle, *Callosobruchus maculatus*, and the bean weevil, *Acanthoscelides obtectus*. These species laid their eggs loose (*A. obtectus*) or glued (*C. maculatus*) to the grain legume, and the emerging larvae burrow directly into the legume. The control of these pests relies mainly on the use of chemicals which are mainly directed to kill the egg stage. However, the need for repeated fumigations when eggs are present (at intervals of 25 to 30 days), that promotes the development of resistant populations, the low residue levels allowed in the final food products and the need to be environmentally friendly is making necessary to look for alternatives. Modified atmospheres (MA) with high carbon dioxide (CO_2) content are safe and environmentally friendly pest control methods for stored products. They are effective in controlling a wide range of species, and they can be applied in a variety of food products without leaving any toxic residues. The present study aimed to evaluate the efficacy of MA with high CO_2 to control eggs of different developmental age from the two bruchid pest species mentioned. Three concentrations of CO_2 MAs (50%, 70% and 90%) were tested at 28ºC, to identify the egg stage more susceptible to the treatments. Eggs sensitivity to the MA varied according to their age and species, with the 4 days old eggs of *A. obtectus* being the most tolerant of all eggs tested. A high level of control was achieved with 70% and 90% CO_2 during 3 days of exposure for all egg stages of both species.

Key words: modified atmospheres, carbon dioxide, *Acanthoscelides obtectus*, *Callosobruchus maculatus*, legumes, storage.

INTRODUCTION

Among the dry grain legumes, chickpeas are the second largest commodity in the world after dry beans. *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is the major pest of chickpeas. The infestation can start in the field, when the pods are dry, and continue in the storage, where it causes extensive damage to healthy or whole grains (Durón et al., 2005). The females attach their eggs to the surface of the seeds using a gelatinous glue-liked material. The eggs are milky white, oval, and dome-shaped with a flat floor. From the eggs hatch a larva that burrows into the grain where it develops to adulthood (Cope and Fox, 2003; Shazali et al, 2004).

Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) is one the main pest of dry beans and it also spend most of its development time inside the grain legume. The female lays their eggs inside the pods in the field or on the beans when in storage. Eggs are laid randomly under seeds and batches of eggs can be attached between them only slightly, if at all. First-instar larvae have legs and move around to find a suitable site to enter the seed (Papachristos and Stamopoulos, 2002).

The chemical control of *C. maculatus* and *A. obtectus* is currently based mainly in the use of fumigants in a preventive manner. Quality control is very strict as the legumes must not have eggs or insect-damaged kernels. For example, in Mexico for the export market, legumes are fumigated with hydrogen phosphide (PH_3) usually at intervals of 25 to 30 days. The number of applications of gas depends on the storage time, which sometimes may extend to a year or more depending on the international market prices. Modified atmospheres (MAs) based on high carbon dioxide (CO_2) are effective alternatives to the use of traditional chemical control (methyl bromide, phosphine and other insecticides) as pest control strategy. This is a cleaner environmental technology and toxicologically safe. Its cost is affordable in comparison to other control techniques to treat bags stored in different types of flexible airtight structures.

The objective of this study was to assess the susceptibility of eggs of this two pest species to three high CO_2 MAs. We evaluated the effect on eggs of different developmental status because they can show a different susceptibility to toxics, and it is important to determine at which age the eggs are more resistant.

MATERIAL AND METHODS

Insect colonies and experiments were performed in a climatic chamber at 28±2ºC, 70±15% r.h., and with a photoperiod of 16:8 hours of Light:Dark.

Stock colonies of the two bruchid species were reared on standard diets (chickpeas for *C. maculatus* and kidney beans for *A. obtectus*). A subsample of 50 individuals was reared in 200 g of standard food diet in ventilated plastic cages, in order to daily collect newly deposited eggs. For *A. obtectus* three eggs were deposited inside a gelatin capsule and 5 capsules were deposited in a small ventilated cage, containing also 50 g kidney beans. For *C. maculatus* five chickpeas with a minimum of three eggs that are attached to the pulse were deposited in a small ventilated cage, containing also 10 extra chickpeas. Eggs of one to five days old were treated with the different gas mixtures.

The small ventilated cages were placed inside plastic bags (Cryovac BB4L) of 300 x 210 mm of size and 59 micrometer-thick. The plastic bags had barrier properties to O_2 and CO_2. The plastic bags were filled with the desired atmosphere, which was previously prepared using a gas mixer (Witt KM 100-3M/MEM), using a vacuum packaging machine (Multivac A 300/16). A gas analyzer (Abiss model TOM 12) was used to verify the CO_2 and O_2 contents inside the plastic bags. Gas levels were determined at the start and at the end of the exposure.

After exposure to the MAPs, the plastic bags were subsequently opened to release the modified atmospheres and the cages were removed from the bags. To check the effect of the treatments on eggs, the cages were kept in the climatic chamber for up to 10 days to allow the hatching of eggs.

Three different MAPs were tested: i) 50% CO_2, with a residual of 10% O_2 and a 40% balance of N_2; ii) 70% CO_2, with a residual of 6% O_2 and a 24% balance of N_2; and iii) 90% CO_2, with a residual of 3% O_2 and a 7% balance of N_2. Eggs of all ages from *A. obtectus* were exposed during 3 days and also, eggs of all ages from *C. maculates* during 2 and 3 days to the treatments. Five replicates with 15 eggs each were prepared for all treatments and species. Sets of control cages with the same number of eggs were tested for the different treatments applied in order to determine the percentages of natural mortality.

RESULTS AND DISCUSSION

ANALYSIS OF GASES

The CO_2 contents within the sealed plastic bags during exposure to modified atmospheres remained quite constant throughout the period of exposure. For all three gas mixtures, CO_2 decreased less than 3% to 8% of the initial content. The O_2 levels in the sealed bags increased less than 1% to 3% of the initial content.

EGG MORTALITY

Comparison between species.
Figure 1 shows the mortality from the eggs of different age of both species exposed to the three MAs during 3 days. All eggs from the different age stage of *C. maculatus* were killed at these conditions. In comparison, while very few eggs of *A. obtectus* survive at 90% CO_2, more eggs survive at 70% CO_2 and even more at 50% CO_2. Therefore, the eggs of *C. maculatus* were more sensitive to the MAs tested.

Comparison among egg developmental stages.
In *A. obtectus*, 4 days old eggs were the most tolerant to 50% and 90% CO_2 MAs. However, at 70% CO_2, eggs of 2, 3 and 4 days old were more tolerant than eggs of one and five days old (Fig. 1). Since at three days exposure 100% mortality was recorded for *C. maculatus* we tested a shorter exposure time of two days in order to assess the sensibility of the different egg ages (Fig. 2). For all three MAs, the 2 days old eggs were the most tolerant. Therefore, the susceptibility of the eggs according to their developmental stage seems to be different in each of the species tested. While for *A. obtectus* mature eggs (4 days) were more resistant, for *C. maculatus* younger eggs (2 days) were more resistant.

As expected, an increase in CO_2 concentration from 50% to 90% produced an increase in egg mortality. This was more evident observed in the case of *A. obtectus* than in *C. maculatus*.

These results indicate that the developmental stage of the eggs has to be considered when testing different strategies for controlling them, because the sensitivity varies according to their age and this also may change for different species. That is, not always mature eggs are more tolerant than young eggs or the opposite. In the present work we have tested two species from the same Bruchidae family, showing an opposite susceptibility pattern. These two species have also different strategies for laying their eggs; while *A. obtectus* located them freely near the pulses, *C. maculatus* glued them onto the surface of the pulse. These differences in egg laying strategies might be related with a difference in the permeability to gas exchanges, and then to susceptibility to be killed by CO_2.

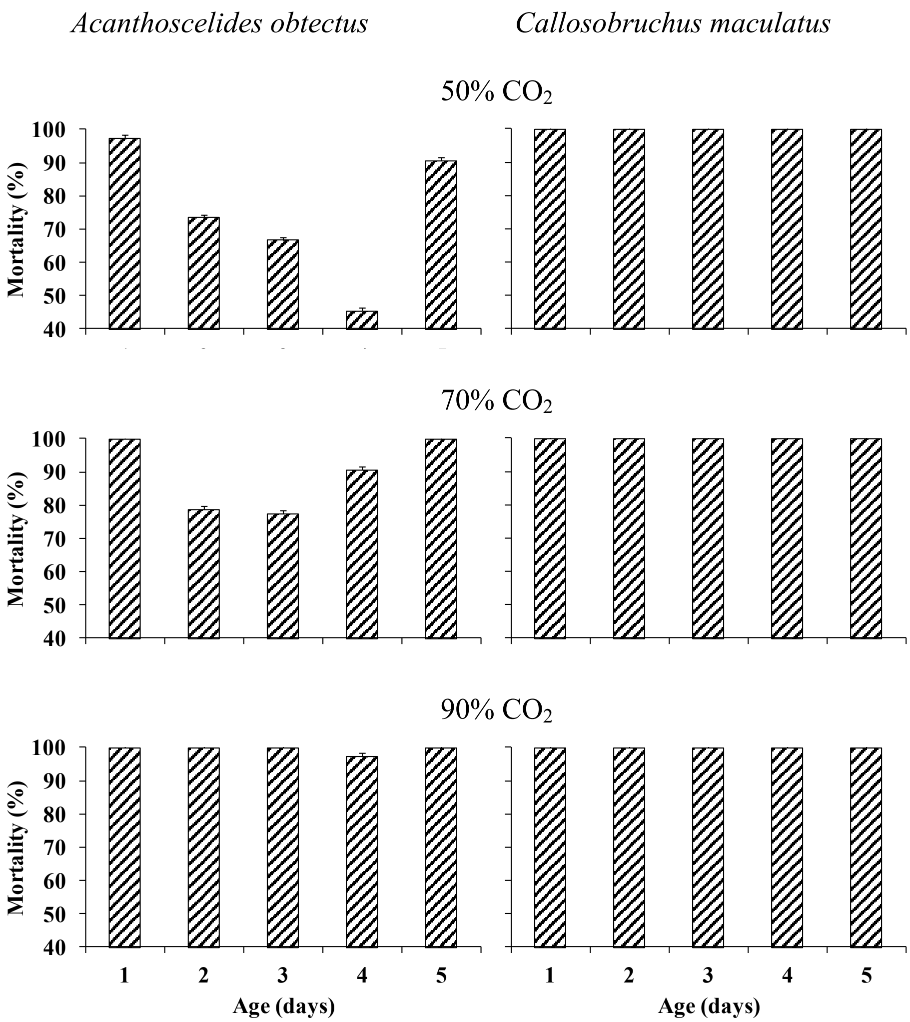

Fig. 1- Percent mortalities of *A. obtectus* (left) and *C. maculatus* (right) eggs of different age after exposure to various concentrations of carbon dioxide (CO_2) for 3 days at 28°C. n=5 of 15 eggs each.

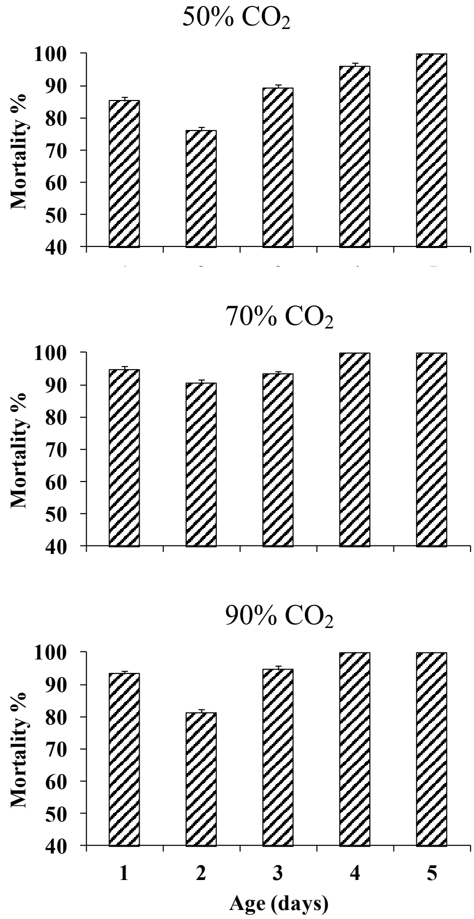

Fig. 2- Percent mortalities of *C. maculatus* eggs of different age after exposure to various concentrations of carbon dioxide (CO_2) for 2 days at 28°C. n=5 of 15 eggs each.

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