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Lethal effects of CO_2-modified atmospheres for the control of three Bruchidae species

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ABSTRACT

Bruchid beetles are important pests of field and stored legumes, causing great economic losses. The main pest species are *Callosobruchus maculatus*, *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. Modified atmospheres (MAs) with high carbon dioxide (CO_2) content are environmentally friendly pest control methods for stored products. They are effective in controlling a wide range of species and can be used to treat different food products without leading to an accumulation of toxic residues. The present study aimed to establish the efficacy of using MAs with high CO_2 to control all developmental stages of these three bruchid pest species. Three high CO_2 MAs (50%, 70% and 90%) were tested at 28 °C. In general, pupae and/or eggs were the most tolerant stages to hypercarbia, while adults were less tolerant. The susceptibility of eggs varied greatly according to their phase of development and the pattern was different in the three species tested. Tolerance of other stages to CO_2 also varied among the three bruchid species. To achieve total mortality of *Z. subfasciatus*, a maximum of 9–11 days were needed, depending on CO_2 concentration, to kill the eggs. This stage and the pupae were the most tolerant stages. However, in *A. obtectus* and *C. maculatus*, only pupae were the most tolerant and required 9 days to be killed, their eggs being more sensitive to CO_2.

1. Introduction

Legumes have great economic importance in Latin America and are usually stored for months after harvest until reaching the consumer. Sometimes, when the price of the product is low, as often occurs with chickpeas, the storage of grain can be extended for more than a year. During this prolonged time, the legumes are susceptible to attacks from arthropod pests that affect their quality and therefore, their marketing value (Singh and van Emden, 1979). Bruchid beetles (Coleoptera: Bruchidae) are important pests of field and stored legumes, causing great economic losses. For example, losses up to about 35% have been reported in Mexico and Central America, 13% in Brazil and 7.4% in Colombia (van Schoonhoven and Cardona, 1986). Any damage caused by these beetles or even the presence of their eggs on legumes is easily visible by the consumer and traders because it affects the visual aspect of the legumes, their odour and palatability. Therefore, they are rejected for purchase. Additionally, significant reduction of the nutritional quality, increased weight loss and significant commercial losses occur in the affected grains due to the presence of insect fragments and remains (Hohmann and Carvalho, 1989).

The predominant pest species are the cowpea bruchid *Callosobruchus maculatus* (Fabricius), the Mexican bean beetle *Zabrotes subfasciatus* (Boheman) and the bean bruchid *Acanthoscelides obtectus* (Say). The cowpea bruchid, *C. maculatus*, is a major pest of stored grain chickpeas and cowpeas, and one of the most widespread species of the genus *Callosobruchus*. It is found wherever its hosts are grown and stored, particularly in tropical and warm temperate regions. The Mexican bean weevil, *Z. subfasciatus*, is one of the main pests of stored beans, causing extensive qualitative and quantitative losses in grains and seeds, mainly in the warmest regions of the world. *Acanthoscelides obtectus* is another of the most important pests of stored beans all over the world (Dendy and Credland, 1991; Giga and Smith, 1997; Sari et al., 2003). Infestation by the three species can start in the field, when the seeds are ready for harvest, and proliferation continues in storage. Females of *C. maculatus* and *Z. subfasciatus* attach their eggs to the surface of the seeds using a gelatinous glue-like material (Shazali, 1990). On the contrary, females of *A. obtectus* lay batches of eggs randomly under the seeds (Umeya and Kato, 1970).

Control of the above three species is currently based on the use of fumigants. For example, in Mexico, legumes are fumigated with

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hydrogen phosphide (PH_3), usually at intervals of 25–30 days, to meet the requirements of the export market. However, the long storage period results in a high number of fumigations that lead to an accumulation of chemical residues in the grain, encourages the appearance of resistant weevil populations and increases costs for the grower. In addition, the number of other synthetic insecticides has recently decreased and options for using those remaining are being greatly restricted, mainly because of their adverse effects on the environment (WMO, 1995) and the development of resistant insect populations (Bell et al., 1977; Nakakita and Winks, 1981). Therefore, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly.

Modified atmospheres (MAs) have been used to disinfest raw or semi-processed food products such as cereal grains and dried fruits while still in storage. Treatments based on reduced oxygen (O_2) and high carbon dioxide (CO_2) or nitrogen (N_2) contents have been tested in the laboratory and under industrial conditions. They are technically and economically suitable alternatives to fumigation for arthropod pest control in durable commodities in a number of countries (Fleurat-Lessard, 1990; Adler et al., 2000). The feasibility in subtropical countries such as Mexico, an important producer of beans, should be investigated. At 27 °C atm rich in CO_2, with more than 40% in air, were faster at controlling pests such as the maize weevil than those with high contents of N_2 (Navarro, 2006). Carbon dioxide has a toxic effect on arthropods and also acts as an inert gas that reduces the O_2 level below that needed to support life. In insects CO_2 poisoning affects the nervous system, the endocrine system, the respiratory and circulatory systems, and general metabolism. Among others, pure CO_2 has an inhibitory effect on the bioelectrical responses of the nervous system (Nicolas and Sillans, 1989). Moreover, in many insects it causes the permanent opening of the spiracles, which induces water loss and may cause mortality. Water loss is higher at high CO_2 concentrations and more pronounced at low relative humidity (r.h.) (Jay et al., 1971). 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 conditions (Banks and Annis, 1990; White et al., 1995; Riudavets et al., 2009, 2010). In gas-tight chambers or silos, CO_2 treatment may take several days to various weeks to be effective, depending on the temperature.

The present study investigated the effect of high CO_2 MAs on the three bruchid species most commonly attacking legumes, *C. maculatus*, *Z. subfasciatus* and *A. obtectus*. More specifically, we evaluated the exposure times needed to kill eggs, larvae, pupae and adults with atmospheres based on 50, 70 and 90% replacement of air with CO_2.

2. Material and methods

2.1. Insect colonies

Stock colonies were maintained and experiments performed in a climatic chamber at 28 ± 2 °C, with 70 ± 15% r.h. and a photoperiod of 16:8 h of light and dark. The three bruchid species were reared on their standard diets: *C. maculatus* with chickpeas, and *A. obtectus* or *Z. subfasciatus* with kidney beans. To collect newly-deposited eggs daily, 50 adults were maintained on 200 g of each standard diet in ventilated plastic cages. After a variable number of days, eggs of known age, larvae, pupae or adults were obtained for treatment with the MAs.

2.2. Mortality of the different developmental stages

Eggs that were one, two, three, four and five days old were separately treated. To do so, different strategies were followed depending on the species considered. For *A. obtectus*, three loose eggs were deposited inside a gelatin capsule, and 5 of these capsules were deposited into a small ventilated cage containing 50 g of whole kidney beans. For *C. maculatus* or *Z. subfasciatus*, five chickpeas or five beans with a minimum of three eggs attached to the pulse were deposited into a small ventilated cage (7 cm diameter × 4 cm high), also containing 10 extra grains. Extra grains were added to allow the absorption of some portion of the CO_2 by the legumes, as would occur in a practical situation. To treat larvae or pupae, a similar methodology was used as with the eggs, but in this case, the eggs of each species were incubated for a variable period of time until reaching the desired stage: 7–9 days for 1st instar larvae; 11–13 days for 2nd instar larvae; 16–18 days for 3rd instar larvae; or 21–23 days for pupae, these times being suitable for all three species at the test conditions. To treat adults, 15 3-day-old individuals of each species were placed in small ventilated cages (7 cm diameter × 4 cm high) with 20 chickpeas in the case of *C. maculatus* or with 20 beans in the case of *A. obtectus* or *Z. subfasciatus*.

These small ventilated cages containing bruchids were individually placed inside plastic bags (Cryovac BB4L; 300 × 210 mm size; 59-μm thick) that had barrier properties to O_2 and CO_2. Before sealing, the bags were filled with the desired MA, which was previously prepared using a gas mixer (Witt KM 100-3M/MEM). A gas analyser (OXYBABY®, www.wittgas.com) was used to verify the CO_2 and O_2 contents inside the plastic bags, and gas levels were determined at the start and end of treatment. The plastic bags were subsequently opened to release the modified atmospheres and cages were removed from the bags. To check the effect of the treatments on adults, the number of living individuals was assessed 24 h later. To check the effect of the treatments on eggs, cages were kept for up to 10 more days in controlled conditions to allow their hatching. Hatched eggs of *C. maculatus* and *Z. subfasciatus* were determined by a change in their colour to creamy. Unhatched eggs were generally transparent, shrivelled and wrinkled. Hatched eggs of *A. obtectus* were identified by the presence of the empty chorion. To check the effect of the treatments on larvae and pupae, cages were kept until the adult emerged.

Three different MAs 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. To assess which was the most tolerant egg age to treatments, eggs were first exposed for 3 days. The exposure time was reduced if mortality was above 90% for all the ages tested. A new set of tests was performed with the most tolerant egg age of each species to determine the time needed to cause 50 or 99% mortality. Larvae, pupae and adults were exposed to MAs for the time required to reach total mortality.

Five ventilated cages each with a minimum of 15 eggs, larvae, pupae or adults were used per treatment. Sets of control ventilated cages with the same number of individuals were maintained in controlled conditions to determine the natural mortality of each developmental stage evaluated.

2.3. Data analysis

Percentages of egg and adult mortality were calculated using the initial number of individuals placed in each cage. In the case of larvae or pupae, the mean number of emerged adults in the control treatments was used as the initial number of individuals when calculating the mortality rate. To determine the most tolerant egg age to treatment, an analysis of variance (ANOVA) followed by the Tukey's test for means comparison was performed for each CO_2 concentration and species tested. Treated eggs from the most tolerant age were analysed by Probit analysis (Poloplus, LeOra