Comparative toxicity of carbon dioxide to two *Callosobruchus* species

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Abstract

Mortalities of eggs, larvae, pupae and adults of two bruchids, *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic), exposed to an inert atmosphere of 100% carbon dioxide were observed at 32°C, 70% r.h. 100% mortality of the eggs and adults of both species. All young larvae of both species died within an exposure period of 48 hours. The older larvae required an exposure period of 72 hours for 100% mortality to be achieved. The pupal stage was the most tolerant stage to carbon dioxide and exposure periods of 5 and 6 days, respectively, were required for 100% mortality of *C. maculatus* and *C. subinnotatus*, respectively.

Introduction

In west African countries, and indeed most other subtropical and tropical countries, grain legumes which are the most important sources of proteins are attacked by two bruchids, *Callosobruchus maculatus* (Fab.) and *Callosobruchus subinnotatus* (Pic). *C. maculatus* is a major pest of stored cowpea, *Vigna unguiculata* L. in these regions while *C. subinnotatus* is a major pest of bambarra groundnuts, *Vigna subterranea* (L.) Verde (Jackai and Daoust 1986; Mbata 1991). The damage is done by the larvae feeding inside the seeds. Severe infestation can lead to losses in weight of up to 30% and render the commodity unfit for human consumption.

Chemical control of bruchids by dusting with insecticides such as pirimiphos-methyl, fenithrothion and synthetic pyrethroids or by fumigation with phosphine and methyl bromide is effective (Cardona and Karel 1990). However, problems associated with the use of chemical insecticides, such as development of resistance by insects, environmental pollution, contamination of grains, etc. are inducing scientists to look for other ways of controlling insect pest populations. Controlled atmosphere storage offers a safe, residue-free method for protecting grains from insect pests (Bailey and Banks 1980). Ofuya and Reichmuth (1992) and Mbata and Reichmuth (1994) have investigated the toxicity of inert atmospheres to *C. maculatus* and *C. subinnotatus*. The present report compared the toxicity of an atmosphere of 100% CO_2 to the eggs, larvae, pupae and adults of the two bruchids.

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Materials and Methods

Eggs, larvae, pupae and adults of both *C. maculatus* and *C. subinnotatus* used in this study were obtained from cultures of the insects maintained at 30°C and 70% r.h. *C. maculatus* was reared on blackeye cowpea while bambarra groundnut was used in rearing *C. subinnotatus*.

The developmental stages tested were as follows: eggs (ca. 2 days old), young larvae (5–9 days old), old larvae (14–17 days old), pupae (1–3 days old) and adults (ca. 24 hours old). The experiments were set up separately for both *C. maculatus* and *C. subinnotatus*.

To obtain eggs of the Bruchids on seeds, 10 pairs of the adults were placed in 500 mL jars containing about 300 cowpea seeds for *C. maculatus*, or bambarra seeds *C. subinnotatus*. These were allowed 24 hours to lay eggs on the seeds. Seeds with egg load of 3 or 4 eggs per seed were selected. Fifty eggs on seeds or fifty larvae or pupae in seeds or 20 adults were placed in separate wire cages of dimensions 5 cm long and 1.5 cm diam. The wire cages were closed with rubber stoppers and exposed to 100% CO_2. The CO_2 was delivered from a pressurised cylinder and the insects were exposed to the gas in gastight connected dressel flasks. The exposure period was from 1–4 days at 32°C. The treatments for each of the stages of the insects were replicated three times and there were three trials for each treatment. Replicated controls were also set up for each of the stages of the insects.

For the adults, mortality was checked 24 hours after the exposure period, sufficient time for insects anaesthetised by the intoxicating effect of the CO_2 to recover. For the eggs, larvae and pupae, the treated seeds were placed in separate Petri dishes and sufficient time allowed for adult emergence at 30°C. Percentage mortality in each stage was calculated and corrected using Abbott's (1925) mortality formula. The mortality values for the different species were compared using two-way analysis of variance.

Results and discussion

There were significant differences in the exposure periods required to obtain 100% mortality among the developmental stages of the bruchids exposed to 100% CO_2 (Table 1). The eggs and the adults were most susceptible: 100% mortalities were recorded in these two stages within 24 hour exposure periods (Figs 1 and 2). These results support earlier observations by Ofuya and Reichmuth (1992) and Mbata and Reichmuth (1994).

The susceptibilities of the larval stages of the bruchids are shown in Figure 3. When the first larval stage was exposed to the inert atmosphere, 100% mortality was recorded after 2 days of exposure. The last larval instar required a longer exposure period of 4 days for 100% mortality to be obtained. The pupae were the most tolerant stages and required exposure periods up to 5 and 6 days for 100% mortality to be

Table 1. Summary of ANOVA for the mortality of different stages of *C. maculatus* and *C. subinnotatus* exposed to 100% CO_2 at 32°C

Source of variation	df	Mean square at different exposure periods (hours)				
		24	48	72	96	144
Insect stage (A)	4	10509.2**	10052.4**	9788.4**	5944.1**	1325.6**
Insect type (B)	1	27.1	19.3	15.3	18.0	15.7
Interaction (A × B)	4	22.5	16.2	12.7	10.6	9.7
Error	20	18.5	17.0	19.3	15.7	16.3
Total	29					

**Significant at $P < 0.01$.

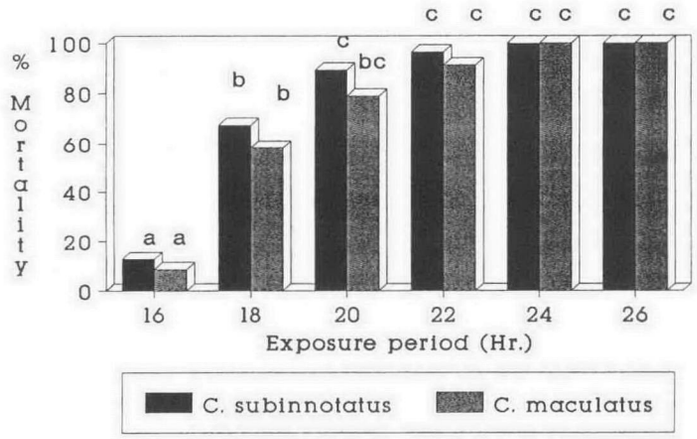

Fig. 1. Mortality of eggs of the two bruchids exposed to 100% carbon dioxide (values bearing different letters are significantly different, $P < 0.05$).

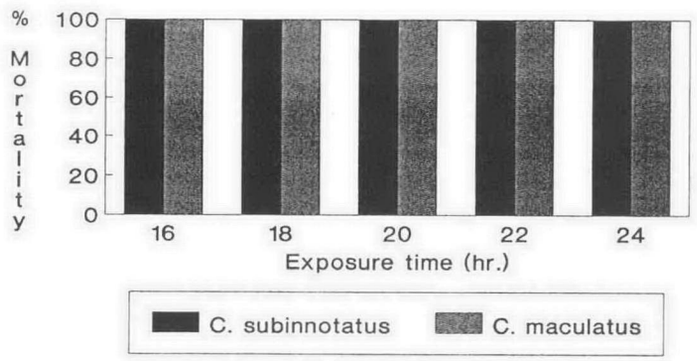

Fig. 2. Mortality of adults of the two bruchids exposed to 100% carbon dioxide (values bearing different letters are significantly different, $P > 0.05$).

recorded in *C. maculatus* and *C. subinnotatus*, respectively (Fig 4).

Oosthuizen and Schmidt (1942) found that the eggs and the adults of *Callosobruchus chinensis* (L), a species related to those used in the present study, were more susceptible than older larvae and pupae when exposed to an atmosphere of CO_2. The high tolerance of the pupae to inert atmosphere might be attributable to the reduced metabolism in the pupae compared with the other stages.

There was no significant difference between the exposure periods required to obtain 100% mortality of similar stages of the two bruchids. Bambarra seeds infested by the two bruchids can therefore be disinfested by exposing them to 100% CO_2 for 6 days. This is in line with the recommended exposure period of 15 days for disinfesting grain with high CO_2 at commodity temperature of 25–29°C (Banks et al. 1990).

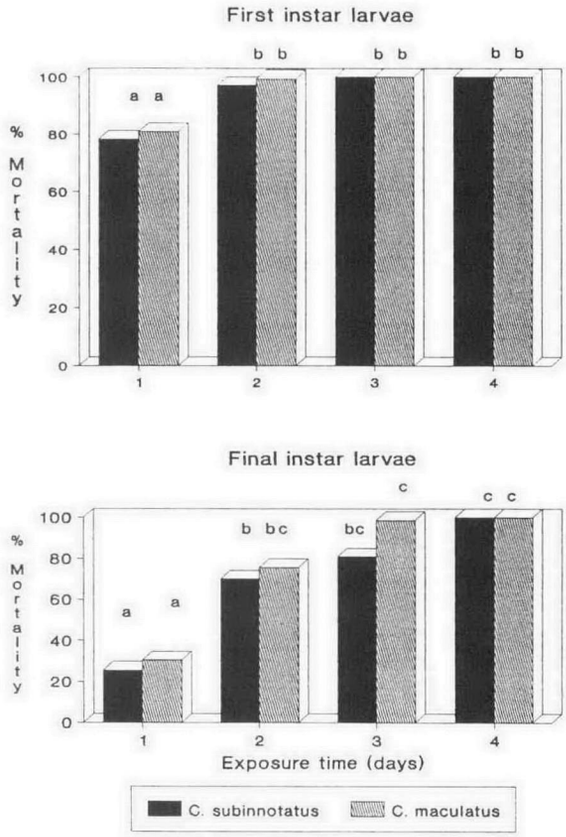

Fig. 3. Mortality of larvae of the two bruchids exposed to 100% carbon dioxide (values bearing different letters are significantly different, $P > 0.05$).

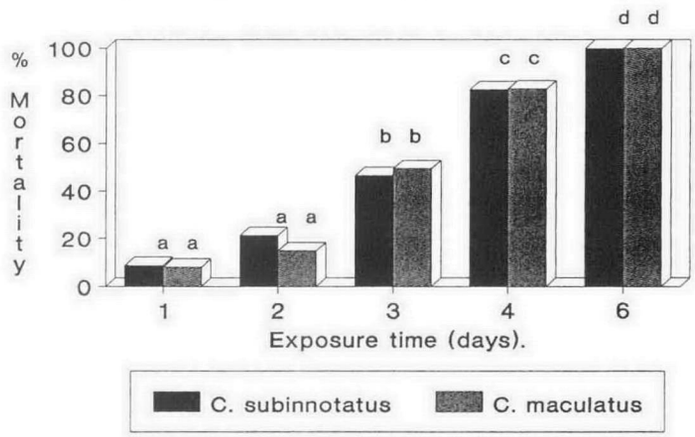

Fig. 4. Mortality of pupae of the two bruchids exposed to 100% carbon dioxide (values bearing different letters are significantly different, $P > 0.05$).

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