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Alternative Protection of Cowpea Seeds Against *Callosobruchus maculat us* (F.) (Coleoptera: Bruchidae) using Hermetic Storage alone or in Combination with *Boscia senegalensis* (Pers.) Lam *ex* Poir

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Abstract

The effect of hermetic storage alone or in combination with *Boscia senegalensis* has been evaluated against *Callosobruchus maculatus.* Analysis of gas concentrations within a 7-day period indicated that O_2 declined from 19.2 to 2.3% and CO_2 rose from 1.2 to 22.8%. Prolonged storage durations increased adult mortality, significantly increased the developmental time and induced 60-80% reduction in the F1 progeny. The use of hermetic storage in combination with *B. senegalensis* fruits, at 1.2 g/1 (flask volume) reduced the emergence of the cowpea beetle, while 2.4-4.8 g/1 completely inhibited the production of a new generation of C. *maculatus.*

Key words: *Callosobruchus maculatus, Vigna unguiculata,* hermetic storage, *Boscia senegalensis.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) seed is a cheap source of protein (Labeyrie, 1981) and is a valuable food in West African countries. Unfortunately, this crop is prone to heavy post-harvest damage by *Callosobruchus maculatus* (F.), commonly known as the cowpea beetle. Caswell (1973) reported on very significant losses due to C. *maculatus* in Nigeria. In Senegal, Seek *et al.* (1991) estimated that up to 90% of stored cowpeas can be damaged after 6 months. In addition, infestation by the cowpea bettle reduces the seed quality and germination ability to under 20% after 4 months (Seek, unpublished results).

The control of C. *maculatus* in developing countries relies heavily on the use of imported chemicals. This dependence on synthetic insecticides is not only a drain on the farmer's limited resources but may lead to many environmental and social side-effects previously well documented (Huignard, 1985; Egwuatu, 1987). On the other hand, insect resistance to one or more insecticides is a growing problem (Georghiou, 1990). This situation has increased the need for less expensive and safer strategies, among which is the use of natural products and hermetic storage.

Studies reported on hermetic storage have shown the technique to be effective against many insect species (Oxley and Wickenden, 1963; Bailey, 1965; Pattinson, 1969; Aliniazee, 1971; O'Dowd, 1971). However, the exposure time required for complete insect control may limit the use of hermetic storage alone, when a high seed quality is required. In the present work we investigated the use of hermetic storage in combination with a natural insecticide from *Boscia senegalensis. B. senegalensis* is a shrub which grows throughout the sahelian regions. It is distributed from Mauritania to Niger, northern Nigeria, the northwest Cameroon and across Africa to Sudan and Ethiopia (Booth and Wickens, 1988). The fruits are used as a famine food in Senegal and Sudan (Becker, 1986; Salih, Nour and Harper, 1991). The leaves are used by many farmers for grain protection. As nothing was known about the biological and chemical backgrounds of this practice, we initiated a programme focusing on these aspects. This research aimed to establish the TL_{50} and CL_{50} values to demonstrate the toxic effect of *B. senegalensis* on *C. maculatus* and four other stored-grain insects (Seek *et al,* 1993; Seek, 1994). The same authors identified that insecticidal activity of *B. senegalensis* is due to the liberation of volatile methylisothiocyanate (MITC) from a glucosinolate precursor contained in plant tissues. These results suggested a practical use of *B. senegalensis* in combination with hermetic storage.

MATERIALS AND METHODS

Three experiments were performed on adults and immature stages of *C. maculatus* (CM). Insects were placed in hermetic atmospheres, with exposures ranging from 1 to 7 days. The insects were reared on cowpea seeds (variety CBE5) under controlled conditions of 30 ±2°C and 80 ± 10% r.h. The varieties, temperature and relative humidities were used for all the trials which were replicated four times.

In the first experiment, 50 unsexed freshly emerged adults were confined in 210 ml glass jars containing 30 g of healthy cowpea seeds. Seeds (30 g) heavily infested with CM larvae (7 days old) and 30 g of non-infested seeds were separately placed in the same conditions.

In the second experiment, 200 CM adults (1 day old) were confined in 850 ml glass jars containing 121 g of cowpea seeds. Similarly, 121 g of non-infested seeds were placed in the same containers. All the jars were hermetically closed using airtight metal caps, for exposures of 1 to 7 days.

Oxygen (O_2) and carbon dioxide (CO_2) concentrations inside the flasks were measured by gas chromatograpy on a Carlo Erba Fractovap 2350 apparatus using two types of stainless steel packed columns:

O_2 determination: Porapak Q (60-80 mesh), 1 m length, 0.125 inch i.d. CO_2determination: Molecular sieve (5Å), 1.75 m length, 0.125 inch i.d.

In both cases, the carrier gas was argon at 30 ml/min flow rate. Injector, oven and catharometric detector (operated at 110 mA) were maintained at room temperature. Gas atmospheres (300 µl) were sampled at 24 h intervals by inserting the needle of a Hamilton 1 ml gas-tight syringe into the jar through a rubber septum and directly injecting into the chromatograph. The chromatographic peak areas were measured automatically with a Hewlet-Packard HP 9122C integration system. Amounts of O_2 and CO_2 were determined (in percentages by volume) by comparing and extrapolating the data obtained with a reference gas calibrated at $5.06 \pm 0.1\%$ CO_2 in extra pure air (21% O_2). After each O_2 and CO_2measurement, the corresponding jar was opened to air and reclosed with a perforated lid. Immediately, it was returned to the rearing chamber until the Fl adults began to emerge. Insects were then removed daily and counted for two weeks. The duration of development was assessed and the percentage of adults which emerged was computed.

The third experiment was conducted using hermetic storage in combination with fresh *B. senegalensis* fruits. The plant material was harvested in the region of Thiès (Sénégal). A sample has been deposited at the Jardin Botanique National de Belgique (BR). Samples of 250 g of cowpea seeds were placed in 850 ml glass jars and thoroughly mixed with fresh *B. senegalensis* fruits, at concentrations ranging from 1.2-4.8 g/1 (flask volume). Twenty-five unsexed CM adults (1 day old) were then placed into the flasks which were immediately sealed with hermetic caps, for an exposure of 69 days. Similarly batches of 250 g of untreated seeds were infested and placed in the same conditions as the treated ones. From 69 days after infestation (DAI), the F1 populations was counted in each jar and the percentage reduction of emergence was calculated in comparison with the control treatment.

RESULTS

The curves in Figs 1(A), (B) and (C) indicate the O_2 depletion and CO_2 build-up in the 210 ml jars. After 1-7 days of airtight storage, O_2 and CO_2 concentrations in flasks containing healthy seeds were almost constant around 21 and 0%, respectively (Fig. 1A). For seeds infested with larvae, O_2 concentration varied from 6.5 to 0%, and CO_2 concentration from 19.2 to 22.8% (Fig. 1B). In the same time, gas concentrations for seeds infested with adults were 16.5 to 2.3% for O_2 and 3.6 to 15.2% for CO_2 (Fig. 1C). The profile of gas concentrations in 850 ml jars is presented in Figs 1(D) and (E). Within the 7-day period, O_2 and CO_2 concentrations in control jars were, respectively, 21 and 0% with little variation (Fig. ID). For seeds infested with adults, O_2 concentration varied from 19.2 to 2.2% and CO_2 from 1.2 to 15.1% (Fig. IE).

Data concerning the lethal effect of hermetic storage on CM indicate 91.1% adult mortality after 4 days and 100% from the 5th day (Fig. 2).

Results from Tables 1 and 2 indicate that hermetic storage significantly prolonged the developmental duration of the cowpea beetle and induced 32-100% reduction in the population that emerged from the sealed jars compared to aerated controls.

The observation of CM daily emergence patterns indicated that prolonged airtight durations reduced the amplitude and delayed the maximum of the curves (Figs 3(A) and (B)).

Data from Table 3 summarize the effect of hermetic storage in combination with *B. senegalensis* on CM emergence following an initial low infestation level of 1 adult per 10 g of seeds. By 69 days after infestation (DAI), 763 adults emerged from the control treatments, but only 300 adults emerged in the presence of *B. senegalensis* fruits at 1.2 g/1 of flask volume. At 2.4 and 4.8 g/1 levels, CM emergence was completely inhibited up to 82 DAI.

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***Fig. 1**. Evolution of oxygen and carbon dioxide concentrations in hermetic jars of 210 ml (A, B, and C) and 850 ml (D and E) containing cowpea seeds infested or not with larvae or adults of Callosobruchus maculatus.*

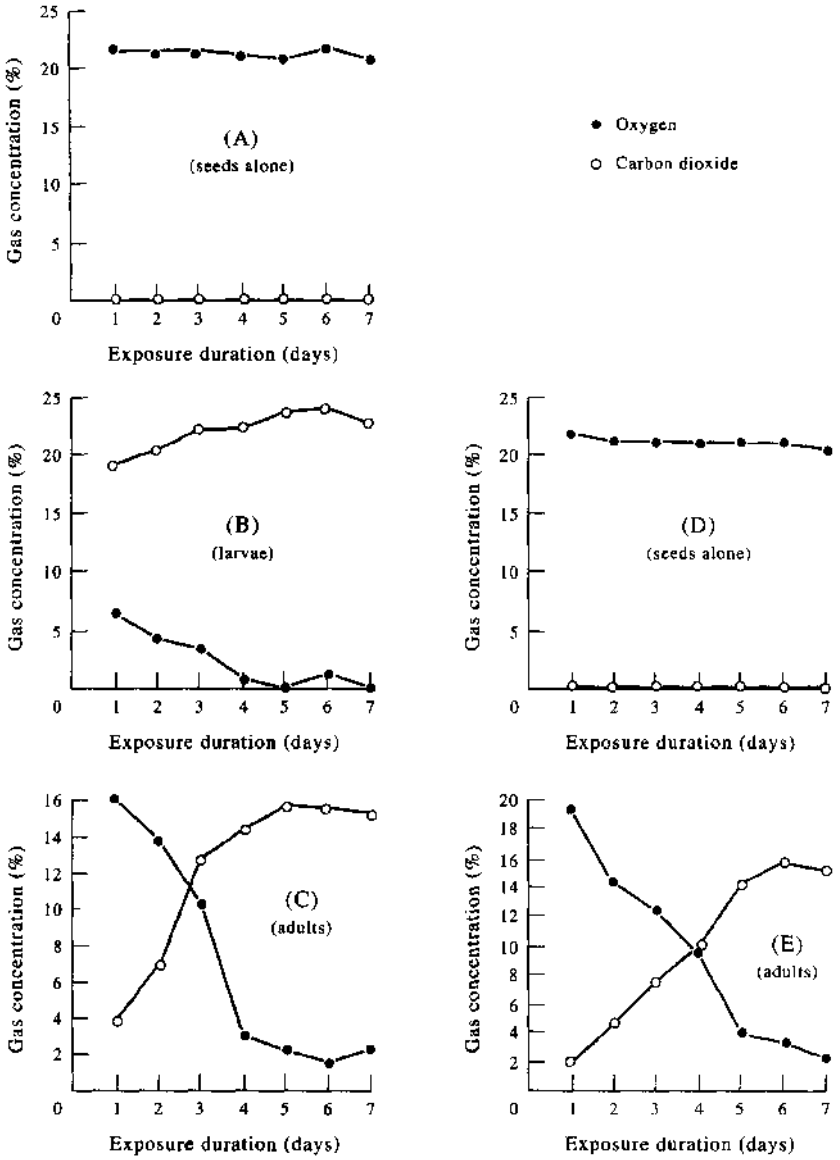

***Fig. 2.** Mortality of Callosobruchus maculatus adults exposed for prolonged durations in 210 ml hermetically sealed jars.*

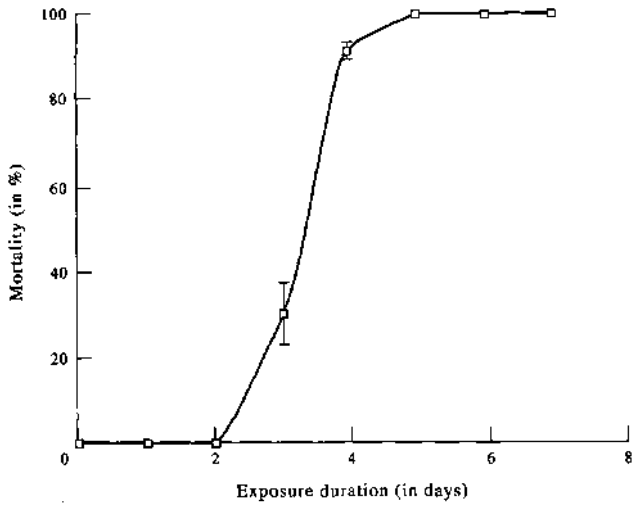

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***Fig. 3**. Emergence pattern of Callosobruchus maculatus Fis from infested cowpea seeds placed in 210 ml (A) or 850 ml (B) jars and hermetically sealed for various durations.*

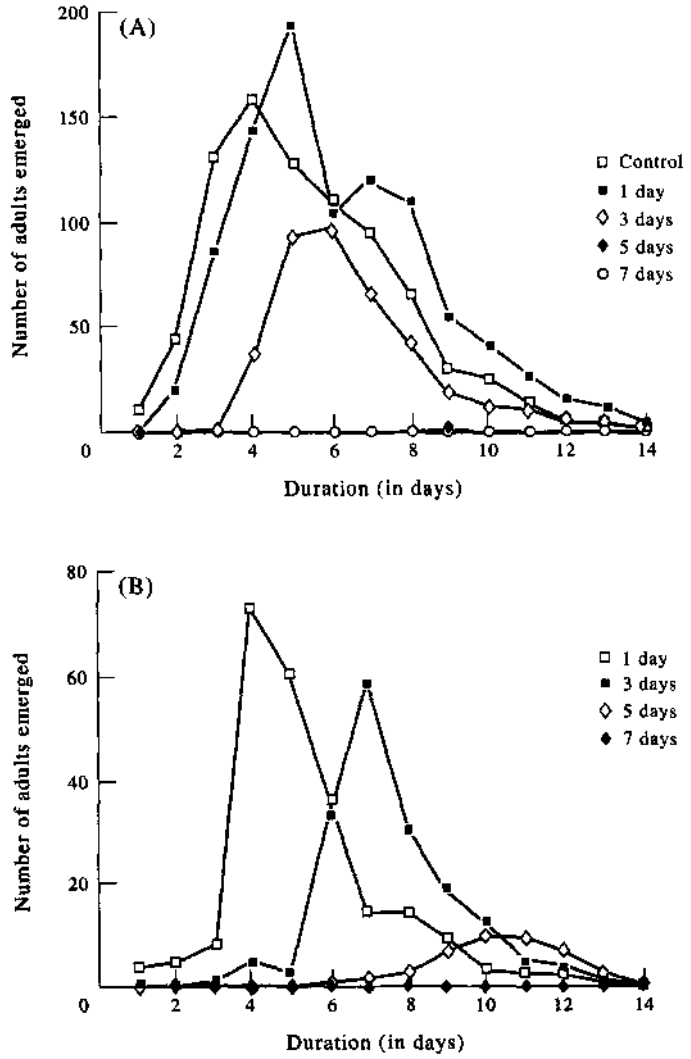

***Table 1**. Effect of hermetic storage in 210 ml (B210) or 850 ml (B850) jars on Callosobruchus maculatus Fl development following exposure of adults*

	B210			B850		
Exposure duration (days)	DD*	nE[b]	PR	DD[a]	nE[b]	PR[C]
0 (control)	18	829	—	20	2729	—
1	18	932	0	20	1846	32
2	18	858	0	20	1604	41
3	20	392	53	21	1241	55
4	23	56	93	21	1103	60
5	26	2	near 100	22	822	70
6	24	3	near 100	24	52	98
7	—	0	100	26	71	97

[a]Developmental duration (in days). [b]Total number of adults emerged.

[c]Percentage reduction in emergence (compared to control treatment)

***Table 2**. Effect of hermetic storage in 210 ml jars on Callosobruchus maculatus F1 emergence following exposure to larvae*

Exposure duration (days)	nE[a]	PR[b]
0 (control)	554	—
1	239	56.9
2	229	58.7
3	177	68.1
4	124	77.6
5	45	91.9
6	48	91.3
7	1	near 100

[a]Total number of adults emerged. [b]Percentage reduction in emergence (compared to control treatment).

***Table 3.** Effect of Boscia senegalensis in combination with hermetic storage on Callosobruchus maculatus F1 emergence*

Treatment	Cumulative numbers of adults emerged (% reduction)		
	69 DAP	72 DAP	82 DAP
Control	190.8 ±76.1	208.8 ± 77.7	306.8 ± 149.7
	(-)	(-)	(-)
1.2 g/1	75.0 ± 16.5	76.3 ± 16.5	96.0 ± 32.2
	(60.7)	(63.5)	(68.7)
2.4 g/1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	(100)	(100)	(100)
4.8 g/1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	(100)	(100)	(100)

[a]Days after infestation.

DISCUSSION

Results obtained from the three experiments clearly demonstrate the effectiveness of hermetic storage alone or in combination with *B. senegalensis.* Studies on hermetic storage alone, using jars of different volumes, indicated a gradual depletion of O_2 and an increase of CO_2 concentrations in relation to the duration of storage, the insect stage and the level of infestation. These results are in agreement with those of many other authors (Williams and Wilbur, 1968, 1969; Aliniazee, 1971; Ofuya and Reichmuth, 1994).

Under our experimental conditions, hermetic storage alone at a high infestation level gave near or complete disinfestation after 5 to 7 days. Subsequently, the duration of development was significantly extended and the emergence of a new generation was reduced or completely inhibited.

Storage of comparatively lightly infested cowpea samples (1 insect/10 g seeds), mixed with fresh ground fruits of *B. senegalensis* (at the level of 2.4 g/1) in hermetically sealed containers, achieved total control of both infestation and damage by the cowpea beetle.

These results give evidence indicating a potential use of the locally available tree *B. senegalensis* in combination with hermetic storage, as an alternative to synthetic pesticides in the sahelian zone of Africa.

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