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Combining *Boscia senegalensis* Lamarck (Capparaceae) Leaves and Augmentation of the Larval Parasitoid *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) for Bruchid Control in Stored Cowpeas

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Abstract: In West Africa, bruchid beetles are responsible for major losses of cowpeas during storage. Small-scale farmers from Burkina Faso often use leaves of a shrub, *Boscia senegalensis* (Lamarck) (Capparaceae), to protect stored cowpeas. The effectiveness of this practice combined with augmentation of the larval parasitoid *Dinarmus basalis* was investigated in two regions of Burkina Faso. Two bruchid species, *Callosobruchus maculatus* (Fabricius) and *Bruchidius atrolineatus* (Pic), emerged from cowpea seeds harvested in both regions of study but only *C. maculatus* developed during the whole period of cowpea storage. The Pteromalid *D. basalis* (Rondani) was the only larval parasitoid in the Bobo-Dioulasso region. In the Ouagadougou region, another larval parasitoid, *Eupelmus vuilleti* (Crawford) (Eupelmidae), appeared and competed with *D. basalis*. Interspecific competition between the two parasitoids species involved low levels of parasitism that favoured the increase in bruchid population. Augmentation of *D. basalis* adults during the first two months of storage reduced bruchid populations and reduced seed weight losses in both regions. The introduction of *B. senegalensis* leaves reduced bruchid numbers during the first three months of storage only, but the pest population drastically increased from this period onwards. The seed losses were finally higher than those of the control after six months of storage. The use of *B. senegalensis* influenced the interspecific relations between parasitoid species at Ouagadougou and could improve the biological control using augmentation of *D. basalis* adults. The results are discussed in a prospect of integrated pest management in stored cowpeas.

Key words: Bruchids, parasitoids, *Boscia senegalensis*, interspecific competition, IPM

INTRODUCTION

In the Sahel region of West Africa, the cowpea, *Vigna unguiculata* (Walper), is an important source of human dietary protein[1]. The storage of cowpea seeds is often very difficult because of losses caused by bruchid beetles[2,3]. In West Africa, *Callosobruchus maculatus* (Fabricius) and *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae) develop in cowpea seeds. Only two generations of *B. atrolineatus* develop in stores because adults of the second generation enter reproductive diapause and leave the granaries[4]. Successive generations of *C. maculatus* adults occur in stores with losses increasing rapidly with time[2,3,5].

At the farmer scale, it is very difficult to store cowpeas for more than 2-3 months because the low income of Small-scale farmers does not give them access to chemicals that also pose a potential health risks and economic losses. These farmers often introduce into their granaries plant products that are thought to have insecticidal properties[6]. Because little is known about the efficacy of these products and their role in cowpea protection, we investigated the role of one of the traditional products, *Boscia senegalensis* (Lamarck) (Capparaceae), in protecting cowpea stores.

In West Africa, several species of parasitoids are associated with bruchids populations in cowpea fields and stores[3,7,8]. Biological control of bruchids by

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augmentative releases of the larvophagous parasitoid *D. basalis* has been investigated in Burkina Faso[3,5]. This species controlled pest populations during six months of storage when its adults were introduced at the beginning of the storage, when the bruchid population was low[5].

The use of *B. senegalensis* in protecting cowpeas in storage is a widespread practice in Burkina Faso[9]. The leaves of this shrub release methylisothiocyanate, which is known to be toxic towards bruchids[10,11]. This compound may also be harmful to parasitoids and then limit effective biological control[12,13]. Furthermore, the outcome of the use of volatiles could vary according to the diversity of parasitic community observed in different zones of Burkina Faso[5]. Under such conditions it is important to determine the actual effects of the volatiles and their role in storage environment. In this study, the effects of *B. senegalensis* leaves on bruchid and parasitoid populations arising from field infestation were analysed in stored cowpeas in two regions of Burkina Faso.

MATERIALS AND METHODS

Location: This study was carried out in the sub-humid region of Bobo-Dioulasso and the sahelian dry region of Ouagadougou from November 1997 to May 1998. The climatic differences between these regions lay in temperature and relative humidity, which were most of the time respectively lower and higher in the Bobo-Dioulasso region throughout the year[3].

Cowpea pods of the local Moussa variety were collected at the end of the rainy season in 1997 from traditionally grown crops around Bobo-Dioulasso and Ouagadougou. They were then brought back to the laboratory, where the seeds were shelled and used for experiments.

Estimate of the initial level of bruchids and parasitoids populations: Six 500 g samples of cowpea seeds were took at random in each region of study and placed into separate Plexiglas boxes (18 by 11 by 3 cm) in local indoor ambient conditions. These samples were observed for 30 days and the emergence of insect adults was recorded daily after removing the seeds. This duration and the local conditions allowed the growth of the first generation of bruchids and associated parasitoids resulting from the insects' activity in the fields[3,14]. Bruchid and parasitoid numbers were then pooled to estimate the initial level of contamination of 3 kg of cowpeas in each region.

Effects of *B. senegalensis* leaves and/or augmentation of *D. basalis* adults on bruchid and parasitoid populations arising from natural infestation

Experimental treatments: Cowpea seeds were stored in batches of 3 kg in 50 L earthenware jars used in the local farming communities. The mouth of each jar was covered with a fine gauze cloth over which a waterproof cover was placed. In each region, these jars were stored below an open barn positioned directly to the sunlight with screens on all four sides, from 8 November 1997 to 7 May 1998. Bruchid and parasitoid populations were monitored in four treatments, each replicated twice at Bobo-Dioulasso and Ouagadougou; (1) In the Control jars, cowpea seeds were placed in the absence of any protection measure against bruchids. (2) In the *D. basalis* jars, two hundred couples of two-day-old *D. basalis* adults were introduced every 30 days during the first two months of storage. (3) In the *B. senegalensis* jars, five hundred grams of crushed *B. senegalensis* leaves were introduced at the beginning of the storage period. (4) In the *B. senegalensis* + *D. basalis* jars, five hundred grams of crushed *B. senegalensis* leaves were introduced at the beginning of the storage period and two hundred couples of two-day-old *D. basalis* adults were monthly added during the first two months of storage.

The adults of *D. basalis* used for these experiments were descendants of insects that emerged from cowpeas harvested in each region and mass-reared in laboratory conditions with *C. maculatus* as host, as previously described elsewhere[3,6].

Parameters determined: The size of bruchid and parasitoid populations was estimated every 15 days from the numbers of dead adults, using the techniques described elsewhere[3]. The jars were emptied and the seeds removed to identify and count the dead adult insects. Seeds and living insects were then put back into the jars. The number of adults of each species was counted at each observation and the data were summed at the end of the study. The intrinsic rate of natural increase, r, of the population of each species was determined according to the formula[15]:

$$r = (1/t)\,\mathrm{Ln}\,(R_0)$$

Where, t is the duration of the storage period considered and R_0 is the basic reproductive rate of the population. The reproductive rate was determined by the formula N_t/N_i where N_i was the initial number of insects present in the jars at the beginning of the storage time and N_t was the number of dead adults counted during the period. The reduction in number of bruchids was estimated by comparing the number of adults counted from jars receiving treatments with numbers obtained from control jars.

Rates of parasitism were estimated by comparing numbers of parasitoids in each jar with total number of insects (bruchids and parasitoids) counted in this jar.

Weight losses of seeds were also estimated at the end of the study. Six samples of 100 seeds were collected at random from each jar and weighed. Their weight was compared with the weight of six samples of healthy seeds stored in the same local ambient conditions. Weight losses in grams per kilogram of seed were then calculated using the formula:

$$(WH-WC/WH)\ 100$$

Where, WH is the weight of the healthy seeds and WC is the weight of contaminated seeds for each set of experimental condition.

Statistical analysis: Data were submitted to multiple analysis of variance (MANOVA) using the STATVIEW software (SAS Institute 92-98). Treatments were considered as factor and locality as independent variable. When the analysis probability was significant, a PLSD Fisher test was used to separate means. Groups were regarded as different if the tests provided discrimination at the 5% level.

RESULTS

Numbers of bruchids and parasitoids at the beginning of storage in both sites: Two bruchid species, *Callosobruchus maculatus* and *Bruchidius atrolineatus*, emerged from cowpeas harvested in both regions of study (Table 1). *D. basalis* was the only larval parasitoid species present at Bobo-Dioulasso. On the opposite, the parasitic community included *D. basalis* and *E. vuilleti* in the Ouagadougou region. The rates of parasitism were then estimated to 8.9% at Ouagadougou and 19.6% at Bobo-Dioulasso.

Increase in bruchid populations and seed losses after 6 months under various treatments

***B. atrolineatus* population:** In both sites, *B. atrolineatus* adults emerged from cowpeas during November and December. No more *B. atrolineatus* adult was recorded in the stores by January onwards (Fig. 1A and B). The intrinsic rate of increase of its population was ≤ 0.2 and did not differ in the 4 experimental treatments ($p>0.05$).

***C. maculatus* population:** After 6 months of storage, the intrinsic rates of natural increase of *C. maculatus* population were reduced in the jars treated by *D. basalis* augmentations alone or combined with *B. senegalensis* leaves (Table 2). The MANOVA revealed significant differences between the 4 treatments and between the localities ($p<0.0001$). The rates of increase of *C. maculatus* population in the jars treated with *B. senegalensis* leaves alone did not differ from those of the control.

Cowpea seed losses: The seed losses also significantly differed between treatments (Table 2). They were higher in the jars treated with *B. senegalensis* leaves and lower in the jars treated by *D. basalis* augmentations alone or combined with *B. senegalensis* leaves.

Rates of parasitism estimated after 6 months in cowpea stores under various treatments: In the Ouagadougou region, global parasitism was a sum of parasitism estimated for both parasitoid species while parasitism was due to *D. basalis* only at Bobo-Dioulasso. The Multiple ANOVA for this parameter indicated significant differences between treatments and localities ($p<0.0001$).

Table 1: Initial number of bruchid and parasitoid adults emerging at the beginning of storage from 3 kg of cowpeas harvested at Ouagadougou and Bobo-Dioulasso

Locality	*C. maculatus*	*B. atrolineatus*	*D. basalis*	*E. vuilleti*	Estimated rate of parasitism (%)
Ouagadougou	463	385	32	51	8.9
Bobo- Dioulasso	451	192	157	0	19.6

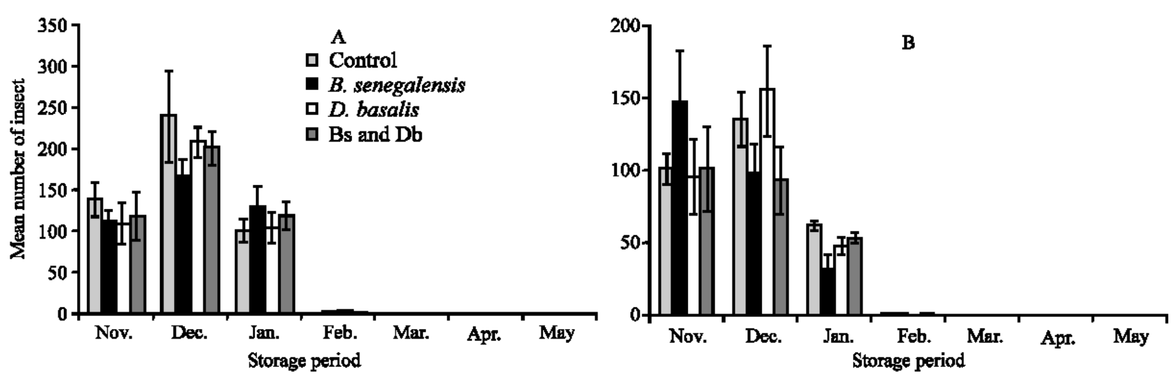

Fig. 1: Monthly variations in *B. atrolineatus* mean numbers into jars under various treatments: control, *B. senegalensis* leaves, *D. basalis* adults, *B. senegalensis* leaves and *D. basalis* adults (*Bs and Db*) at Ouagadougou (A) and Bobo-Dioulasso (B)

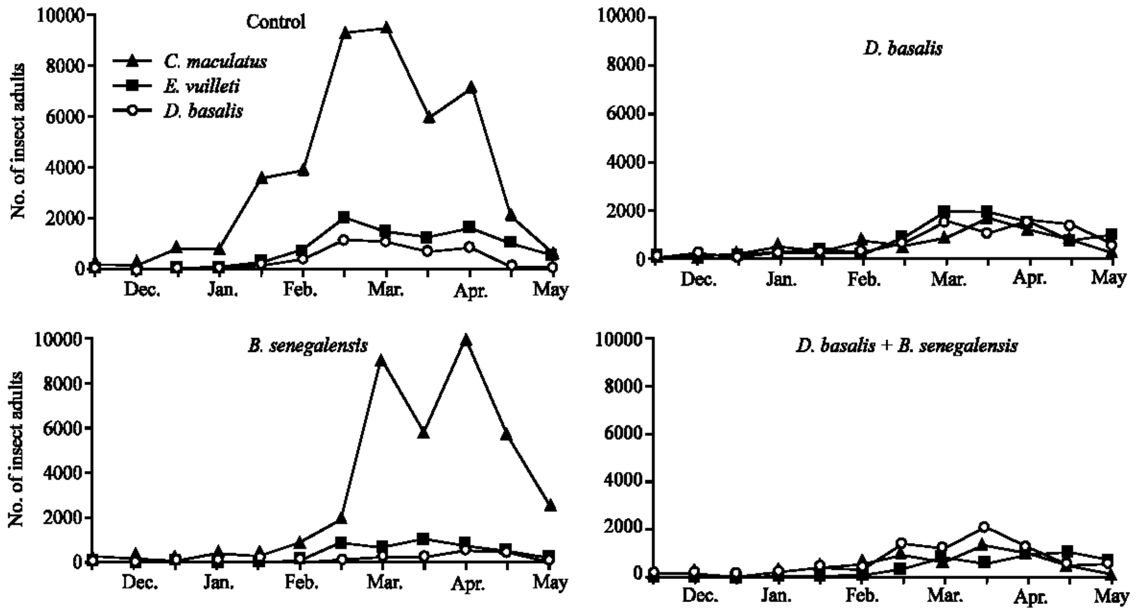

Fig. 2: Monthly variations in bruchid and parasitoid mean numbers in jars under various treatments: control, *B. senegalensis* leaves, *D. basalis* adults, *B. senegalensis* leaves and *D. basalis* adults at Ouagadougou

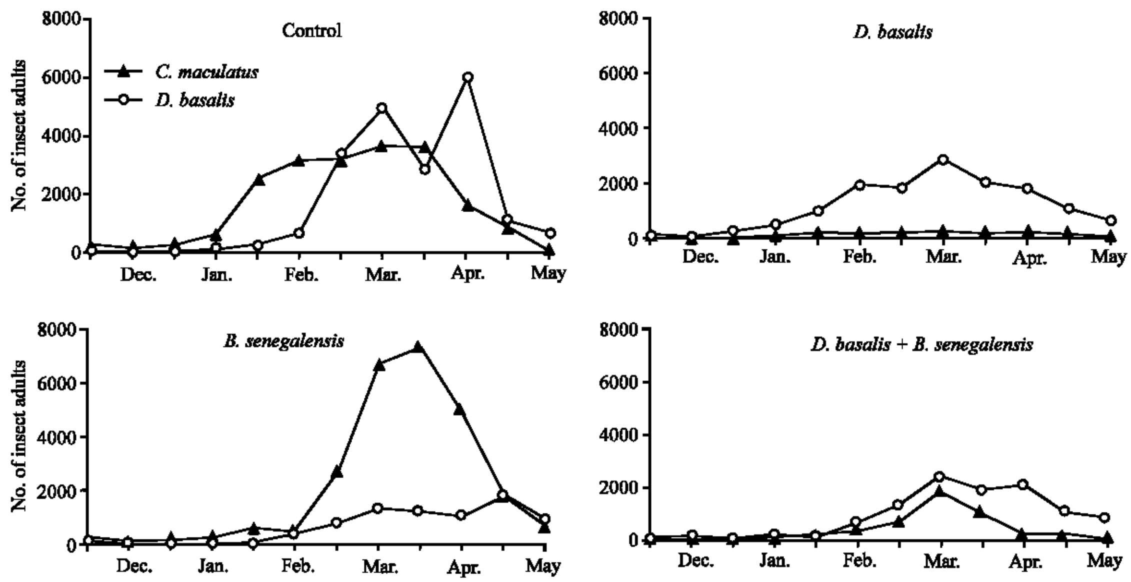

Fig. 3: Monthly variations in bruchid and parasitoid mean numbers in jars under various treatments: control, *B. senegalensis* leaves, *D. basalis* adults, *B. senegalensis* leaves and *D. basalis* adults at Bobo-Dioulasso

The interaction between treatments and localities was also significant (p=0.0005). The rates of parasitism were lower in the jars treated with *B. senegalensis* alone and differed from that of the control jars (Table 2). Higher rates of parasitism were recorded in the jars treated by *D. basalis* augmentations alone.

Variations in the rates of increase of *C. maculatus* population during the storage: During the first 3 month period of storage, the intrinsic rates of natural increase of *C. maculatus* population were highly reduced in the jars treated with *B senegalensis*, *D. basalis* and the combination of *D. basalis* augmentations with *B. senegalensis* leaves (Table 3). During this period even the use of *B. senegalensis* leaves allowed a reduction in bruchid numbers by up to 78% in comparison to control jars. The reduction was 90 and 87%, respectively in the jars treated by *D. basalis* augmentations alone or combined with *B. senegalensis* leaves.

However during the second 3 month period of storage, the intrinsic rate of natural increase for *C. maculatus* population significantly increased in the jars

Table 2: Estimate of intrinsic rates of increase in *C. maculatus* populations, global rates of parasitism and seed losses under various treatments after 6 months of storage

Treatments	*C. maculatus*	Seed losses (g/kg)	Globalparasitism(%)
Control	4.1±0.5a	491.3±53.5b	38.5±7.1c
B. senegalensis	4.2±0.2a	557.3±42.2a	18.7±5.5d
D. basalis	2.0±0.4c	105.0±27.4c	79.8±7.8a
B. senegalensis and D. basalis	2.5±0.2b	96.0±13.0c	67.5±3.8b

Means (±SD) followed by different letter are significantly different using a PLSD Fisher test, $p<0.05$

Table 3: Estimate of intrinsic rates of increase in bruchid populations per 3 month period

Treatments	1st 3 month period	2nd 3 month period
Control	3.2±0.2a	0.9±0.5c
B. senegalensis	1.5±0.2b	2.7±0.2a
D. basalis	1.0±0.1c	1.0±0.2c
B. senegalensis and D. basalis	1.0±0.2c	1.5±0.3b

Means (±SD) followed by different letter are significantly different using a PLSD Fisher test, $p<0.05$

treated with *B. senegalensis* leaves, while decreasing or remaining at a low level in the other treatments, including the control (Table 3).

Temporal variations in bruchid and parasitoid numbers in stores: In the control jars of Ouagadougou, numbers of the two parasitoid species remained at a low level during the whole storage period and did not prevent the increase in *C. maculatus* numbers from the end of January onwards (Fig. 2). In the presence of *B. senegalensis* leaves, the population of *C. maculatus* was low from November to February but increased after this period while the parasitoids population remained at a low level throughout the storage period. When *D. basalis* adults were augmented either alone or in combination with *B. senegalensis* leaves, bruchids did not really increased during storage and parasitoid numbers were always higher than bruchid numbers. Estimated parasitism rates were >70% at each observation date.

In the control jars of Bobo-Dioulasso, the *D. basalis* population increased by February onwards and subsequently limited the development of *C. maculatus* population (Fig. 3). As at Ouagadougou, when *B. senegalensis* leaves were used alone, *C. maculatus* populations were maintained at low levels during at least the first three months of storage, but drastically increased after this period in the presence of a limited number of parasitoids. In the two other treatments, *C. maculatus* populations were reduced throughout the storage time.

DISCUSSION

The cowpea seeds used in the current study were naturally infested by two bruchid species, *C. maculatus* and *B. atrolineatus* and their larval parasitoids, *D. basalis* and/or *E. vuilleti* in relation to the zone of study[4,5,7]. During the early part of storage time, the *B. atrolineatus* population slightly increase and disappeared probably because adults of the new emerging generation were in reproductive diapause[4]. Therefore *C. maculatus* was the main bruchid species developing during storage[3,5].

The initial rates of parasitism were relatively limited and varied according to the region of study. At Ouagadougou two parasitoid species, *D. basalis* and *E. vuilleti* were present and the rates of parasitism were low. At Bobo-Dioulasso, *D. basalis* was the only parasitoid species and the initial rates of parasitism were higher. Augmentation of *D. basalis* adults at the beginning of storage strengthened the initial parasitic population and limited the bruchid damages during the whole storage time. This study confirmed that the biological control of bruchids using augmentation of *D. basalis* adults could be an efficient control method[3,5,14] even when in a situation of interspecific competition with *E. vuilleti*.

The introduction of *B. senegalensis* leaves in the jars, as small-scale farmers of Burkina Faso do, proved to be toxic towards bruchid and parasitoids adults during the first three months of storage in both regions of study. The volatile compounds released by the plant affected the insects emerging at the beginning of storage but their concentration probably decreased over time[10]. Bruchid larvae inside the seeds were slightly affected by the treatments and new bruchid generations could develop in the presence of a low concentration of volatiles and a very limited number of parasitoids, which are more susceptible[13]. Considering the whole storage period, *B. senegalensis* did not significantly protect the stores and after six months of storage the cowpea seed losses were higher than those of the control. In certain conditions the use of *B. senegalensis* may be very self-defeating and could favoured the bruchid population as suggested by several authors[12,13]. These findings are consistent with field observations because most of the farmers using *B. senegalensis* do not store their cowpea more than three months. Authors analyzing the interactions between pests, insecticides and natural enemies demonstrated that insecticidal treatments in storage systems brought about the elimination of parasitoid or at least a reduction in their numbers, which allowed an explosion of a fraction of the host population[16,17]. However in the Ouagadougou region the combination of *D. basalis* and *B. senegalensis* was particularly effective in controlling the bruchid population. In fact, *B. senegalensis* eliminated the two parasitoids species and therefore interspecific competition, at the beginning of the storage. Then further releases of *D. basalis* adults occurred in the presence of a low concentration of *B. senegalensis* volatiles and a low bruchid population. Therefore *D. basalis,* which is an excellent biological control agent, could reproduce and prevent the increase in bruchid population.

The results of this study suggest that the effects of *B. senegalensis* volatiles in the storage environment may be very complex. Knowing the consequences of the treatments on the pest population and their antagonist is required[12] for an efficient integrated pest management of stored cowpeas. Research on the judicious way of combining *B. Senegalensis* volatiles and biological control agents will be of great interest in the future.

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