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Potential of combining the parasitoid *Dinarmus basalis* (Hymenoptera : Pteromalidae) with varietal resistance for the control of the cowpea bruchid *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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ABSTRACT

In this study, the potential of combining *D. basalis* with a resistant cowpea variety IT81D–994 for the control of *C. maculatus* populations was analyzed, with the use of TN 5-78, a susceptible variety as control. The result obtained showed that, in absence of *D. basalis*, the development time and the percentage of eggs mortality were not significantly different for both varieties. However, the variety IT81D–994 reduced significantly the bruchids larval development by causing higher mortality of larvae. The rate of larval mortality was 80% for this variety in the absence of the parasitoid *D. basalis*. The dynamic study of the bruchids populations showed that the combination of *D. basalis* reduced significantly the evolution of bruchids at storage. In fact, for both treatments, the number of bruchids was more important in jar with *D. basalis* than those without this parasitoid. For the IT81D–994, the evolution of bruchids population was completely inhibited when the parasitoid was associated to the variety. Thus, the strategy combining *D. basalis* with varietal resistant was a potential way to control bruchids population in the field and in the storage system.
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Keywords: Cowpea; varietal resistant; *D. basalis*; storage system; reproductive capacities; biological control

INTRODUCTION

The production of cowpea – *Vigna unguiculata* (L.) Walp. is restricted by a number of biotic and abiotic factors both in the field and the seed in storage. Among the constraining biotic factors are insect pests.

In west Africa, the bruchid beetle *Callosobruchus maculatus* (F.) develops during its postembryonic growth inside seeds of *Vigna unguiculata* (Walp.) and causes high losses of seeds during storage in traditional granaries (Haines, 1991; Oue´draogo et al., 1996; Sanon et al., 1998; Ofuya, 2001; Doumma et al., 2006).

The adults of *C. maculatus* colonize the cowpea crops in August at the end of the main rainy season, and the females oviposit on the developing pods (Huignard et al., 1985;

Zannou et al., 1997). The new generation of *C. maculatus* adults emerges in the granaries after seeds harvesting and reproduce on the stored seeds. Thus, 4-5 generations of *C. maculatus* develop in the stores from November to June during the dry season, causing high damages. At the end of the storage, the seed losses were estimated at 800-900 g/kg in different zones of West Africa (Oue´draogo et al., 1996). Their damage causes loss of weight, nutritional value and viability of stored grains.

In Africa, store fumigation with synthetic insecticides is used to control insects in commercial storage centers, but the availability and the cost of these chemicals limit their use by small-scale African farmers (Ketoh et al., 2002; Kabeh and Lalo, 2008). On the other hand, On-farm stored product pest control needs to be safe for both the user and the consumer (Dorn, 1998). This implies, in the case of insecticide use, proper handling of the contact chemical or fumigant during application, and a zero or low residue level at the time of product consumption.

As these requirements are difficult to fulfill on small-scale farms, alternative control methods are required (Dorn, 1998). Such methods have been identified but have not been thoroughly tested (e.g. reviewed by van Schoonhoven, 1978; Lienard and Seck, 1994; Abate and Ampofo, 1996).

The main difficulties associated with the introduction of alternative control methods are low acceptance by farmers and consumers, e.g. treatment of the grain with oil or ashes, or their high costs, e.g. storing of the grain in metal bins (*C. Cardona*, pers. obs.).

Two biologically based methods of control, host-plant resistance and parasitoid use, have been developed and described recently. Breeding of resistant bean varieties has been successful against many species of bruchid (Cardona and Kornegay, 1999; Doumma et al., 2006, Kabeh and Lalo, 2008) and parasitoids have been found to be promising for the control of theses insects (Sanon et al., 2005).

In west Africa, the parasitoid *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) has been reported to be a promising control agent of *Callosobruchus maculatus* (F.) and *Bruchidius atrolineatus* (Pic) on cowpea (Ouedraogo et al., 1996; Sanon et al., 1998; Doumma and alzouma, 2000).

Until now host resistance to *C. maculatus* and biological control of this insect have been studied separately, and the potential of an integrated control strategy has not yet been addressed (Dorn, 1998). In this study we investigate whether the use of host plant resistance beans is compatible with biological control of *C. maculatus* by the parasitoid *D. basalis*.

MATERIALS AND METHODS
Cowpea varieties

For this study, we chose bean variety IT81D–994 characterized by the International Institute of Tropical Agricultural (IITA) as resistant to *Callosobruchus maculatus*. A commonly cultivated bean variety TN 5-78 selected by the National Institute for Agronomic Research of Niger (INRAN) was used as a control in relation to its susceptibility to bruchids (Doumma et al., 2006).

Insect rearing

Callosobruchus maculatus and *Dinarmus basalis* were collected from cowpea seeds bought at the local market of Niamey. They were brought back to the laboratory and mass reared under conditions of the laboratory.

Bruchids rearing

Fifty pairs of 2- or 3-days-old *C. maculatus* adults were placed for 48 h in rearing boxes containing seeds of *V. unguiculata* of the TN 5-78 variety. The females laid eggs on these seeds and their offspring completed development within the seed. The adults were isolated after emergence

and used either for the production of a new generation or for experiments.

Parasitoids rearing

One or 2 day-old adults of *D. basalis* were placed in rearing box (18 by 11 by 3 cm) in the presence of seeds containing *C. maculatus* fourth instars because *D. basalis* females preferentially oviposit on this developmental stage (Nishimura, 1993). After 24 h, parasitoids adults were removed from box and seeds containing bruchids larvae, whether parasitized or not, were maintained in laboratory under the standard rearing conditions. Females emerged from seeds were used in the experiments

Experimental design

Our experiments were based on experimental design used by Schmale et al. (2003).

Effect of the two varieties on reproductive capacities of C. maculatus in presence or absence of D. basalis

Treatments were arranged in a completely randomized design (CRD) and each was replicated ten times. Cowpea seeds of the two varieties tested were disinfested by keeping them in a deep freezer at a temperature of –2 °C for 48 hours. The seeds were then conditioned to room temperature before being used for experimental purposes.

For each variety twenty Petri dishes containing 20 seeds were prepared. One pair of *C. maculatus* adults were placed in each Petri during 3 days. 5 days after, eggs that failed to hatch and first instar larvae that failed to penetrate the seeds were counted.

Then the Petri dishes were shared in two batches of 10 dishes**.**

For the first batch, cowpea seeds were maintained in laboratory until adult emergence in order to study the effect of the variety on *C. maculatus* development. For this beans were checked daily in order to count and remove all adults emerging. Ten replicates were conducted for each bean variety.

For the second batch cowpea seeds were maintained until 15 – 16 days at the four instar larvae. Then, one female of *D. basalis*

was placed in each Petri dish. After, 24 h the adults were removed and the seeds parasite or not were maintained in laboratory until *D. basalis* adults emergence.

Data collected

Data collected included:

- Development time: It is the time between eggs deposited and the emergency of the adults derived from this egg;
- Egg mortality rate: total number of eggs – number of hatched eggs / total number of eggs
- Larval mortality rate:: Number of hatched eggs – number of emerged adults/number of hatched eggs
- Adult emergence rate: total number of eggs emitted – number of emerged adults/ number of eggs emitted.
- *D. basalis* rate of parasitism: = **Nh / Nh+Nb**

Nh= number of *D. basalis* adults emerged.

Nb= number of *C. maculatus* adults emerged.

Evolution of bruchids populations during storage

The storage system used in this experience was a jar of about 20 l of volume which was filled with 3 kg of cowpea healthy seeds. Two replicates were conducted for each cowpea variety. To simulate the level and distribution of bruchid infestation at harvest time, seeds were consecutively infested 50 L2, 50 L3 and 50 L4 of *C. maculatus* (Schmale et al., 2003).

Every month, 1 kg of seeds was sampled and the numbers of live and dead *C. maculatus* and *D. basalis* adults were counted. The experience was carried out on 7 month (November to May).

Data analysis

For statistical analysis of both insect population development and bean damage, the data were log10 transformed to attain a normal distribution. Differences were

analyzed using a one-way ANOVA followed by Fisher's test.

RESULTS
Effect of beans varieties on the development of *C. maculatus*
The development time

The time until bruchid emergence was not significantly prolonged by the two tested beans varieties (Table 1). The average of the development time observed was approximately 23 days for TN 5-78 and 29 days for the bean variety IT81D–994. This means that the resistance mechanism of IT81D– 994 was not in the induction of retard immature development.

Egg mortality

The results pointed out that the two varieties did not avoid the infestation by *C. maculatus*. In fact, for both cowpea varieties, the mean number of eggs deposited by female was low and was approximately 36 eggs for TN 5-78 and 55 eggs for the resistant variety IT81D-994 (Table 2).

In addition, egg mortality before seed penetration was low in both treatments and was not significantly different. For the two cowpea varieties, almost 87% of eggs were hatched. These results showed that the resistance mechanism of IT81D-994 is not in the induction of sublethal effects on *C. maculates* eggs.

Larval mortality

The analysis of results showed that *C. maculatus* larval mortality after bean penetration depended on the variety (Table 3). Indeed, the percentage of larval mortality observed on IT81D-994 was significantly more important than those of the control variety TN 5-78; the percentage of larval mortality was approximately 32% while in the resistance bean variety this parameter reached a level of 80%. Thus, IT81D-994 induced significantly sub lethal effects on *C. maculatus* larval development.

In presence of the parasitoid *D. basalis*, the larval mortality for both varieties was significantly affected by increasing the level of this parameter. In fact, in this situation, the larval mortality observed on the two varieties was 67.6% and 92.32% respectively for TN 5-78 and IT81D-994.

Emergence of C. maculatus adults

The analysis of bruchid adults emerged from the seeds showed that the percentage of emergence was significantly more important in the resistant variety than in the control one. In fact, in IT81D–994, only 20% of *C. maculatus* adults (Table 4) were emerged from the eggs emitted while more than 60% of adults emerged in the control variety.

The use of *D. basalis* in combination with the varieties resulted in reduction of *C. maculatus* adults. But this reduction was more effective on the resistant variety where only 5% of emerged adults were observed.

Population dynamics of bruchids

Analysis of the results (Fig. 1) showed that, for both treatment, the number of bruchids was significantly more important in jars filled with TN 5-78 than those filled with IT81D–994. In addition, when TN 5-78 was used alone, the population of bruchids increased progressively during the storage period. However, when *D. basalis* was combined to the variety, the evolution of bruchids populations during the storage was very low.

For the variety IT81D–994, the number of bruchids was not important for both treatments. But, when this variety was combined with the parasitoid *D. basalis*, bruchids population became very negligible.

Evolution of *D. basalis* adults

The analysis of Figure 2 pointed out that the evolution of *D. basalis* adults during the storage period depended on the treatment. Indeed, for both treatments, parasitoids number was more important in the jar with TN 5-78 than in those with IT81D–994. The parasitoids number, low at the first observation, increased very significantly in

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Table 1 : Development of *C. maculatus* observed on the two varieties.

varieties	Time of development	
	Intervalle of time (days)	**Average ± standard error**
TN5-78	18-29	23.5 ± 3.6
IT81D-994	22-35	28.5 ± 3.89

In each column, values followed by different letters were significantly different.

Table 2: Egg mortality observed on the two varieties.

Varieties	Mean number of eggs by female	Mean number of non hatched eggs	% egg mortality
TN5-78	36 ± 27.34[a]	12[abcd]	33.33[abcd]
IT81D-994	55 ± 23.44[a]	13[abcd]	23.63[abcd]

The values in the column followed with the same letters are not significantly different.

Table 3: Percentage of larval mortality observed on the two varieties.
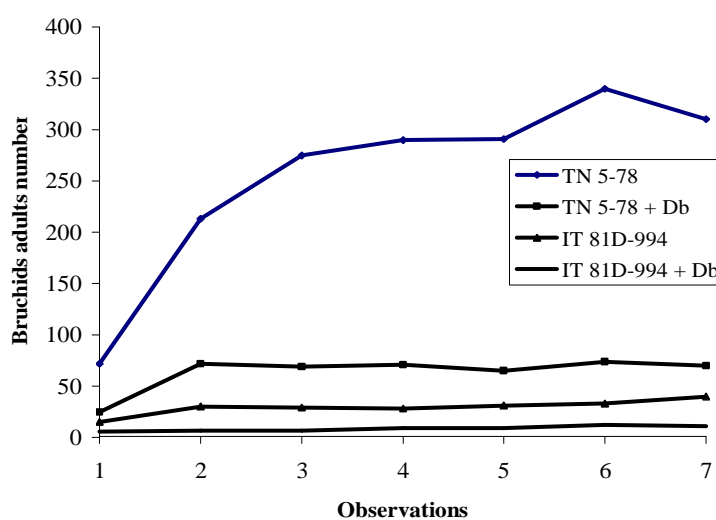
Variétés	Percentage of larval mortality	
	In absence of *D. basalis*	**In présence of *D. basalis* (%)**
TN5-78	32.22[ab]	67.61
IT81D-994	80[a]	92.32

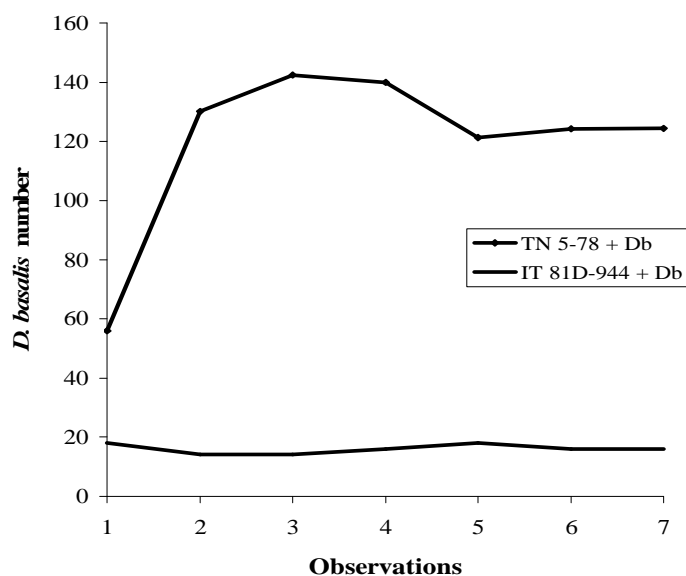In each column, values followed by different letters were significantly different.

Table 4: Percentage of *C. maculatus* emergence in presence or not of *D. basalis*.

Variétés	Percentage of *C. maculatus* emergence	
	In absence of *D. basalis*	**In présence of *D. basalis***
TN5-78	62.22[cab]	32.3[ab]
IT81D-994	20.51 [d]	5.78[b]

In each column, values followed by different letters were significantly different.

Figure 1: Bruchids evolution during the storage period in function of the treatment.

Figure 2: Evolution of the number of *D. basalis* adults during the storage period.

the jar with TN 5- 78 until the fourth month of storage where the number became relatively constant.

In the jar with IT81D–994, the evolution of parasitoids number was low during the storage period.

DISCUSSION

The results obtained during this study showed that, the resistant variety IT81D–994 did not affect the time until bruchid emergence and the rate of egg mortality. Indeed, for both varieties, the development time and the egg mortality rate were not significantly different.

Our results were different with those of Lale and Kolo (1998) who showed *that C. maculatus* egg viability were significantly reduced on seeds of the resistant cultivars. These authors observed that the proportion of unhatched eggs laid on resistant cultivars was 98.2, 81.5 and 50% for Kanannado, IT89KD-391 and Dan'ila, respectively, while 9.2, 13.1 and 14.8% of eggs remained unhatched on the susceptible cultivars Bausse local, Babura-4 and IT89KD-374, respectively.

These results implied that the resistance mechanism of IT81D–994 was not in the induction of retard immature development and inhibition of larvae penetration of *C.*

maculatus. However, this variety inhibited significantly the bruchids larvae development in the seeds by causing a mortality rate of 80%. This rate of larvae mortality is more important than the rate observed by Schmale et al. (2003) who showed that the arcelin containing variety RAZ 36, the bean variety with the highest resistant inhibited the development of only 23% of *Zabrotes subfasciatus* larvae. Our data showed that IT81D–994 seemed to have an antibiosis effects on the development of *C. maculatus*. The consequence of this higher mortality caused by this variety was a significant eradication of *C. maculatus* adults. In our experimental conditions only 17% of bruchids adults emerged with the resistant variety while a rate of emergence of 63% was observed on the control variety TN 5-78.

Data on bruchids dynamic in storage system pointed out that the evolution of bruchids populations was relatively inhibited by the resistant variety during all the storage period comparatively to the control variety.

This result confirmed the efficiency of the use of varietal resistant in the control of *C. maculatus* populations as described by many authors.

Studying the behaviour of twenty cowpea varieties on the evolution of bruchids

in storage system, Doumma et al. (2006) showed that some varieties reduced significantly the population dynamics of bruchids during storage.

Similar observations have also been made by Singh et al. (1985) on three resistant cowpea lines (TVu 2027, TVu 11952 and TVu 11953) to *C. maculatus* compared to the very susceptible Nigerian variety "Ife Brown".

In a previous study on the testing of a part of the Senegalese cowpea gene pool, Seck (1993) indicated that the varieties: 59-12; 58-28; 66-50; 66-5; 58-16-Dl; 59-26 have resistance to attack by *C. maculatus* while 58-57 which is the most cultivated variety is sensitive.

When *D. basalis* was combined with the varieties a significant reduction of *C. maculatus* adults was observed. But this reduction was more effective on the resistant variety where only 5% of emerged adults were recorded. Our result confirmed the efficiency of these parasitoids in the control of bruchids populations as indicated by many authors (Sanon et al., 1998; Doumma and Alzouma, 2000; Schmale et al., 2003; Sanon et al., 2005).

Data on the evolution of parasitoïds populations pointed out that parasitoids population dynamic was relatively low during all the period of experience. In our conditions, this situation was not in relation with an eventual antibiosis effect of the resistant variety by affecting *D. basalis* development as mentioned by van Emden (1997) who indicated that resistant variety can affect natural antagonists via their prey. According to our data, this result can be explained by the low number of bruchids larvae which were not sufficient to allow *D. basalis* development.

This study pointed out that host plant resistance could play a significant role in reducing infestation by bruchids in storage system, with respect to number of eggs on seeds and adult bruchids that emerged as well as the proportion of the total bruchid populations that developed in seeds.

As mentioned by Schmale et al. (2003) the data in the present study indicated that an IPM system for the control of *C. maculatus* combining host resistance and parasitoids should be based on bean varieties with low

resistance, rather than those with maximal resistance. This work showed the promise of a combination of biological control and resistant plant varieties for pest management in storage systems. The combination proved to be of significant advantage even for situations where *C. maculatus* has to be controlled as the only bruchid pest.

Conclusion

In this study, it is clearly demonstrated that the use of host plant resistance beans is compatible with biological control of *C. maculatus* by the parasitoid *D. basalis*. Thus, the potential of this combination can be proposed to cowpea producers as an integrated control strategy against bruchids in storage system.

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