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Full Length Research Paper

Potential of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) as biocontrol agent of *Callosobruchus maculatus* (F.) (Coleopetera: Bruchidae)

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The bruchid *Callosobruchus maculatus* (F.) (Coleopetera: Bruchidae) is a major pest of stored cowpea *Vigna unguiculata* (Walp.) in Africa and particularly in northern Cameroon. *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitoid of its larval stages, could be used in the biological control of this grain pest. In field conditions, 5 months samples in small holder granaries established the phenological relationship between this parasitoid and its host. Large amount of *A. calandrae* may efficiently control *C. maculatus* infestations. Laboratory assays made an evidence of the preference of *A. calandrae* to parasitise 4th instar larvae of *C. maculatus*. To estimate the suitable density of the infesting population of parasitoid, other experiments pointed out that one mated female induced reduction of 4.97% of emergence of *C. maculatus* while 4 females performed more. The reduction of emergence observed was 42.34%. In suitable density, *A. calandrae* may play an important role in the biological control of *C. maculatus* on cowpea during storage.

Key words: *Anisopteromalus calandrae,* biological control, Cameroon, *Callosobruchus maculatus,* Cowpea.

INTRODUCTION

Insect pests are the major constrain in cowpea production and storage Singh and Allen (1980), Youdeowei (1989). Damages due to insects affect especially the quality, the quantity, the commercial and agronomic values of the product Bell et al., (1998). In general, stored grain insect larvae usually bore into the grain feeding preferentially on the germ with large amount of the protein and vitamins Dal Bello et al. (2001).

In tropical countries, the cowpea *Vigna unguilata* (Walp.) (Fabaceae) is an important source of proteins. In northern Cameroon 78% of farmers produce cowpea which is highly susceptible to insect attacks, particularly of the weevil *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae), (Kitch et al., 1992; Boeke et al., 2001; Boeke et al., 2004). Many methods have been used to prevent these post harvest losses. Chemicals insecticides are currently used also some plant materials as insecticides or repellent agents (Amatobi, 1995; Boeke, 2002).

Synthetic insecticides are subject to dynamic trends. Target insect are able to develop resistance against single insect pesticide, (Boeke, 2002) or of the residual and side effect of the agent on consumers and environment. This can also enhance the development of non target pests, allowing a secondary pest in the past to be an important one with high tolerance towards popular pesticides. Therefore, more sustainable measures are required to reduce pests' resurgence and to enhance the incidence of natural enemies in crop protection strategies.

Several researches have shown that the parasitoid Hymenoptera, could serve as biological control agent of rice weevil *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae), (Perez-Mendoza et al., 1999; Lucas and Riudavets, 2002) *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) and *Sitophilus zeamais* (Helbig, 1998).

In northern Cameroon, *C. maculatus* is currently parasitized by the parasitoid *Anisopteromalus calandrae*

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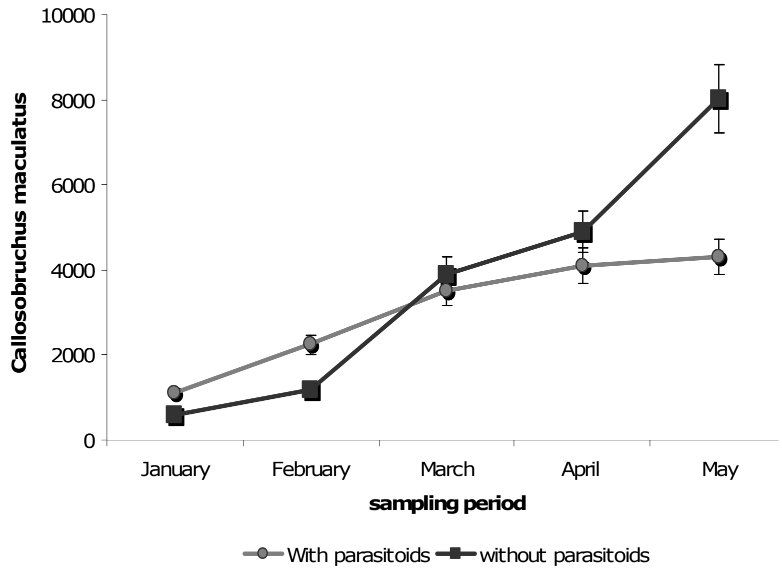

Figure 1. Relationship between emergence of *Callosobruchus maculatus* in presence and in absence of its parasitoid *Anisopteromalus calandrae*.

(Hymenoptera: Pteromalidae). The present study was conducted both at the Centre of Research of the Biodiversity of the Catholic University of Louvain at Louvain-la-Neuve in Belgium first to synchronise biological cycles of parasitoid and of its host, in order to evaluate the real impact of *A. calandrae* on the reduction of the emergence of *C. maculatus*, and in Ngaoundéré in Cameroon for field observation in small holders granaries to estimate the effectiveness of the reduction of the pest population due to its natural enemy.

MATERIALS AND METHODS

Collection of grains from farmers' granaries

From November 2003 to January 2004 in the 3 regions of Northern Cameroon, 12 samples of grains of black-eyed cowpea were taken on the farmers resources destined to be introduced into the granaries. The samples collected were taken to the laboratory where they were weighted and 500 g introduced separately in 1200 ml glass flasks. Thereafter they were check once a month during 5 months period. Each time all the emerged insects were removed and counted in order to establish the diversity of the entomofauna and phenology of pests in relationship with that of its antagonists

The emerging parasitoids were identified in the Research Center on the Biodiversity of the Catholic University of Louvain-la-Neuve (Belgium).

Rearing in laboratory conditions

Insects used emerged from cowpea sampled in peasants granaries in northern Cameroon. The rearing of *C. maculatus* has been maintained on *V. unguiculata* in laboratory conditions. In the glass jars, 200 g of clean cowpea grains were introduced and infested with 48 h-old *C. maculatus*. Two days after this infestation all the insects were removed and introduce into other jars containing also 200 g of clean grains. The grains that receive this oviposition were observed till the emergence of a new generation of bruchid.

In the same way, *A. calandrae* was reared on *C. maculatus*. One and 4 pairs, male and female of parasitoids were introduced for a delay of 48 h on 15 g of cowpea infested by at least 6 eggs of *C. maculatus* every 2 days till the beginning of the emergence of the bruchids. This allows the synchronisation of the life cycles of the parasitoids and of its host. For this work, 15 replications were made for the rearing of bruchid alone and 10 with parasitoids.

The rearing was conducted in a climatic darkroom where temperature and relative humidity were recorded every hour with Hobo onset H8 series.

Evaluation of parasitism inside cowpea grain

Every two days, half of the grains previously parasitized by *A. calandrae* were removed put into ethanol in order to halt the development of parasitoid larvae inside cowpea. They were therefore dissected. The number of clean and parasitized bruchid larvae was estimated. The stage of development at which *A. calandrae* parasitizes its host was hence determined.

RESULTS AND DISCUSSION

Phenological relationship between the population of bruchid and that of its parasitoid

Pteromalids and Ichneumonids were the two main families of parasitoids occurring on insect grain pests, they emerged from cowpea infested by *C. maculatus*. But Ichneumonids were in very low numbers, the most observed genus was *Eupelmus.*

On infested cowpea the occurrence of Pteromalids was important and continuous (Figure 1). *A. calandrae* was the most important parasitoid observed. Each month, more than 100 parasitoid insects emerged from infested

Table 1. Variation in the amount of emerging *Callosobruchus maculatus* in relationship with the duration of observation and the presence of its parasitoid *Anisopteromalus calandrae*.

	Amount of *C. maculatus* emerging in presence of parasitoid	Amount of *C. maculatus* emerging without parasitoid	Khi 2
At the first month	1120	577	173.85***
Five month later	4307	8032	1124.62***
Khi 2	1871.22***	6341.50 ***	

*** p< 0.001

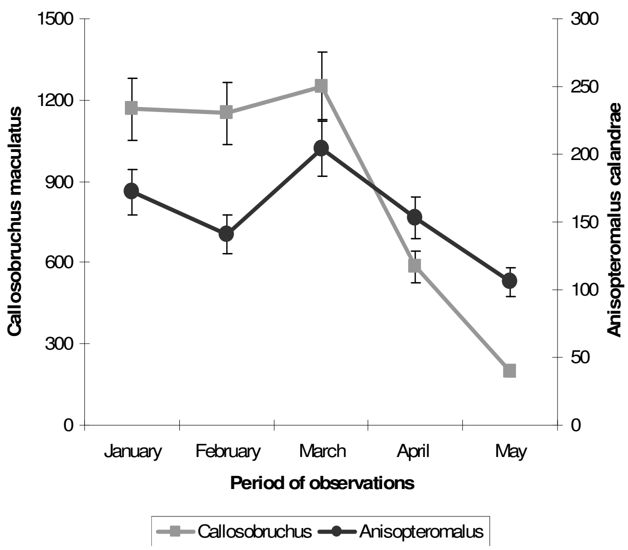

Figure 2. Dynamic of emergence of *Callosobruchus maculatus* and its parasitoids *Anisopteromalus calandrae* during 5 months.

cowpea.

Only 5 of the 12 samples collected infested by *C. maculatus* were parasitized by *A. calandrae*. The first month of observation, 1120 *C. maculatus* emergerged form the groups where parasitoid occurred, only 577 were observed in the other groups. Five months later, 4307 bruchids emerged from the group where parasitoids were present and 8032 emerged from where they were absent (Table 1). These differences are significant. In the presence of parasitoids, the amount of emerging bruchid is was reduced compare to that observed in sample where parasitoids were absent.

The infestation by the parasitoid was important in the most infested cowpea. In this most infested group, 5 months later was a very low level of infestation was observed compare to that of the lowly infested group. A biological control is therefore made by *A. calandrae* on *C. maculatus.* Without control, the augmentation of the amount of emerging bruchid was continuous during all the 5 months period.

Another observation of the relationship between the dynamic of the population of bruchid and that of *A. calandrae* was made by counting of the emerging insect per month (Figure 2). During the first 3 months, the 2 populations develop in the same way, after this period, an important reduction of the host population is observed. That of the parasitoid is also observed. No correlation was found between the phenological development of the 2 populations (r = 0.86; n = 5; ns).

This expresses the potential of biological control that *A. calandrae* is able to perform on *C. maculatus*. It is imaginable that if the number of parasitoid is high at the beginning of the storage, an efficient control could be made before the delay of 3 months. In the present observations, the natural infestation of grain provide very low amount of parasitoid, in consequence only the first generation of parasitoid, installed with the pest performed the control. The natural control observed is to be improved by an augmentation of the parasitoid population at the beginning of the storage.

Synchronisation of life cycles of *Anisopteromalus canlandrae* and *Callosobruchus maculatus* inside cowpea grains

The parasitoid *A. calandrae* develops into *C. maculatus* larva. The stage by which *A. calandrae* entered the host life cycle is the fourth instar larva. This stage was achieved 14 ± 0 days after the oviposition. The development of *A. calandrae* was completed after 18 ± 1.97 days. The developmental stages observed into the fourth instar larva of the host were the egg, the larva and the nymph (Figure 3).

After dissection of cowpea grains infested, microscopic observations showed all stages of the development of *A. calandrae* and its host *C. maculatus*.

Impact of parasitism on emergence of *Callosobruchus maculatus*

Without presence of parasitoids almost all the bruchid infesting grains developed and emerged to a new generation of *C. maculatus*. With 1-mated female parasitoid, 95.13% of the bruchid emerged (Table 2). This 4.97% reduction did not differed significantly from the complete emergence observed without parasitoid. With 4-mated female parasitoids staying 48 h on infested grains,

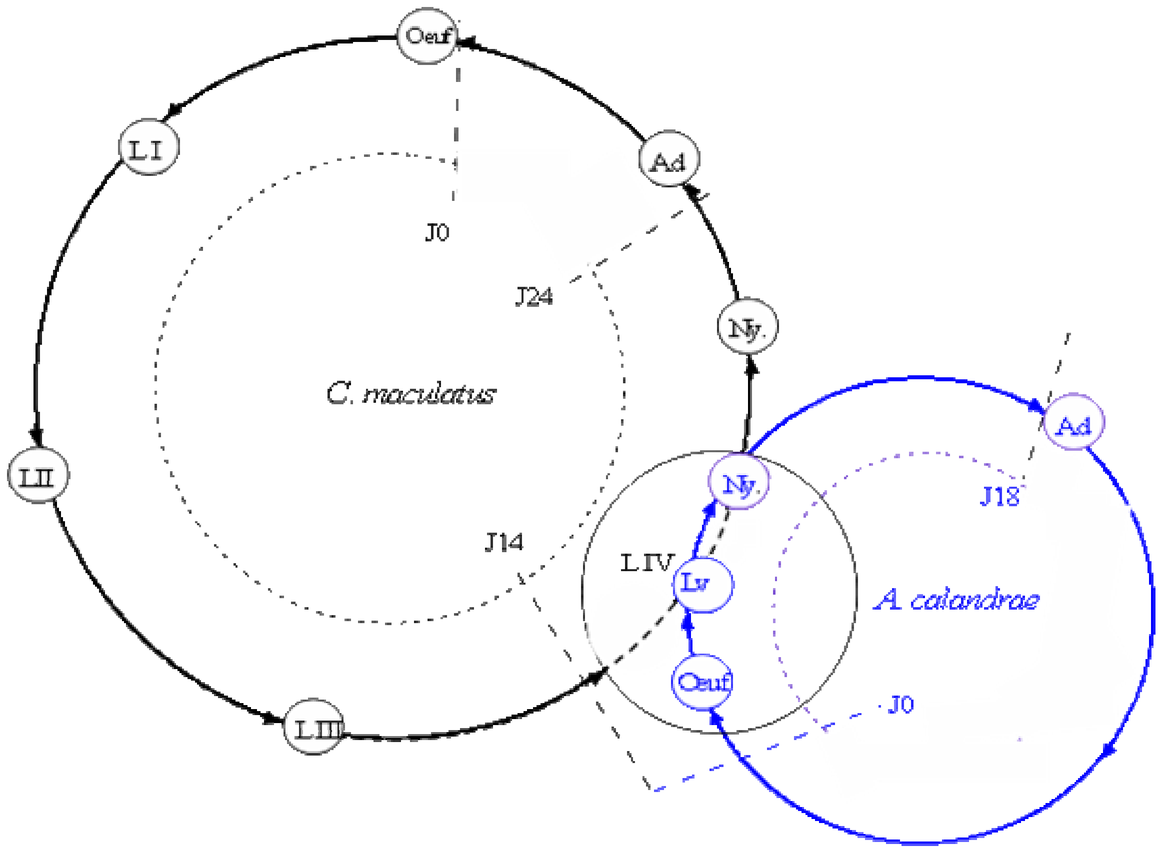

Figure 3. Life cycle of *Callosobruchus maculatus* and of its parasitoid *Anisopteromalus calandrae.* J: days; LI-IV : larvae of instars I to IV; Ny: nymph, Ad: adult

Table 2. Reduction of the emergence of *Callosobruchus maculatus* due to parasitism by *Anisopteromalus calandrae.*

	Number of mated female parasitoids		
	0 (Control)	1	4
Replications	35	10	10
Emergence of buchid (P<0.001***)	100 % a	95.13 %a	58.46 % b
SD	0	4,45	7,01

(Within the line, rates followed by the same letter do not differ significantly, p<0.001).

only 58.46% of *C. maculatus* emerged. This 42.34% reduction of emergence was significantly different from that observed in with only 1-mated female.

It comes clear that *A. calandrae* could control the infestation of cowpea by bruchid. The success of this control depends on the amount of females introduce in the infested cowpea. Previous work pointed out that this parasitoid was a biological control agent of stored grains pests able to control the rice weevil *S. oryzae* (L.) (Coleoptera: Curculionidae). (Perez-Mendoza et al., 1999; Lucas and Riudavets, 2002). It could also be an efficient biological control agent of *S. zeamais* and *Prostephanus truncates* (Helbig, 1998). Other works are to be carry out to precise the conditions of application of this parasitoid and how to take it into account in integrated pest management of stored grain pests using chemicals or other biopesticides.

Acknowledgement

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