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Original Research Article

Response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) to variation in densities of its host *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) within stored grains of *Vigna subterranea* (L.) Verdc (Fabaceae)

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Abstract: Bambara groundnut [*Vigna subterranea* (L.) Verdc (Fabaceae)] is an important source of protein for rural populations in sub-Saharan Africa. During storage, this commodity is destroyed by *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) which have *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) as parasitoid of its larval stages. Analysis of the numerical response among seeds, bruchids and its parasitoids demonstrated that oviposition of *C. maculatus* is great on low mass of seeds. The parasitoid that prefers the fourth larva stage of *C. maculatus* is abundant at the lowest densities of the host. *A. calandrae* could therefore control bruchids on Bambara groundnuts at very low densities, as from their infestation.
Keywords: *C. maculatus, A. calandrae*, Bambara groundnut, biological control, stored products, tritrophic relationship.

INTRODUCTION

Bambara groundnut [*Vigna subterranean* (L.) Verdc (Fabaceae)] seeds are the source of cheaper protein for most African populations in off season. This off season crop is a highly caloric plant (387 kcal / 100 g); rich in vitamins, minerals and protein, its seeds contain most of the amino acids necessary for human nutrition. Indeed it is the richest legume lysine and methionine even if for the moment it does not have important consideration in the malnutrition reduction strategies [1, 2].

The most important difficulty producers are facing to provide Bambara groundnut permanently to malnourished and needy population is noxious activities of stored grain insects pests, mostly bruchids (Coleoptera: Chrysomelidae) [3]. Bruchids larvae bore inside grains, in shelter, feeding on cotyledons. *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in the Bambara groundnut seed stocks, is a parasitoid associated to weevils' larvae. Theoretically, their densities are important when large amount of seeds are infested by weevils. Abundance of weevils provides hosts for parasitoids. Preference of parasitoids depends on the quality and the quantity of the host. The female parasitoid may prefer a specific stage of development of its host from which it synchronizes its life cycle.

It was already established that *A. calandrae* is a parasitoid larvae of the corn borer, maize weevil [6] and cowpea weevil *C. maculatus*[4]. Researches carried out on stocks of cowpea shown that parasitoid coexists with its host and its ability to discover and parasitize it is a criterion to make him a good candidate for biological control of the pest. The present work evaluates the response of a parasitoid to the variation in the density of the population of its host on Bambara groundnut. The numerical response made in this research is estimated at 2 levels of the tritrophic relationship targeted. The first level numerical response concentrates on the variation of intensity of weevils' damages on seeds in relationship with the mass of Bambara groundnut seeds. The second level of estimation of numerical response is on the variation of intensity of parasitisation by *A. calandrae* depending on the variation in host densities.

MATERIAL AND METHODS

The strain of Bambara groundnut seeds used for experiments is WHITE CREAM. It was harvested in a smallholder farm in Borongo, Vina division, in the Adamawa region of Cameroon. Grains used were first sifted and sorted to retain only safe and clean grains without any weevils' infestation. These seeds were thereafter kept for three weeks in a freezer at -10 ° C to kill any possible initial infestation.

The strain of *C. maculatus* used comes from infested Bambara groundnut seeds collected from farmer granaries in the Far North region Cameroon. These infested seeds were sieved and all 24hours –aged adult of *C. maculatus* removed and transferred into glass jars containing approximately 300g of clean Bambara groundnut seeds. The rearing took place in a ventilated incubator monitor at 30° C.

As it was the case for *C. maculatus*, *A. calandrae* used for the experiments was extracted from infested seed collected from smallholder granaries in August 2014 in Mayo Danay division, Far North region. They were reared in laboratory on Bambara groundnut seeds in a ventilated incubator monitor at 30°C.

Analysis of the Numerical Response of Bruchids on Bambara Groundnut

Damages of the bruchid*C. maculatus* were estimated taking into account increase of the biomass of seed offer to the pest. Amount of 5g, 10g, 15g, 20g, and 25g of Bambara groundnut seeds were infested by 2 mated females of *C. maculatus* of 24 hours age. Five replicates were and the set up introduce in the incubator. After 48 hours, the females were removed and their eggs layed counted. The seed were again introduced in the incubator and 50 days after the amount of adult weevils emerged (F1) is counted. Other parameters of weevil damages of such as weight loss, weight of the drill flour, number holes on seeds, parasitism rates were noted. The susceptibility of infection (SI) was calculated from the formula of Dobie [5].

$$\mathbf{SI} = \frac{\mathbf{logF_1}}{\mathbf{D}}\mathbf{X100}$$

Where F1 is the total number of the insects emerging at the first generation and the period D corresponds to the emergence of 50% of that F1.

Analysis of the numerical response of parasitoids on Bruchids

Determination of the larval stage of bruchid preferred by parasitoid

The ventilated incubator allowed the standardization of rearing of bruchid such to have at 30°C a fixed life cycle delay of 28 days. In the permanent rearing put in place, infested seeds were removed at the 4^{th}, 9^{th}, 13^{th}, 18^{th} and 24^{th} days. These estimated delays correspond to different larval and nymph of *C. maculatus* on Bambara groundnut seeds.

To confirm the linkage between the duration and the larval stage expected, some seeds were dissected, the larvae present removed and measured. The preference of the parasitoid was achieve by use of a four-arm olfactometer. For the first trial, infested seeds whose ages correspond to any stage of larval development were tested. Three larval stages and a blank were tested a time. The closer stages were in opposite ends and last opposite the blank. The parasitoid female was put in the middle of the olfactometer. At each end, larval stages were exposed three samples plus reference to the selection of parasitoid females. In the second set of trial, odors sources put into the end of olfactometer were larvae and their cell walls removed from dissected grains. An amount of 10 g of matter were put in the end branch of the olfactometer at each trial.

Intensity of parasitism of *A. calandrae* in relationship with variation in abundance of preferred larval stage of *C. maculatus*

A rearing of parasitoid was made on seeds carrying the larval instar preferred. Two females of *A. calandrae* were introduced on 5g, 10g, 15g, 20g and 25g, of suitable seeds for a delay of 48 hours, five replications were made. The rearing was carrying out in an incubator monitor at 30 ° C. After 35 days, emergence of beetles and parasitoids occurred; they were removed and were counted. The rate of parasitism was calculated with the following formula:

$$\mathbf{Rate\ of\ parasitism} = \frac{\mathbf{Amount\ of\ emerged\ parasitoids}}{\mathbf{Amount\ of\ host\ available}} \times \mathbf{100}$$

RESULTS

Numerical response of *Callosobruchus maculatus* to Bambara groundnut

Large amount of eggs, 23, is laid on the lowest weigh of seeds tested 5g (fig. 1). From 10 to 25g of seeds, oviposition is around 5.55 eggs. The amount of hole where bruchids emerge from the seed is the same at 5g, 10 and 25g around 35 holes per seed.

Damages of *Callosobruchus maculatus* on Bambara groundnut

Analysis of damage parameters indicate that the most abundant losses are observed in the lowest mass of seeds (table1), but the statistical analysis did not show significant differences (P = 0.125> 0.05 between weight loss; P = 0, 4094> 0.05 between the number of adult emergence of weevils).

Analysis of the relationship between the amount of Bambara groundnut, larval stages of bruchids and parasitoid (*A. calandrae*)

Preferential Larval stage of parasitoids

The larval stage of *C. maculatus* preferred by the parasitoid *A. calandrae* accessed by choices made throughout a 4-armed olfatometer made clear in the first round of experiment testing all the existing larval stages that, the 3^{rd} and the 4^{rth} instar larvae are more chosen by the parasitoid for its oviposition(p<0.001***). A second round of tests concentrated on the 3 aged instar larvae pointed out that the 4^{rth} instar larvae is the most preferred by *A. calandrae* for this oviposition (P > 0, 05).

Numerical Response of the parasitoid to variation of density of its host

The parasitoid *A. calandrae* is able to locate and parasitize its host even at lowest densities. The capability of parasitizing by *A. calandrae* increases with the augmentation of the density of 4-insta larvae of *C. maculatus*.

Table 1: Susceptibility of damages of *Callosobruchus maculatus* on Bambara groundnut seeds expressed by the mean emerging *C. maculatus* and mean holes/seed

Seed mass (g)	**5**	**10**	**15**	**20**	**25**
Mean emerging *C. maculates* after 30 days	9,6	8,6	3,4	4,8	4
Mean holes/seed	9,95	6,75	3,4	4,9	4
Dobie Index (%)	**7.12**	5.79	**6.88**	6.56	4,62

(P = 0.125> 0.05 between weight loss; P = 0, 4094> 0.05 between the number of adult bruchids emergence).

Table 2: Number of choice of two female's *A. calandrae* on seeds infested by four stages larvae of *C. maculatus* in four arm olfactometer

Larval instars of *C. maculatus*	Preference of *A. calandrae*			
	First round (n=5)		Second round (n=5)	
	Mean choice	Percentage (%)	Mean choice	Percentage (%)
1rst	1	12,01	/	/
2nd	1,33	15,97	4	17,3
3rth	**3,33**	**40,01**	5	21,7
4rth	**2,66**	**31,92**	**14**	**61**

(First round n=5, p<0.001).
(Second round n=5, P > 0, 05).

Table 3: Evaluation of the number of parasitoids emergence depending on the abundance of larvae and the amount of seeds

Mass of seeds	**5g**					
Mean host number/gr of seed	5	6	7	8	9	10
Mean emergence of parasitoids	3,75	3	3,25	2,75	3,5	3,5
Mass of seeds	**10g**					
Mean host number/gr of seed	5	6	7	8	9	10
Mean emergence of parasitoids	3,25	3,5	2,25	2,5	3	3,5
Mass of seeds	**15g**					
Mean host number/gr of seed	5	6	7	8	9	10
Mean emergence of parasitoids	1,75	2,5	2,25	3	2,75	2,75

(n=5, p>0,05)

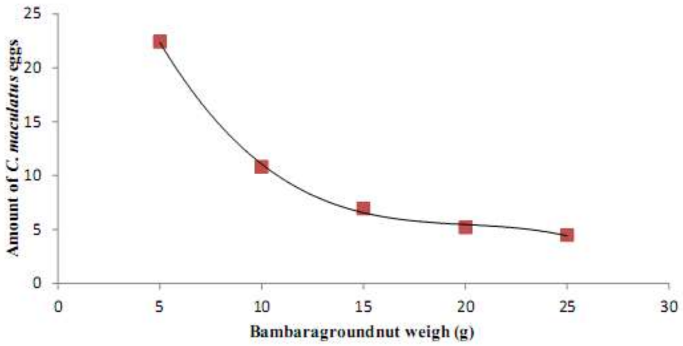

Fig. 1: Evaluation of the number of *C. maculatus* eggs depending on the amount of seeds of Bambara groundnut

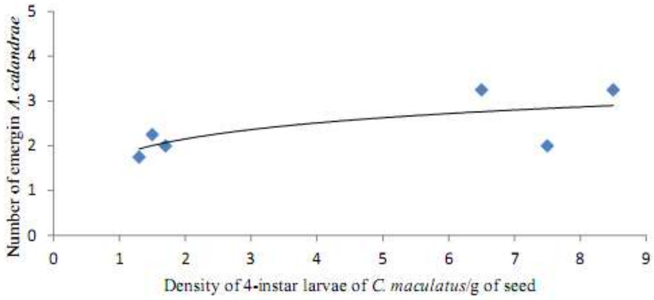

Fig. 2: Evaluation of the number of parasitoids emergence depending on the abundance of larvae

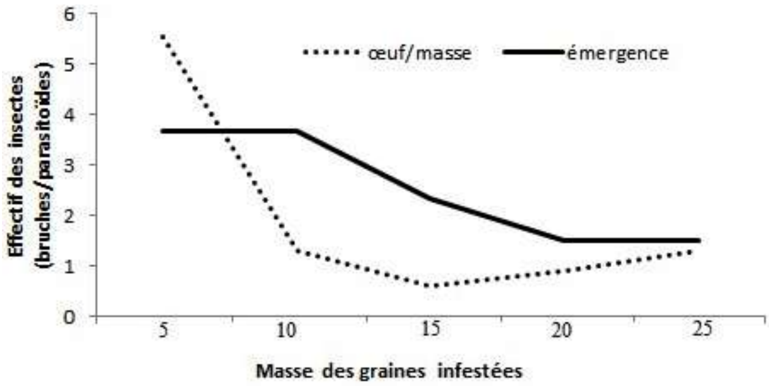

Fig. 3: Evolution of parasitoids emergence depending from the abundance and the dispersion volume larvae on seeds of Bambara groundnut

DISCUSSION

Analysis of the relationship between the dynamics of biomass of Bambara groundnut and *C. maculatus*

To ensure good survival to its offsprings, females of bruchids or of parasitoid have the task to make a trade of between the quality of the food and the amount of eggs to lay. The female *Callosobruchus* species lays few eggs on clean seeds to minimize intraspecific competition between larvae [6]. Females' ovipositing behavior reducing competition among larvae does not suggest uniform distribution of the eggs on the seeds but some changes may occur in the mass of seeds increases. The amount of seeds influences the eggs number laid by *C. maculatus*. Females gather more their eggs on certain seeds when the amount is lowest leaving healthy seeds. The number of eggs is low when the amount of seeds is increasing (fig. 1). Danho, [7] observed that females increase their oviposition in relationship with the number of seeds available till the optimum clutch size. Burlando and Fava [8] show that *Sitophilus granarius* tends to lay egg on seeds as they are available. Longstaff reported that females tend to group their eggs, but the factor mentioned here and seed size; when the size is normal distribution is random. Pests (*C. maculatus*) present in Africa have differences in biological and behavioral level, among them: the dispersal of eggs [9]. The chance is that females avoid any competition between females minimizing competition between their offspring. Indeed, when a female operating a *patch*, gathers eggs leaving healthy seeds. The Dobie index shows a high susceptibility of this cultivar Bambara groundnut on low masses. This high susceptibility results in a decrease in the duration of emergence of adult *C. maculatus*, the emergence is more rapidly when the amount of seeds or quantity of resources is low. This shortening of development time in *C. maculatus* seems to be due to an evolutionary process that is related to the low amount of resources. There exist a genetic factor in *C. maculatus* acting in the physiology of these insects which allows them to adapt to conditions such that a small quantity and poor quality of resources that individuals differ from most of those months there whose income conditions are suitable. Which is justified by the passage of the non-sailer shape the sailer form including intermediate forms that is controlled mainly by the conditions in inventories (quality of the food substrate, larval density in seeds, seed moisture, temperature variation Thermo period) [10].

Analysis of the response of the parasitoid to the availability of their host

The presence of *A. calandrae* on larvae of different stages shows a more efficient emergence of larvae 18 days (fig. 2). The location good hosts for parasitoids assume that females perceive certain chemical cues. The choice of nesting site requires a precise size and age of the host that the female parasitoid must calculate this cycle to synchronize with that of its offspring which she recognizes a probability of better survival of the larvae. In our experimental conditions, the affinity of parasitoid females to recognize and exploit a specific stage of *C. maculatus* for its development proves the effectiveness of the development of parasitoids on Bambara groundnut seeds

The way to distribute the eggs by female *C. maculatus* can influence the probability of female parasitoids find the larvae; By varying the mass of seeds and the number of hosts of 4^{th} larvae's, the emergence of parasitoids is higher on the populations of larvae in quantities of seeds and the number of hosts are lowest. The hypothesis of parasitism based on the number of hosts did not seem to be supported with certainty. However, it is the amount of seed by dispersal of the hosts in combination with the abundance of larvae that influences the rate of parasitism. The concentration of larvae in a small amount of Bambara groundnut promotes female parasitoids find hosts, females are less likely to hosts on the seeds of the great masses whose larvae are less concentrated. One can understand from the results that there is a minimum rate of *C. maculatus* on Bambara groundnut seeds below which their parasitoids cannot access.

CONCLUSION

During this study, the distribution strategy of *C. maculatus* eggs is identified. The results of this work show that the quantity of seeds influences the number of eggs laid by *C. maculatus*. The number of eggs is low when the amount of seeds is increasing. To accomplish our purpose, we first evaluated the affinity of parasitoid females to recognize their host. We noted that the parasitoid recognizes 4^{th}stage of its host. Second, we evaluated the rate of parasitism on the amount of Bambara groundnut according to abundance of larvae. Our results indicate that the distribution of *C. maculatus* eggs on Bambara groundnut seeds influences the probability of females meeting the hosts. The concentration of larvae in a small amount of Bambara groundnut promotes female parasitoids find hosts, females are less likely to hosts on the seeds of the great masses whose larvae are less concentrated. The validity of these results will allow the implementation of strategies for using *A. calandrae* in biological control against *C. maculatus*, pests Bambara groundnut.

REFERENCES

1. Minka DR, Bruneteau M; Partial chemical composition of Bambara pea (Vigna subterranea L. Verdc.). Food Chem., 2000; 68: 273–276.
2. Amarteifio JO, Tibe O, Njogu RM; The mineral composition of bambara groundnut (Vigna subterranea (L.) Verdc) grown in Southern Africa. African Journal of Biotechnology, 2006; 5:2408-2411.
3. Costa RR, De Sousa1 AH, Faroni LRDA, Dhingra OD, Pimentel MAG ;Toxicity of mustard essential oil to larvae and pupas of Sitophiluszeamais (Coleoptera: Curculionidae). 9^{th} International Working Conference on Stored Product Protection, 2006 ; 908-913.
4. Ngamo TLS, Kouininki H, Ladang YD, Ngassoum MB, Mapongmetsem PM Hance T;Pontentiel of Anisopteromalus calandrae (Hymenoptera: Pteromalidae) as biocontrol agent of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) African journal of agricultural research, 207; 2(4): 168-172.
5. Dobie P; The laboratory assessment of the inherent susceptibility of maize varieties to post harvest infestation by Sitophiluszeamais Motsch. (Coleoptera: Curculionidea) infesting field corn. Journal of Entomological Science, 1974; 21:367-375.
6. Giga DP, Smith RH; Varietal resistance and intraspecific competition in cowpea weevils Callosobruchus maculatus and Callosobruchus chinensis (Coleoptera: Bruchidae). Journal of Applied Entomology, 1981; 106: 451-464.
7. Danho M ;Strategie reproductive du charançon du maïs, Sithophiluszeamays (Coleoptera: Curculionidae), Thèse de Doctorat, Faculté universitaires des sciences Agronomiques de Gembloux, 2003.
8. Fava A,Burlando B; Influence of femelle age and grain availability on the ovipositioal pattern of the wheat weevil Sithophilusgranarus (Coleoptera: Curculionidae). European Journal of Entomology, 1995; 92:421-426.
9. Alebeek FAN, Van Bezemer TM, Huis A, Van Lenteren J, Van C ; Foragingbehavior of the eggparasitoidUscanalariophaga. Towardsbiological control of bruchidspests in storedcowpea in West Africa. Chap1996; 6 : 95- 110.
10. Patounn O ; Le déterminisme du polymorphisme imaginal chez callosobruchusmaculatus (fab), coléoptère Bruchidae. Son importance sur la biologie des populations de ce Bruchidae. Thèse de doctorat en Sciences biologiques et fondamentales appliquées. Psychologie à Tours, 1991.