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Dynamics of parasitoid-host interaction: Application of the case of *Callosobruchus maculatus* (Chrysomelidae) and *Dinarmus basalis* (Pteromalidae)

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Abstract: This paper carried out the dynamics of the interaction between the parasitoid *Dinarmus basalis* and its host the cowpea weevil *Callosobruchus maculatus*. We test directly the effect of nutrients on the biological parameters of *D. basalis*. A correlation investigation was carried out between the biochemical composition of *C. maculatus* fourth instar larvae and the performances of its parasitoid *D. basalis*. Results showed that biological performance of *D. basalis* was closely related to their host *C. maculatus*. This study revealed the importance of protein and lipids contents on the increase of parasitism rate.

Keywords: *Callosobruchus maculatus*; biochemical composition; dynamics of interaction; *Dinarmus basalis*; strain; cowpea; chickpea

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1. Introduction

Some theoretical and experimental studies focused on the influence of parasites on their host populations [1, 2]. Parasitoid insects develop at the expense of others organism. The quality of the hosts will directly influence the development and survival of parasitoids. The number of offspring produced by females therefore depends on their ability to locate hosts and determine their quality [3]. Parasitoid insects reproduce by laying their eggs on other insect hosts. In fact, these hosts then represent the only nutrient resource available for the development of the larva. Furthermore, the host quality directly influences the developmental success of the larva parasitoid [4]. *Dinarmus basalis* (Rondani, 1877) (Pteromalidae) is the most used in biological control against cowpea weevil *Callosobruchus maculatus* (Fabricius, 1775) (Chrysomelidae) [5, 6]. This parasitoid is effective against the larvae of 4th instar and nymphs of its host [7]. In this stuy, we carried out a detailed physiological investigation of biochemical composition of proteins, lipids, sugars and glycogen in whole body extract of *C. maculatus* L4 larvae. In addition, the main objective of this study is to understand the egg-laying strategies of *D. basalis* females in order to enhance their parasitism potential in the control against the weevil *C. maculatus* during storage.

2. Materials and Methods

2.1. Insect rearing

2.1.1. *Callosobruchus maculatus* rearing colony

C. maculatus rearing colony was initiated from infested chickpea since 2011 and maintained in controlled condition (at 27°C and 70 % RH in a 12/12 h L/D) in Laboratory of Biotechnology Applied to Agriculture at the National Agricultural research Institute of Tunisia (INRAT). In this study, three strains of the cowpea weevil were used. A laboratory colony maintained on chickpea (*Cicer arietinum* L.) for 45 generations; then reared on ancestral host cowpea (*Vigna unguiculata* L.) for 15 generations. Additionally, a wild colony of *C. maculatus* collected from infested chickpea crops was employed.

2.1.2. *Dinarmus basalis* rearing colony

D. basalis colony was reared on *C. maculatus* larvae (L4). After 16-18 days the bruchid oviposition period, the 4th larval instars were collected and used.

2.2. Whole host body extracts

From each *C. maculatus* strains reread on *C. arietinum*, *V. unguiculata* seeds and the wild strain, sixty cowpea weevil larvae L4 were extracted. Fourth instar (L4) larvae were weighed and individually placed in Eppendorf tube (1.5 mL) and placed in ice. To extract whole body, the larvae was crushed with a micro-pestle. The extract was analyzed individually for each larva using 60 samples for sugar, lipid, glycogen and protein analysis.

2.2.1. Lipid, sugar and glycogen analyses

The colorimetric techniques following Van Handel & Day [8] and Giron et al. [9] were used for the total lipids, sugar and glycogen quantification. For this purpose, 30 samples were used. To each sample, sodium sulphate (2%) and chloroform-methanol (1: 2) were appended. After centrifugation, the supernatant were used for the lipids and sugars quantification, althought, the precipitate were utilized for the glycogen.

For the lipid analysis, the supernatant was putted into a borosilicate tube. To evaporate the solvent, the tube was placed in an ethylene-glycol heating block at 90 °C. Then, 40 µL of 95 % sulphuric acid were added, and the tube reheated at 95 °C for 2 minutes. After cooling, the vanillin reagent was appended to the tube and read in a spectrophotometer at 525 nm.

For the sugar analysis, as above, the supernatant was transferred into an ethylene-glycol heating block at 90°C to evaporate the solvent. The tubes were placed at 90 °C for 15 min, after adding 1 mL anthrone reagent, and read in a spectrophotometer at 625 nm.

The precipitated was used for glycogen quantification. The precipitated was washed with 400 µL of 80 % methanol. Samples were then vortexed and centrifuged for 5 min. The supernatant was eliminated, 1 mL of anthrone reagent was added and tubes were placed at 90°C for 15 min. The samples were filtered and read in the spectrophotometer at 625 nm after cooling. Calibration cruves were obtained using vegetable oil for lipids and glucose for sugars and glycogen

2.2.2. Protein analyses

For Protein analysis, 30 samples were used. This investigation was carried out via the Bradford assay procedure [10]. 800 µL of physiological water (0.15M NaCl) containing 0.001% Triton X-100 was added to each sample and then placed 5 days in the fridge to allow time for the Triton-X to dissolve the proteins. 200 µL of Bradford Reagent reactive were then added. Samples were read at 595 nm. Calibration cruves were achieved using bovine serum albumin.

2.3. The effect of biochemical nature on reproductive parameters and demographic traits of Dinarmus basalis

The effect of biochemical nature on the reproductive parameters and demographic traits of *D. basalis* were carried out. The adults longevity was determined. Furthermore, the total number of eggs laid, the fertility rate, the emergence rate and parasitism rate were determined.

2.4. Statistical analysis

Statistical analyses were performed using SPSS statistical software version 20.0 (IBM Corporation, New York, USA). All values given were the mean of three replications and were expressed as the mean ± SD. One-way ANOVA followed by Duncan test were used for biochemical composition. Where necessary, data were transformed by common logarithm or square root to meet the assumptions of normality. For each reproductive parameters and demographic traits of *D. basalis* data were subjected to two-way ANOVA. The means were separated using the Least Significant Difference (LSD) ($P < 0.05$). Pearson's correlation coefficient were established between biochemical nature and reproductive parameters and demographic traits of *Dinarmus basalis*. A hierarchical cluster analysis based on Ward's method and Euclidean distances was made.

3. Results

3.1. Biochemical composition of whole body extracts

The composition of proteins, lipids, sugars and glycogen in whole body extract of *C. maculatus* L4 larvae are represented in Figure 1.

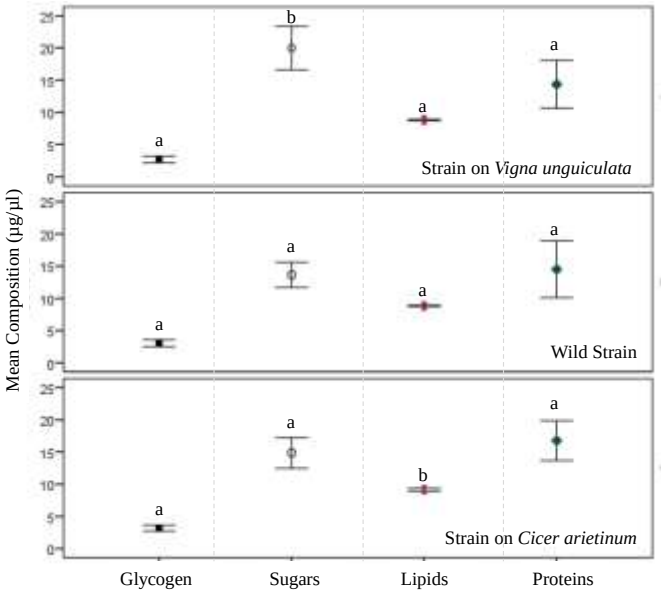

Figure 1. Proteins, lipids, sugars and glycogen quantification in whole host body extract of *Callosobruchus maculatus*. Different letters indicate significant differences (at $P<0.05$), for each parameters (proteins, lipids, sugars and glycogen) comparisons were made among strains. Each value is the mean ± SD of three replicate.

Results show that a biochemical nature varied according *C. maculatus* strains. Furthermore, the glycogen content reached 2.7, 3.04 and 3.19 µg/µL respectively for cowpea, chickpea and wild strain. There were no statistical differences in the amount of glycogen between *C. maculatus* strain ($F = 1.24$; $P = 0.302$). Additionally, the protein content showed no significant variation between *C. maculatus* strain ($F = 2.04$; $P = 0.140$). Proteins content varied from 13.14 to 16.91 µg/µL respectively for cowpea and wild strain. In contrast, sugar and lipids content widely different among *C. maculatus* strains. In fact, the highest lipids content were recorded in wild strain 9.15µg/µL, against 8.82 and 8.83 µg/µL for chickpea and cowpea strain respectively ($F = 7.41$; $P = 0.002$). The sugar content was significantly affected by cowpea weevil strains ($F = 7.37$; $P = 0.002$).

3.2. Hierarchical cluster analysis (CA)

In order to investigate the relationship between biochemical natures of whole host body extract of the three strains of *C. maculatus*. The hierarchical cluster analysis based on

Euclidean distances was presented in Figure 2. Three clusters were distinguished at a Euclidean distance of 10.0. According to the silhouette index. In the first group (Cluster A), represented by *C. maculatus* strain reared on cowpea which was characterized by the highest sugar content and the lowest glycogen content. Cluster B with included *C. maculatus* strains reared on chickpea and wild strain, which were characterized by similar levels of sugar, and these two strains were reared on chickpea.

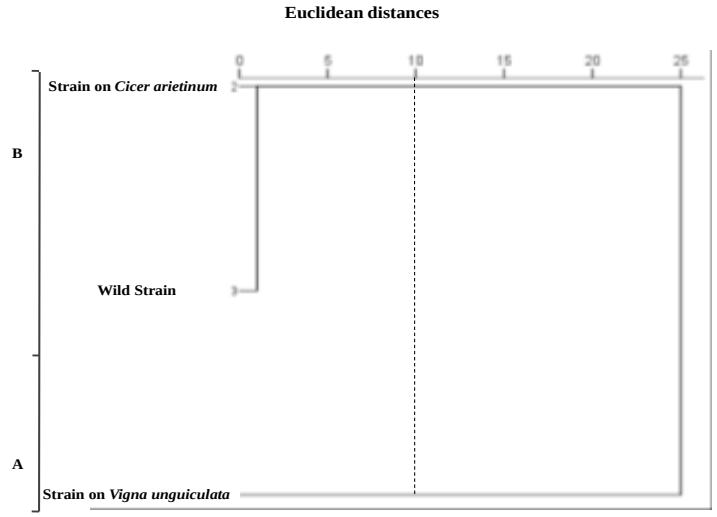

Figure 2. The hierarchical cluster analysis using Euclidean distance based on biochemical nature of whole host body extract of *Callosobruchus maculatus*.

3.3. The effect of biochemical nature on reproductive parameters and demographic traits of Dinarmus basalis

To determined the effect of biochemical nature of *C. maculatus* strains on reproductive parameters and demographic traits of *D. basalis* a correlations analyses were performed (Table 1). Therefore, weight of *C. maculatus* larvae was correlated with sugars content (r = 0.5), *D. basalis* longevity (r = 0.631), parasitism rate (r = 0.503) and means growth rate (r = 0.396). Further, the relative proportion of lipids varied significantly and a positive high correlation were recorded with parasitism rate (r = 0.481), means growth rate (r = 0.521) and with development period (r = 0.54). Moreover, the protein content correlated with parasitism rate (r = 0.258). Nevertheless, no correlation was observed with glycogen content.

Table 1. Correlations analyses between biochemical nature and reproductive parameters and demographic traits of *Dinarmus basalis*

	Strains	**weight**	**Glycogen**	**Sugars**	**Lipids**	**Proteins**	**L‡**	**PR‡**	**DP‡**
Sucre	-0,408**	0,500**	-0,130	1					
Lipids	0,470**	0,305	0,152	0,080	1				
Proteins	0,121	-0,015	0,062	-0,043	0,315	1			
L‡	-0,569**	0,631**	-0,180	0,519**	-0,037	0,119	1		
PR ‡	0,500**	0,503**	0,093	0,069	0,481**	0,258*	0,427**	1	
SR‡	-0,679**	0,582**	-0,205	0,535**	-0,117	0,085	0,990**	0,296*	
DP‡	0,866**	0,220	0,194	-0,193	0,540**	0,219	-0,082	0,866**	1
MGR‡	0,674**	0,396*	0,139	-0,042	0,521**	0,250	0,223	0,977**	0,953**

* Significant at 5% level. ** Significant at 1% level.

‡L=longevity, PR=Parasitism rate, SR= sex-ratio, DP=Development period, MGR=Means growths rate

These results suggest that the differences in the relative chemical composition of larvae L_4 had a significant effect on reproductive parameters and demographic traits of *D. basalis*. Thus, the higher weight of *C. maculatus* forth larvae, the higher the performance of *D. basalis* increases. *D. basalis* female can advance in fitness in terms of increased of longevity, mean growth rate and parasitism rate. In general, an increase of parasitism rate with an increase in host size was observed.

4. Discussion

Endoparasitoids usually lay and develop their eggs in the bodies of host, and their offspring grow by consuming host tissues or hemolymph [11]. Previous research demonstrated that the performance of parasitoid wasps was affected by differences in the quality of the host's diet. Ours results revealed that biochemical composition of whole body extracts of *C. maculatus* larvae varied according the diet (cowpea, chickpea seeds) which is in accordance with results obtained by Spitzen and Van Huis, [12]. On the other hand, host-quality dependent sex allocation assumes that host quality (e.g. size, age, or nutritional quality) differentially influences the reproductive success of offspring [12]. In this context, previous research demonstrated that fecundity and longevity varied according different adult diets. Furthermore, the proteins content acquired from host-feeding provide to meet the high amino acid demands associated with egg production [9, 13]. The two fundamental characteristics of the life-history traits were the nature of nutritional resources and the pattern of allocation of nutrients. This aspects have critical consequences for fitness [9]. Indeed, the longevity of insects, specifically parasitoids was affected directly or indirectly by specific sugar [14]. This study showed that parasitoids longevity increase and offspring was in favor of females when host was rich in sugars content. In order to understand an organism's ecology, nutritional physiology, behaviour, life-history and population dynamics were dependly studied.

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